Tumor Formation and β -Glucuronidase Expression in *Phaseolus vulgaris* Inoculated with *Agrobacterium tumefaciens*

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Abstract. Ten common bean (*Phaseolus vulgaris* L.) lines—including cultivars, breeding lines, and one wild line—were evaluated for susceptibility to *Agrobacterium tumefaciens* strain C58 by stab-inoculating intact shoot tips of germinating seeds. Significant differences for tumor frequency and size were found on the resulting 3-week-old seedlings. UW 325, a wild bean, had the highest rate of tumorigenesis; ‘Olathe’, a dry bean cultivar, had the lowest. Uninoculated excised shoot tips cultured in media with BA or BA plus NAA exhibited differences in phytohormone sensitivity, as evidenced by callusing and root initiation. The cultivar Montcalm seemed to be highly sensitive, while ‘Olathe’ was relatively insensitive. Fluorometric GUS assays of shoot tips from germinating seeds inoculated with the disarmed GUS-containing *A. tumefaciens* strain C58C1(pGV3850/pKIWI105) showed that UW 325 had the highest level of GUS activity. ‘Montcalm’ had a high rate of tumorigenesis but a low level of GUS activity; this anomaly was attributed to its high phytohormone sensitivity. The use of the virulence-inducing compound acetosyringone in the inoculum culture medium did not alter genotypic differences (ranks) in susceptibility. Histochemical GUS assays of inoculated UW 325 shoot tips showed that 60% of the apexes exhibited one or more transformation events. Chemical names used: β -glucuronidase (GUS); α -naphthaleneacetic acid (NAA); *N*-(phenylmethyl)-1*H*-purin-6-amine (BA).

Grain legumes, including common bean (*Phaseolus vulgaris* L.), are somewhat recalcitrant to *Agrobacterium tumefaciens*-mediated transformation and regeneration. Franklin et al. (1993) produced transformed GUS-positive callus from inoculation of a kidney bean cultivar with *Agrobacterium tumefaciens* strain EHA101. Putative transformants have been reported in one common bean cultivar (Mariotti et al., 1989); however, no molecular evidence of transformation or data on transmission through the seed was presented. Axillary bud tissue culture systems for whole-plant regeneration in bean have been developed (Franklin et al., 1991; McClean and Grafton, 1989). However, they have yet to be adapted successfully for use in an *Agrobacterium*-based transformation system (McClean et al., 1991). The transformation method of infecting the meristem of germinating seeds with *Agrobacterium* was used successfully in soybean (Chee et al., 1989). This procedure has the advantage of not requiring regeneration from tissue culture.

Genotypic differences in susceptibility of grain legumes to *A. tumefaciens* have been reported for pea (Hobbs et al., 1989; Puonti-Kaerlas et al., 1989, 1990) and soybean (Byrne et al., 1987; Delzer et al., 1990; Owens and Cress, 1985), but the basis for these differences is not well understood. Most susceptibility studies have involved scoring for tumorigenesis subsequent to inoculation. The possible association of phytohormone sensitivity and *Agrobacterium*-induced tumorigenesis has been suggested to ex-

plain differences in susceptibility (Van Wordragen et al., 1992). The use of the naturally occurring compound acetosyringone in a pre-inoculation culture step has been shown to increase the level of virulence in *Agrobacterium* (Vernade et al., 1988) and lead to higher transformation rates (Sheikholeslam and Weeks, 1987).

McClean et al. (1991) were able to induce a relatively high tumor frequency on seedling hypocotyls of 19 bean lines using *A. tumefaciens* strains A208, A281, and LBA 4001. However, those results were difficult to assess quantitatively because infection rates were reported only as the number of plants with at least one tumor. Using a GUS marker gene, particularly a construct such as that in pKIWI105 (Janssen and Gardner, 1989), that does not express GUS in *Agrobacterium* and therefore cannot lead to false background readings would provide a more direct means of assessing transformation rates. The objectives of this research were to examine aspects of susceptibility of common bean to oncogenic (tumor-forming) and disarmed-engineered (GUS-containing) strains of *A. tumefaciens* using the stab-inoculation technique.

