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Transformation and regeneration in sugar beet (*Beta vulgaris* L.) induced by 'shooter' mutants of *Agrobacterium tumefaciens*

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Summary

Beta vulgaris plants were found to be susceptible to *Agrobacterium tumefaciens* strains carrying octopine Ti-plasmids after wounding of GA_3 elongated stems or of hypocotyls. Tumors could be isolated and cultured aseptically. The tumor marker, octopine synthase (Ocs) activity, was present demonstrating the applicability of the *Agrobacterium* system for transfer of genetic information. For the production of transgenic plants two procedures were tested: inoculation of explants derived from cotyledons and hypocotyls of two weeks old seedlings and a leaf-disc procedure. The first method yielded both octopine positive calli as well as shoot regeneration on the six genotypes tested. In most cases, regeneration occurred from pre-existing, non-transformed meristems. The presence of Ocs activity could not be demonstrated in these shoots, although in one case octopine positive callus was formed at the base of the shoot, suggesting a chimeric structure of the plantlet or T-DNA genes, which were silent within the shoot and became active again in proliferating callus. The leaf-disc method did not give rise to direct or indirect regeneration, but transformed callus proliferated on the leaf edges. Optimal transformation frequencies were dependent on *B. vulgaris* genotype and *Agrobacterium* strain. The use of *Agrobacterium* shooter mutants or strains carrying an isolated cytokinin gene in order to influence endogenous phytohormone ratios did not result in the formation of shoots nor did it increase levels of regeneration in the first method. Further optimization is in order and in progress.

Abbreviations: BAP – 6-Benzylaminopurine; GA_3 – Gibberellic acid; 2iP – 2-isopentenyladenine

Introduction

In many laboratories nowadays research is aimed at applying genetic manipulation techniques developed with model plant species to agriculturally important crop species. Often adaptations and further optimization of protocols are required. A potent instrument for the transfer of well-defined pieces of genetic information into plants is the soil bacterium *Agrobacterium tumefaciens* (for reviews see Schilperoort, 1986 and Schell, 1987). For the production of transgenic plants many suitable vector-types (An et al., 1985; Bevan, 1984; Hoekema et al., 1985; Simoens et al., 1986; Van der Elzen et al., 1985) have been designed and also different inoculation strategies are now available such as co-cultivation (Marton et al., 1979) and the leaf-disc method (Horsch et al., 1985). This latter method has the advantage of a very short tissue culture period, thus reducing the chance of the occurrence of somaclonal variation (Gould, 1986; Scowcroft et al., 1987). However, a prerequisite is regeneration

from leaf explants, if necessary inducible by plant hormones . The sugar beet (*Beta vulgaris* L.) has been found to be a recalcitrant species in this respect (Atanassov, 1986; Ford-Lloyd & Bhat, 1986) as are many economically important crops. Regeneration from callus, which was either directly induced or derived from protoplasts, occurred at very low frequencies or not at all (De Greef & Jacobs, 1979; Saunders & Doley, 1986; Tétu et al., 1987; Krens & Jamar, manuscript in preparation). Here, plant hormones, which were used for shoot induction, were supplied exogenously by addition to the growth medium. By using *Agrobacterium tumefaciens* 'shooter' mutants, in which the auxin locus (Ooms et al., 1981) of the T-DNA has been inactivated or removed, one can increase endogenous levels of cytokinins in the transformed tissues, leading to the 'shooty' phenotype in some plant species. Steffen et al. (1986) have reported regeneration in previously recalcitrant species after transformation with 'shooter' mutants. The susceptibility of sugar beet to *Agrobacterium tumefaciens* wild type nopaline strains has already been demonstrated by Wang et al. (1985) and to *A. rhizogenes* by Paul et al. (1987).

