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Note

Evaluation of anti-herbivory genes using an *Agrobacterium*-mediated transient expression system

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Abstract A high-throughput system for the evaluation of anti-herbivory genes in plant is proposed, in which genes are transiently expressed in a small area of leaf via *Agrobacterium*-infiltration, and the results can be obtained within 1 week. Transient expression of the *cry1Ab* gene, a well-known Bt toxin gene, in the leaves of tobacco (*Nicotiana tabacum* cv. Samsun NN) exhibited a lethal effect on both cotton cutworm (*Spodoptera litura*) and cabbage armyworm (*Mamestra brassicae*) larvae. In *Arabidopsis* leaves, this gene also provided potent inhibition of feeding of diamondback moth (*Plutella xylostella*) larvae. The expression of another anti-herbivory gene, *MLX56*, in tobacco and tomato (*Solanum lycopersium* cv. Micro-Tom) leaves resulted in enhanced mortality of *M. brassicae* and *S. litura* larvae. Significantly higher growth inhibition of *S. litura* larvae and feeding inhibition of *P. xylostella* larvae were also observed in tobacco and *Arabidopsis* leaves expressing the *MLX56* gene. All results could be obtained within 5–7 days, indicating that this *Agrobacterium*-mediated transient gene expression system enables a high throughput evaluation of anti-herbivory genes.

Key words: Anti-herbivory gene, BT toxin, Lepidoptera, MLX56, transient assay

To date, many types of genetically modified (GM) crops have been grown commercially worldwide. All GM soybean and canola hybrids are herbicide tolerant, whereas some GM corn and cotton are resistant to insect pests. The most well-known insect resistant GM crops are transformed with the insecticidal crystal (*cry*) gene from *Bacillus thuringiensis*, which encodes a protein called Bt toxin with strong insecticidal activity against lepidopteran insects. However, Cry proteins are very selectively active against insects and no Bt toxins have been found to be available for agriculturally important insect pests such as hemipteran and orthopteran insects, and the emergence of pests resistant to Bt toxins has also been reported (Janmaat and Myers 2003; Tabashnik et al. 2008; Wang et al. 2003). Therefore, other novel genes with anti-herbivory effects are being sought for agricultural pest control (Foissac et al. 2000; Fowler et al. 2009; Gatehouse 2008; Mochizuki et al. 1999; Wang et al. 2005; Wang and Constabel 2004; Yarasi et al. 2008).

Although searching of new anti-herbivory genes is needed for the development of herbivory resistant transgenic plants, the evaluation of such genes is labor intensive and time consuming. For example, testing the toxic or resistance activity of candidate anti-herbivory proteins requires large amounts of the purified protein. However, it is sometimes very difficult to purify candidate anti-herbivory proteins from a source organism or to produce candidate proteins using ectopic expression system (Pechan et al. 2004; Mohan et al. 2006). Carrying out bioassays with the protein is more difficult for insect species for which an artificial diet has not been established. Furthermore, the evaluation of anti-herbivory genes in stable transgenic plants requires substantial time, labor and cost for the introduction of candidate anti-herbivory genes into a crop, and then the selection and maintenance of strains exhibiting stable expression levels, before the bioassay for herbivory resistance can be performed.

Agrobacterium-infiltration methods enable the transient and local expression of genes of interest in the infiltrated area of a leaf, in a broad range of plants, including *Nicotiana tabacum*, *N. benthamiana*, *Arabidopsis thaliana*, *Linum usitattissium*, *Pisum sativum*, and *Lactuca sativa* (Kapila et al. 1997; Van

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der Hoorn et al. 2000; Wroblewski et al. 2005). This *in planta* transient expression system using *Agrobacterium* has been applied to analysis of genes function, promoter analysis and protein production (Bendahmane et al. 2000; Sawers et al. 2006; Vaquero et al. 1999; Yang et al. 2000). A strikingly successful example of the application of transient expression systems is the characterization of signaling events involved in the induction of the hypersensitive reaction, which is associated with plant resistance against pathogens (e.g. Van der Hoorn et al. 2000).

