Transient Heterologous Gene Expression Methods for Poison Ivy Leaf and Cotyledon Tissues

Christopher C. Dickinson

Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, 220 Ag Quad Lane, Blacksburg, VA 24061

Alexandra J. Weisberg

Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331

John G. Jelesko¹

Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, 220 Ag Quad Lane, Blacksburg, VA 24061

Additional index words. *Toxicodendron radicans*, agroinfiltration, luciferase, biolistic transformation

Abstract. Poison ivy [*Toxicodendron radicans* (L.) Kuntz] is a widely recognized native plant species because of its production of urushiol, which is responsible for delayed contact dermatitis symptoms in humans. Poison ivy is predicted to become both more prevalent and more noxious in response to projected patterns of climate change. Future studies on poison ivy chemical ecology will require reverse genetics to investigate urushiol metabolism. A prerequisite for reverse genetic procedures is the introduction and expression of recombinant DNA into poison ivy tissues. Poison ivy leaves and cotyledons were marginally susceptible to vacuum- and syringe-agroinfiltration and expression of two firefly luciferase (*LUC*)-based reporter genes. The efficacy of agroinfiltration and transient *LUC* expression was dependent on leaf age and plant growth environmental conditions, with young leaves grown in magenta boxes showing highest transient *LUC* expression levels. Agroinfiltrated leaves showed an *Agrobacterium*-dependent accumulation of brown-colored pigments. Biolistic transformation of a *LUC* reporter gene did not show brown pigment accumulation and readily displayed transient *LUC* bioluminescence in both leaves and cotyledon tissues. These studies establish best practices for introducing and transiently expressing recombinant DNA into poison ivy leaf and cotyledon tissues, on which future reverse genetic procedures can be developed.

Poison ivy [*Toxicodendron radicans* (L.) Kuntz] is a native North American species best known for its capacity to induce allergic dermatitis symptoms after contact with human skin. The natural product responsible for the dreaded skin rashes are alk(en)yl-catechol congeners generically called urushiol, which is found in all members of the *Toxicodendron* genus (Gross et al., 1975; Hill et al., 1934; Kurtz and Dawson, 1971; Majima, 1922; Markiewicz and Dawson, 1965; Symes and Dawson, 1953,

1954). Urushiol chemical ecology is largely enigmatic because humans appear to be uniquely sensitive to the allergenic effects of urushiol, whereas wild and domesticated animals appear to be largely unaffected on contact with poison ivy. In fact, deer (Martin et al., 1951) and goats (Popay and Field, 1996) eat poison ivy foliage with no apparent ill effects. There are few studies focused on poison ivy physiology and/or ecology. Two studies are noteworthy as they establish that poison ivy responds to elevated atmospheric CO₂ levels by growing faster, accumulating more biomass, and shifting urushiol congener levels toward more allergenic forms of urushiol (Mohan et al., 2006; Ziska et al., 2007). Given the current pattern of increasing anthropogenic CO₂ emissions, poison ivy is poised to become more prevalent and more noxious, yet most aspects of urushiol metabolism and ecology are largely unexplored. For example, Dewick (1997) postulated that urushiol is derived from fatty acid metabolism beginning with a C₁₆ fatty acid–CoA starter molecule that is extended by a presumed polyketide synthase activity into a tetraketide, that is subsequently modified to form

alk(en)yl-catechol (urushiol) congeners. With that said, none of the proposed urushiol metabolites, enzyme activities, enzymes, or genes have been empirically validated to date. DeWick's hypothesized formation of a fatty acid tetraketide that is cyclized into an alkylphenol by a polyketide synthase activity is likely because a number of plant polyketide synthases use fatty acid–CoA starting molecules in the production of alkylphenols (Abe et al., 2004; Kim et al., 2010, 2013; Matsuzawa et al., 2010; Taura et al., 2016, 2009). The capacity to investigate poison ivy metabolism and chemical ecology was recently advanced with the publication of the poison ivy (*T. radicans*) leaf and root transcriptome (Weisberg et al., 2017).

The capacity to express recombinant DNA molecules in plants enables detailed investigations of the role of both endogenous and foreign genes. Recombinant genes in plants can be expressed either as transient unintegrated transgenes or as transgenes that are stably integrated into the plant nuclear or plastid genomes. Understanding the molecular basis for urushiol biosynthesis and urushiol chemical ecology will require a variety of molecular genetic methods, all of which require the introduction and expression of recombinant DNA in poison ivy plant cells and tissues. The principle methods of introducing recombinant DNA constructs into plant cells are biolistic and *Agrobacterium*-based transformation procedures.