Here, we report the results of our investigations on transformation and subsequent regeneration of sugar beet using different *Agrobacterium tumefaciens* strains (wild-type and shooter mutants). Several genotypes, including a monosomic addition , AN5 (Speckmann et al., 1985), with a high regenerative capacity, were used in transformation and inoculation optimization studies. Optimal transformation frequencies appeared dependent on genotype and *Agrobacterium* strain. The influence on regeneration of endogenous hormone levels or differences in hormone sensitivity in different parts of the sugar beet plant are discussed.

Materials and methods

Bacterial strains

Bacterial strains are listed in Table 1. Bacteria were cultured at 29° C in TY medium (Koekman et al., 1980) or LB medium (Hooykaas et al., 1977). A bacterial colony was suspended in 10 ml culture medium and grown overnight to the stationary phase. Bacterial concentrations were determined by measuring the optical density (O.D.) at 666 nm; at an O.D. of 1.5 the bacteria are at a density of approx. 4.10^9/ml (Krens et al., 1985a). These suspensions were used as such for inoculations or after dilution as indicated in the text. For *in planta* tumor induction colonies were taken directly from the 1.8% (w/v) agar solidified culture plates.

Plant material

Seed coats were removed mechanically with a mill or were permeabilised by hot water treatment (15 min. at 55° C). Sterilization was achieved by subsequent submersion in: 95% (v/v) ethanol (few seconds); 1% (w/v) Na-hypochlorite solution (15 min.); 3 washes in sterile H_2O (5 min.; 10 min.; 15 min. respectively). After germination on water/agar (1.5% (w/v) Daichin agar, Brunschwig Chemie, in distilled H_2O) the germinating seeds were placed in tubes on half strength MS (Murashige & Skoog, 1962) medium supplemented with 30 g/l sucrose and solidified with 0.8% (w/v) agar (1/2MS). Culture was performed at 22° C with a 16 hours photoperiod (1000 lux from cool white FTD58W/33 fluorescent and Philinea lamps). The *B. vulgaris* genotypes that were used in this study, are presented in Table 2.

Tumor induction in planta

In order to allow wounding of the stem in the rosette plants of *B. vulgaris* vegetative stem elongation was induced first by addition of 5 mg/l GA_3 to the medium (Huizing et al., 1988). Stems were then wounded with the needle of a syringe and inoculated with a colony of *A. tumefaciens* LBA 4001 or LBA 1501. Visible tumor formation occurred after 2 weeks. Tumors were dissected and placed on culture medium (1/2MS + 10 μM BAP) 4 weeks after inoculation. In a later stage seedlings with well developed hypocotyls were selected; wounding and inoculation was carried out at the hypocotyl, thus saving the time needed for induction of elongation (approx. 4 weeks).

Transformation procedures

A. After approx. 2 weeks seedlings (Fig.1) had

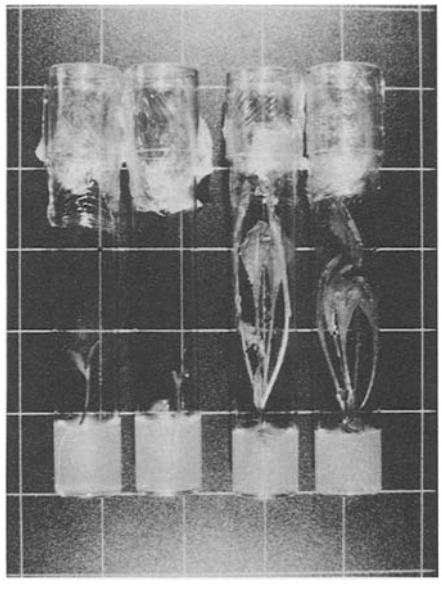

Fig. 1. Aseptically grown *Beta vulgaris* seedlings.
Left: two weeks after seed sterilization.
Right: six weeks after seed sterilization.

developed 2 cotyledons, a hypocotyl and roots. After removal of the root system the rest of the seedlings was chopped up into 5 mm segments and incubated in 10 ml of an overnight culture of *A. tumefaciens* strains LBA 4001 or LBA 1501 for 10 minutes. The plantlet cuttings were rinsed two times with liquid 1/2MS medium and subsequently placed on agar-solidified 1/2MS + 10 μM BAP in order to stimulate shoot formation. After 7 days the explants were transferred to fresh medium supplemented with 500 mg/l cefotaxime in order to eliminate the bacteria. Controls were placed on medium immediately after cutting.