Materials and Methods

Plant materials. Ten bean lines reflecting a portion of the variation present in common bean for market class (seed type), growth habit, frequency of *Rhizobium* nodulation, and the presence or absence of phaseolin (the major seed storage protein) were studied. These included line UW 325 (wild *P. vulgaris* var. *mexicanus*), ‘Puebla 152’ (prostrate, highly nodulating), ‘Rio Tibagi’ (poor nodulator), ‘Tendergreen’ and ‘Montcalm’ (both upright determinate), ‘C-20’ (upright indeterminate), ‘Olathe’ (prostrate), and lines BMC 3352, 11-33, and 13-41 (all phaseolin deficient).

Agrobacteria strains and inoculum preparation. The oncogenic strain C58-st, obtained from J. Slightom (Upjohn Molecular Biology Division, Kalamazoo, Mich.), was used in tumor-formation

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studies. The disarmed-engineered strain C58C1(pGV3850/pKIWI105) (obtained from A. Dandekar, Univ. of California–Davis) contains the *nptII* and *uidA* (GUS) marker genes. The GUS gene of pKIWI105 (Janssen and Gardner, 1989) cannot be expressed within the bacterium due to an alteration of the ribosome binding site. The disarmed strain C58C1(pGV3850) (also obtained from A. Dandekar) has no T-DNA region; it was used in some cases as a negative control.

A loop of freshly plated bacteria was used to initiate liquid cultures. Luria-Bertani medium (Sambrook et al., 1989) was used in solid- and liquid-phase culture systems for growing inoculum for the tumorigenesis assay; YEP medium (pH 7) (per liter: 10 g peptone, 10 g yeast extract, 5 g NaCl) was later found to promote faster growth (data not shown) and was used in all subsequent experiments. Liquid orbital shake cultures (200 rpm, $28 \pm 1.0^\circ\text{C}$) were grown overnight with the appropriate antibiotics and resuspended in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 2% sucrose (MS20) at pH 5.8 to an optical density of 1.3 at 600 nm (usually a 50% dilution of the bacteria). In experiments subsequent to the tumorigenesis trial, a resuspension medium pH of 5.2 was used. In one transformation experiment, an overnight culture of strain C58C1(pGV3850/pKIWI105) was resuspended in MS20 medium (pH 5.2) with 100 μM acetosyringone and 1 mM betaine phosphate (Vernade et al., 1988). The concentration of the bacterial resuspension was set at an optical density of 1.3 at 600 nm and returned to rotary shake culture for 6 h. Strain C58C1(pGV3850), used as a control, also was prepared in the same manner.

Tumorigenesis assay. Seedling shoot tips were stab-inoculated similarly to the method of Chee et al. (1989). Seeds of the 10 lines were mildly surface-sterilized (agitated at 150 rpm for 10 min in 20% chlorine bleach) and rinsed four times with sterile deionized water. Seeds with testae that remained smooth were chipped to promote uniform imbibition, and all seeds were germinated at 25°C in petri plates on Whatmann no. 3 filter paper moistened with a half-strength MS salt solution. After 1 or 2 days, when the seeds had swollen and the radical had begun to emerge, the seed coat and one cotyledon were removed. Under stereomicroscope magnification, the bases of the unifoliate leaves were partially removed to expose the shoot apical meristem. A 30-gauge needle attached to a 1-cm³ syringe containing liquid *Agrobacterium* culture was used to wound the meristem region and deliver the inoculum. The inoculated “half-seedlings” were returned to the petri plates and incubated for 2 more days at 25°C in darkness. Controls were inoculated with sterile half-strength MS20 medium.

Subsequently, the seedlings were transferred to pots containing a standard soil mix and grown in environment chambers with a 16-h photoperiod of $\approx 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $25/17^\circ\text{C}$ (day/night), and 50%/70% (day/night) relative humidity. Plants were scored for tumor frequency and tumor size 3 weeks after inoculation. Tumors were assigned a size score of 1 (<1 mm), 2 (1 to 4 mm), or 3 (>4 mm). Frequency was calculated by recording the number of distinct sites with tumors on each plant and dividing by seven, the maximum number of tumor sites found among the inoculated plants (tumors often occurred on plant parts not directly inoculated). For example, a plant having a tumor at the unifoliate node and on a unifoliate leaf (either one or both leaves) would receive a score of 2/7, or 29%. Absolute tumor number could not be determined accurately because of the frequent inability to distinguish individual tumors.