Agrobacterium tumefaciens was initially investigated as a soil-borne phytopathogen responsible for crown gall tumor formation. The etiology of pathology requires segments of bacterial tumor-inducing (Ti) plasmid DNA transfer, stable integration, and expression of these bacterial DNA sequences within the plant cell nucleus (Drummond et al., 1977). This inherent plant gene transformation capability of *A. tumefaciens* was reengineered to replace the tumor formation regions on the Ti plasmid (i.e., disarmed binary Ti plasmids) with recombinant “genes of interest.” The potential utility of *Agrobacterium*-mediated transient transformation was first demonstrated by expressing high levels of transient *GUS* reporter gene activity in bean, tobacco, and poplar leaves by simply infiltrating *A. tumefaciens* harboring a *GUS* reporter gene on a binary T-DNA plasmid directly into the leaf mesophyll interstitial space (Kapila et al., 1997). The general applicability of the technique is shown by transient reporter gene expression in the leaves of diverse plant species such as switchgrass (VanderGheynst et al., 2008), habanero pepper (Arcos-Ortega et al., 2010), cowpea epicotyl (Bakshi et al., 2011), rice (Andrieu et al., 2012), soybean (King et al., 2015), and persimmon (Mo et al., 2015), to name a few.

Biolistic transformation is often used when *Agrobacterium*-mediated transformation proves problematic. Biolistic transformation is the acceleration of nucleic acid-coated

Received for publication 4 Oct. 2017. Accepted for publication 23 Dec. 2017.

This work was supported by funds from the Virginia Agricultural Experiment Station and the USDA National Institute of Food and Agriculture, U.S. Department of Agriculture (Washington, DC). C.C.D. performed the syringe-agroinfiltration, biolistic transformation, and luciferase-imaging experiment data analysis, and writing and editing of the manuscript. A.J.W. performed the vacuum-agroinfiltration, luciferase imaging, and editing of manuscript. J.G.J. conceived and supervised the experiments, performed data analyses, and wrote the manuscript.

¹Corresponding author. E-mail: jelesko@vt.edu.

microparticles to high velocity (using a so-called gene gun) resulting in entry into the plant cell cytoplasm, where the nucleic acid subsequently uncoats from the microparticle, and is either transiently expressed or stably integrates into the plant nuclear genome (Klein et al., 1987). Alternatively, if combined with appropriate plastid DNA sequences the transgene can integrate into, and be expressed from, the plastid genome (Daniell et al., 1990). Biolistic transformation is the method of choice for stable transformation of major cereal crops (Klein et al., 1988; Wang et al., 1988) that are otherwise recalcitrant to *Agrobacterium*-mediated stable transformation.

Development of reverse genetic methods in poison ivy will enable molecular genetic investigations of poison ivy ecophysiology and chemical ecology, and provide foundational molecular genetic approaches in other members of the *Toxicodendron* genus (e.g., poison oak and poison sumac). As an initial step toward the implementation of such molecular genetic studies, the present report describes the introduction and transient expression of recombinant firefly *LUC* reporter gene constructs in poison ivy cells and tissues through agroinfiltration and biolistic transformation.

Materials and Methods

Plant material. Poison ivy (*T. radicans* subsp. *radicans*) drupes were sourced from the RoaCo-1 liana located in Catawba VA (Benhase and Jelesko, 2013). Drupes were mechanically and chemically scarified (Benhase and Jelesko, 2013). Axenic seedlings were germinated on petri plates of 0.5X Murashige & Skoog (MS) basal salts media (Plant Natural, Bozeman, MT), in the dark for four nights. Germinating seedlings were then transferred to either sterile 0.5X MS media in magenta boxes or pots containing nonsterile Sunshine Mix 1 (Plant Natural). The plants were grown at 28 °C under 16 h light/day cycle. Poison ivy leaves were typically agroinfiltrated with a syringe at the three to four true leaf stage. *Nicotiana benthamiana* plants were directly germinated and grown in pots containing Sunshine Mix, under the same environmental conditions as the poison ivy seedlings. *Nicotiana benthamiana* plants were syringe agroinfiltrated 4 weeks postgermination.

