Theor Appl Genet (1988) 76:656–664

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

W. Lange [1], Th. S. M. De Bock [1], J. P. C Van Geyt [2,3] and M. Oléo [2]

[1] Foundation for Agricultural Plant Breeding, SVP, P.O. Box 117, NL-6700 AC Wageningen, The Netherlands
[2] Vrije Universiteit Brussel, Laboratorium Plantengenetica, Paardenstraat 65, B-1640 St. Genesius Rode, Belgium
[3] Present address: Phytotec, Pl. L. Pasteur 1, B-1348 Louvain-La-Neuve, Belgium

Received March 17, 1988; Accepted March 29, 1988
Communicated by H. F. Linskens

Summary. Alien monosomic additions in beet (*Beta vulgaris*), each carrying one of the nine chromosomes of *B. procumbens*, were grown in vivo and in vitro to study the effect of the alien chromosomes on plant development. All additional chromosomes caused a reduction of the growth rate in vivo, which, in one case was so strong that some of the plants died as seedlings. In general, the morphological plant characteristics were not very useful to distinguish the addition types; this could have been the results of the wide variation in the recipient parent. However, some developmental characteristics proved to be highly chromosome-specific; for plants in vivo this was annuality, in combination with early or late flowering. If grown in vitro, chromosome specificity was observed for growth type (rosette or elongated stem), occurrence and rate of vitrification, occurrence and morphology of wound callus, formation of additional meristems on the midribs of leaves, formation of roots and a specific reaction to benzylaminopurine (BAP) the medium. Two chromosome types of *B. procumbens* caused resistance to the beet cyst nematode.

Key words: *Beta vulgaris* – *Beta procumbens* – Alien monosomic additions – Plant development in vivo – Development in vitro

Introduction

Alien monosomic additions are characterized by having an extra chromosome of a related species added to the full chromosome complement of the recipient parent. Such material can be used for transferring small segments of the alien chromosome into the genome of the recipient species. Generally, plant morphology of monosomic additions differs from the recipient species, more or less in the same way as in trisomics, the differences often being quantitative in nature (for references see Khush 1973). Also, traits such as days to flowering and plant fertility are reported to be affected by the extra chromosome. As in trisomics, the ensemble of differences between the monosomic additions and the recipient species has been used to identify the different extra chromosomes. Recently, Lazar et al. (1987) reported chromosome-specific effects of certain rye chromosomes in wheat-rye additions on callus formation and plant regeneration in vitro, and Higgins and Mathias (1987) studied such chromosome-specific effects in intervarietal substitutions in wheat involving chromosome 4B.

In sugar beet, both Butterfass (1964) and Romagosa et al. (1986) developed and described a full series of primary trisomics, the former series having a heterozygous background and the latter series based on homozygous or inbred material. In both cases, however, it was possible to distinguish between the trisomic plants on the basis of plant morphological traits, especially the size and shape of leaves of plants in the vegetative stage. It was shown that the environment could complicate the classification and that the specific morphological features of both series only partly matched each other (Romagosa et al. 1986).

Savitsky (1975) was first to produce monosomic additions in beet (*Beta vulgaris* L.) carrying an extra chromosome of *B. procumbens* Chr. Sm. The alien chromosome was selected to contain a gene(s), rendering the recipient species resistant to the beet cyst nematode (*Heterodera schachtii* Schm.). Also, Speckmann and De Bock (1982), Speckmann et al. (1985), Heijbroek et al. (1983) and Löptien (1984a) reported the development of resistant monosomic additions carrying alien chromosomes of each of the three species of the section *Patellares* of genus *Beta*. From this material, gene(s) for resistance were

transferred to diploid sugar beet (Savitsky 1978, Yu 1981, Jung and Wricke 1987, Heijbroek et al. 1988, Lange and De Bock, unpublished). Based on plant morphology (Löptien 1984b, Speckmann et al. 1985) and chromosome studies (De Jong et al. 1985), it was concluded that the species of section *Patellares* each have at least two non-homologous chromosomes carrying a gene(s) for nematode resistance.

Jung et al. (1986) investigated resistant monosomic additions by means of electrophoretical techniques. Three types of resistant additions were studied in relation to three isozyme systems. Two types were found to be associated with certain bands, but the banding patterns were not consistent; the third type did not show association with any band at all. Part 1 of the present paper (Van Geyt et al. 1988) reported the identification of the alien chromosomes in monosomic additions of beet carrying an extra chromosome of *B. procumbens*. Eleven isozyme systems were used to study 33 additions. Nine groups could be distinguished, obviously representing the nine different chromosome types of *B. procumbens*. Only two groups were associated with resistance to the beet cyst nematode, one of them showing no association with any band at all. The specific banding patterns found by Jung et al. (1986) for resistant additions could not be confirmed.

The present study aims at describing the effects of the nine different chromosomes of *B. procumbens* on plant morphological and physiological traits in the recipient species, *B. vulgaris*. To this end the monosomic additions were studied both in vivo and in vitro.