B. Leaves of 6 to 8 weeks old seedlings (Fig.1) were cut into pieces of 0.25–0.5 cm^2 and placed on a nylon sieve (mesh 297 um) fitting a 10 cm diameter plastic petri dish. At t = 0 the nylon sieve carrying the leaf segments was dipped in 20 ml *Agrobacterium* suspension (overnight cultures, 1:10 or 1:100 dilutions) and removed after variable incubation times. The leaf segments were blotted dry with sterile filter paper and placed upside down on the culture media to reduce curling. Different media with variable BAP concentrations were tested for optimal shoot formation. Two days later the leaf explants were transferred to the same medium supplemented with 200 mg/l cefotaxime. Controls were treated identically except that incubation was performed in liquid LB medium without bacteria.

The transformed nature of calli that were obtained, was established by growth on phytohormone-free medium or on medium with 100 mg/l kanamycin as well as by octopine synthase (Ocs) assay if appropriate. Calli were grown at 22° C and a 16 hours photoperiod (500 lux).

Octopine synthase activity assay

The presence of lysopine dehydrogenase (= octopine synthase,Ocs) activity in plant material was demonstrated as described by Otten & Schilperoort (1978) or according to a modified procedure using precoated cellulose thin-layer chromatography plates as described by Huizing et al. (1988).

Results

Table 2 provides a survey of the combinations of *B.vulgaris* genotypes, bacterial strains and transformation procedures that were tested.

Table 1. Agrobacterium tumefaciens strains

Strain	Chromosomal background	Plasmid	Tumor morphology	Reference or source
LBA 4001	oct, Ach 5	pTiAch5 wild type	unorganized, Ocs+	R.H. Hamilton
LBA 1501	nop, C58	pTiB6::Tn1831	shoots, Ocs+	Ooms et al. (1981)
LBA 4508b	oct, Ach 5	(pAL 4404, pRAL 3108b)	shoots, Km^r, Ocs−	B.S. de Pater
LBA 4509	oct, Ach5	(pAL 4404, pRAL 3109)	shoots, Km^r, Ocs−	De Pater et al. (1987)

N.B. Strains LBA 4508b and 4509 differ in orientation of the cytokinin gene (T-cyt) with respect to the Km^r gene.

In planta tumor formation

In order to study the susceptibility of the *B. vulgaris* genotypes to *Agrobacterium tumefaciens* and the phenotype of the tumors formed, in planta inoculations were performed at first. Tumor formation could be achieved quite readily both on elongated stems and on well-developed hypocotyls (Fig. 2). Tumors induced by LBA 4001 as well as by LBA 1501 showed an unorganized morphology. No shoot formation occurred on the tumors induced by the shooter mutant LBA 1501. Removal of the tumors from the plant and culture on hormone-free 1/2MS medium or on 1/2MS containing BAP also did not stimulate the formation of shoot primordia irrespective as to what *Agrobacterium* strain was used. The transformed nature of the calli was demonstrated by the continued growth on hormone-free medium and by the presence of the tumor-specific Ocs enzyme.

Transformation via explants (method A)

All genotypes were susceptible to *Agrobacterium tumefaciens* leading to the development of octopine positive, fast growing callus as shown in Table 2. Continued growth on medium (1/2MS) supplemented with 10 uM BAP for approx. 8 weeks resulted in the formation of shoots again on both LBA 4001 and LBA 1501 induced calli with low frequencies (approx. 1–2% of the subcultured calli; Fig. 3). All regenerants scored negative in Ocs activity assays. In one case (Bella/LBA 4001) after subculturing several times one of the regenerants formed callus at its base. This callus was octopine positive.

