Evaluation of a Herbicide-resistant Trait Conferred by the Bar Gene Driven by Four Distinct Promoters in Transgenic Blueberry Plants

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ABSTRACT. Four chimeric bialaphos resistance (*bar*) genes driven by different promoters were evaluated for production of herbicide-resistant ‘Legacy’ blueberry plants (73.4% *Vaccinium corymbosum* L. and 25% *Vaccinium darrowi* Camp) through *Agrobacterium tumefaciens* (Smith & Towns.) Conn.-mediated transformation. When the *bars* were used as selectable marker genes, different promoters yielded different transformation frequencies. Three chimeric *bar* genes with the promoter nopaline synthase (*nos*), cauliflower mosaic virus (CaMV) 35S, or CaMV 34S yielded transgenic plants, whereas a synthetic (Aocs)₃AmasPmas promoter did not lead to successful regeneration of transgenic plants. In addition, herbicide resistance in *bar*-expressing plants was influenced by the promoter strength. Under controlled environmental conditions, 3-month-old plants from six single-copy transgenic events with 35S::*bar* or *nos*::*bar*, as well as those nontransgenic plants, were sprayed with herbicide glufosinate ammonium (GS) at five levels (0, 750, 1500, 3000, and 6000 mg·L⁻¹). Evaluations on leaf damage 2 weeks after spraying indicated that all transgenic plants exhibited much higher herbicide resistance than nontransgenic plants. Additionally, the transgenic plants with the 35S::*bar* showed a higher herbicide resistance than those with the *nos*::*bar*. After application of 6000 mg·L⁻¹ GS, over 90% of the leaves from plants with the 35S::*bar* and 19.5% to 51.5% of the leaves from plants with the *nos*::*bar* showed no symptom of herbicide damage, whereas only 5% of leaves from the nontransgenic had no damage. One-year-old, field-grown plants from four transgenic events with the *nos*::*bar* were evaluated for herbicide resistance after spraying with 750 mg·L⁻¹ GS. Transgenic plants survived with variations in the level of foliar damage; in contrast, all nontransgenic plants died. This study is the first investigation of different promoters for engineering transgenic blueberry plants.

Blueberry is an important fruit crop in the genus *Vaccinium* L. (for reviews, see Ratnaparkhe 2007; Song and Sink, 2005). Blueberry fruit are one of the richest sources of antioxidant phytonutrients among the fresh fruit (Conner et al., 2002; Ehlenfeldt and Prior, 2001). According to U.S. Department of Agriculture-National Agricultural Statistics Service (USDA, 2007), the United States ranks first in world production of blueberry, supplying 124,976 Mg of blueberry fruit on 21,376 ha in 2006.

Molecular genetic and genomic approaches will enable localization and isolation of genes controlling blueberry traits such as fruit size, fruit quality, disease resistance, and various environmental tolerances. Recently, several genes associated with cold hardiness have been identified and isolated from highbush blueberry using a large expressed sequence tag library, forward and reverse subtracted cDNA libraries, or microarrays (Dhanaraj et al., 2004, 2007; Naik et al., 2007). Once genes of interest are isolated, genetic engineering and transformation of blueberry will be a powerful approach to

complement traditional breeding by rapidly introducing individual traits without changing the inherent desirable characteristics of existing cultivars (Ratnaparkhe, 2007; Song and Sink, 2005). To date, transformation methodologies for blueberry cultivars have been developed by several research groups (Cao et al., 1998, 2003; Graham et al., 1996; Rowland, 1990; Song and Sink, 2004, 2006; Song et al., 2007a).

There are some particularly persistent perennial weeds adapted to low pH soils of blueberry fields. To control weeds, nonselective and broad-spectrum herbicides, such as glyphosate and glufosinate ammonium, are preferable; however, they can be applied only as a directed spray under the bushes to avoid contact with green tissues. Blueberry plants withstanding broad-spectrum herbicides would add flexibility to weed control programs while providing a simple and effective management tool. Broad-spectrum herbicide resistance genes have been introduced into many crop species (Castle et al., 2004; James, 2007). In 2006, herbicide tolerance, deployed in soybean [*Glycine max* (L.) Merr.], maize (*Zea mays* L.), canola (*Brassica napus* L.), cotton (*Gossypium* L.), and alfalfa (*Medicago sativa* L.) continued to be the most dominant trait, occupying 68% (69.9 million hectares) of global biotechnology crops (James, 2007). In *Vaccinium* species, transformed cranberry (*Vaccinium macrocarpon* Ait.) plants with the bialaphos resistance gene (*bar*) showed resistance to 1000 mg·L⁻¹ GS (Polashock and Vorsa, 2002). Using our established transformation protocol (Song and Sink, 2005), four constructs, including *nos*::*bar*, 35S::*bar*, (Aocs)₃AmasPmas::*bar*, and 34S::*bar* were previously used to produce herbicide-resistant