In vitro culture of excised shoot tips. Germinating seedlings were stabbed in a manner similar to that described previously to test for phytohormone sensitivity, except that only sterile resuspension medium (no *Agrobacterium*) was used as an inocu-

lum. After mock inoculation and 2 days of incubation at 25°C in the dark, shoot apices were excised directly above the cotyledonary node and cultured singly in 30-ml polystyrene blood sample vials (Fisher Scientific, Pittsburgh) on a medium containing MS salts, B5 vitamins (Gamborg et al., 1968), 3% sucrose, cefotaxime (Claforan) at 500 mg·liter⁻¹, and 0.7% Phytagar (TC grade; Gibco, Grand Island, N.Y.) at pH 5.8 with 1) 11.1 μM BA, 2) 4.4 μM BA and 10.7 μM NAA, or 3) 22.2 μM BA and 2.7 μM NAA. These growth regulator concentrations approximated those used by Kartha et al. (1984) (nos. 1 and 2) and Allavena and Rossetti (1986) (no. 3), which were found to promote callus formation, multiple budding, or both in bean shoot apex explants. These cultures were maintained at 24°C under a 16-h photoperiod of $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by cool-white fluorescent bulbs. Explants were transferred to fresh medium after 2 and 9 days and evaluated for root and callus formation after 3 weeks.

Stab-inoculation with C58C1(pGV3850/pKIWI105). Germinating half-seeds were inoculated as previously described with the GUS-containing strain C58C1(pGV3850/pKIWI105). Control half-seedlings were inoculated with strain C58C1(pGV3850). After 1 day of incubation in petri plates on moistened filter paper in the dark, the inoculated half-seedlings were placed on cotton squares saturated with a MS salts solution in GA-7 vessels (Magenta Corp., Chicago) and exposed to a 16-h photoperiod at 24°C as previously described. After 6 days, shoot apices were excised just below the unifoliate node, and the unifoliate leaves, along with most of the petiole, were removed. This unifoliate node tissue was used for the fluorometric GUS analysis.

In a separate experiment, germinating seeds of line UW 325 were inoculated with strain C58C1(pGV3850/pKIWI105). The histochemical GUS assay was used to examine whole shoot tips for locating transformation events in the meristem. GUS-transformed walnut somatic embryos (obtained from G. McGranahan, Univ. of California–Davis) were used as positive controls in each fluorometric and histochemical GUS assay.

Biochemical assays for transformation. Tumor tissue from different plant sites of each line from the susceptibility evaluation experiment was assayed for the production of nopaline using paper chromatography as described by Otten and Schilperoort (1978). Fluorometric assays for GUS activity in unifoliate node tissue were conducted with the substrate 4-methylumbelliferyl β -D-glucuronide (MUG) (Sigma Chemical Co., St. Louis) as described by Jefferson (1987). The reaction mixture was incubated at 37°C for 100 min plus an additional 10 min to allow for equilibration. To account for background fluorescence, part of the reaction mixture of six randomly chosen samples for each line was transferred to the stop buffer immediately after the reaction buffer was added. Histochemical GUS assays of UW 325 shoot tips were done using the substrate 5-bromo-4-chloro-3-indolylglucuronide (X-GLUC; Jersey Lab Supply, Livingston, N.J.). The excised shoot apex was sliced in half longitudinally and incubated in 1 mM X-GLUC for 6 h at 37°C (Jefferson, 1987). Localized regions of transformed cells, as indicated by a blue precipitate, were detected visually under stereomicroscope magnification.

Statistical methods. Data were analyzed by using MSTAT 1.4-C (Michigan State Univ.). The C58 10-line evaluation was done twice; a randomized complete-block analysis of variance was performed on the combined data set, with each trial designated as a block. The F test for tumor size was done by hand (Snedecor, 1946) because of the unequal size of data sets among lines; a size score was assigned only if a tumor existed. Absolute GUS activity readings of the control (providing a measure of intrinsic GUS activity) and of GUS-inoculated materials were first subjected to

within-line paired *t* tests to determine if a significant amount of GUS activity was present over the controls. Comparisons among lines were made possible by subtracting the mean control GUS activity measurement from the individual GUS-inoculated readings of that particular line, thus producing a corrected GUS activity. These data were transformed by the function $\log(X + 1)$ to correct for a mean–variance relationship (Gomez and Gomez, 1984) before analysis of variance as a completely randomized design.