Material and methods

Plant material consisted of the 32 monosomic additions listed in Table 1 of Van Geyt et al. (1988) and of accession IRS 1719, made available through Dr. W. Heijbroek, Bergen op Zoom, the Netherlands, carrying the extra chromosome isolated by Savitsky (1975). Disomic sib plants of the monosomic additions served as controls.

Plants were grown in a greenhouse and were studied for a series of morphological and developmental characteristics, as listed in Table 1 (nos. 1–22). The data were recorded about 3 months after sowing, when most plants were still in the vegetative stage, except for the data of No. 3 and No. 4, which were taken just before the vernalisation period. The data were used in a discriminant analysis (see Dillon and Goldstein 1984) to detect which variables are useful to discriminate between the groups of monosomic additions, and also to what extent such a discrimination is possible. Details of this analysis will be given in the text. After vernalisation the flowering plants were studied only superficially, because variation was too extensive to allow for a more detailed comparative study.

Most monosomic additions were also grown and studied in vitro. To that end the top of the flower stalks were cut off prior to flowering, and sterilised by subsequent submersion in ethanol (70%, for 3 min) and $HgCl_2$ (0.5%, for 1 min). After rinsing in Na_2EDTA (5 m*M*) there followed a further sterilisation in KClO (8%, for 5 min) and three subsequent rinses in sterile bidistilled water. The explants were allowed to elongate on PG_{OB} medium (De Greef and Jacobs 1979), supplemented with benzylaminopurine (BAP, 0.3 mg/l), for 3 weeks, with only the cut side of the explant in contact with the medium. Next the top of each explant was cut in pieces, each piece containing at least one meristem. The pieces were planted out in petri dishes, on the same medium as mentioned before, to allow the development of meristems and plantlets. After 3 weeks a series of subsequent subculturing was started, with 3 week intervals and using 1 litre Weck pots and the same medium.

At the end of a 3 week period of subculturing a series of data was recorded on characteristics in vitro, as listed in Table 1 (nos. 23–35). Some clones looked rather yellow at the beginning of each subculturing period, therefore chlorophyll content at the end of the 3 week interval was measured to see whether the effect could still be detected, although it was no longer visible. Because of the BAP in the medium, root formation often was strongly inhibited. The so-called BAP-effect (no. 35) is the formation of very tiny leaves with thick, swollen, highly parenchymatic petioles.

Table 1. List of characteristics studied on monosomic additions in beet (*Beta vulgaris*) carrying an extra chromosome of *B. procumbens*, when grown in vivo or in vitro

1. Growth rate (3 months after sowing; scale 1–9)
2. Transition to generative stage (3 months after sowing)
3. As 2 (6 months after sowing, before vernalisation)
4. Gall formation (6 months after sowing)
5. Position of leaves (erect, semi-erect, flat)
6. Length of total leaf (in cm)[a]
7. Length of leaf blade (in cm)[a]
8. Length of petiole (in cm)[a]
9. Location of greatest width (from leaf tip; in cm)[a]
10. Maximum width of leaf blade (in cm)[a]
11. Leaf index (width/length; calculated from 7 and 10)
12. Relative length of petiole (calculated from 6 and 8)
13. As 9; relative value (calculated from 7 and 9)
14. Leaf colour (scale 1–9)
15. Red colouration in petiole (none, weak, intermediate, strong)
16. Gloss of leaves (dull, intermediate, glossy)
17. Form of leaf tip (descriptive)
18. Form of leaf base (descriptive)
19. Undulation of leaf blade (weak, intermediate, strong)
20. Undulation of leaf margin (weak, intermediate, strong)
21. Rolling up of young leaves (descriptive)
22. Rolling up of full-grown leaves (descriptive)
23. Length of plantlets (in mm)
24. Greatest width of clusters (in mm)
25. Number of newly formed meristems
26. Growth type (rosette, elongated)
27. Length of leaf (in mm)
28. Maximum width of leaf (in mm)
29. Leaf index (width/length; calculated from 27 and 28)
30. Chlorophyll content (according to Moran 1982)
31. Vitrification (scale 0–5)
32. Wound callus (formation and morphology; descriptive)
33. Additional meristems on midribs of leaves
34. Root formation
35. BAP-effect