In the present study, we attempted to establish a transient evaluation system for anti-herbivory genes using the *Agrobacterium*-infiltration system (*Agrobacterium*-mediated transient expression system). We expressed two anti-herbivory genes in the leaves of tobacco, tomato and *Arabidopsis* by *Agrobacterium*-infiltration and then assessed the effect of transient expression on lepidopteran larvae fed on the leaves. A CaMV 35S promoter-driven, codon-optimized version of a synthetic *cry1Ab* gene (pBIK102mcbt, Shinoyama et al. 2003) was infiltrated into leaves of *N. tabacum* cv. SamsunNN using *Agrobacterium* (GV3101), as described by Kobayashi et al. (2011). Native *cry1* genes are barely expressed in plant systems because of their prokaryotic type codon usage with highly AT-rich sequences. Thus, we used a synthesized *cry1Ab* gene with codons optimized to the chrysanthemum codon usage for expression in higher plants (Shinoyama et al. 2003). The negative control consisted of *Agrobacterium* transformed with pIG121-Hm, as described by Ohta et al. (1990), which has a 35S::int-GUS construct. Liquid-cultured *Agrobacterium* containing the T-DNA vectors ($O.D._{600}$=0.4 to 0.8) were harvested and resuspended in buffer containing acetosyringone (10 mM MES (pH 5.6), 10 mM $MgCl_2$, 20 μM acetosyringone) at O.D.=0.1. The suspension of *Agrobacterium* was left on bench at room temperature for 3 h to induce virulence and then infiltrated into the intercellular space of leaves using needleless syringe (Figure 1A–C). Quantitative RT-PCR analysis was used to monitor transgene expression with the use of primers 5′-AAC TTC TCC AAC GGG TCC TCT G-3′ (*Cry1Ab*5′) and 5′-TGG CAC GAA CTC GAT CCT GTC -3′ (*Cry1Ab*3′) as described previously (Kobayashi et al. 2010) (Figure 2A). The transcript of the *cry1Ab* gene was detected at 1 day post infiltration (DPI), its level peaked at 2 DPI and remained distinct until 5 DPI. Protein gel blot analysis using a polyclonal antibody against *Bacillus thuringinesis* Cry1Ab toxin (Abcam, Cambridge UK) was used to confirm the accumulation of the *cry1Ab* gene product (Bt protein) (Figure 2B). One day after infiltration, 10 newly hatched larvae of *Mamestra brassicae* (cabbage armyworm) (eggs were kindly provided by the Japan Plant Protection Association, Ibaraki, Japan) or *Spodptera litura* (cotton