Results

Tumorigenesis study. The susceptibility study uncovered significant differences among the 10 bean lines for tumor frequency and size (Table 1 and Fig. 1). Tumors occurred at up to seven different plant sites: the hypocotyl, cotyledonary node, epicotyl internode, unifoliate node, unifoliate petiole, unifoliate leaf, and the first trifoliate node. Although most plants (87% to 100%) had at least one tumor, tumorigenesis scores ranged from a high of 58.7% for UW 325 to a low of 24.4% for ‘Olathe’. The line $\times$ replicate interaction was not significant. The range of differences for tumor size was smaller; UW 325 and ‘Montcalm’ had the largest tumors, while 13-41 and ‘Puebla 152’ had the smallest. Control stabbed seedlings (two per line) formed no tumors.

The lines that had the highest overall frequency of tumor formation also had high rates of tumorigenesis at the unifoliate node (Table 2). In the case of ‘Tendergreen’, a high rate of tumor formation also occurred at the trifoliate node. Due to occasional disruption of the meristem by tumor growth, trifoliate nodes were sometimes absent (especially on UW 325). These plants were not included in the tabulation of the data for trifoliate node tumor frequency. The unifoliate leaf site had the highest mean frequency of tumors (86%) over all lines. Tumors rarely formed on the hypocotyl and epicotyl (7% and 9%, respectively). Tissue samples of tumors from different positions on the plant from a random sample of plants of each line tested positive for the production of nopaline without exception. Control shoot tips and in vitro-induced ‘Tendergreen’ callus tested negative for nopaline (data not shown). The three most tumorigenic lines (UW 325, ‘Tendergreen’, and ‘Montcalm’) and the two lines that formed tumors least frequently (‘Puebla 152’, which also had the second lowest tumor size score, and ‘Olathe’) were used in further studies.

Table 1. Tumor formation on 10 common bean lines 3 weeks after stab-inoculation with *Agrobacterium tumefaciens* strain C58.

Bean line	Total no. of plants	Plants with at least one tumor (%)	Tumor frequency (% of seven sites)	Tumor size score ^z
UW 325	22	100.0	58.7 a ^y	2.1 a
Tendergreen	24	100.0	52.4 a	2.0 a
Montcalm	22	100.0	42.6 b	2.1 a
11-33	23	100.0	40.6 bc	2.0 a
C-20	22	100.0	34.3 cd	1.7 b
Rio Tibagi	24	91.7	28.9 de	1.9 ab
13-41	24	100.0	28.0 de	1.3 c
BMC 3352	23	87.0	27.8 de	1.7 b
Puebla 152	24	91.7	26.2 de	1.4 c
Olathe	24	95.8	24.4 e	1.7 b
Mean		96.6	36.4	1.8

^zTumor size: 1 is < 1 mm, 2 is 1 to 4 mm, 3 is > 4 mm in diameter.

^yMean separation by Duncan’s multiple range test, $P \leq 0.05$.

Hormone sensitivity of excised shoot tips. Shoot tips of the five lines cultured in media with BA or BA plus NAA displayed significant differences for callus and root formation (Table 3). The means of the three growth regulator combinations were pooled because there were no significant changes in rank among media treatments. ‘Montcalm’ and ‘Tendergreen’ shoot tips consistently had the largest basal calli (overall means of 13.6 and 12.6 mm, respectively). ‘Olathe’ seemed to be fairly recalcitrant to callusing (mean basal callus diameter of only 4.6 mm). Phytohormone sensitivity was also reflected in the propensity to form roots in response to auxin (NAA) in the medium. The rate of root formation paralleled the tendency to callus with one exception: UW 325 had the highest frequency of explants with roots and only an intermediate callus size. The lines that seemed to have the highest phytohormone sensitivity, ‘Montcalm’ and ‘Tendergreen’, were also among the most highly tumorigenic (Table 1). ‘Olathe’ had low tumorigenesis scores (Table 1) and also was insensitive to phytohormone-induced callusing.

GUS analysis of inoculated seedlings. Of the five lines inoculated with the GUS-containing strain C58C1(pGV3850/pKIWI105), only ‘Olathe’, a line with a low frequency of tumor formation, had no significant level of fluorometric GUS activity over the control (Table 4). The higher levels of endogenous GUS activity in the ‘Tendergreen’ and ‘Olathe’ controls may have been due to the presence of a GUS-like enzyme or to microbial endophytes with GUS activity. After accounting for intrinsic GUS

Fig. 1. Tumors on the unifoliate node region of ‘Tendergreen’ 3 weeks after stab-inoculating the germinating seed with *Agrobacterium tumefaciens* strain C58.

