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A full set of monosomic addition lines in *Beta vulgaris* from *Beta webbiana*: morphology and isozyme markers

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Summary. Nine different monosomic additions in *Beta vulgaris* from *Beta webbiana* were characterized through morphological characters and isozyme markers. The effect of the alien chromosome on the morphology of the recipient species is chromosome specific, and nine morphotypes could be distinguished. The added chromosome caused a growth reduction in the recipient plants. Eleven isozyme systems were used as marker systems. A 6PGDH band was found as a marker for chromosome 7, which contains a resistance gene for the beet cyst nematode in monosomic additions from *Beta procumbens* and *Beta webbiana*. A difference in the 6PGDH zymogram pattern between the two species with respect to this chromosome has been noted.

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

Introduction

The wild species of Section Procumbentes (*Beta procumbens* 2n=18, *Beta webbiana* 2n=18 and *Beta patellaris* 2n=36) have primarily been of interest to sugar beet breeders because their genomes contain the genes for resistance to the beet cyst nematode *Heterodera schachtii*. Also of economic importance are their monogermity and resistance to Cercospora leaf spot and curly top virus (Coons 1954). Efforts to hybridize these species to sugar beet have been reviewed by Van Geyt et al. (1990a).

Another area of interest is the question of whether *B. procumbens* and *B. webbiana* should be classified as two distinct species or as two extremes of a single ecospecies (Curtis 1968). No difference in 11 isozyme patterns has been found between the two species; thus, raising further doubt as to their classification as two different species (Wagner et al. 1989).

Monosomic addition lines, wherein a single chromosome of a wild species of Procumbentes is added to the diploid complement of *B. vulgaris*, have been an important means for breeding nematode-resistant beets (Savitsky 1975; Speckmann and de Bock 1982; Speckmann et al. 1985; Heijbrook et al. 1983, 1988; Löptien 1984a, b; De Jong et al. 1985). This approach could, however, be further utilized for the localization of specific genes, especially desirable ones, for ease in transferring them. For instance, resistance to the Rhizomania vector *Polymyxa betae* in monosomic additions from *B. procumbens* has been localized on chromosomes 4 and 8 (Paul et al., in preparation). Addition lines could, also be used to compare chromosomes of different genomes, for example, those of *B. webbiana* and *B. procumbens*, for taxonomic clarification.

Nine types of monosomic additions in *B. vulgaris* representing the nine different chromosomes of *B. procumbens* have been morphologically described (Lange et al. 1988), and their corresponding isozyme markers except for chromosome 7 have been reported (Van Geyt et al. 1988). In this article we report on the morphology and isozyme markers of the nine types of monosomic additions in *B. vulgaris* from *B. webbiana* including an isozyme marker for chromosome 7, which is also known to contain the gene for resistance to the beet cyst nematode. A difference in zymogram pattern with respect to this chromosome between monosomic additions from *B. procumbens* and *B. webbiana* has also been noted that could possibly serve as a demarcation line between the two species.

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Materials and methods

The monosomic additions used in this study were progenies of the first backcross of triploid hybrids, resulting from a cross between colchicine-tetraploidized *B. vulgaris* and *B. webbiana*, to diploid sugar beet. Germination was very poor, and from among those germinated, some were very weak and seemed unable to form roots. To recover more plants, those weak ones were grafted to cultivated beet (Löptien 1984a).

Three lines were the major source of the monosomic additions ($2n=18+1$), and their distribution is shown in Table 1. The chromosome number was determined according to the technique described in Löptien (1984a). In addition to the monosomic additions, however, plants having 2 or more added chromosomes were considered for electrophoretical study. BC_2 progenies of some monosomic additions were also included for further isozyme analysis. Existing types a, b and c monosomic addition lines in Hanover were used to confirm results.

The enzymes were extracted from mature leaves. The extractions were carried out with extraction equipment using 250 µl of a 5% sucrose solution with 0.1% mercaptoethanol for every 200–600 g of leaf material. The extract was centrifuged at 14,000 rpm for 15 min at 4 °C. For ADH induction, mature leaves were soaked in water in sealed glass containers for 48 h in the dark before extraction.

Eleven isozyme systems were used for identifying the alien chromosome. ICD, MDH, ACO, PRX, 6PGDH and ADH were separated on horizontal gel electrophoresis for 3 h at 300 V. LAP, ACP and GOT were electrophoresed on 8% vertical polyacrylamide flat gels for 3 h at 500 V, while SOD and PGM on 6% vertical polyacrylamide flat gels for 2 h at 360 V. The separation conditions and staining procedure for ACO, ACP, GOT, ICD, LAP, MDH and PGM were adapted from Wagner (1990) and Wagner and Wricke (1991); for ADH, 6PGDH and SOD, from Vallejos (1983); and for PRX, from Endo (1972). For every run at least two samples of *B. vulgaris* and *B. webbiana* were used as controls. Where necessary, samples of *B. procumbens* or *B. patellaris* were also included for comparison.