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Fig. 1. Schematic representation of the transfer DNA (T-DNA) region of the four bialaphos resistance (*bar*) gene constructs. T-nos = nos gene terminator, pAg7 = gene7 (*pAg7*) polyadenylation signal, P-nos = nopaline synthase (*nos*) promoter, *bar* = *bar* gene coding region, P-34S = cauliflower mosaic virus (CaMV) 34S promoter, P-(Aocs)₃AmasPmas = (Aocs)₃AmasPmas promoter, T-ags = agropine synthase (*ags*) terminator, P-35S = CaMV 35S promoter, HPT = hygromycin phosphotransferase coding region, RB = right T-DNA border, LB = left T-DNA border, *Bam*HI = unique restriction endonucleases at T-DNA region.

Table 1. Summary of transformations of ‘Legacy’ blueberry using the bialaphos resistance (*bar*) gene driven by different promoters.

Construct ^z	Explants inoculated (no.)	Explants yielding shoots with resistance to 0.1 mg·L ⁻¹ GS ^y (no.)	Explants yielding shoots with resistance to 0.5 mg·L ⁻¹ GS (no.)	Explants yielding PCR-positive shoots (no.)	Avg transformation frequency ^x (% ± SD)
35S::bar	202	32	16	16	7.9 ± 0.6
34S::bar	201	6	3	3	1.5 ± 0.6
(Aocs) ₃ AmasPmas::bar	209	8	0	0	0.0 ± 0.0
Nos::bar	300	30	21	21	7.0 ± 2.2

^z35S = cauliflower mosaic virus (CaMV) 35S promoter, 34S = CaMV 34S promoter, (Aocs)₃AmasPmas = (Aocs)₃AmasPmas promoter, Nos = nos gene promoter.

^yGS = glufosiate ammonium.

^xBased on results of PCR amplification of the presence of the *bar* for the shoots showing resistance to 0.5 mg·L⁻¹ GS. Transformation frequency = number of leaf explants with at least one PCR-positive shoot/total number of inoculated explants × 100.

Fig. 2. ‘Legacy’ blueberry shoots on chlorophenol red assay medium after 2 weeks. NT = nontransgenic shoots; L18 = a representative transgenic clone with the *nos*::*bar*; S1, S2, and S3 = transgenic clones with the 35S::*bar*; 34S = a transgenic clone with the 34S::*bar*.

blueberry plants. Our preliminary results demonstrated that the *nos*::*bar* gene can serve as a selectable marker for transformation, as well as being a source for herbicide resistance in blueberry plants (Song et al., 2007a).

Expression levels of a transgene are known to be greatly influenced by promoters and the host plant species (Ni et al., 1995; Song et al., 2007b; Wilmink et al., 1995). Evaluation of different promoters for genetic engineering blueberry plants has not been reported. The aims of this study were to evaluate four promoters for directing *bar* expression in ‘Legacy’ blueberry plants and to test their effect on herbicide resistance in the laboratory and field.

Materials and Methods

CONSTRUCTS AND AGROBACTERIUM STRAIN. Four *bar*-containing vectors were used (Fig. 1). PGPTV-BAR (a gift from D. Becker, Max-Planck-Institut, Köln, Germany) has *bar* under the *nos* promoter and gene7 (*pAg7*) polyadenylation signal (Becker et al., 1992). PDHB321.1 (a gift from D. Bouchez, Institut National de la Recherche Agronomique, Versailles, France) contains the *bar* gene under the 35S promoter and the *nos* terminator. A polymerase chain reaction (PCR)-amplified 5′-HindIII-*bar*-XbaI-3′ fragment covering the coding region of *bar* was inserted into multiple cloning sites located between (Aocs)₃AmasPmas promoter and an agropine synthase (*ags*) terminator in pUC119 to generate a *bar* expression cassette (Aocs)₃AmasPmas::*bar*::*ags*. Similarly, a PCR-amplified 5′-HindIII-*bar*-XbaI-3′ fragment was inserted into multiple cloning sites located in front of the *nos* terminator in pUC19 to yield a cloning vector with multiple cloning sites plus *bar*::*nos*. Subsequently, a 5′-BamHI (blunted)-*bar*-*nos*-EcoRI-3′ fragment was ligated with a 1.1-kb 5′-XbaI-BamHI (blunted)-3′ fragment, derived from pMS34b, containing the cauliflower mosaic virus (CaMV) 34S promoter. The 5′-XbaI-34S::*bar*::*nos*-EcoRI-3′ fragment derived was inserted into multiple cloning sites of pUC19 to create a *bar* expression cassette. The HindIII (blunted)-34S::*bar*::*nos*-SmaI and PvuII-(Aocs)₃AmasPmas::*bar*::*ags*-PvuII were then inserted into the blank T-DNA region of pGPTV-BAR at EcoRI (blunted)-XmnI, respectively, to generate vectors with *bar* expression cassettes. All plasmids were checked by restriction mapping and the PCR-amplified *bar* was confirmed by sequencing.