[a] Measured on two fully developed leaves

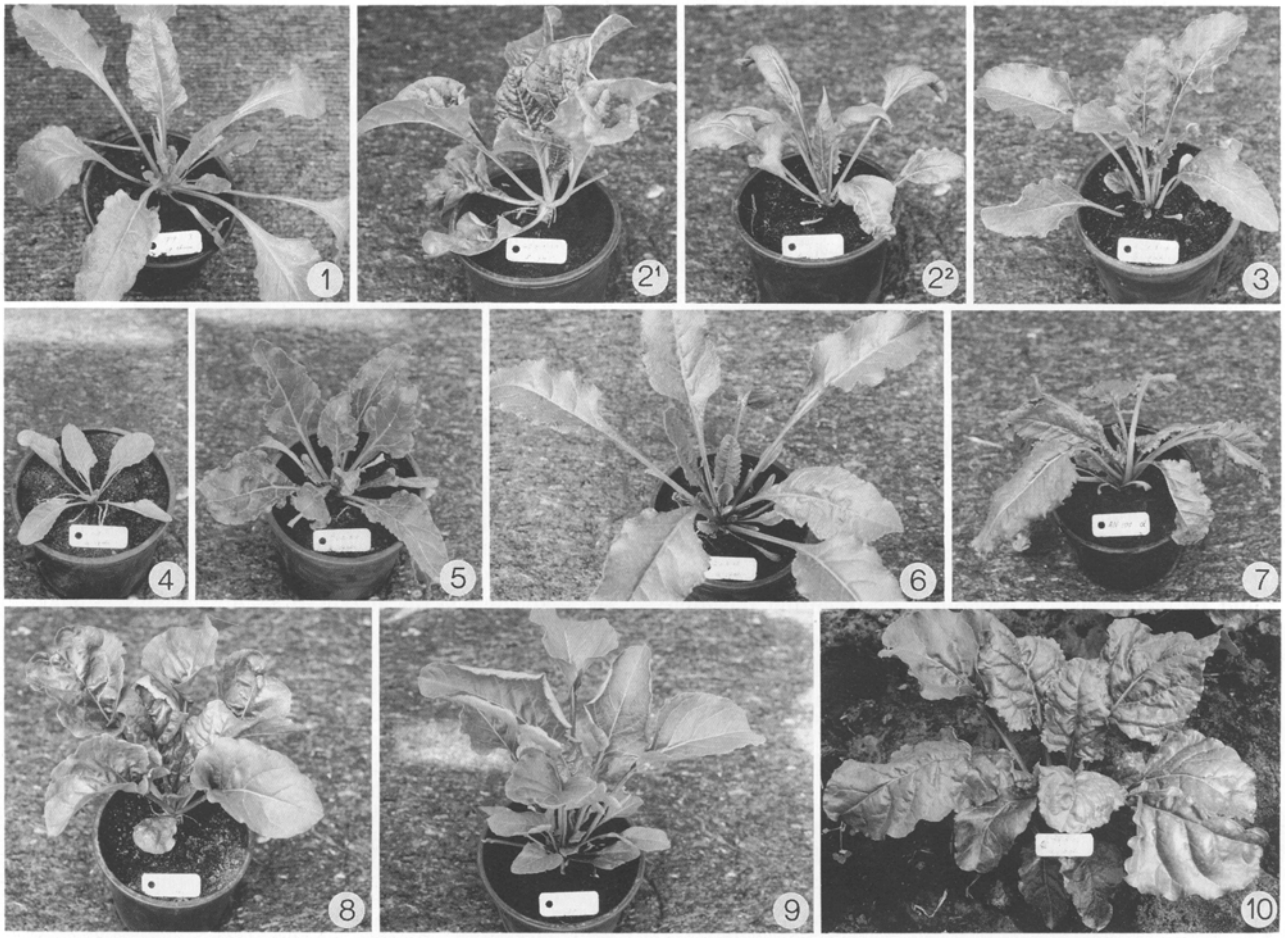

Fig. 1. Monosomic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *B. procumbens*. Numbers *1–9* correspond to chromosomes 1–9 (chromosome 2 having two morphotypes), no. *10* is disomic control. Length of label is 6.5 cm

Results and discussion

Plant development and morphology in vivo

For most of the 22 characteristics studied (Table 1), the data showed a wide variation, both within and between groups of families with the same alien chromosome type. As an example, the results of measurements on the leaves are summarized in Table 2. Three items will be discussed: (1) a striking effect of all alien chromosome types on growth rate of the monosomic additions; (2) the results of a canonical variate or discriminant analysis; and (3) a description of some chromosome-specific effects. Finally, the last paragraph of this paper comprises a more general description of the groups of monosomic additions carrying the same alien chromosome type.

All types of alien chromosomes caused a considerable reduction of growth of the monosomic additions, especially in the vegetative stage (Table 2; Fig. 1). Growth rate was recorded visually, scale 1–9. The disomic sibs generally received figure 9, whereas the monosomic additions on average were rated as 4.4, ranging from 1–7. The effect is illustrated in the 'Leaf length' columns of Table 2, showing that the leaves of the monosomic additions were in general only half as long as the leaves of the disomic plants, as well as in the column 'Growth rate'. This general reduction in growth rate, which is caused by only one copy of an alien chromosome, cannot easily be explained. Autotrisomic plants often show a slower growth than their disomic sibs (Khush 1973), indicating that an extra dose of a chromosome of the same species is enough to bring about some sort of disturbance of the general metabolism of the plants. In alien additions, the disturbance can be much more dramatic, as in the case of chromosome 4 of the present study, indicating that something extra happens. From the isozyme patterns it can be concluded that the expression of genes on the alien chromosomes is not being hampered (see also Oléo et al. 1986), so that one could speculate that certain alien gene products cause the disturbance. However, possible effects of