Results and discussion

Chromosome 1

The enzyme isocitrate dehydrogenase (ICD, Threo-D_s isocitrate: $NADP^+$ oxidoreductase (decarboxylating) E.C.1.1.1.42) was localized in this chromosome. Monosomic additions with chromosome 1 showed one or two extra bands on the fast zone as well as the gene products between the parental species (Fig. 1). The ICD bands of *B. webbiana* varied from one to four bands and migrated faster than those of *B. vulgaris*. In *B. vulgaris*, three types of ICD zymograms – two with three bands and one with five bands – were observed, which is in agreement with previous results (Van Geyt et al. 1988; Smed et al. 1989; Wagner 1990). The ICD system in *B. vulgaris* is controlled by two genes (Smed et al. 1989). Monosomic additions from *B. procumbens* and *B. patellaris* of this type also exhibited the same extra bands. Interspecific heterodimers between the gene products of *B. procumbens* and *B. vulgaris* have likewise been reported (Van Geyt et al. 1988; Smed et al. 1989).

Plants with an extra chromosome 1 have been previously described as type a and resistant to the beet cyst nematode (Löptien 1984b). They are annuals, have narrow leaves and erect growth (Fig. 3a).

Table 1. Monosomic additions from *Beta webbiana* used in the electrophoretical study

Chromosome number	Isozyme markers	Number of plants			
		3153	3155	3156	Total
1	ICD	–	5	–	5
2	MDH, PGM	1	12	7	20
3	LAP	1	21	1	23
4	ACP, GOT Band 6	–	–	1	1
5	GOT Band 7	–	–	5	5
6	ICD, PRX	–	4	–	4
7	6PGDH fast zone	–	–	3	3
8	SOD, ACO, 6PGDH slow zone	2	12	2	16
9	ADH	–	2	3	5

Chromosome 2

Markers for chromosome 2 are malate dehydrogenase (MDH; L-Malate: NAD^+ oxidoreductase E.C. 1.1.1.37) and phosphoglucomutase (PGM; α-D-glucose 1,6 phosphomutase E.C. 5.4.2.2). Monosomic additions containing chromosome 2 had one or two extra bands that corresponded to the two fastest bands of *B. webbiana* (Fig. 1). No hybrid bands were formed, a result similar to findings in chromosome 2 monosomic additions from *B. procumbens* (Van Geyt et al. 1988). On the *Mdh1* locus, or the fastest zone, we observed three phenotypes with the homozygotes having one band and the heterozygotes three bands. Five loci have been reported for the MDH system (Aicher and Saunders 1990), and *Mdh1* codes for a dimeric enzyme (Aicher and Saunders 1990; Van Geyt et al. 1990b; Wagner 1990).

The PGM marker band for chromosome 2 was visualized on the slow zone of *B. vulgaris* and in the same position as that of the faster of the two bands of *B. webbiana* (Fig. 1). In *B. vulgaris*, two zones of activity were observed within the fast zone, one fast or one slow band, indicating that this locus codes for a monomeric enzyme with two alleles (Smed et al. 1989; Aicher and Saunders 1990). The slow zone was not polymorphic: one band or two thin bands could be observed. No hybrid bands were formed in the monosomic additions.

The effect of the alien chromosome on the recipient plant is very specific, and only one morphotype was observed (Fig. 3a, b). In monosomic additions from *B. procumbens* two morphotypes have been found (Lange et al. 1988). Plants have semi-erect growth, rather flat leaves that are leathery and glossy, and in maturity the petioles tend to fold at the base to form a rosette pattern. Com-