Agroinfiltration transformation. Three *LUC*-containing plasmids were used in this study. Plasmid pJGJ204 contains an Arabidopsis *RBCS1B-LUC* gene fusion comprised the *RBCS1B* promoter-exon 3, resulting in an in-frame *RBCS1B-LUC* chimeric fusion protein (Jelesko et al., 1999). The firefly *LUC* was polymerase chain reaction (PCR) amplified from pJGJ102 (Jelesko et al., 1999) using oligonucleotide primers (oJGJ234 5'-GGG-GAGAAGTTTGTACAAAAAGCAGGC-TATGGAAGACGCCAAAAACATA-3'; and oJGJ235 5'-GGGGACCACTTTGTACAA-GAAAGCTGGGTATTTTACAATTTGGA-CTTTC-3') that incorporated 5'-*attB1* and 3'-*attB2* sequences. The resulting *LUC* PCR fragment was BP subcloned into pDONR221

using BP Clonase II (ThermoFisher, Waltham, MA) to yield plasmid pJGJ404. The *LUC* gene from pJGJ404 was subcloned into pMCD32 (Curtis and Grossniklaus, 2003) using LR Clonase II (Invitrogen, Carlsbad, CA) to yield plasmid pJGJ410 expressing the *LUC* gene from a double CaMV35S promoter. Plasmid pJGJ411 containing a *LUC-INT* gene was similarly subcloned, except using pLUK07 (Mankin et al., 1997) as the template in the initial PCR reaction. Plasmid p19 (Voinnet et al., 2003) was used for enhancing transient expression levels of the transgenes in plant cells. *Agrobacterium tumefaciens* strain GV3101 containing either p19 (Voinnet et al., 2003), pJGJ204, pJGJ410, or pJGJ411 were grown overnight (16 h) in 5 mL Luria-Bertani medium supplemented with 50 µg·mL⁻¹ kanamycin and 50 µg·mL⁻¹ gentamycin. The culture optical density (OD) at 600 nm (OD₆₀₀) was estimated from a 1:10 culture dilution before initial centrifugation (15 min at 4 °C, 3000 × g). Cultures were resuspended in MMA buffer [10 mM 2-N-Morpholino ethanesulfonic acid (MES), 10 mM MgCl₂, and 20 µM acetosyringone] by vortexing for either final OD₆₀₀ of 4.0 or 0.4. The *Agrobacterium* strains containing a *LUC*-containing plasmid (pJGJ204, pJGJ410, or pJGJ411) were mixed with GV3101/p19 at equivalent concentration and incubated at 22 °C for 1 h before agroinfiltration (Kapila et al., 1997). Leaf tissues were either vacuum-agroinfiltrated or syringe-agroinfiltrated using a 3 mL Luer Lok syringe (Becton Dickinson, Franklin Lakes, NJ), on the leaf underside (Kapila et al., 1997). Plants were returned to a 28 °C growth chamber for 24 h before assay of *LUC* activity.

Biolistic-mediated transformation. Plasmid pJGJ411 was isolated from *Escherichia coli* OmniMax strain first by large scale alkaline lysis (Ausubel et al., 2006), then purification using a QIAprep Miniprep kit (Qiagen, Valencia, CA). Purified pJGJ411 plasmid DNA was coated on (0.6 µm) gold particles, accelerated using a 1100 psi rupture disk (Analytical Scientific Instruments US Inc., Richmond, CA) in a Bio-Rad (Richmond, CA) Biolistic PDS-1000 He Particle Delivery System. Poison ivy cotyledon/first true leaf-stage seedlings in magenta boxes were subjected to particle bombardment at a distance of ≈13 cm. After bombardment, plants were returned to a 28 °C growth chamber for 24 h before assay of *LUC* activity.

Luciferase assay. Before *LUC* imaging, transformed poison ivy tissues were placed onto water agar plates and then sprayed with luciferin and subjected to in vivo *LUC* imaging, as previously described (Jelesko et al., 1999). Qualitative photon emission imaging was obtained from “slice” images. Quantification of vacuum-agroinfiltration and biolistic-transformed poison ivy cotyledon and first true leaf in vivo-*LUC* activity (photon emission) was estimated using “gravity” images. A fixed area defined by the area that encompassed either the largest cotyledon (6358 pixels²) or largest first true leaf (30,458 pixels²) was used to measure the number of emitted photons for each subsequent

cotyledon, leaf, and background. The background heat photon emission was estimated for each specified tissue by randomly measuring the apparent gravity photon emission in 10 regions corresponding to the absence of poison ivy tissue in the reflected light images, and the upper 95% confidence interval value was calculated. The upper 95% confidence interval value established the upper boundary of background heat photon emission levels corresponding to cotyledon and leaves, respectively. The respective upper background photon emission value was subtracted from the value of the respective tissue's highest photon emission level, and the resulting difference was equally divided into five bins, thereby providing a rough distribution of photon emission levels for each transformed tissue.