Table 2. Results of measurements on leaves of monosomic additions in beet (*Beta vulgaris*) carrying an extra chromosome of *B. procumbens*

Identification		No. of plants	Leaf length (cm)		Length of leaf blade (%)[a]	Leaf index (%)[b]	Location greatest width (%)[c]	Growth rate (1–9)
chro-mosome	monosmic addition		total leaf	leaf blade				
1	IRS 1719	6	26	17	64	46	61	7.0
2	AI 2-2-30	1	25	19	74	62	60	5.0
	AU 5-1-2	2	21	14	69	50	52	3.0
	AU 5-1-7	2	25	18	70	43	56	3.5
	D 2-2-27	3	29	20	71	61	67	5.7
	J 5-1-1	2	37	23	63	39	47	5.0
	L 1-1-32	1	43	31	71	45	68	7.0
	total/mean[d]	11	29	20	69	50	58	4.7
3	AH 1-2-5	4	22	14	66	50	62	3.3
	AI 2-2-16	3	24	15	62	51	62	3.3
	AI 2-2-61	4	24	16	65	52	55	3.5
	D 2-2-185	2	18	13	73	48	69	2.5
	D 3-2-17	4	31	20	64	50	63	5.5
	H 1-1-6	2	26	17	64	50	65	5.0
	L 1-1-30	1	24	16	65	58	61	3.0
	total/mean	20	25	16	65	51	62	3.9
4	I 3-2-1	2	9	6	65	57	52	1.5
5	AU 6-1-5	4	30	21	69	42	54	3.8
	I 3-2-24	4	21	16	75	45	54	3.5
	J 4-1-12	4	27	19	71	49	61	4.5
	N 2-2-13	1	23	15	67	44	58	3.0
	total/mean	13	26	18	71	45	56	3.8
6	D 3-2-35	2	33	23	68	41	63	6.0
	F 2-2-1	2	32	18	58	48	59	6.0
	K 3-1-17	3	27	16	61	53	67	4.7
	total/mean	7	30	19	62	48	63	5.4
7	A 6-2-6	4	29	19	68	50	63	5.3
	AU 6-1-4	1	26	16	62	35	56	2.0
	D 2-2-211	4	23	17	74	49	64	4.0
	total/mean	9	26	18	70	48	62	4.3
8	D 3-2-13	2	23	18	77	63	66	4.5
9	C 6-1-3	2	30	19	66	50	58	4.0
	E 3-1-3	2	31	20	64	53	64	6.0
	J 4-1-1	3	23	16	69	53	62	2.7
	total/mean	7	27	18	67	52	62	4.0
–	disomic[e]	3	51	33	64	60	65	9.0

[a] Relative to total leaf length
[b] Leaf width/length of leaf blade
[c] Relative to length of leaf blade; measured from tip of leaf
[d] Calculated from total of all plants per chromosome type
[e] Three plants with representative appearance, for comparison only

Table 3. Results of allocation of monosomic additions to chromosome types, with the help of two discriminant functions based on seven selected plant characteristics

Chromosome type	No. of plants	Plants allocated to chromosome type[a]								
		1	2	3	4	5	6	7	8	9
1	6	6								
2	11		3		1	2	1		2	2
3	20		2	5	3	6			1	3
4	2				0	1			1	
5	13		1	1		11				
6	7						7			
7	9	1				2		3	3	
8	2		1					1	0	
9	7			1		1		3	1	1
Total	77	7	7	7	4	23	8	7	8	6

[a] Correctly allocated numbers of plants are underlined

the alien genes on regulatory processes cannot be ruled out.

A discriminant analysis was applied to detect variables, or linear combinations of variables, by which the individuals belonging to a certain group of monosomic additions could be classified as such. One or two simple expressions are sought, whose values classify unknown individuals as members of a group. The analysis should identify those linear functions of the original variables, which maximise the amount of variance between groups in proportion to the variance within the groups. The first discriminant function provides the greatest possible separation between the groups; it is called the most explaining linear combination. The second discriminating function in the most explaining linear combination of the remaining variance between the groups, etc. It is hoped that one or two discriminant functions suffice for a reasonable explanation, i.e. 80% of the original variance between the groups. In addition, it is desired that as few original variables as possible constitute the discriminant functions; the variables that do not add to the discriminating power can be discarded. The quality of the discrimination is measured by the proportion of the individuals allocated correctly to the group to which they belong, on the basis of the scores of the individuals on the discriminant functions.