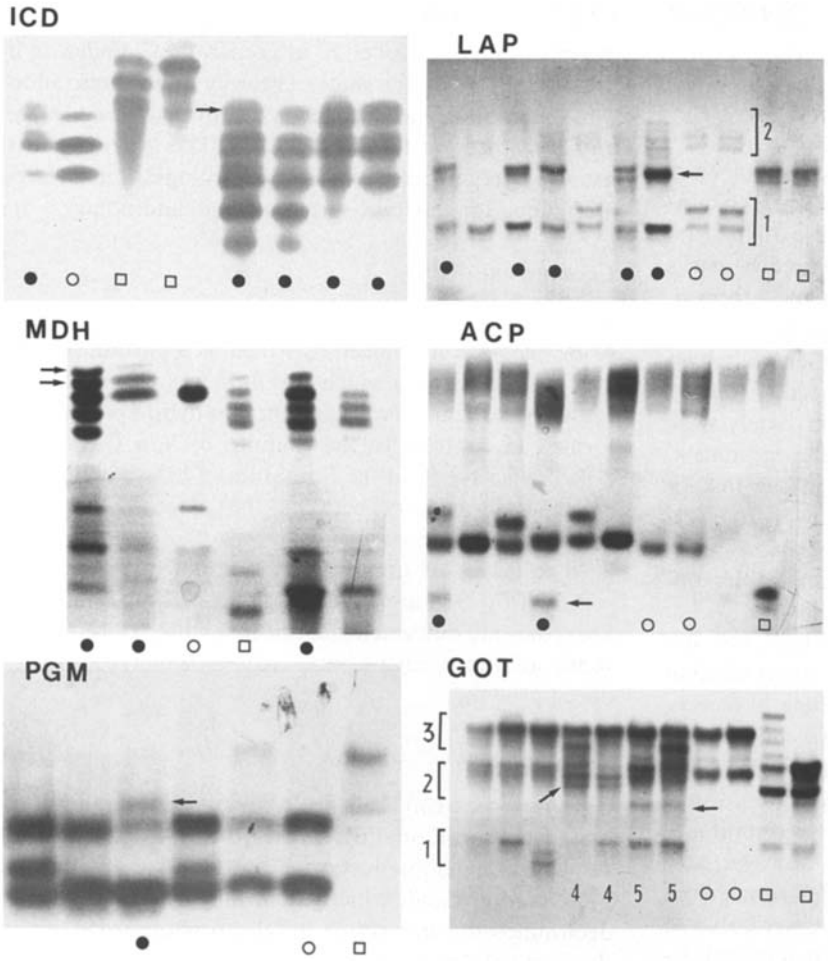

Fig. 1. The enzyme systems *ICD*, *MDH*, *PGM*, *LAP*, *ACP* and *GOT*. *Arrows* point to the marker band(s). ● Monosomic additions, ○ *Beta vulgaris*, □ *Beta webbiana*. The *numbers* correspond to the added chromosome for the particular isozyme system. *Unmarked* zymograms are monosomic additions negative for the isozyme stained

pared to the other monosomic additions, they are among the smallest and weakest. A very characteristic chromosome effect could be noticed at the flowering stage where the plants showed ribbon-like flowering stalks.

Chromosome 3

The enzyme system leucine aminopeptidase (LAP; α-Aminoacyl peptide hydrolase E.C. 3.4.11.1) serves as the marker for plants with an extra chromosome 3. The LAP marker is shown as one or two bands of *B. webbiana* migrating at intermediary position between the two activity zones of *B. vulgaris* (Fig. 1). Monosomic additions with chromosome 3 exhibited the sum of bands seen in both species, and no evidence of a hybrid band was found. Three alleles were observed in the LAP1 zone of *B. vulgaris*. The LAP2 zone where two or more bands were usually found is generally weakly stained or no bands appear at all.

The zymogram of monosomic additions from *B. webbiana* was compared with those monosomic additions from *B. procumbens* and *B. patellaris* developed in our institute. In comparison to *B. webbiana* and *B. procumbens*, *B. patellaris* showed two extra bands migrating in the same region as that of LAP1 of *B. vulgaris* (not shown). More plants have to be tested, however, to confirm this finding. But what seems to be interesting is that the monosomic additions from *B. webbiana* and *B. procumbens* exhibited the first two slower bands relative to the four bands noted in the position between the two zones of activity in *B. vulgaris*, while the monosomic additions from *B. patellaris* exhibited the next two faster bands. This again confirms that *B. patellaris* is a distinct species from *B. webbiana* and *B. procumbens*. With this enzyme system no difference between *B. webbiana* and *B. procumbens* could be seen with respect to chromosome 3 monosomic addition.

Even without the LAP marker, monosomic additions with chromosome 3 from *B. webbiana*, *B. procumbens* and *B. patellaris* could be easily identified through morphological characters. Monosomic addition lines from any of these three species look basically the same (Fig. 3a, b). They have the glossiest leaves among the additions, which could possibly be a specific effect of the

alien chromosome. Lange et al. (1988) found no specific morphotype with this type of monosomic additions.