Results

Transient *LUC-INT* gene expression in poison ivy leaves using syringe agroinfiltration. Poison ivy leaves were subjected to syringe-agroinfiltration using *A. tumefaciens* strain GV3101 harboring plasmid pJGJ411 containing a double CaMV promoter driving the expression of firefly *LUC* gene containing an artificial intron (*LUC-INT*). The intron in the *LUC-INT* gene abolished low expression levels in *Agrobacterium* cells relative to the continuous *LUC* open reading frame in pJGJ410 (Supplemental Fig. 1), and thus all *LUC* activity was derived from transient *LUC* expression in poison ivy cells. Poison ivy leaves from potting soil-grown plants subjected to syringe-agroinfiltration generally did not display *LUC* activity greater than background heat photon emission levels in qualitative pseudocolor superimposed slice photon emission images (Fig. 1A). On very few occasions weak photon emission more than background levels of syringe-agroinfiltrated leaves was observed. In contrast, syringe-Agroinfiltrated leaves on poison ivy plants grown axenically in magenta boxes displayed small patches of consistent photon emission over regions that were syringe-agroinfiltrated (Fig. 1A). Thus, magenta box-grown poison ivy leaves were more susceptible to syringe-agroinfiltration-mediated transient DNA transformation. However, not all leaves from Magenta box grown poison ivy plants were equally susceptible to syringe-agroinfiltration. Older leaves of magenta box-grown plants were less susceptible to syringe-agroinfiltration transient *LUC-INT* expression compared with younger leaves (Fig. 1B). Younger leaves more readily took up the *Agrobacterium*-infiltration solution delivered from the blunt syringe. Consequently, young poison ivy leaves in magenta box-grown plants consistently showed higher transient *LUC* expression levels than older leaves from the same plants, whereas changing the bacterial concentration 10-fold had little effect on transient *LUC* expression levels (Supplemental Fig. 4). With that said, even the best poison ivy transient *LUC* expression levels were dramatically lower than *N. benthamiana* leaves syringe-agroinfiltrated with pJGJ411 (Fig. 1C). The poison ivy

Fig. 1. Imaging of agroinfiltrated leaves. Panels A–C single photon pseudocolor images (panel A inset is pseudocolor step gradient with high photon emission shown as red to low photon emission as dark blue) superimposed on reflected light image (grayscale). Panel D, digital color image of poison ivy leaves infiltrated with either MMA buffer or MMA buffer with *Agrobacterium* containing the firefly *LUC* construct. Arrows indicate browning at site of agroinfiltration. (A and B) Composite pseudocolor single photon imaging on reflected light image of poison ivy leaves agroinfiltrated with firefly *LUC* construct. (C) *Nicotiana benthamiana* leaf similarly agroinfiltrated and imaged.

leaves did not take up as much of the *Agrobacterium* solution as the *N. benthamiana* leaves with each syringe-agroinfiltration attempt. However, these differences in relative infiltration volumes were far less than the difference in magnitude of photon emission between poison ivy and *N. benthamiana* (Fig. 1A and C).

Syringe-agroinfiltrated poison ivy leaves displayed brown discoloration within the regions that were effectively infiltrated with bacteria (Fig. 1D). This brown discoloration was dependent on the presence of the *Agrobacterium*, because syringe infiltration with just the MMA media lacking *A. tumefaciens* did not acquire the brown pigmentation. When plants are infiltrated with bacteria, many plant species induce the synthesis and accumulation of phytoalexin compounds as part of a plant defense response (Hammerschmidt, 1999). Thus, the overall low transient *LUC* expression levels may have been a consequence of reduced *Agrobacterium* vigor in response to a biotic stress response, however this possibility was not further investigated in this study.

Transient *AtRBCS1B-LUC* gene expression in poison ivy leaves using vacuum-agroinfiltration. Vacuum-agroinfiltration was investigated as an alternative method for transient heterologous plant gene expression in poison ivy. An *Arabidopsis* chimeric

AtRBCS1B-LUC chimeric gene fusion (pJGJ204 comprised the *AtRBCS1B* promoter, exons I, intron I, exon II, intron II, and partial exon III fused in-frame to the firefly *LUC* open reading frame), was used to vacuum-agroinfiltrate excised poison ivy leaves and cotyledons from plants grown in magenta boxes. Vacuum-agroinfiltrated leaves were placed on 0.5 × MS media plates, sprayed with luciferin at 24 h postinfiltration, and then imaged for three sequential 1 h-imaging sessions beginning at ≈48 h post agroinfiltration. The *AtRBCS1B-LUC* chimeric gene produces a low rate of photon emission in transgenic *Arabidopsis* plants. Superimposed “slice” images with complete subtraction of background photons displayed a few blue spots over leaves/cotyledons (Supplemental Fig. 2A), whereas superimposed “slice” imaging that retained some background photons displayed higher qualitative photon accumulation over leaves vacuum-agroinfiltrated with the plasmid containing the *AtRBCS1B-LUC* chimeric gene, relative to vacuum-agroinfiltrations with the vector control plasmid pSLK7292 (Supplemental Fig. 2B) indicating low but demonstrable *AtRBCS1B-LUC* recombinant protein expression over background heat photons. Photon emission levels were significantly higher in pJGJ204 (*AtRBCS1B-LUC*) vacuum-agroinfiltrated leaves and cotyledons compared with pSLK7292 vacuum-agroinfiltrated leaves and cotyledons