The discriminant analysis on the 22 characteristics mentioned eventually resulted in two discriminant functions consisting of linear combinations of seven of the original variables, viz. the characteristics nos. 1, 2, 3, 14 and 16 for the first function, and nos. 2, 4 and 20 for the second function (see Table 1). Thus, the majority of the studied morphological characteristics did not discriminate at all between the groups of monosomic additions, and were of no use in a discriminant function. The 2 discriminating functions accounted for 92.6% of the between groups variance of the 7 original variables. Though this percentage was very high, only approximately half of the individuals were allocated correctly (Table 3). The best results were obtained for chromosomes 1, 5 and 6. Because the discriminating functions were not capable of classifying more than half of the individuals, it can be concluded that most of the monosomic additions are hard to identify, even on the basis of some selected characteristics. This result indicates that the effects of the alien chromosomes have a more general nature, as was already mentioned above in relation to the reduced growth rate. On the other hand, it cannot be excluded that possible chromosome-specific effects are masked by the variability of the recipient parent. Relative to the primary aim of producing the monosomic additions, viz. the transfer of genetic material from the donor to the recipient species, it was thought that a wide genetic variability might be favourable. Therefore, both the original female parents (Table 1 in Van Geyt et al. 1988) and the populations used for backcrossing represented a wide range of beet types.

Three chromosome-specific effects were observed. In plants carrying the alien chromosome 4 the reduction of growth was very drastic. In the only family with this chromosome (I 3-2-1) two plants were very small (rated as 1 or 2 on the scale for plant growth), whereas the other two plants died as seedlings. The second and third chromosome-specific effects concerned annuality. At the first date of scoring for transition to the generative stage all monosomic additions with chromosome 6, as well as one plant out of family AU 5-1-2 (chromosome 2), were developing a generative stem; flowering started a little later. These monosomic additions combined annuality and early flowering. At the second date of scoring for generative plants, all additions carrying chromosome 1 and the one plant of family AU 6-1-4 (chromosome 7) were also generative. These monosomic additions were annual and late flowering. The annual character of additions carrying chromosome 1 has already been reported by Speckmann et al. (1985). At the second date of scoring for the transition to the generative stage, most vegetative plants were still growing as a rosette, except for one plant of AI 2-2-61 (chromosome 3), one plant of C 6-1-3, and both plants of E 3-1-3 (all with chromosome 9), all of which showed elongation of the vegetative stem.

A final striking effect of the extra chromosomes was the growth of gall-like malformations on the roots, and later also in the inflorescenses. Such malformations were previously mentioned by Speckmann et al. (1985). Root galls were not observed on plants with chromosomes 6 or 9. On plants with chromosomes 2 or 5 root galls occurred only rarely. For the remaining monosomic additions root galls were formed on about half of the plants. Thus, no clear correlation between root galls and specific chromosomes was found.

Table 4. Some data collected during in vitro culture of monosomic additions in beet (*Beta vulgaris*) carrying an extra chromosome of *B. procumbens*

Identification		No. of original plants	Growth in 3 weeks (cm)		Leaf index	Chlorophyll content
chromosome	monosomic addition		length	width		
2	AU 5-1-2	1	2.5	5.0	27	28
	AU 5-1-7	2	1.6	1.3	27	40
	D 2-2-27	2	2.8	1.7	42	35
	J 5-1-1	2	2.0	1.3	53	29
	L 1-1-32	1	0.7	1.0	47	42
	total/mean	8	2.0	1.8	40	35
3	AI 2-2-61	1	5.0	6.0	44	73
	D 2-2-185	2	4.8	6.3	44	31
	D 3-2-17	3	4.3	6.8	35	61
	H 1-1-6	1	2.0	5.0	40	37
	L 1-1-30	1	3.0	5.0	56	46
	total/mean	8	4.1	6.1	42	48
4	I 3-2-1	2	3.8	5.0	56	34
5	AU 6-1-5	2	5.0	4.0	81	26
	I 3-2-24	3	4.8	4.2	48	33
	J 4-1-12	4	3.0	3.5	61	15
	N 2-2-13	1	3.0	4.5	57	58
	total/mean	10	3.9	3.9	61	26
6	D 3-2-35	2	3.5	3.2	14	80
	F 2-2-1	2	2.6	4.9	27	35
	K 3-1-17	2	3.8	3.7	30	37
	total/mean	6	3.3	3.9	24	51
7	AU 6-1-4	1	3.0	5.0	38	41
8	D 3-2-13	2	2.5	2.7	75	17
9	C 6-1-3	2	2.8	4.3	23	28
	E 3-1-3	2	1.9	1.1	39	66
	J 4-1-1	2	1.9	1.1	42	43
	total/mean	6	2.2	2.2	35	45

Development in vitro

In general, the monosomic additions reacted well to in vitro culture by showing a high rate of success of the cultures, as also was demonstrated by Miedema (1982), as well as a growth rate in the same order of magnitude as experienced for sugar beet. In the same way as for the data collected on plants in vivo, only a selection of the data recorded during in vitro culture is presented in Table 4. Growth rate can be deduced from the columns 'Growth in three weeks'. Although these figures show considerable variation, they suggest differences of effect on growth rate between e.g. chromosome 3 (highest growth rate) and chromosomes 2 and 9 (lowest growth rate). It also should be mentioned that the strong negative effect of chromosome 4 on growth in vivo did not occur in in vitro culture. The number of meristems formed in a 3 week period mostly was in the same order of magnitude as for sugar beet, about 10–30, the only exception being the explants with chromosome 7, on which an average of only two meristems were formed.