Fig. 3. Southern hybridizations for the bialaphos resistance (*bar*) gene in blueberry plants. DNA samples were digested with *Bam*HI and hybridized with a 438-bp fragment containing the *bar* coding region. NT = nontransgenic plant; S1, S2, and S3 = transgenic events with the *35S::bar*; 34S = transgenic event with the *34S::bar*; M = molecular marker.

All four *bar*-containing vectors were introduced into *A. tumefaciens* strain EHA105 using the freeze-thaw transformation method (An et al., 1988; Hood et al., 1993).

PLANT TRANSFORMATION. Transformation of 'Legacy' blueberry using four *bar*-containing vectors was performed according to our previous reports (Song and Sink, 2006). For each

Fig. 4. PAT activity in leaf extracts of transgenic blueberry. Glufosinate ammonium (GS) is acetylated by the bialaphos resistance (*bar*) gene product under the presence of acetyl-coenzyme A, yielding acetyl-GS. S1, S2, and S3 = three transgenic events with the *35S::bar*; EB = extract buffer for crude protein extract; 34S = transgenic event with the *34S::bar*; NT = nontransgenic plant.

vector, 200 to 300 leaf explants were used in two transformation experiments. The number of regenerating explants was recorded after 10 weeks. Morphologically normal-appearing plants of three events transformed with *35S::bar* and one event

Fig. 5. Response of 'Legacy' blueberry plants 2 weeks after application of four levels (750, 1500, 3000, and 6000 mg·L⁻¹) of herbicide glufosinate under controlled environmental conditions. NT = nontransgenic blueberry; S1, S2, and S3 = transgenic events with the *35S::bar*; L18 and L21 = transgenic events each with a single copy of the *nos::bar*.

with *34S::bar* were selected and subjected to the chlorophenol red (CR) assay, Southern blot analysis, and phosphinothricin acetyltransferase (PAT) assay. Transgenic events with *nos::bar* were previously reported using these assays (Song et al., 2007a).

CHLOROPHENOL RED ASSAY. CR assay was performed according to our previous report (Song et al., 2007a). Nontransgenic and transgenic shoots, 0.8 to 1.2 cm in length, were placed individually into wells of a 24-well (6 × 8) microliter plate containing 1 mL of assay medium. The plates were placed at 25 °C under a 16-h photoperiod of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and were inoculated for 2 weeks.

SOUTHERN BLOT ANALYSIS. Total DNA was isolated from 2 g of fresh leaf tissue of newly obtained transgenic events by the CTAB method (Doyle and Doyle, 1987). *Bam*HI-digested DNA, 20 μg per sample, was electrophoresed in a 0.8% agarose gel and was then transferred to N^+ -nylon membranes (Amersham, Arlington Heights, IL). A 438-bp fragment containing the *bar* coding region was used as the probe after labeling with [^{32}P]-dCTP using Random Primers DNA Labeling System (Invitrogen, Carlsbad, CA). Hybridization and membrane washing was conducted according to our previous report (Song and Sink, 2004).

PHOSPHINOTHRICIN ACETYLTRANSFERASE ASSAY. Crude protein was extracted as described (De Block et al., 1987) from 200 mg of leaf tissue. The protein was quantified using a protein assay reagent (Bio-Rad Laboratories, Hercules, CA). The PAT reaction mixture (20 μL : 50 mM Tris-HCL, 5.6 mM ^{14}C acetyl-CoA, 100 μM GS, and 30 μg of sample protein) was incubated at 37 °C for 30 min, spotted (10 μL) onto a silica gel thin-layer chromatography plate, and separated by ascending chromatography in a 3:2 (v/v) mixture of 1-propanol and NH_4OH (De Block et al., 1987). ^{14}C was visualized by autoradiography.