Chromosome 4

Acid phosphatase (ACP; Orthophosphoryl monoester phosphohydrolase E.C. 3.1.3.2) and glutamate oxaloacetate transaminase (GOT; L-Aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1.) have been used as marker systems for additions carrying chromosome 4 from *B. webbiana*. In the ACP system the products of *B. webbiana* migrated faster than those of *B. vulgaris*. Generally, two densely stained bands of *B. webbiana* appeared on the gel, but there were runs where four thin bands were noted. These bands serve as markers for chromosome 4. Four different alleles have been found in the fast or major zone of ACP in *B. vulgaris*. The slower zone was usually smeared. Additions contained the bands from both parental species, and no hybrid bands were observed (Fig. 1).

The sub-lethal effect of chromosome 4 in *B. procumbens* has been documented (Lange et al. 1988) wherein monosomic additions with this chromosome have very much reduced growth and plants die at the seedling stage. This could possibly be true in the case of *B. webbiana* for we have only succeeded in obtaining one monosomic addition carrying chromosome 4 that survived until maturity, and that due to grafting. We were, however, able to obtain plants with two or more added chromosomes whereby chromosome 4 was present that were able to reach maturity, due perhaps to the masking of the sub-lethal effect of chromosome 4. These plants were used to confirm the isozyme markers for this chromosome type.

GOT band 6 (Fig. 1) of *B. webbiana* could also be used to mark additions with chromosome 4. Similar to the findings of Van Geyt et al. (1988) in *B. procumbens*, we found at least seven bands in *B. webbiana*, which we also numbered consecutively in order of increasing mobility. In additions with chromosome 4, the bands could be readily seen at GOT2 zone (Fig. 1) with two bands coming from *B. vulgaris* and one band corresponding to GOT band 6 of *B. webbiana*. The *B. vulgaris* bands in GOT2 were usually fused and appeared as one thick band. There was no indication of the presence of hybrid bands.

For reasons not yet understood, the GOT system in *B. vulgaris* is not an easy one to study. Bands observed in first studies did not appear in subsequent testing, and seasonal change could not be discounted as a possible influencing factor. It was only after much testing that we could be truly sure of our results. The ACP marker for chromosome 4, however, is usually expressed and is in our opinion, the preferred enzyme marker system.

Despite the sub-lethal effect of chromosome 4, monosomic additions of this type form *B. procumbens* have been reported to be resistant to the Rhizomania vector, *Polymyxa betae* (Paul et al. in preparation) rendering it interesting for further study. The only monosomic addition we obtained has deep green and oblong ovate leaves and is leathery in texture (Fig. 3b). The leaf margin is usually finely serrated. These morphological characters are unique for this type of monosomic addition.

Chromosome 5

Monosomic additions positive for GOT band 7 (Fig. 1) of *B. webbiana* have been classified as a chromosome 5 type. In *B. webbiana* which was used as a control and is shown here, band 7 is not present. No hybrid bands were formed in contrast to the findings of Van Geyt et al. (1988), who reported the formation of heterodimers migrating intermediary between the parental bands in monosomic additions from *B. procumbens*.

The leaves of monosomic additions with chromosome 5 (Fig. 3b) are rather flat and with an acute leaf apex; they are dull green and fine in texture. These monosomic additions have a semi-erect growth type and reduced growth.

Chromosome 6

Chromosome 6 can be identified in monosomic additions by means of cathodal peroxidase (PRX, Donor: hydrogen peroxide oxidoreductase; E.C.1.11.17) and ICD. The PRX marker band, which could only be diagnostically determined through starch gel electrophoresis (SGE), is the major and fastest band of *B. webbiana*. We found that no other band co-migrated with the *B. webbiana* PRX band. Polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing (IEF) gave, however, different results whereby monosomic additions could not be distinguished from the sugar beet. Whether the discrepancy was due to the electrophoresis conditions or to the pH of the gel could not be determined. The monosomic additions exhibited the PRX marker band and the sugar beet band, which is also the major and fastest band. (Fig. 2). In *B. vulgaris*, the fastest migrating zone (PRX1) was not polymorphic and was characterized by a single band, but the next fastest zone (PRX2) behaved as a monomer (not shown). Both zones could be observed in mature leaves. Only the fastest zone could be observed in young plants. No hybrid bands were observed between parental species.