Table 2. Frequency of tumors at seven sites on plants of five common bean lines 3 weeks after stab-inoculation with *Agrobacterium tumefaciens* strain C58.

Bean	No. of	Tumor sites ²						
line	plants	hy	cn	ep	un	up	ul	tn
<i>Percentage of plants with tumors</i>								
UW 325	22	32	82	0	100	82	64	22 ^y
Tendergreen	24	8	62	25	71	38	100	67
Montcalm	22	0	14	18	91	59	91	25
Puebla 152	24	4	12	4	46	12	83	21
Olathe	24	4	21	0	54	12	71	4
All lines		7	28	9	65	36	86	19

^zAbbreviations: hy = hypocotyl, cn = cotyledonary node, ep = epicotyl, un = unifoliate node, up = unifoliate petiole, ul = unifoliate leaf (the pair was regarded as only one site), tn = first trifoliate node.

^yDue to frequent disruption of UW 325 meristems by a unifoliate node tumor, trifoliate nodes were often absent. The figure of 22% represents only two of the nine plants having trifoliate nodes.

Table 3. Basal callus diameter and frequency of root formation on excised cultured shoot tips of five common bean lines 3 weeks after mock stab-inoculation.

Bean line	No. of explants	Callus diam (mm)	Proportion of tips forming roots (%)
Montcalm	27	13.6 a ^z	52
Tendergreen	27	12.6 a	56
UW 325	27	7.2 b	63
Puebla 152	27	7.6 b	30
Olathe	26	4.6 c	23

^zMean separation by Duncan's multiple range test, $P \leq 0.05$.

activity, it was apparent that line UW 325 (169.6 pM/mg per min 4-MU) and 'Tendergreen' (93.6 pM/mg per min 4-MU) had the highest levels of GUS activity. The level of GUS activity in 'Montcalm', one of the more highly tumorigenic lines (Table 1), was moderately low and was no higher than in 'Puebla 152', a weakly tumorigenic line. Pretreating bacteria with acetosyringone led to a general decrease in GUS activity (data not shown) except in UW 325, which seemed to have a dramatically increased level of expression (434.5 pM/mg per min 4-MU).

The shoot apices of UW 325 seedlings stab-inoculated with the GUS-containing strain had one or more regions of GUS expression in the area of the meristem (determined by histochemical analysis) in 13 of 25 (after 6 days) and 11 of 15 (after 15 days) shoot tips examined. These apices (total of 24) had an average of ≈ 2.5 GUS-positive regions, which appeared as blue zones. Stabbed seedlings were assayed at 6 days, corresponding to the time period used previously for the fluorometric analysis, or 15 days, by which time many shoot apices had recovered from the wounding caused by stabbing and resumed growth. In addition, a fluorometric analysis done on 6-day-old shoot apices of 12 seedlings stab-inoculated with the GUS-containing strain (plus 8 controls) had a mean level of GUS activity (185.2 pM/mg per min 4-MU) similar to the reading obtained in an earlier experiment (Table 4). Although transformation occurred in the meristem region, no continuing cell lineages that might have led to the formation of a transformed shoot were seen.

Discussion

Stab-inoculating seedlings is an attractive method of transformation because it does not require tissue culture regeneration. Further, it creates a wound site, which is necessary for infection

(Kado, 1991), and exposes the interior cell layers of the meristem to *Agrobacterium*. Transformation of these layers would allow a subsequently arising germline cell to pass on the acquired transgene through the seed. The procedure is rapid, taking only 3 weeks from inoculation to date of scoring.

The tumor frequency score indicated susceptibility to *A. tumefaciens* strain C58. Nodal tumor frequency relates more directly to the probability of producing a transformed shoot from inoculations with a disarmed-engineered strain. There did not seem to be an association of *Agrobacterium* susceptibility with any particular bean line attribute, such as region of ancestral origin, growth habit, or nodulation capability. For example, UW 325 (Mesoamerican origin, indeterminate climbing growth habit) and 'Tendergreen' (Andean origin, upright determinate growth habit) were highly susceptible. The rate of tumor formation for 'Puebla 152' (highly susceptible to nodulation by *Rhizobium*; Bliss, 1987) was similar to that of 'Rio Tibagi' (less susceptible to *Rhizobium* nodulation; F.A. Bliss, unpublished results).