HERBICIDE RESISTANCE IN PLANTS GROWN UNDER CONTROLLED ENVIRONMENTAL CONDITIONS. One-month-old rooted plants were grown in planting medium (sphagnum moss:soil = 1:1, v/v) in six-cell trays. They were watered as needed, fertilized weekly using 0.2 $\text{g}\cdot\text{L}^{-1}$ 21N–3.1P–5.8K fertilizer (Scotts, Marysville, OH), and grown for 3 months at 25 °C under a 16-h photoperiod of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Control plants, along with plants of six

Fig. 6. Evaluation of herbicide resistance in 'Legacy' blueberry plants 2 weeks after application of four levels (750, 1500, 3000, and 6000 $\text{mg}\cdot\text{L}^{-1}$) of herbicide glufosinate under controlled environmental conditions. Injury ratings, 0 to 4 are classified according to the percentages of injured area for individual leaves: 0 = no injury, 1 = 0% to 20% injury, 2 = 20% to 50% injury, 3 = 50% to 80% injury, and 4 = 80% to 100% injury. NT = nontransgenic blueberry; S1, S2, and S3 = transgenic events each with a single copy of the *35S::bar*; L18, L21, and L22 = transgenic events each with a single copy of the *nos::bar*.

single-copy transgenic events, three new events with *35S* and three reported events with *nos* promoter (Song et al., 2007a), were evaluated. Nondamaged plants with 10 to 30 leaves were sprayed with five levels (0, 750, 1500, 3000, and 6000 $\text{mg}\cdot\text{L}^{-1}$) of GS (Rely; Bayer CropScience, Research Triangle Park, NC), respectively, using a track sprayer. The sprayed plants were subsequently grown under the same environmental conditions

as described above. Herbicide injury to sprayed plants was recorded after 2 weeks by scoring all of the sprayed leaves of each plant, excluding three most newly emerging leaves. Each leaf was scored from 0 to 4 according to the percentage of leaf area with visible injury: 0 = no injury, 1 = 0% to 20% injury, 2 = 20% to 50% injury, 3 = 50% to 80% injury, and 4 = 80% to 100% injury.

These experiments were conducted using a completely randomized design with six plants as replications for each treatment. The experiments were performed twice.

HERBICIDE RESISTANCE IN FIELD PLANTS. Field trials were performed on four previously reported transgenic events with the *nos::bar*, of which L21, L22, and L18 each has a single copy of the *nos::bar* and L25 has three copies of the *nos::bar* (Song et al., 2007a). One-year-old plants, six for each transgenic event as well as four non transgenic plants, were sprayed on 8 Aug. 2006 with herbicide GS at 750 mg·L⁻¹. Additionally, two plants for each of the transgenic and nontransgenic events were sprayed with water. Herbicide injury to sprayed plants was recorded after 3 weeks. Two branches per plant, each with 10 to 20 leaves, were randomly selected and each leaf on the selected branches was scored as above from 0 to 4. No field trials were conducted on transgenic plants with *35S::bar* due to the lack of permission from the USDA-Animal and Plant Health Inspection Service.

Results

TRANSFORMATION AND REGENERATION. GS-resistant shoots emerged from *A. tumefaciens*-inoculated leaf explants after transformation with all of the four constructs, *nos::bar*, *35S::bar*, *(Aocs)₃AmasPmas::bar*, and *34S::bar*. However, not every GS-resistant shoot showing resistance to 0.1 mg·L⁻¹ GS proliferated on medium containing 0.5 mg·L⁻¹ GS. Only 16 individual transgenic events with *35S::bar* and three events with *34S::bar* proliferated on the medium containing 0.5 mg·L⁻¹ GS, whereas no shoots showed resistance to 0.5 mg·L⁻¹ GS after transformation with *(Aocs)₃AmasPmas::bar* (Table 1). All of the 19 transgenic events were verified to be positive by PCR amplification of the *bar*-coding region (data not shown). The *35S::bar* and the *nos::bar* produced higher herbicide resistance (GS at 0.5 mg·L⁻¹) in shoots than the *(Aocs)₃AmasPmas::bar* or the *34S::bar* during the

Fig. 7. Response of field grown plants of 'Legacy' blueberry 2 weeks after application of herbicide glufosinate at 750 mg·L⁻¹ to the foliage. (A) Response of plants to herbicide. (B) Field of transgenic blueberry plants. NT-spray = nontransgenic plants sprayed with herbicide; L21, L22, and L18 = transgenic events each with a single-copy of the *nos::bar*; L25 = a transgenic event with three copies of the *nos::bar*.

selection step, indicating that the promoter of a selectable marker gene is important for efficient production of transgenic plants.