Explants of sugar beet normally grow as a rosette when cultured on the vegetative multiplication medium. In contrast, the species of section *Patellares* of the genus *Beta*, as well as the hybrids between these species and *B. vulgaris*, show an elongated growth type (unpublished results). In the present series of monosomic additions it appeared that both chromosomes 6 and 9 caused the elongated growth. Leaf index (Table 4) showed considerable variation and showed no relation with the figures for

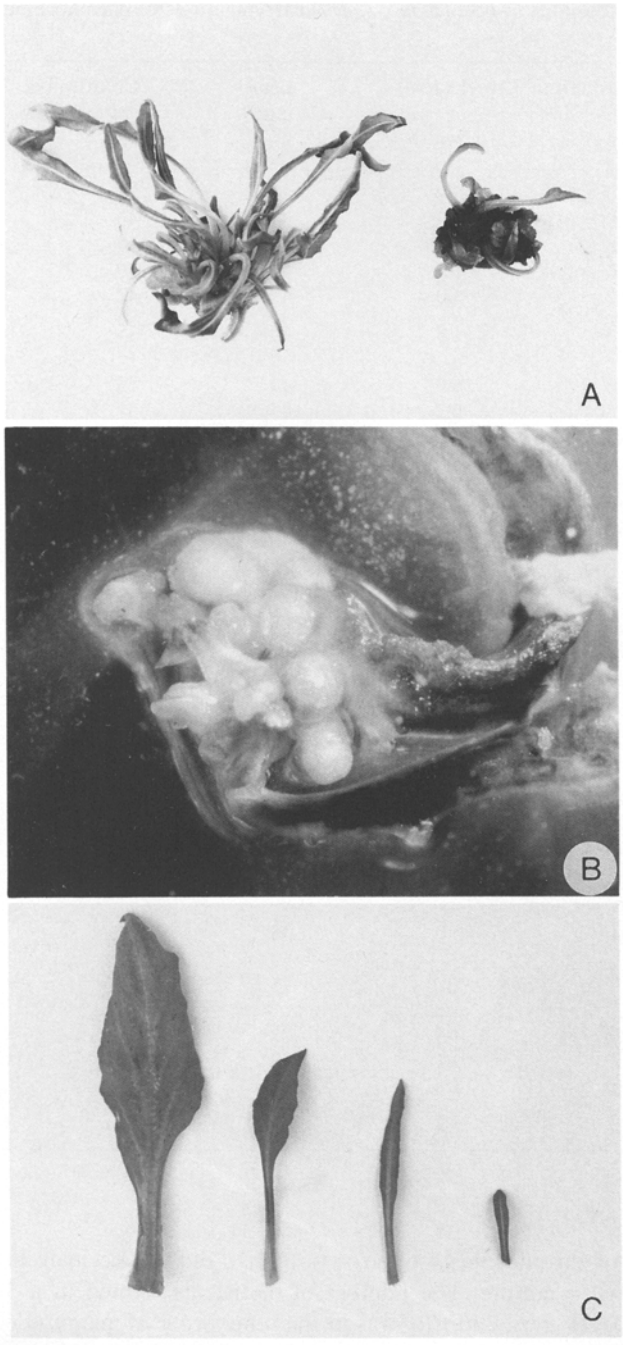

Fig. 2 A – C. Some special features of development in vitro of monosmic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *B. procumbens*. **A** vitrified (*right*) and not vitrified (*left*) plantlet; **B** formation of adventitious meristems and plantlets on midrib of leaf; **C** various degrees of reaction to BAP, compared with normal leaf (*left*)

the same characteristic, as obtained from the same plants in vivo (Table 2). Additions with chromosomes 6 or 9 had leaf indices much lower than those in vivo. This probably was the result of the BAP in the medium and will be discussed later.

Chlorophyll content was measured, and the figures are listed in Table 4. It showed considerable variation, which did not bear any relation to a specific yellow discolouration that was observed in certain cultures, just after the beginning of a new subculturing. Neither was this variation related to specific alien chromosomes. Also, the rate of vitrification showed variation (Fig. 2). Plantlets with the alien chromosomes 6 or 9 were highly vitrified, and additions with chromosomes 3 or 8 did not show any vitrification at all. In the other groups most plantlets showed vitrification of those parts that were in direct contact with the medium.