(P value < 0.05), during the first two of three sequential 1 h-imaging sessions quantified using “gravity” imaging (Fig. 2). There was a consistent trend of gradually lower total photon emission levels over the course of three hours suggesting that luciferin substrate levels were a limiting factor during the imaging session. The vacuum-agroinfiltrated leaves and cotyledons also showed browning of infiltrated tissues similar to the syringe-agroinfiltrated leaves. This was particularly apparent in cases where the cotyledon or leaf segments were not fully vacuum-agroinfiltrated, and showed a lighter green color (see arrows in Supplemental Fig. 2C) typical of uninfiltrated leaves. These results demonstrate that a heterologous *Arabidopsis* genomic *RBCS1B* promoter and intron containing *RBCS1B-LUC* gene fusion was expressed in poison ivy leaves and cotyledons.

Biolistic transient *LUC* gene expression in poison ivy leaves and cotyledons. The induction of an apparent biotic stress response (i.e., accumulation of brown pigmentation) in agroinfiltrated poison ivy leaf tissue might limit the efficiency of agroinfiltration transient DNA transformation. To avoid such a possibility, biolistic transformation of isolated pJGJ411 (*LUC-INT*) plasmid DNA directly into poison ivy leaves and cotyledons was investigated. Using superimposed slice photon imaging, punctuate foci of photon emission were observed on both cotyledons and leaves (Supplemental Fig. 3). The distribution of *LUC* expression was not uniform, both across tissue type and between replicated tissues on the same targeting field. Likewise, quantification of gravity images demonstrated that the amount of photon emission from any given leaf or cotyledon was variable, ranging from background heat photons to quite intense photon emission (Fig. 3). This variability is likely a consequence of the uneven and unpredictable spreading of plasmid-coated gold particles during particle acceleration. Unlike all poison ivy tissues transformed by agroinfiltration, the biolistic transformed poison ivy cotyledons and leaf tissues did not produce demonstrable brown pigmentation.

Discussion

These studies lay the foundation for future transgenic reverse genetic approaches to investigate various poison ivy physiologies by demonstrating that exogenous DNA can be effectively introduced and expressed in poison ivy cotyledon and leaf tissues. Because the *CaMV35S* promoter is weakly active in *A. tumefaciens* (Supplemental Fig. 1; Mankin et al., 1997) it was essential to use intron-containing firefly *LUC* gene constructs (*CaMV35S-LUC-INT* and *AtRBCS1B-LUC*) to validate poison ivy-specific transient *LUC* activity. This was particularly germane in the case of the *AtRBCS1B-LUC* construct used in the vacuum-agroinfiltrated leaves and cotyledons because this reporter gene construct is expressed at relatively low levels in plant cells (Jelesko et al., 1999, 2004). The two introns in the *AtRBCS1B-LUC* gene ensured that all

Fig. 2. Transient *AtRBCS1B-LUC* expression in vacuum-agroinfiltrated poison ivy cotyledons and leaves. Leaves were imaged at 48 h post vacuum-agroinfiltration for three consecutive 1 h-imaging sessions. The experiment was replicated two times. The mean photon emission values were plotted with standard error, and asterisks indicates P value ≤ 0.05 in two-sample t test comparing vector control (pSLK7292) with *AtRBCS1B-LUC* binary plasmid pJGJ204.

Fig. 3. The number of cotyledons or leaves with a specified range of photons emitted on a per leaf or per cotyledon basis. The dagger (†) indicates the background photon emission bin for cotyledons (panel A) and leaves (panel B), see Materials and Methods section for calculation of background photon emission levels.

LUC activity was due to expression in transiently transformed poison ivy cells and not within the *A. tumefaciens* strain GV3101.