All 16 transgenic plants with *35S::bar* appeared similar with no obvious differences compared with the nontransformed plant. Of the three transgenic events with *34S::bar*, rooted plants of one event appeared morphologically normal; in contrast, plants of the other two events had shortened internodes or reduced apical dominance.

CR ASSAY. We previously found that the CR assay was a reliable method to identify *nos::bar*-expressing transgenic blueberry shoots (Song et al., 2007a). Similarly, nontransgenic shoots were sensitive to GS in this study. They became necrotic

and led to the red-orange color of the CR assay medium (Fig. 2). In contrast, transgenic shoots of three events (S1, S2, and S3) with *35S::bar*, one representative event with *nos::bar*, and one event with *34S::bar* continued normal growth and medium acidification, resulting in the yellowing of the assay medium over a 2-week period (Fig. 2).

SOUTHERN BLOT ANALYSIS AND PAT ASSAY. Southern hybridization confirmed stable integration of *bar* in all four independent transgenic events analyzed, including three with *35S::bar* and one with *34S::bar*. One band was observed for each, whereas no signal was detected in the nontransgenic plant, as expected (Fig. 3). Because *Bam*HI is a unique cleavage site in the T-DNA region in vectors *35S::bar* and *34S::bar* (Fig. 1), the variation in the band pattern amongst transgenic events most likely represents a single copy of randomly inserted T-DNA. This supports our previous observation that mostly low copy number transgenic plants are generated using *A. tumefaciens*-mediated transformation (Song and Sink, 2004).

35S::bar or *34S::bar* yielded active PAT in all of the four Southern-confirmed transgenic events. The PAT metabolically detoxified GS and produced an inactive product, *N*-acetyl-GS; in contrast, *N*-acetyl-GS was not detected in nontransgenic plants (Fig. 4).

HERBICIDE RESISTANCE IN PLANTS GROWN UNDER CONTROLLED ENVIRONMENTAL CONDITIONS. Due to the high sensitivity of blueberry leaves to GS, herbicide injury symptoms were initially observed on transgenic and nontransgenic plants 2 d after application of herbicide at all rates as red spots or red areas on leaves. On the nontransgenic plants, the initial moderate injury symptoms developed into extensive leaf necrosis, whereas most of the injury symptoms disappeared on transgenic plant leaves (Fig. 5). At high levels GS (1500, 3000, and 6000 mg·L⁻¹), transgenic plants with *35S::bar* showed less herbicide damage than those of with *nos::bar* (Fig. 5).

The data on final damage to the leaves indicated that transgenic plants with *35S::bar* or *nos::bar* showed much higher resistance to the herbicide than nontransgenic plants (Fig. 6). In addition, at high-dose GS (1500, 3000, and 6000 mg·L⁻¹), transgenic plants expressing *35S::bar* had higher percentages of nondamaged leaves than those expressing *nos::bar*. The widest variations in herbicide resistance were

observed at GS 6000 mg·L⁻¹, in which nontransgenic plants were seriously damaged and were dying, whereas plants of all three transgenic events with the *nos::bar* (L18, L21, and L22) remained alive but had 20% to 50% nondamaged leaves, and transgenic plants of all three events with the *35S::bar* (S1, S2, and S3) showed little damage with 92% to 97% nondamaged leaves (Fig. 6).

HERBICIDE RESISTANCE OF FIELD-GROWN PLANTS. One day after the application of 750 mg·L⁻¹ GS, nontransgenic and transgenic leaves of field-grown plants exhibited herbicide injury symptoms as red spots. Subsequently, the red areas in nontransgenic leaves became necrotic, whereas the transgenic leaves returned to normal green. Two weeks after spraying with GS at 750 mg·L⁻¹, nontransgenic plants died, as did the surrounding weeds (Fig. 7). Plants of all the four transgenic events survived, confirming herbicide resistance in *bar*-expressing plants (Fig. 7).