Most explants formed a wound callus at the base of the cluster of plantlets, with the exception of additions with chromosomes 4 or 5, on which no or very little callus developed. The monosomic additions with chromosomes 6, 7 or 8 formed a non-vitrified and non-friable callus, which was very rigid and rounded. In the additions with chromosomes 3 or 9 most calli had a hard kernel surrounded by brown to black friable callus; whereas in additions with chromosome 2, the morphology of the wound callus varied from hard and rounded to friable and completely vitrified.

Some genotypes of *B. vulgaris*, as well as a hybrid between *B. vulgaris* and *B. patellaris*, showed the ability in vitro to develop adventitious meristems on shoots and petioles (unpublished results). This characteristic might be of interest in relation to the regeneration ability of genotypes. Plantlets of only one of the two additions in each of the three families with the alien chromosome 9 formed meristems and plantlets on the midribs of the leaves (Fig. 2). All other additions did not show this characteristic.

Root formation in vitro on explants of sugar beet is often strongly inhibited by BAP in the medium, and can be induced by omitting BAP and replacing it with kinetin or auxins. Also, in the present material root formation was inhibited on the medium with BAP, except for most additions with chromosome 5 and one with chromosome 2 (L 1-1-32). BAP also is known to exert a strong effect on the morphology of plantlets of sugar beet in vitro, especially regarding the development of leaves and petioles (unpublished results). The leaves remain very small and almost no leaf blade is formed. Instead, the petioles become thick, swollen and highly parenchymatic (Fig. 2). If the BAP is omitted from the medium, the plantlets retain their changed morphology for about 1 month, followed by a gradual disappearance of the effect. In the present material this so-called BAP-effect occurred strongly in all additions with chromosomes 6, 7 or 9, and in one of the additions with chromosome 4. About one third of the remaining additions was only weakly affected and the others were normal. On the basis of the alien chromosomes involved in some of these characteristics, one could speculate on a relationship between the reaction to BAP

(strong vs weak or absent), the growth type in vitro (elongated vs rosette), and the level of vitrification (high vs low or absent). This relationship might be causal, through the hormonal balance.

Conclusions and description per chromosome addition

The addition of chromosomes of *B. procumbens*, in single dose, to the diploid chromosome complement of *B. vulgaris* resulted in a more general reduction of the growth rate of the plants in vivo, which effect did not appear if the plants were grown in vitro. This might indicate that the application of hormones in the medium of the in vitro culture would mask or remove the supposed disturbance of the general plant metabolism in vivo.

Some characteristics were found to be specific for a certain type of alien chromosome in vivo as well as in vitro. These characteristics will be summarized below, together with a more general description of the plants. However, the morphological plant characteristics were not very useful to identify the addition types. That identification could be carried out only with isozyme analysis (Van Geyt et al. 1988), and therefore the results of that analysis are included in the descriptions of the additions. It seems unlikely that the expression of the alien isozymes and the chromosome-specific effects in the monosomic additions have any causal relation. The enzymes are supposed to be involved with housekeeping functions, and the alien types are probably variants of the original enzymes of the recipient parent, having the same functions.

Chromosome 1. Isozyme marker: NADP specific isocitrate dehydrogenase, gene Icd^{Pro}. Plants annual and late flowering with a reduced growth rate, a semi-erect growth type, and rather narrow and flat leaves. Chromosome 1 carries a gene(s) for resistance to the beet cyst nematode. It appears to be the same chromosome as isolated by Savitsky (1975), as well as the same chromosome as in monosomic additions type a of Löptien (1984b). This chromosome was also shown to carry the nucleolus organiser region (NOR) of the donor species (De Jong et al. 1986). These additions were not studied in vitro.

Chromosome 2. Isozyme markers: glutamate dehydrogenase, gene Gdh^{Pro}; NAD specific malate dehydrogenase, genes Mdh_1^{Pro} and Mdh_2^{Pro}; phosphoglucomutase, gene Pgm_2^{Pro}. Most plants biennial, with variable phenotype and reduced growth rate. Two of the families looked more or less like the recipient parent, with broad, undulating and glossy leaves. In the other four families the growth rate was slightly more reduced and the leaves were rather narrow, dull and weak, giving the plants a droopy appearance. These differences in phenotype did not show up in in vitro culture. In that case the plantlets grew as a rosette, and the average growth rate was lowest among the groups tested. Plantlets showed vitrification of those parts that were in direct contact with the medium, and varied considerably in the morphology of the wound callus.

Chromosome 3. Isozyme marker: leucine aminopeptidase, gene Lap_1^{Pro}. Plants biennial, generally rather small, with an erect growth type and rather flat leaves. In fact, these monosomic additions did not have a specific phenotype. In contrast to the rather low growth rate in vivo the growth rate in vitro on average was highest among the monosomic addition types. All explants grew as a rosette, formed a wound callus with a hard kernel surrounded by friable callus, and none of the clusters showed vitrification.