Leaf disc transformation (method B)

Table 3 and Figure 5 summarize the inoculation conditions that were tested using the leaf disc method for the production of transgenic plant material (Fig. 4). Parameters that were varied, were: incubation time, inoculation density and bacterial strain. Also four different growth media were used based on 1/2MS medium (Murashige & Skoog, 1962) or on PGo medium (De Greef & Jacobs, 1979) with variable amounts of the cytokinin BAP (10 μM or 20 μM). All experiments were performed with *B. vulgaris* cv. Monohil except where in-

Table 2. Transformation and regeneration of *B. vulgaris* genotypes

Genotype	Agrobacterium strain	Transformation procedure	Tumor		Shoot	
			formation	Ocs	regeneration	Ocs
K3-1-17[1] (2x + 1)	LBA 4001	in planta/GA_3	+	+	–	
cv. Monohil (3x)	LBA 4001	in planta	+	+	–	
	LBA 1501	in planta	+	+	–	
cv. Regina (3x)	LBA 4001	A	+	+	+	–
cv. Arigomono (3x)	LBA 4001	A	+	+	+	–
cv. Primahill (2x)	LBA 4001	A	+	+	+	–
AN5-90[1] (2x + 1)	LBA 4001	A	+	+	–	
cv. Bella (3x)	LBA 4001	A	+	+	+	+/–
	LBA 1501	A	+	+	+	–
cv. Monohil (3x)	LBA 4001	A	+	+	+	–
	LBA 1501	A	+	+	+	–
cv. Monohil (3x)	LBA 1501	B	+	+	–	
	LBA 4508b	B	+	–	–	
	LBA 4509	B	+	–	–	
SVPno 31-188 (NF)	LBA 1501	B	+	+	–	
(2x)	LBA 4508b	B	+	–	–	
AN5[1] (2x + 1)	LBA 1501	B	+	+	+	–

[1] Monosomic additions.

Fig. 2. In planta crown gall formation on well-developed sugar beet hypocotyls after wounding and incubation with *A. tumefaciens* LBA 1501.

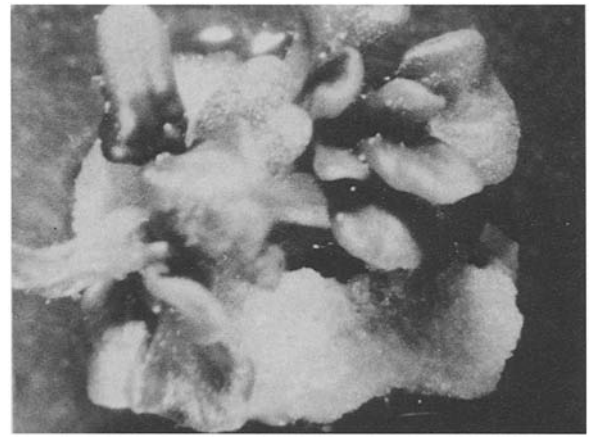

Fig. 3. Shoots regenerating from callus obtained after cutting of two weeks old seedlings (method A) and subsequent inoculation with *Agrobacterium* strain LBA 4001.