The age of poison ivy leaves was an important factor for the relative susceptibility of syringe agroinfiltration transient transformation. Leaves from potted soil-grown poison ivy plants were mostly recalcitrant to syringe-agroinfiltration transient transformation. On the other hand, young poison ivy leaves from plants grown in magenta boxes showed consistent syringe-agroinfiltration transient *LUC-INT* expression. Moreover, overall plant age was not the critical determinant, but rather the relative age of target leaves. As shown in Fig. 1B, the fifth emerged leaf (young leaf) showed greater syringe-agroinfiltration transient *LUC* expression levels than that of the second emerged leaf (older leaf) on the same plant. The qualitative levels of *LUC-INT* expression levels in poison ivy leaves were dramatically lower than the levels observed in *N. benthamiana* leaves syringe-agroinfiltrated with the same reporter gene construct. Poison ivy cotyledons and leaves were also susceptible to vacuum-agroinfiltration transient transformation using a chimeric *AtRBCS1B-LUC* transgene. Leaves infiltrated with *A. tumefaciens* strain GV3101 displayed discoloration consistent with an inducible plant biotic stress response. Both syringe- and vacuum-agroinfiltrated leaves demonstrated the accumulation of uncharacterized brown pigments (Fig. 1D; Supplemental Fig. 2C). This pigment accumulation was not observed for leaves infiltrated with MMA buffer lacking *Agrobacteria* (Fig. 1D). The relative *LUC* expression levels in the brightest poison ivy leaves was dramatically less than that observed in control *N. benthamiana* leaves syringe agroinfiltrated with the same construct. This difference could have been due to a variety of parameters including the quantity of *Agrobacteria* penetrating into the apoplastic space of leaves, and/or a biotic stress response that inhibited bacterial vigor or T-DNA transfer into the plant cells. Nevertheless, poison ivy leaves and cotyledons showed significant transient *LUC-INT* and *AtRBCS1B-LUC* expression levels over controls indicating the feasibility of using agroinfiltration transient poison ivy transformation.

As expected, poison ivy cotyledons and leaves were readily transiently transformed using a biolistic method. Biolistically transformed poison ivy leaves and cotyledons did not produce brown pigmented regions and thus averted a poison ivy biotic stress response against bacterial pathogen associated molecular patterns. As is typical of biolistic transformation of plant tissues, the transient *LUC-INT* expression levels were highly variable among both leaf and cotyledon tissues. This is in large part to previously recognized inconsistencies in both plasmid DNA coating of gold micro particles (Sanford et al., 1993) and heterogenous particle spread on the macro carrier disk, resulting in heterogeneous distribution during particle acceleration toward the target tissue.

These results demonstrate the feasibility of poison ivy leaf and cotyledon transient transformation with recombinant reporter

gene constructs using either agroinfiltration or biolistic methods. Both poison ivy DNA transformation methods are important technical advancements enabling the introduction of recombinant DNA constructs designed for a variety of reverse genetic (e.g., RNA interference or viral induced gene silencing) and genome-editing methods (e.g., zinc finger, TALEN, or CRISPR-CAS9) to enable molecular genetic investigations of poison ivy ecophysiology and metabolism.

Literature Cited

- Abe, I., Y. Utsumi, S. Oguro, and H. Noguchi. 2004. The first plant type III polyketide synthase that catalyzes formation of aromatic heptaketide. *FEBS Lett.* 562:171–176.
- Andrieu, A., J.C. Breitler, C. Sire, D. Meynard, P. Gantet, and E. Guiderdoni. 2012. An *in planta*, *Agrobacterium*-mediated transient gene expression method for inducing gene silencing in rice (*Oryza sativa* L.) leaves. *Rice (NY)* 5:23.
- Arcos-Ortega, G.F., R.A. Chan-Kuuk, W.A. Gonzalez-Kantun, R. Souza-Perera, Y.E. Nakazawa-Uejii, E. Aviles-Berzunza, G. Godoy-Hernandez, M.A. Lawton, and J.J.Z. Aguilar. 2010. *Agrobacterium tumefaciens*-transient genetic transformation of Habanero pepper (*Capsicum chinense* Jacq.) leaf explants. *Electron. J. Biotechnol.* 13:10.
- Ausubel, F.A., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 2006. Current protocols in molecular biology. Wiley, New York, NY.
- Bakshi, S., A. Sadhukhan, S. Mishra, and L. Sahoo. 2011. Improved *Agrobacterium*-mediated transformation of cowpea via sonication and vacuum infiltration. *Plant Cell Rep.* 30:2281–2292.
- Benhase, E.B. and J.G. Jelesko. 2013. Germinating and culturing axenic poison ivy seedlings. *HortScience* 48:1525–1529.
- Curtis, M.D. and U. Grossniklaus. 2003. A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol.* 133:462–469.
- Daniell, H., J. Vivekananda, B.L. Nielsen, G.N. Ye, K.K. Tewari, and J.C. Sanford. 1990. Transient foreign gene expression in chloroplasts of cultured tobacco cells after biolistic delivery of chloroplast vectors. *Proc. Natl. Acad. Sci. USA* 87:88–92.
- Dewick, P.M. 1997. Medicinal natural products: A biosynthetic approach. Wiley, Chichester, UK.
- Drummond, M.H., M.P. Gordon, E.W. Nester, and M.D. Chilton. 1977. Foreign DNA of bacterial plasmid origin is transcribed in crown gall tumours [*Agrobacterium tumefaciens*]. *Nature* 269:535–536.
- Gross, M., H. Baer, and H.M. Fales. 1975. Urushiol of poisonous anacardiaceae. *Phytochemistry* 14:2263–2266.
- Hammerschmidt, R. 1999. Phytoalexins: What have we learned after 60 years? *Annu. Rev. Phytopathol.* 37:285–306.
- Hill, G.A., V. Mattacotti, and W.D. Graha. 1934. The toxic principle of the poison ivy. *J. Amer. Chem. Soc.* 56:2736–2738.
- Jelesko, J.G., K. Carter, W. Thompson, K. Kinoshita, and W. Gruissem. 2004. Meiotic recombination between paralogous *RBCS* genes on sister chromatids of *Arabidopsis thaliana*. *Genetics* 166:947–957.
- Jelesko, J.G., R. Harper, M. Furuya, and W. Gruissem. 1999. Rare germinal unequal crossing-over leading to recombinant gene formation and gene duplication in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 96:10302–10307.