There were significant differences in response (sensitivity) of mock-inoculated cultured shoot tips to growth regulators. It was anticipated that perhaps the highly tumorigenic nature of 'Tendergreen' and 'Montcalm' was the result of a high phytohormone sensitivity, as shown by the tendency to form large amounts of callus in vitro. *Agrobacterium*-induced tumors result from cell proliferation induced by the products of the T-DNA phytohormone oncogenes. Still, it was possible that these lines were highly susceptible to *Agrobacterium* and highly sensitive to phytohormones. 'Olathe' had the lowest phytohormone sensitivity and the lowest overall tumorigenesis score. But it was conceivable that the paucity of tumor formation on this line was due simply to an insensitivity to the phytohormones produced, and that it might actually be reasonably susceptible to *Agrobacterium*. The wild line UW 325 did not follow the same pattern of response as the other four lines. While being highly tumorigenic, it had only a moderate level of in vitro callus formation.

Tumorigenesis assays are simple to perform. However, tumor formation, as a measure of susceptibility, depends on expression of the oncogenic transgenes and on the phytohormone sensitivity of the plant. In contrast, the GUS marker gene allows for direct determination of the transformation rate as a measure of susceptibility. The GUS enzyme that is encoded by the *uidA* gene does not depend on plant substrates for expression. Further, the GUS gene found in pKIWI105 is not expressed within the *Agrobacterium* itself. This provides an opportunity for early transformation events to be assayed without concern for residual *Agrobacterium* GUS activity.

Table 4. Fluorometric GUS activity of excised shoot tips from seedlings of five common bean lines 6 days after stab-inoculation with *Agrobacterium tumefaciens* strain C58C1(pGV3850/pKIWI105).

Bean line	Treatment	No. of shoot tips	GUS activity (pM/mg per min 4-MU)	
			Absolute	Corrected
UW 325	Control	6	11.8	
	pKIWI105	36	181.4**	169.9 a ^z
Tendergreen	Control	6	31.7	
	pKIWI105	36	124.7**	93.6 b
Montcalm	Control	5	13.8	
	pKIWI105	32	36.8**	23.7 c
Puebla 152	Control	5	14.9	
	pKIWI105	36	36.6**	21.8 c
Olathe	Control	6	35.6	
	pKIWI105	35	39.8 ^{NS}	NS

^zMean separation by Duncan's multiple range test, $P \leq 0.01$. Statistical analysis was performed with $\log(X + 1)$ transformed data; original data are shown.

^{NS,**} Nonsignificant or significant at $P \leq 0.01$ as determined by paired *t* tests.

The C58 tumorigenesis tests were useful for measuring susceptibility with one exception. 'Montcalm', a highly tumorigenic line, turned out to be no more susceptible than 'Puebla 152', a weakly tumorigenic line, as measured by the GUS activity of pKIWI105-inoculated shoot tips. It seems that 'Montcalm' was merely more phytohormone-sensitive than 'Puebla 152'. UW 325 had a high tumorigenesis score despite being only moderately phytohormone-sensitive because of high susceptibility to *Agrobacterium*, as shown by a high level of GUS expression. The pre-induction of virulence did not raise the level of transformation in four of the five lines. This provided evidence that differences in susceptibility among the lines were not due to differences in the amounts or types of virulence-inducing compounds produced by the plant.

A histochemical assay of putatively GUS-transformed UW 325 shoot apexes detected expression of GUS at a fairly high frequency. The results of the fluorometric assay of the five lines inoculated with the GUS-containing strain did not reveal where in the shoot tip transformation was occurring. Since UW 325 has a high frequency of tumors on the petiole (82%), it was conceivable that a significant amount of the GUS fluorometric activity was a result of transformation in the remaining petiole stub, rather than in the meristem. However, most of the shoot tips in the histochemical assay had one or more transformation events in the meristem.

Because of the position of the blue-staining zones in the shoot apex, it was apparent that none of the transformation events was in continuing cell lineages of the meristem. Perhaps if more seeds were inoculated, cells in the meristem leading to a transformed shoot may be infected by *Agrobacterium*. On the other hand, it is possible that a meristem exclusion mechanism, such as that occurring in the case of many viral infections, may be functioning in *Agrobacterium* infection of the meristem (Potrykus, 1991).

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