Table 3. Percentages of callus formation under varying conditions

Medium Hormone BAP (μM)		PGo+ 10	1/2MS+ 10	PGo+ 20	1/2MS+ 20	Mean
Strain	Conc.					
LB medium alone		21.8	16.0	23.9	20.0	20.4
LBA 1505	1:0	0.0	39.3*	20.0	75.4	35.7
LBA 1501	1:10	10.7	52.8*	49.2	59.8	44.9
LBA 1501	1:100	4.1	33.8*	29.5	47.9	31.2
Mean		5.3	41.4*	34.0	59.9	37.5
LBA 4508b	1:0	14.6	15.5*	9.4	3.6	9.8
LBA 4508b	1:10	31.0	7.3*	23.3	10.4	16.1
LBA 4508b	1:100	43.6	33.3*	32.0	5.5	24.5
Mean		29.5	18.3*	21.0	6.4	16.5
LBA 4509	1:10	3.9	19.6	23.8	9.7	13.1
LBA 4509	1:100	12.5	10.9	23.6	10.7	14.3
Mean		8.7	15.3	23.7	10.2	13.7
LBA 1501#	1:0		21.7		28.8	27.1
LBA 1501#	1:10		29.4		9.5	19.8
Mean			27.5		19.9	22.9

cv. Monohil was used except where marked * (NF) or # (AN5).

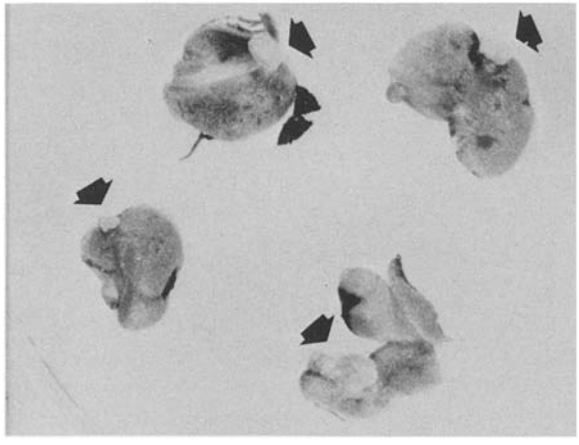

Fig. 4. B. vulgaris NF leaf explants showing the formation of fast-growing callus, mainly where a vein was hit. Inoculation conditions were 30 sec. with 1:10 dilution of an o/n culture of *A. tumefaciens* LBA 1501. Growth medium: 1/2MS + 10 μM BAP.

dicated in Table 3. Here the diploid SVP accession no. 31-188 (NF) was used. LBA 1501 was also taken to transform the monosomic addition AN5 (Speckmann et al., 1985), which has a high regeneration capacity from callus.

As can be seen from the controls (dipping in LB without bacteria) there was no clear difference in callus formation between the four media. Apparently, the composition of the media, that were tested here, did not influence normal callus formation with cv. Monohil (this was found for accession NF as well, Krens & Jamar, manuscript in preparation). However, after incubation with agrobacteria the medium composition did have an effect, but not an uniform one. With LBA 1501 the data showed a better response on 1/2MS over PGo and of 20 μM BAP over 10 μM (Note that in the case of 1/2MS + 10 μM BAP NF was used instead of Monohil). Except for PGo + 10 μM BAP the percentage of callus formation after incubation with LBA 1501 was considerably higher than in the controls. This phenomenon was not found with strain LBA 4508b nor with LBA 4509. Here, frequencies were the same or lower as compared to the controls. For LBA 4508b the data showed that a better response was obtained on PGo basal medium with 10 μM BAP being superior to 20 μM. For LBA 4509 no general rule could be extracted from the data; PGo + 20 μM BAP gave rise to the highest percentage of callus formation. In later experiments with LBA 1501 and a *B.vulgaris* monosomic addition plant (AN5) the effect of medium composition on callus formation as found earlier with cv. Monohil did not appear to be a general rule, but to be dependent on the genotype.

Bacterial cells were grown overnight to stationary phase (for average densities see Table 4) and used at different dilutions. Taking all media together a 1:10 and a 1:100 dilution appeared optimal for LBA 1501 and LBA 4508b resp. (an exception was 1/2MS + 20 μM BAP for both). For LBA 4509 no preference could be found on cv. Monohil. On this cultivar the effect of different inoculation periods was tested. Generally, as shown in Figure 5, no clear differences in callus formation was found with variable incubation times for LB, LBA 4508b and LBA4509 (Note that frequencies of the two bacterial inoculations did not differ from the controls). LBA 1501 showed increasing numbers of calli formed with increasing incubation times.