Chromosome 4. Isozyme marker: glutamate oxaloacetate transaminase, band 6^{Pro}. Plants biennial, with very strongly reduced growth rate in vivo: half of the plants died as seedlings. Growth rate in vitro was rather good, and all plantlets grew as a rosette. One of the two additions combined a high level of vitrification with a strong reaction to BAP in the medium, whereas the other full-sib additions lacked both these characteristics. No wound callus was formed.

Chromosome 5. Isozyme marker: glutamate oxaloacetate transaminase, band 7^{Pro}. Plants biennial, having a reduced growth rate and a semi-erect growth type. Leaves often rather flat with undulated leaf margin and rather short petioles. Development in vitro was quite good. The plantlets grew as a rosette and the leaf blades were relatively broad. The explants formed no, or sometimes very little, wound callus. As a special feature, additions carrying chromosome 5 showed the ability of the plantlets to form roots on a medium containing BAP.

Chromosome 6. Isozyme marker: cathodal peroxidase, gene Pod_1^{Pro}. Plants annual and early flowering, having a reduced growth rate and a semi-erect growth type. Leaves had a relatively long petiole. Development in vitro was quite good. The explants formed a hard and rounded wound callus. Special features were stem elongation instead of formation of a rosette, a high level of vitrification, and a strong reaction to BAP in the medium, resulting in tiny leaves and swollen petioles.

Chromosome 7. No isozyme marker. Most plants biennial with a reduced growth rate and a rather flat growth type, apparently as a result of weak petioles. Chromosome 7 carries a gene(s) for resistance to the beet cyst nematode. It appears to be the same chromosome as in monosomic additions type b of Löptien (1984b). The development in vitro was good, with very few meristems developing on the explants as a special feature. The wound callus was rigid and rounded and the plantlets showed a strong reaction to BAP in the medium.

Chromosome 8. Isozyme markers: aconitase, genes Aco_1^{Pro} and Aco_2^{Pro}, superoxide dismutase, two bands, and 6-phosphogluconate dehydrogenase, zone 2^{Pro}. Plants

biennial, with reduced growth rate and an appearance more or less like the recipient parent. Leaves were broad and undulated with short petioles. In vitro the plantlets grew as a rosette and were among the slowest growing addition plants. The leaf index was very high, there were neither vitrification nor a reaction to BAP in the medium, and the wound callus was rigid and rounded.

Chromosome 9. Isozyme marker: alcohol dehydrogenase, gene Adh^{Pro}. Plants biennial, showing a tendency to elongation of the vegetative stem before vernalisation, and with a reduced growth rate and erect growth type. The leaf margins were often bent or rolled upwards. In vitro, most plants were among the slowest growing additions. The explants grew a wound callus with a hard kernel, surrounded by friable callus. Special features were stem elongation instead of growing as a rosette, a high level of vitrification and a strong reaction to BAP in the medium, whereas some of the plantlets showed the ability to grow secondary meristems and plantlets on the midribs of the leaves.

Acknowledgements. Thanks are due to Dr. F. A. van Eeuwijk, SVP, Wageningen, for his indispensable help regarding design and realisation of the canonical variate analysis, and to Mr. L. Dua, VU, Brussels, for skilful assistance. The financial support of IWONL, Belgium, is gratefully acknowledged.

References

Butterfass T (1964) Die Chloroplastenzahlen in verschiedenartigen Zellen trisomer Zuckerrüben (*Beta vulgaris* L.). Z Bot 52:46–77

Dillon WR, Goldstein M (1984) Multivariate analysis, methods and applications. Wiley, New York, 587 pp

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

Greef W De, Jacobs M (1979) In vitro culture of the sugar beet: description of a cell line with high regeneration capacity. Plant Sci Lett 17:55–61

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

Higgins P, Mathias RJ (1987) The effect of the 4B chromosomes of hexaploid wheat on the growth and regeneration of callus cultures. Theor Appl Genet 74:439–444

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

Lazar MD, Chen THH, Scoles GJ, Kartha KK (1987) Immature embryo and anther culture of chromosome addition lines of rye in Chinese Spring wheat. Plant Sci 51:77–81

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 of resistance to *Heterodera schachtii* Schm. in wild species of the section *Patellares*. Z Pflanzenzücht 93:237–245

Miedema P (1982) A tissue culture technique for vegetative propagation and low temperature preservation of *Beta vulgaris*. Euphytica 31:635–643

Moran R (1982) Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol 69:1376–1381

Oléo M, Geyt JPC Van, Lange W, Bock ThSM De (1986) Investigations on an interspecific hybrid involving three species of the genus *Beta*, with special reference to isozyme polymorphism. Theor Appl Genet 73:261–266

Romagosa I, Hecker RJ, Tsuchiya T, Lasa JM (1986) Primary trisomics in sugar beet. I. Isolation and morphological characterisation. Crop Sci 26:243–249

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

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

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