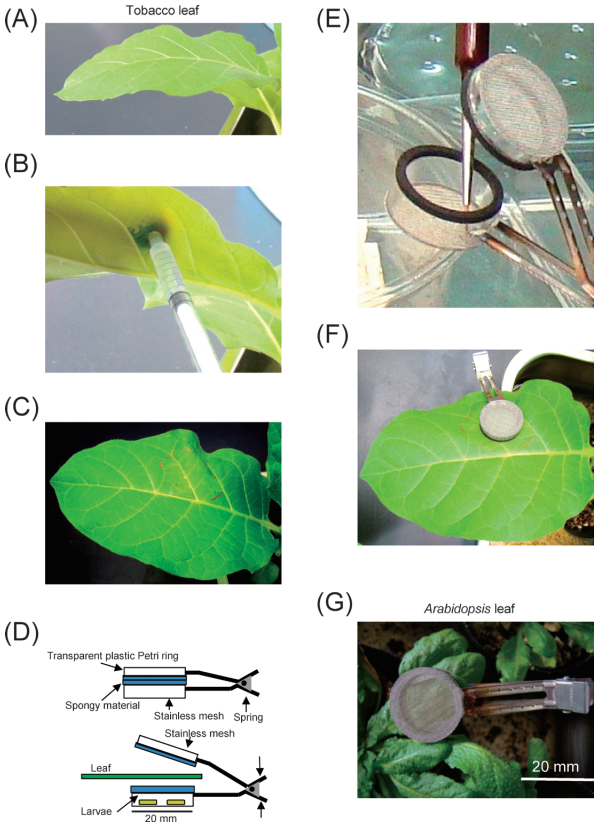

Figure 1. Procedures of the *Agrobacterium*-mediated transient assay for insect resistance. (A) Photograph of a tobacco (*Nicotiana tabacum*) leaf. (B) Infiltration of an *Agrobacterium* suspension into a tobacco leaf. (C) The margin of the infiltrated tobacco leaf area was marked. (D) Schematic figure of the miniature chamber. The chamber containing a larva was positioned onto each infiltrated tobacco leaf area. (E) Larvae were placed into the miniature chamber. (F) Photograph of a tobacco leaf, part of which was infiltrated with an *Agrobacterium* suspension. One day after infiltration, larvae were inoculated onto the area and enclosed in a miniature chamber (20 mm diameter). All plants and insects were kept at 25±1°C, LD 16:8 h. (G) Photograph of an *Arabidopsis* leaf, part of which was infiltrated with an *Agrobacterium* suspension and then inoculated with one first instar diamondback moth (*P. xylostella*) larva (neonate larvae). The larva on the leaf was enclosed in a 20-mm diameter chamber. *Arabidopsis* and *P. xylostella* larva were kept at 25±1°C, LD 16:8 h.

cutworm) (Sumika Technoservice Co., Takarazuka, Japan) were inoculated onto each infiltrated tobacco leaf area and kept within a miniature chamber to inhibit escaping the larvae from the area of inoculation (Figure 1D–F). At 4 days after inoculation, the numbers of surviving *M. brassicae* larvae on the leaves expressing *cry1Ab* gene decreased significantly compared with those on the control leaves (Figure 2C). All larvae of *S. litura* on the leaves expressing *cry1Ab* gene died within 4 days, whereas most larvae on the control leaves survived (Figure 2D).

Plutella xylostella larvae (diamondback moth) were also used to assess the effect of expression of *cry1Ab* in *Agrobacterium*-infiltrated leaves of *Arabidopsis*. Rosette leaves of *Arabidopsis thaliana* ecotype Columbia were

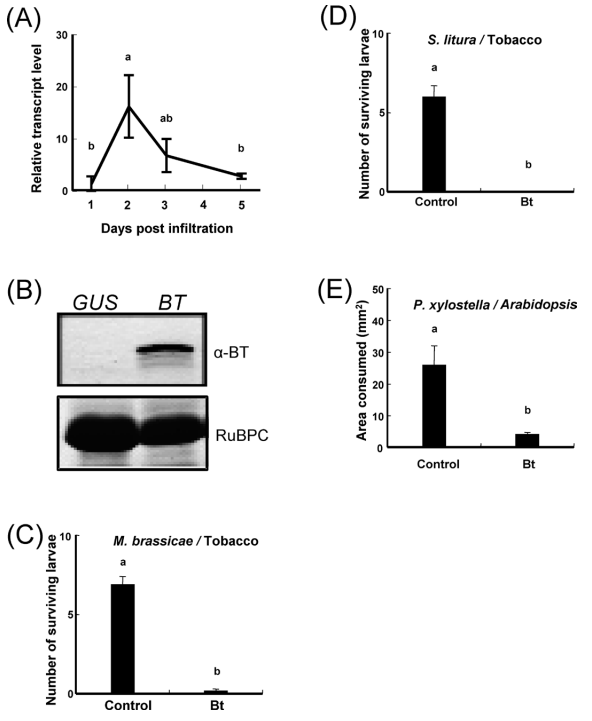

Figure 2. *Agrobacterium*-mediated transient expression of a *cry1Ab* gene was used to induce resistance to lepidopteran larvae. (A) Quantitative PCR was used to assess transcript levels of the *cry1Ab* gene in *Agrobacterium*-infiltrated leaves of tobacco. The transcript levels of *cry1Ab* were normalized using those of the *actin* gene. Values are means±SE. The assay was replicated three times ($df=3$, $F=11.2562$, $p=0.0030$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (B) Upper panel: Immunoblotting analysis of a tobacco leaf infiltrated with *Agrobacterium* containing the *cry1Ab* (*BT*) gene or *GUS*. Infiltration of the *Agrobacterium* suspension was performed as described by Kobayashi et al. (2010). After 2 days, a leaf extract was prepared from the infiltrated area and subjected to immunoblotting analysis using an anti-Cry1Ab antibody (α-BT), as described by Shinoyama et al. (2008). Lower panel: Signal of RuBPC large subunit, which was visualized by Coomassie brilliant blue staining, as the standard for gel loading. (C) Transient expression of the *cry1Ab* gene reduced the survival rate of larvae of cabbage armyworm moth (*M. brassicae*) on tobacco leaves. Ten first-instar larvae of *M. brassicae* were inoculated onto the leaf region infiltrated with the *Agrobacterium* suspension, and the number of larvae surviving after 4 days was counted. Values are means±standard error (SE). Control: *GUS* expressed leaf. Bt: *cry1Ab* expressing leaf. The assay was replicated ten times ($df=1$, $F=179.56$, $p<0.0001$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (D) Transient expression of the *cry1Ab* gene reduced the survival rate of cotton cutworm (*S. litura*) larvae on tobacco leaves. The method was the same as described above. Values are means±SE. The assay was replicated ten times ($df=1$, $F=95.30$, $p<0.0001$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (E) Transient expression of the *cry1Ab* gene reduced the area of *Arabidopsis* leaf consumed by a first-instar larva of *P. xylostella*. The area consumed by the larva was measured 3 days after inoculation. Values are means±SE. The assay was replicated twenty-four times ($df=1$, $F=13.12$, $p=0.0007$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA.