Confirmation of transformed character. From the LBA 1501 induced calli which were growing vigorously on medium without phytohormones, 10 were chosen at random and assayed for the presence of the tumor-specific enzyme Ocs. All were positive. Calli obtained after dipping in LB (Controls) or LBA 4508b (lacking the Ocs gene in the T-DNA) were negative (Fig. 6). In order to get an indication of transformation frequencies after LBA 4509 incubation twenty calli were placed on growth medium supplemented with 100 mg/l kanamycin. Two of them showed sustained growth in the presence of the selective agent, whereas all control calli died. This suggested that, although frequencies were

Table 4. Average bacterial densities after o/n culture

Strain	Density (bact./ml)
A. tumefaciens	
LBA 1501	16.2×10^9
LBA 4508b	13.0×10^9
LBA 4509	10.5×10^9

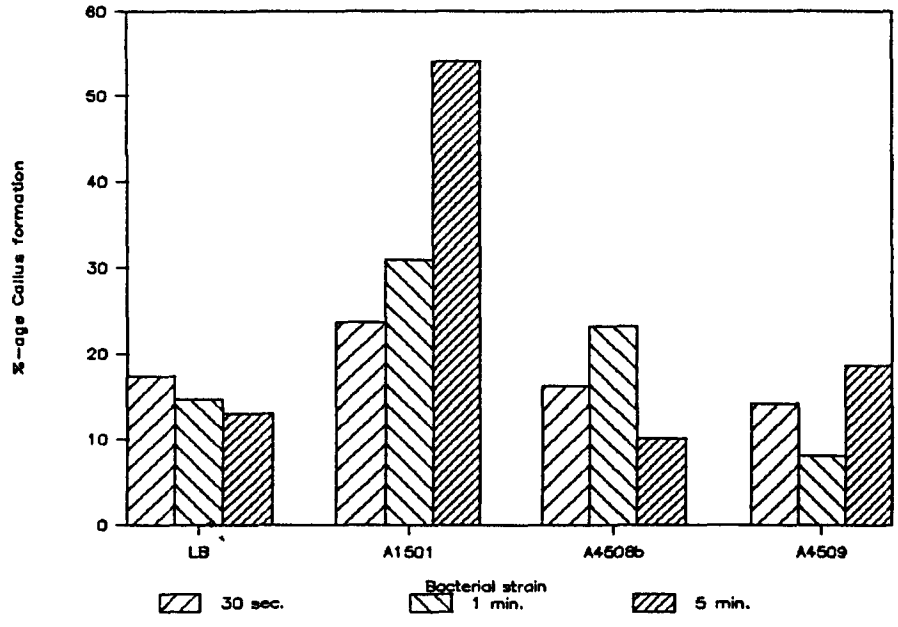

Fig. 5. The percentage of callus formation related to bacterial strain and incubation time.

much lower as compared to LBA 1501 and did not differ from the controls, still transformation did take place with LBA 4509.

Regeneration. No direct regeneration via shoot formation from the cut edges of the leaf discs was ever observed with any of the *Agrobacterium* shooter mutants on any of the media that were tested. Also monosomic addition AN5, which has a high regenerative ability from normal callus tissue, yielded mostly unorganized calli after transformation with LBA 1501 with two exceptions. However, these shoots were negative when tested for Ocs activity. Approx. 600 calli, which were induced under different conditions including the Ocs positive calli, were transferred to and subcultured on media supplemented with different cytokinins at high concentrations (2iP: 3 or 10 mg/l; zeatin: 2 or 5 mg/l; kinetin: 3 or 10 mg/l and BAP: 20 or 40 μM). However, this did not result in the formation of shoots.

