11. Genetic and Molecular Analysis of Hybrids between Transgenic Sugar Beet and Related Beet Varieties

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In 1993 and 1994 transgenic sugar beet (*Beta vulgaris* ssp. *vulgaris*) plants containing the coat protein (CP) gene from beet necrotic yellow vein virus (BNYVV) and the *bar* gene from *Streptomyces hygroscopicus* (mediating resistance to the herbicide Basta®) were released in a field experiment conducted by the German company PLANTA.

Transfer of virus and herbicide resistance genes from the transgenic sugar beet to other *Beta vulgaris* varieties might result in the emergence of more compatible weed beets (Newbury *et al.*, 1989). Reciprocal gene exchange between cultivated sugar beet and wild beets in seed production areas was demonstrated by Santoni and Bervillé (1992) and is probably causing the occurrence of weed beets in production fields (Boudry *et al.*, 1993).

As the genetic constitution of a plant is one of the factors determining the pattern of transgene expression, the expression of newly introduced genes is not readily predictable after transfer to another genetic background. Also, transgenes can be lost or poorly transmitted to subsequent generations (Spencer *et al.*, 1992). In order to evaluate the consequences of outcrossing of the transgenes, we used the transgenic sugar beet plants in sexual crossings with two wild beet (*Beta vulgaris* ssp. *maritima*) accessions and with mangel (*Beta vulgaris* ssp. *vulgaris*). The resulting hybrids were analyzed in the greenhouse with the following objectives:

- Do transgenes show Mendelian inheritance after outcrossing, or are there instabilities?
- Does outcrossing to a different genetic background lead to changes in the expression pattern of the transgenes?
- Is Basta® application appropriate to quantify the transfer frequency of the *bar* gene to related plants in field experiments or does possible gene inactivation lead to an underestimation of transfer frequencies?

As Table 1 shows, transgenes are inherited essentially according to Mendelian law. Despite the 31 : 16 ratio found for mangel x L4 hybrids, there is no significant deviation from the expected 3:1 ratio.

Leaf as well as root expression of the BNYVV coat protein gene in the transgenic F 1 hybrids is in the same range as in the transgenic sugar beet lines. Some of the mangel X L4 hybrids (3 out of 43), however, were found to express the coat protein at a very low level in leaves. Based on equal total protein, root expression is about 8 to 10 fold lower than leaf expression. Table 2 gives an overview of the results of two gene expression

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experiments.

All the transgenic F1 hybrids tested (about 30 plants per hybrid line) did express the *bar* gene. Still, dot application of 1% Basta® can occasionally lead to weak herbicide symptoms (necroses) which do not occur when only the active ingredient D,L-Phosphinothricin is applied. Spraying with 5 l/ha Basta® , however, is an appropriate means to discriminate between transgenic and non-transgenic plants.

References

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Table 1 Segregation of the bar gene in F2 plants derived from selfing of different transgenic F1 hybrids.

F1 hybrid	Transgenic	Non-transgenic	Expected ratio	χ^2	P
(Ma×L3)-3	37	12	3:1	0.007	0.9
(Ma×L4)-1	31	16	3:1	1.6	0.2
(51421×L4)-8	22	8	3:1	0	1
(57733×L4)-7	16	8	3:1	0.5	0.4

PCR followed by Southern hybridization as well as a non-destructive BASTA test (dot application) was used for the analysis. Ma: mangel; 51421 and 57733: wild beet accessions from Greece and Portugal, respectively; L3 and L4: transgenic sugar beet lines differing in the length of the CP leader sequence and in the plant integration site.

Table 2 Expression of the BNYVV coat protein gene in transgenic F1 hybrids.

F1 hybrid	Experiment A					Experiment B				
	Number of plants	ELISA (A405) Leaves		ELISA (A405) Roots		Number of plants	ELISA (A405) Leaves		ELISA (A405) Roots	
		Mean	SD	Mean	SD		Mean	SD	Mean	SD
H3	7	0.43	0.053	0.03	0.022	11	0.167	0.072	0.022	0.016
H4	5	0.38	0.065	0.027	0.01	4	0.154	0.042	0.024	0.019
Ma×L3	9	0.52	0.088	0.047	0.025	7	0.176	0.057	0.027	0.026
Ma×L4	5	0.37	0.202	0.035	0.013	10	0.155	0.052	0.022	0.013
51×L3	14	0.4	0.071	0.04	0.027	ND	ND	ND	ND	ND
51×L4	10	0.39	0.048	0.042	0.024	ND	ND	ND	ND	ND
57×L4	10	0.38	0.1	0.044	0.033	ND	ND	ND	ND	ND

For each plant, two protein extracts of the third leaf from the top were prepared. Root data are based on one extract per plant. Each extract was tested twice in the ELISA. H3 and H4: L3 and L4 crossed with non-transgenic sugar beet, 51 and 57: wild beet accessions 51421 and 57733; SD: standard deviation.