infiltrated with *cry1Ab* transformed *Agrobacterium* or the control, and one *P. xylostella* larva was inoculated on the infiltrated area in the same manner as *M. brassicae* (Figure 1G). Because the first instar of *P. xylostella* larva is very small (approximately 1 mm in length) and they tend to mine within the plant mesophyll tissue to feed, larval mortality is difficult to assess. We considered that the area of leaf consumed reflected the degree of larval performance; therefore, we measured the area consumed by *P. xylostella* larva. At 3 days after inoculation, the area consumed by *P. xylostella* larvae in leaves expressing the *cry1Ab* gene was significantly less than that in the control leaves (Figure 2E). These results indicated clearly that activity of the well-known anti-herbivory gene *cry1Ab* can be demonstrated with the use of our *Agrobacterium*-infiltration system.

To further assess the present method, we analyzed the effect of transient expression of a second anti-herbivory gene, *MLX56*. MLX56 is a 56-kDa protein contained in the latex of cultivated mulberry, *Morus alba cv*, Shin-Ichinose, and it acts a defensive protein against lepidopteran insects (Wasano et al. 2009). When the larvae of the cabbage armyworm, *M. brassicae*, and of Eri silkworm, *Samia ricimi* ate an artificial diet containing MLX56 protein, larval growth was retarded. However, MLX56 showed no activity against silkworm larvae, *Bombyx mori*, the mulberry specialist, suggesting the adaptation of *B. mori* to this mulberry defensive protein.

The *MLX56* gene was inserted into binary vector pEl2Ω (Ohtsubo et al. 1999) under the control of a modified 35S promoter and introduced into *Agrobacterium* GV3101 and then infiltrated into tobacco, tomato or *Arabidopsis* leaves. Quantitative RT-PCR analysis of infiltrated tobacco leaves using primers 5′-AAC TTC TCC AAC GGG TCC TCT G-3′ (MLX565′) and 5′-TTT CCG AGG GCT CTT CCA CAT C-3′ (MLX563′) identified expression of the *MLX56* gene (Figure 3A). The numbers of surviving larvae of *M. brassicae* or *S. litura* on the areas of tobacco leaves infiltrated with *Agrobacterium* containing the 35S::MLX56 construct was significantly reduced compared with those on control areas (Figure 3B, C). The numbers of surviving *S. litura* larvae on tomato (cv. micro Tom) leaf areas expressing *MLX56* gene also decreased significantly (Figure 3D). Thus, the transient expression of the *MLX56* gene in tobacco and tomato leaves decreased to the survival of *M. brassicae* and *S. litura* larvae. One 3rd instar larvae of *S. litura* onto each tobacco leaf area expressing the *MLX56* gene in order to assess the effect of expression of *MLX56* gene on the growth of larvae. Two days after inoculation, the weight gain of the larvae was significantly suppressed compared with those exposed to a control area (Figure 3E). To assess the effect of expression of the *MLX56* gene on the rate of consumption by larvae, one 1st instar *P.*