- Kapila, J., R. DeRycke, M. VanMontagu, and G. Angenon. 1997. An *Agrobacterium*-mediated transient gene expression system for intact leaves. *Plant Sci.* 122:101–108.
- Kim, S.S., E. Grienberger, B. Lallemand, C.C. Colpitts, S.Y. Kim, A. Souza Cde, P. Geoffroy, D. Heintz, D. Krahn, M. Kaiser, E. Kombrink, T. Heitz, D.Y. Suh, M. Legrand, and C.J. Douglas. 2010. *LAP6/POLYKETIDE SYNTHASE A* and *LAP5/POLYKETIDE SYNTHASE B* encode hydroxyalkyl alpha-pyrone synthases required for pollen development and sporopollenin biosynthesis in *Arabidopsis thaliana*. *Plant Cell* 22:4045–4066.
- Kim, S.Y., C.C. Colpitts, G. Wiedemann, C. Jepson, M. Rahimi, J.R. Rothwell, A.D. McInnes, M. Hasebe, R. Reski, B.T. Sterenberg, and D.Y. Suh. 2013. *Physcomitrella* PpORS, basal to plant type III polyketide synthases in phylogenetic trees, is a very long chain 2'-oxoalkylresorcinol synthase. *J. Biol. Chem.* 288:2767–2777.
- King, J.L., J.J. Finer, and L.K. McHale. 2015. Development and optimization of agroinfiltration for soybean. *Plant Cell Rep.* 34:133–140.
- Klein, T.M., M. Fromm, A. Weissinger, D. Tomes, S. Schaaf, M. Sletten, and J.C. Sanford. 1988. Transfer of foreign genes into intact maize cells with high-velocity microprojectiles. *Proc. Natl. Acad. Sci. USA* 85:4305–4309.
- Klein, T.M., E.D. Wolf, R. Wu, and J.C. Sanford. 1987. High-velocity microprojectiles for delivering nucleic-acids into living cells. *Nature* 327:70–73.
- Kurtz, A.P. and C.R. Dawson. 1971. Synthesis of compounds structurally related to poison ivy urushiol. 4. 3-(1-Alkyl)alkylcatechols of varying side-chain shape and flexibility. *J. Med. Chem.* 14:733–737.
- Majima, R. 1922. Über den hauptbestandteil des Japan-lacs, VIII. Mitteilung: Stellung der doppelbindungen in der seitenkette des urushiols und beweissführung, dab das urushiol eine mischung ist. *Ber. Dtsch. Chem. Ges.* 55 (b):172–191.
- Mankin, S.L., G.C. Allen, and W.F. Thompson. 1997. Introduction of a plant intron into the luciferase gene of *Photinus pyralis*. *Plant Mol. Biol. Rpt.* 15:186–196.
- Markiewitz, K.H. and C.R. Dawson. 1965. On the isolation of the allelogenically active components of the toxic principle of poison ivy. *J. Org. Chem.* 30:1610–1613.
- Martin, A.C., H.S. Zim, and A.L. Nelson. 1951. American wildlife & plants. Dover Publications, Inc., New York, NY.
- Matsuzawa, M., Y. Katsuyama, N. Funa, and S. Horinouchi. 2010. Alkylresorcylic acid synthesis by type III polyketide synthases from rice *Oryza sativa*. *Phytochemistry* 71:1059–1067.
- Mo, R.L., Y.M. Huang, S.C. Yang, Q.L. Zhang, and Z.R. Luo. 2015. Development of *Agrobacterium*-mediated transient transformation in persimmon (*Diospyros kaki* Thunb.). *Scientia Hort.* 192:29–37.
- Mohan, J.E., L.H. Ziska, W.H. Schlesinger, R.B. Thomas, R.C. Sicher, K. George, and J.S. Clark. 2006. Biomass and toxicity responses of poison ivy (*Toxicodendron radicans*) to elevated atmospheric CO₂. *Proc. Natl. Acad. Sci. USA* 103:9086–9089.
- Popay, I. and R. Field. 1996. Grazing animals as weed control agents. *Weed Technol.* 10:217–231.
- Sanford, J.C., F.D. Smith, and J.A. Russell. 1993. Optimizing the biolistic process for different biological applications. *Methods Enzymol.* 217:483–509.
- Symes, W.F. and C.R. Dawson. 1953. Separation and structural determination of the olefinic components of poison ivy urushiol, cardanol and cardol. *Nature* 171:841–842.
- Symes, W.F. and C.R. Dawson. 1954. Poison ivy “urushiol”. *J. Amer. Chem. Soc.* 76:2959–2963.
- Taura, F., M. Iijima, E. Yamanaka, H. Takahashi, H. Kenmoku, H. Saeki, S. Morimoto, Y. Asakawa, F. Kurosaki, and H. Morita. 2016. A novel class of plant type III polyketide synthase involved in orsellinic acid biosynthesis from *Rhododendron dauricum*. *Front. Plant Sci.* 7:1452.
- Taura, F., S. Tanaka, C. Taguchi, T. Fukamizu, H. Tanaka, Y. Shoyama, and S. Morimoto. 2009. Characterization of olivetol synthase, a polyketide synthase putatively involved in cannabinoid biosynthetic pathway. *FEBS Lett.* 583:2061–2066.
- VanderGheynst, J.S., H.Y. Guo, and C.W. Simmons. 2008. Response surface studies that elucidate the role of infiltration conditions on *Agrobacterium tumefaciens*-mediated transient transgene expression in harvested switchgrass (*Panicum virgatum*). *Biomass Bioenergy* 32:372–379.
- Voinnet, O., S. Rivas, P. Mestre, and D. Baulcombe. 2003. An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant J.* 33:949–956.
- Wang, Y.C., T.M. Klein, M. Fromm, J. Cao, J.C. Sanford, and R. Wu. 1988. Transient expression of foreign genes in rice, wheat and soybean cells following particle bombardment. *Plant Mol. Biol.* 11:433–439.
- Weisberg, A.J., G. Kim, J.H. Westwood, and J.G. Jelesko. 2017. Sequencing and de novo assembly of the *Toxicodendron radicans* (poison ivy) transcriptome. *Genes (Basel)* 8:317.
- Ziska, L.H., R.C. Sicher, K. George, and J.E. Mohan. 2007. Rising atmospheric carbon dioxide and potential impacts on the growth and toxicity of poison ivy (*Toxicodendron radicans*). *Weed Sci.* 55:288–292.