Discussion

Sugar beet is susceptible to agrobacterial infection. Wang et al. (1985) reported nopaline containing crown galls on a large number of *Beta* genotypes. Paul et al. (1987) demonstrated that by using *A. rhizogenes* hairy roots containing agropine were formed which could be used for the in vitro production of beet cyst nematodes (*Heterodera schachtii*). In order to study the possibilities of transferring genetic information into sugar beet plants via *Agrobacterium tumefaciens* we examined transformation related to regeneration. In *Beta vulgaris* shoot formation from different types of callus has been observed only in a very limited number of cases (De Greef & Jacobs, 1979; Saunders & Doley, 1986; Tétu et al., 1987; Krens & Jamar, manuscript in preparation). The formation of roots on the other hand can be very abundant in tissue culture. Minor injuries on hypocotyls result in roots formed in the vicinity of the wound area; suspension cell cultures can be rendered phytohormone autotrophic (also auxin autotrophic) with relative ease (Kevers et al., 1981). This taken together with our observation that both wild type and shooter mutant LBA 1501 generated unorganized callus growth both after in planta inoculation as well as after inoculating explants, may suggest that at least in some parts the sugar beet plants contained high

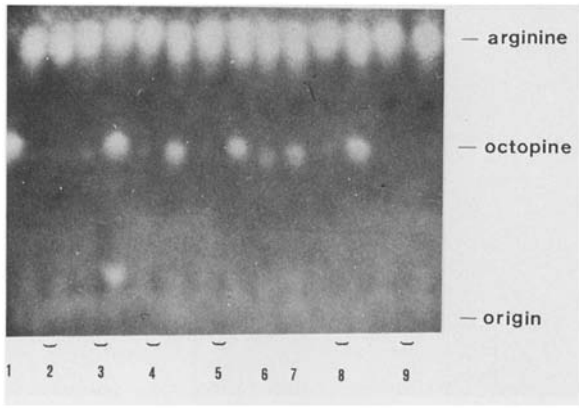

Fig. 6. Octopine (Ocs) activity assay. Lane 1: octopine standard 10 ng; lane 2: negative control (LB dip). Next lanes represent transformed calli. Lane 3: in planta/LBA 1501 (t = 0; t = 75 min. cv. Monohil); lanes 4&5: method B/LBA 1501 (t = 0; t = 75 cv. Monohil); lanes 6&7: method B/LBA 1501 (t = 75 only, accession NF); lane 8: in planta/GA_3/LBA 4001 (t = 0; t = 75 cv. Arigomono); lane 9: method B/LBA 4508b (t = 0; t = 75 cv. Monohil).

levels of auxin or an elevated sensitivity to this hormone. Direct biochemical measurements of auxin and cytokinin levels in sugar beet callus and suspension cultures first confirmed this hypothesis (Kevers et al., 1981), but more recent reports presented hormone levels in leaves and stems (Terry et al., 1986) which do not deviate significantly from endogenous hormone levels reported for tobacco stems (Akiyoshi et al., 1983). Tobacco shows a shooty phenotype after incubation with LBA 1501. Apparently, the endogenous hormone levels naturally present within the plant stems are not solely responsible for the observed difference in response to the shooter mutant LBA 1501. In this respect it is interesting to compare the response of shooter mutants on different plant species: shoot formation on *N. tabacum* and *Kalanchoe daigremontiana*; no tumors at all on tomato or pea and normal sized crown galls on *N. rustica* and *N. debneyi* (Ooms et al., 1981; Van Slogteren et al., 1984a). From *N. paniculata* and *Physalis minima*, which are recalcitrant in regenerating from protoplast-derived calli, shoots were obtained after incubation with shooter mutants (Steffen et al., 1986). We investigated the effect of increasing endogenous cytokinin concentrations on regeneration in sugar beet. For this we used the cytokinin locus from the T-DNA of octopine type *Agrobacterium tumefaciens* strains. LBA 1501 carries a transposon insertion inactivating the auxin locus leaving the other T-DNA loci intact. The other strains we used , possessed a binary vector system with on one vector the isolated T-DNA cytokinin gene together with a kanamycin resistance marker. In tobacco the cytokinin locus alone is sufficient to allow growth on media devoid of externally added phytohormones (Krens et al., 1985b) and therefore this T-cyt gene can be used as an selectable marker.