Lange et al. (1990) reported that monosomic additions with chromosome 1 from *B. procumbens* were positive for ICD but not for PRX, which distinguished it from addition lines with chromosome 6. They also differ from each other by their resistance to the beet cyst nematode: monosomic addition lines with chromosome 1 are resistant, while those with chromosome 6 are susceptible. We also found monosomic additions from *B. webbi-*

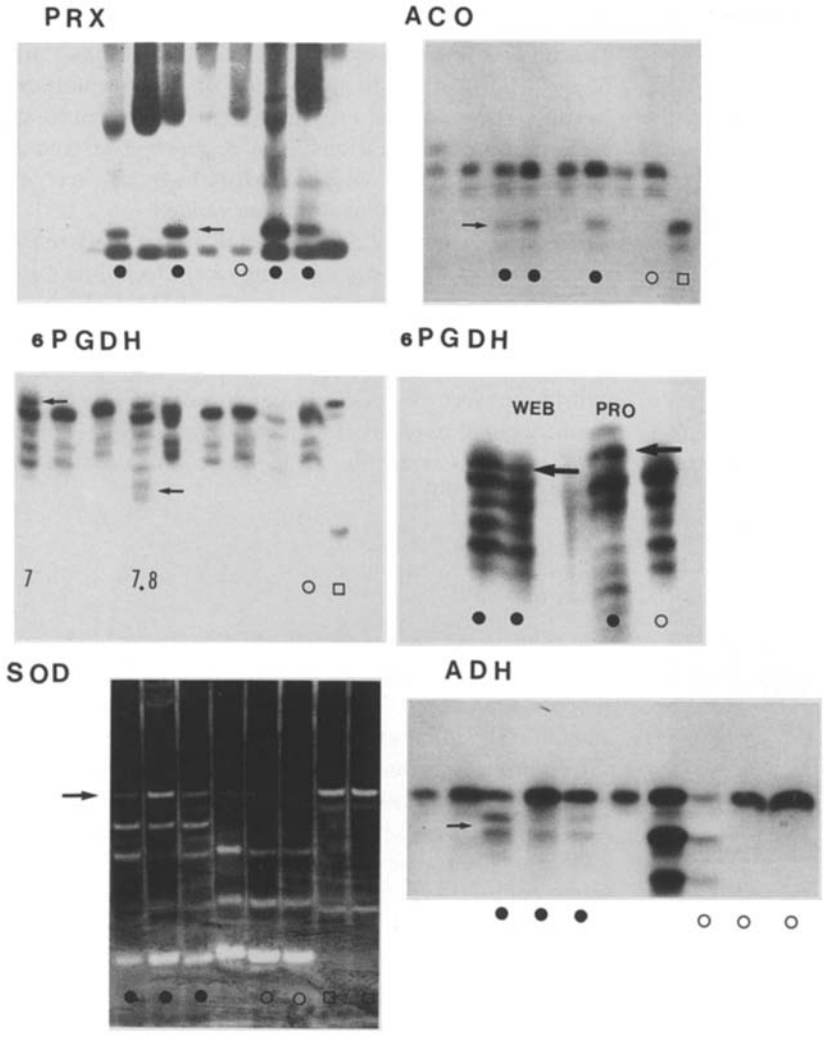

Fig. 2. The enzyme systems *PRX*, *6PGDH*, *SOD*, *ACO* and *ADH*. *Arrows* point to the marker band(s). ● Monosomic additions, ○ *Beta vulgaris*, □ *Beta webbiana*. The *numbers* correspond to the added chromosome for the particular isozyme system. *Unmarked* zymograms are monosomic additions negative for the enzyme stained

ana that stained positive for ICD and PRX and classified these plants as chromosome 6 types. These plants characteristically have longer petioles than the so-called chromosome type 1 monosomic addition. The ICD marker for both monosomic additions 1 and 6 are the same, which could be accounted for perhaps by the duplication of chromosome segments in the course of evolution. The duplication of chromosomes or chromosome segments in *Brassica oleracea* has been reported (McGrath et al. 1990). What we did not understand, however, is that we also found monosomic additions of the supposed type a from all three species of Procumbentes that are resistant to the beet cyst nematode and clearly positive for both the ICD and PRX markers. Preliminary results also showed that fragment lines from type a *B. patellaris* monosomic addition have the PRX marker. Further investigations are being carried out.

Chromosome 7

This monosomic addition has been previously described as type b and is known to be resistant to the beet cyst nematode (Löptien 1984b). It has reduced growth and narrow leaves that droop at maturity in a most remarkable manner. No isozyme marker has been reported so far to identify this type (Jung et al. 1986) nor in the monosomic addition with chromosome 7 from *B. procumbens* (Van Geyt et al. 1988).