Supplemental Fig. 1. Superimposed photon emission images of *Agrobacterium tumefaciens* strains GV3103/pJGJ411 (*LUC-INT* reporter gene, wells C5–6) and GV3101/pJGJ410 (*LUC* reporter gene, wells E5–6) duplicate cultures placed in a 96-well microtiter plate. Pseudocolor step gradient is the same as Fig. 1A.

Supplemental Fig. 2. Poison ivy cotyledons and leaves vacuum-infiltrated with *Agrobacterium tumefaciens* harboring either empty vector (pSLK7292) or *AtRBCS1B-LUC* (pJGJ204). (A) Composite pseudocolor photon image with background heat photon levels subtracted. Pseudocolor step gradient is the same as Fig. 1A. (B) Composite pseudocolor photon image with background heat photon levels displayed. (C) Digital color image displaying mostly water-soaked infiltrated tissues and noninfiltrated tissues (indicated by arrows pointing toward lighter green noninfiltrated tissues).

Supplemental Fig. 3. Superimposed photon emission of cotyledons (A and B) and true leaves (C and D) on reflected light image. Pseudocolor step gradient is the same as Fig. 1A.

Supplemental Fig. 4. Box plots of photon emission levels from poison ivy leaves of different age and Agrobacterium inoculum concentrations. Leaves were from magenta box-grown poison ivy plants. Three leaves were independently imaged for each treatment combination. The line bisecting each box is the median value. Agrobacterium inoculum concentration was either 0.2 OD or 2.0 OD at 600 nm.