In analogy to the leaf-disc method (Horsch et al., 1985) the sugar beet leaf explants were placed after incubation with agrobacteria on medium supplemented with a cytokinin, BAP, for induction of shoots. No regeneration was observed, suggesting that hormone levels or ratios were not the most important factor in induction of shoot formation in *B. vulgaris*, but that sensitivity of hormone receptors might be involved. The complex nature of factors playing a role in regeneration, was also demonstrated by the fact that AN5, a monosomic addition plant capable of regenerating high numbers of shoots, tended to loose this capacity after transformation even with a shooter mutant.

In experiments where 2 weeks old seedlings were cut and incubated, shoot formation did occur albeit with very low frequencies. However, it is important to realise that with this method meristems remained present. Both *A. tumefaciens* strains (wild type LBA 4001 and shooter LBA 1501) gave regeneration with similar frequencies on all tested *B. vulgaris* genotypes. Shoots could have arisen from meristems or meristem parts present in the proliferating tumorous tissues. Ocs activity could not be demonstrated in any of the shoots. This suggested that the transformed callus tissues that developed, were chimeric in nature (Peerbolte et al., 1986). The formation of Ocs positive callus at the base of one of the shoots might be considered as an indication for the chimeric character of the shoots as well, but it might also suggest that at least some of the shoots were actually transformed but that the Ocs gene was not expressed. This has been reported earlier in tobacco LBA 1501 derived calli too (Van Slogteren et al., 1984b). The incubation

of cut meristems with disarmed *Agrobacterium* strains might be a method in *B. vulgaris* to introduce genetic information and to result in transgenic plants (chimaeras).

Transformed, Ocs positive calli were also produced by the leaf-disc method. In our experimental approach our aim next to regeneration was to establish optimal inoculation conditions. The culture media tested in this study did not show a difference in the growth of normal callus as demonstrated by the controls. However, after transformation with LBA 1501 higher numbers of calli could be isolated on 1/2MS medium while after inoculation with LBA 4508b or 4509 no preference for a particular medium was found. It should be noted that the frequency of callus formation with the latter two strains did not rise above the level of the controls. This suggested that tumorous tissue proliferated better on 1/2MS and that LBA 1501 had a higher T-DNA transfer capacity on sugar beet than the other strains. The observed difference in virulence was confirmed by the results obtained with the Ocs assays and with the culture under selective conditions. LBA 1501 induced calli, that were tested, showed 100% positive in octopine synthesis, while only 10% of the LBA 4509 induced calli gave sustained growth on medium supplemented with kanamycin. The decrease in transformation frequency was accompanied by an increased toxicity of the strains. More diluted suspensions of LBA 4508b and 4509 gave better responses than undiluted overnight cultures. This phenomenon was not found with LBA 1501. An important difference between the strains which might be responsible for the observed effects, resides in the chromosomal background. LBA 1501 has a nopaline C58 chromosomal background, while the other strains possess an octopine Ach5 chromosomal background. Also in other species the C58 background was reported to play a positive role in virulence, e.g. in *Brassica* spp. (Holbrook & Miki, 1985).

No generally applicable protocol could be established for *Beta vulgaris*, since optimal conditions for transformation (bacterial density, incubation time, culture medium) appeared to be dependent on genotype and *Agrobacterium* strain. Additional specific requirements will have to be met in leaf-disc procedures before obtaining routinely high yields of transformed shoots. As stated earlier, in sugar beet alternative plant explant types might prove to be superior to leaf-discs in obtaining transgenic plants (see also Krens & Jamar, manuscript in preparation).

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