We found, however, that the enzyme system 6-P-gluconate dehydrogenase (6PGDH, 6-Phospho-D-gluconate: $NADP^+$-2-oxidoreductase (decarboxylating) E.C.1.1.1.44) could be utilized to mark such chromosome types. Plants having this extra chromosome showed a band corresponding to the fastest band of *B. webbiana* (Fig. 2). In *B. webbiana*, we observed two zones of activ-

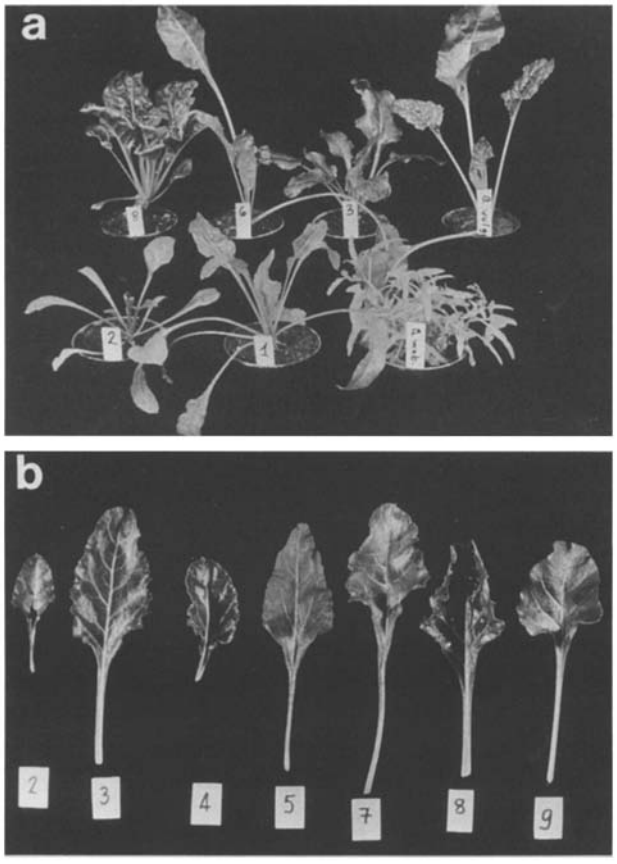

Fig. 3a, b. Morphology of the different monosomic additions. The *numbers* correspond to the added chromosome

ity: the faster zone exhibiting two bands, the slower one, one or two bands. The slower zone has been earlier reported to be a marker for chromosome 8 from *B. procumbens* (Van Geyt et al. 1988). Three or four bands have been observed in *B. vulgaris*, confirming previous results (Van Geyt et al. 1990b; Aicher and Saunders 1990). Monosomic additions with chromosome 7 showed the marker band from *B. webbiana* and bands from *B. vulgaris*. No hybrid band was noted.

If this marker band could indeed identify chromosome 7, it would also perhaps hold true for type b from *B. procumbens*, which has similar characteristics from the monosomic addition from *B. webbiana*, i.e., morphologically similar and resistant to the beet cyst nematode. We tested type b lines from both *B. webbiana* and *B. procumbens* that are maintained at our institute and confirmed our finding: monosomic additions from *B. procumbens* had the 6PGDH marker for chromosome 7. There was also a difference in the banding pattern of this chromosome between the two species. Monosomic additions from *B. procumbens* showed one more faster band and the two extra bands were more widely spaced from each other compared to the bands from *B. webbiana* (Fig. 2). This observation suggests that perhaps *B. webbiana* and *B. procumbens* differ in some small or point mutations. No differences in other enzyme systems have been found when monosomic additions from *B. procumbens* and *B. webbiana* have been compared. More tests, however, especially at the molecular level, are needed.

Previous workers did not find a difference between *B. procumbens* and *B. webbiana* using recent techniques and methods such as restriction analysis of ctDNA (Kishima et al. 1987; Fritzche et al. 1987) and isozymes (Wagner et al. 1989). The morphology and chromosome pairing in hybrids between *B. patellaris* and *B. webbiana* or *B. procumbens* could not provide evidence whether *B. webbiana* or *B. procumbens* are the same species or two different ones (Sobek 1991).

Chromosome 8

The enzyme systems aconitase [ACO, citrate (isocitrate) hydrolyase) E.C. 4.2.1.3], superoxide dismutase (SOD, superoxide: superoxide oxidoreductase E.C. 1.15.1.1) and 6PGDH differentiate monosomic additions carrying chromosome 8 from the other additions. They showed two aconitase bands of *B. webbiana* and also the *B. vulgaris* bands (Fig. 2), which migrated faster than the *B. webbiana* bands. The aconitase marker bands for monosomic addition type c from both *B. procumbens* and *B. webbiana* (Jung et al. 1986) and chromosome 8 from *B. procumbens* (Van Geyt et al. 1988) have been reported.