Quantitative analyses of scores confirmed herbicide resistance in transgenic plants (Fig. 8). In addition, field-grown blueberry plants showed greater sensitivity to GS than those grown under controlled environmental conditions (Fig. 8). There were no nondamaged mature leaves on nontransgenic plants 2 weeks after herbicide application. In contrast, the percentage of nondamaged leaves in transgenic plants sprayed with herbicide GS at 750 mg·L⁻¹ were 4% for L25, 21% for L21, 45% for L22, and 61% for L18, respectively. GS at 750 mg·L⁻¹ caused little damage to plants of the two most highly resistant events (L18 and L22) compared with the water-sprayed transgenic plants that had about 50% nondamaged leaves (Fig. 8), indicating adequate expression of *nos::bar* for commercial application.

Discussion

As one of the three current, major selectable marker genes (Miki and McHugh, 2004), *bar* is also a desirable herbicide-resistance trait in crop plants. Many factors, such as promoters, culture conditions, and metabolic status of plant tissue, have impact on expression of *bar*, and thus correspondingly affect selection of transgenic tissues or subsequent level of herbicide resistance in the transgenic plants (De Block et al., 1995; Li et al., 2001).

Constitutive and inducible promoters have previously been used successfully to drive the *bar* gene (De Block et al., 1995; Li et al., 2001). Transformation with (*Aocs*)₃ *AmasPmas::bar* versus *nos::bar* in peppermint (*Mentha × piperita* L. cv. Black Mitcham) yielded no significant difference in transformation frequency (Li et al., 2001). In this study, when *bar* genes driven by four different promoters were tested for production of transgenic blueberry plants, expression of *bar* at in vitro stages was influenced by the promoter (Table 1). The (*Aocs*)₃ *AmasPmas* promoter was previously shown to successfully drive β-glucuronidase expression in transgenic blueberry plants (Song and Sink,

Fig. 8. Evaluation of herbicide resistance in field grown plants of 'Legacy' blueberry 2 weeks after application of herbicide glufosinate at 750 mg·L⁻¹. Sprayed leaves in two randomly selected branches for each plant were scored. Injury ratings, 0 to 4 are classified according to the percentages of injured area for individual leaves: 0 = no injury, 1 = 0% to 20% injury, 2 = 20% to 50% injury, 3 = 50% to 80% injury, and 4 = 80% to 100% injury. NT-spray = nontransgenic plants sprayed with herbicide; L21, L22, and L18 = transgenic events each with a single-copy of the *nos::bar*; L25 = a transgenic event with three copies of the *nos::bar*; L-control = two nonsprayed plants for each event of L21, L22, L18, and L25.

2004). In this study, low activity of the promoter under in vitro conditions or misplacement of promoter region during gene construction or transformation might be responsible for the failure in production of transgenic shoots with (*Aocs*)₃*AmasPmas::bar*. The (*Aocs*)₃*AmasPmas::bar* was more likely than the *nos::bar* to yield higher herbicide resistance in peppermint (Li et al., 2001). We found that the *35S::bar* and the *nos::bar* had similar transformation frequencies; however, the plants expressing the *35S::bar* showed higher herbicide resistance than those expressing the *nos::bar* under controlled environmental conditions (Figs. 5 and 6). Therefore, the transformation frequency with different promoters is not a good criterion for predicting herbicide resistance in *bar*-expressing plants.

Using single-copy transgenic plants, we demonstrated that herbicide resistance in blueberry plants can be strengthened by using better promoters. Under controlled environmental conditions, the *nos* or the *35S* promoter is efficient to direct expression of the *bar* in blueberry plants (Figs. 5 and 6). Sensitivity of nontransgenic and transgenic plants to herbicide is also influenced by plant age or environmental conditions (Figs. 6 and 8). Unlike the application of nonselective, systemic herbicides in commercial fields where contact with the blueberry foliage is avoided, in our field trials, the *nos::bar*-expressing plants were sprayed directly with herbicide. Because *bar*-expressing plants quickly recovered after GS application, this type of expression may work well in the commercial industry. In addition, a directed spray that might hit lower leaves and the canes but not drench the plant with glufosinate would be better as it would not set plants back as far and reduce recovery time. Additional field trials are still needed to evaluate those *bar*-expressing plants, in particular, how the transgene affects fruit quality and yield.

In summary, this article demonstrates that when the *bar* gene is used as a selectable marker, transformation frequency is influenced by promoter strength, that herbicide resistance in blueberry plants can be enhanced by using stronger promoters, and that it is possible to engineer blueberry plants with resistance to herbicide using the *bar* gene.

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