SOD showed three zones of activity in *B. vulgaris* that were not polymorphic. Monosomic additions with chromosome 8 could be identified by having the slowest bands of *B. webbiana* and also that of *B. vulgaris* (Fig. 2). A hybrid band was formed that migrated at a intermediary position between the parental bands. There was no difference in banding pattern between monosomic additions carrying chromosome 8 from *B. webbiana* and *B. procumbens*.

The slower 6PGDH bands of *B. webbiana* are also markers for chromosome 8 (Van Geyt 1988; Jung et al. 1986; Fig. 2). *B. webbiana* had two bands migrating more slowly than that of *B. vulgaris*. The monosomic additions exhibited the bands of the parental species as well as the hybrid bands formed between them.

This monosomic addition has been known as type c (Jung et al. 1986; Jung and Wricke 1987), which is the third type resistant to the beet cyst nematode. The plants are characterized by their broad, undulating leaves and thickened petioles (Fig. 3a, b). The leaf surface is rugged. We observed similar morphological features in monosomic additions from *B. procumbens*. In monosomic additions from *B. procumbens*, chromosome type 8 is resistant to *Polymyxa betae* (Paul et al. in preparation).

Chromosome 9

Alcohol dehydrogenase (ADH, alcohol: $NADP^+$ oxidoreductase E.C. 1.1.1.1), a marker system for chromosome 9 from *B. procumbens* (Van Geyt et al. 1988), could also be used to mark this chromosome from *B. webbiana*. The ADH system in *B. vulgaris* is controlled by one genetic locus inherited in a Mendelian way and behaves as a dimer (Van Geyt and Jacobs 1986). In a heterozygous condition, three bands could be observed in *B. vulgaris* and the single *B. webbiana* band migrated at the same position as the intermediate band of *B. vulgaris*. Additions with chromosome 9 exhibited the parental as well as the newly formed hybrid bands (Fig. 2). Morphologically, monosomic additions with chromosome 9 have flat broad leaves (Fig. 3b), which differentiated them from the rest of the monosomic additions.

Other enzyme systems are being tested as markers for chromosome 9 since ADH is a difficult system to use, especially when seeds are not available as material. ADH needs to be induced under anaerobic conditions for at least 12 h if leaf materials are to be tested; thus limiting the practicability of this system.

Conclusions

The nine chromosomes of *B. webbiana* can be differentiated from each other using isozyme markers. The markers used in identifying the alien chromosome in monosomic additions from *B. procumbens* are basically the same as those from *B. webbiana*, suggesting a close relationship between the two species. Each chromosome has also a very characteristic morphological effect on the recipient species, *B. vulgaris*. Another remarkable effect is the reduction in growth in all the nine types.

Monosomic additions could be utilized in answering basic questions of taxonomy and likewise find application in plant breeding. Monosomic addition lines make it possible to characterize further each of the nine chromosomes of *B. webbiana* and to compare them to other monosomic additions from other species in Procumbentes, for example, *B. procumbens*, to determine exactly whether these two species are distinct and to be classified as such or to be considered as two extremes of a single ecospecies. We found differences in the 6PGDH zymogram pattern between the monosomic addition with chromosome 7 from *B. procumbens* and *B. webbiana*, but further tests are still needed for conclusive evidence. Furthermore, the characterization of a complete series of monosomic additions from both *B. procumbens* and *B. webbiana* may help determine whether *B. patellaris* is an autotetraploid or amphidiploid. Preliminary results with two monosomic additions from *B. patellaris* showed the same isozyme marker bands as those from *B. procumbens* and *B. webbiana*. Another monosomic addition, i.e., type 3, shared two bands exhibited by the two other species, but in another manner had two more extra bands. It could have been possible that earlier in evolution the three species had the same basic complement, but *B. patellaris* had undergone further polyploidization.

The monosomic additions could also be used to localize genes such as the genes for resistance to the beet cyst nematode, Cercospora leaf spot, Rhizomania and curly top virus. In this manner, the transfer of these desired genes to the cultivated beet through conventional breeding or genetic engineering is facilitated. They could also elucidate the number of genes containing resistance to the beet cyst nematode: in *B. procumbens* two have been reported (Van Geyt et al. 1988; Lange et al. 1988), while in *B. procumbens* and *B. webbiana* three have been reported (Löptien 1984b; Jung and Wricke 1987; Jung et al. 1986, 1992).

Attempts to identify and characterize alien chromosomes in monosomic addition have been made (Löptien 1984b; De Jong et al. 1985). However, isozymes have been proven to be a fast and easy method to identify the added chromosomes. The 6PGDH marker for chromosome 7 could facilitate the tedious screening of plants of this type that are resistant to the beet cyst nematode.

Further characterization of the monosomic additions, especially at the molecular level are deemed desirable to be able to detect more polymorphisms that could be useful in the genetic improvement of beet.

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References

Aicher LD, Saunders JW (1990) Inheritance studies and clonal fingerprinting with isoenzymes in sugarbeet. Crop Sci 30:1064–1072

Coons GH (1954) The wild species of *Beta*. Proc Am Soc Sugar Technol 18:142–147

Curtis GJ (1968) Observations of fruit shape and other characters in the species of the section *Patellares* Genus *Beta*. Euphytica 17:485–491

De Jong JH, Speckmann GJ, De Bock Th SM, Van Voorst A (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

Endo T (1972) Application of the Nadi reaction to rice peroxidase isozyme stain. Bot Mag 85:147–151

Fritzche K, Metzlaff M, Melzer R, Hagemann R (1987) Comparative restriction endonuclease analysis and molecular cloning of plastid DNAs from wild species and cultivated varieties of the genus *Beta* (L.) Theor Appl Genet 74:589–594

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

Heijbroek W, Roelands AJ, De Jong JH, Van Hulst C, 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

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

Jung C, Koch R, Fischer F, Brandes A, Wricke G, Hermann RG (1992) DNA markers closely linked to nematode resistance genes in sugar beet (*Beta vulgaris* L.) using chromosome additions and translocations originating from wild beets of the Procumbentes section. Mol Gen Genet 232:271–278

Kishima Y, Mikami T, Hirai A, Sugiura M, Kinoshita T (1987) Beta chloroplast genomes: analysis of Fraction I protein and chloroplast DNA variation. Theor Appl Genet 73:330–336

Lange W, De Bock Th SM, Van Geyt JPC, Oleo M (1988) Monosomic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *B. procumbens* II. Effects of the alien chromosomes on in vivo and in vitro plant development. Theor Appl Genet 76:656–664

Lange W, Oleo M, Wagner H (1990) Identifizierung von Wildarten – Chromosomen in monosomen Additionstypen von *Beta vulgaris*. Vortr Pflanzenzücht (Arbeitsgemeinschaft Pflanzenzüchtung Gesellschaft für Pflanzenbauwissenschaften) 18:210–218

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

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

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

McGrath JM, Quiros CF, Harada JJ, Landry SB (1990). Identification of *Brassica oleracea* alien chromosome addition lines with molecular markers reveals extensive gene duplication. Mol Gen Genet 223:198–204

Smed E, Van Geyt JPC, Oleo M (1989) Genetical control and linkage relationships of isozyme markers in sugar beet (*B. vulgaris* L.) Theor Appl Genet 78:97–104

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

Speckmann GJ, De Bock Th SM, De Jong JH (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

Sobek R (1991) Untersuchungen zur Meiose von Arten und Artkreuzungen aus der Sektion Procumbentes der Gattung *Beta*. Diplomarbeit Universität Hannover

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding (part A). Elsevier Sci Publ, Amsterdam, pp 469–515

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

Van Geyt JPC, Oleo M, Lange W, De Bock Th SM (1988) Monosomic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *Beta procumbens* I. Identification of the alien chromosomes with the help of isozyme markers. Theor Appl Genet 76:577–586

Van Geyt JPC, Lange W, Oleo M, De Bock Th SM (1990a) Natural variation within the genus *Beta* and its possible use for breeding sugar beet: a review. Euphytica 49:57–76

Van Geyt JPC, Smed E, Oleo M (1990b) Genetical control on linkage relationships of isozyme markers in sugar beet (*B. vulgaris* L.) 2. NADP- and NAD- specific malate dehydrogenases, 6-P-gluconate dehydrogenase, shikimate dehydrogenase, diaphorase and aconitase. Theor Appl Genet 80:593–601

Wagner H (1990) Genetische Untersuchungen und Kopplungsanalysen von Isoenzymen und Morphologischen Markern bei *Beta vulgaris*. Thesis, University of Hanover

Wagner H, Wricke G (1991) Genetic control of five isozyme systems in sugar beet (*Beta vulgaris* L.) Plant Breed 107:124–130

Wagner H, Gimbel EM, Wricke G (1989) Are *Beta procumbens* Chr. Sm. and *Beta webbiana* Moq different species? Plant Breed 102:17–21