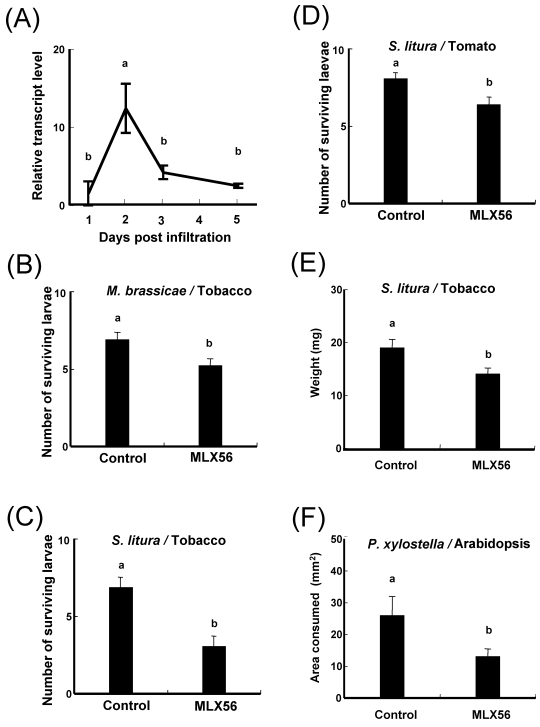

Figure 3. *Agrobacterium*-mediated transient expression of the *MLX56* gene was used to induce resistance to lepidopteran larvae. (A) Quantitative PCR was used to assess transcript levels of the *MLX56* gene in *Agrobacterium*-infiltrated leaves of tobacco. The assay was replicated three times ($df=3$, $F=5.5330$, $p=0.0237$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (B) Transient expression of the *MLX56* gene reduced the survival rate of larvae of cabbage armyworm moth (*M. brassicae*) on tobacco leaves. Ten first-instar larvae of *M. brassicae* were inoculated onto a leaf region infiltrated with an *Agrobacterium* suspension, and the number of larvae surviving after 4 days was counted. Values are means±SE. Control: *GUS* expressing leaf. MLX56: *MLX56* expressing leaf. (C) Transient expression of the *MLX56* gene reduced the survival rate of cotton cutworm (*S. litura*) larvae on tobacco leaves. The method was the same as described above. Values are means±SE. The assay was replicated ten times ($df=1$, $F=179.56$, $p<0.0001$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (D) Transient expression of the *MLX56* gene reduced the survival rate of cotton cutworm (*S. litura*) larvae on tomato leaves. The method was the same as described above. Values are means±SE. The assay was replicated ten times ($df=1$, $F=179.56$, $p<0.0001$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (E) Transient expression of the *MLX56* gene reduced the weight of cotton cutworm (*S. litura*) larva on tobacco leaves. Values are means±SE. The weight of one third-instar *S. litura* larva on a tobacco leaf expressing the *MLX56* gene was measured at two days after inoculation. The assay was replicated ten times ($df=1$, $F=179.56$, $p<0.0001$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA. (F) Transient expression of the *MLX56* gene reduced the area of *Arabidopsis* leaf consumed by one first-instar *P. xylostella* larva. Values are means±SE. The assay was replicated twenty-four times ($df=1$, $F=5.475$, $p=0.0244$). Means with different letters are significantly different by Tukey-Kramer HSD test ($p<0.05$) after one-way ANOVA.

xylostella larvae was inoculated onto each *Arabidopsis* leaf area expressing the *MLX56* gene. At 3 days after inoculation, the area of *Arabidopsis* leaves expressing the *MLX56* gene consumed by *P. xylostella* larvae was significantly less than those for control areas (Figure 3F). BT toxin is known to have strong insecticidal activity against lepidopteran insects, whereas MLX56 showed only growth inhibition against the larvae of the *M. brassicae* and *S. ricimi* (Wasano et al. 2009). However, in present transient assay system, the expression of *MLX56* gene in the leaves exhibited not only a growth inhibition effect but also induced feeding inhibition and lethality. This may have been achieved by a higher level of accumulation of the *MLX56* gene product as a result of *Agrobacterium* infiltration or by a combination of MLX56 and unidentified anti-herbivory agents in the test plants.

In the present study, the inhibitory effect on lepidopteran larvae of *Agrobacterium*-mediated transient expression of the *cry1Ab* gene was verified in tobacco, tomato and *Arabidopsis*. Furthermore, our transient assay demonstrated that expression of the gene for the anti-herbivory protein MLX56 enhanced plant resistance against some lepidopteran larvae fed on tobacco, tomato and *Arabidopsis*. Consequently, we are now trying to produce stable transgenic plants with enhanced herbivore resistance by introduction of the *MLX56* gene. The *Agrobacterium* infiltration method is a powerful tool for plant molecular biology, because it enables high throughput expression of a gene of interest. Furthermore, our current system involving the combination of *Agrobacterium*-mediated transient expression and a specialized miniature inoculation chamber permit the high throughput evaluation of anti-herbivory genes within 1 week, thereby reducing the time and effort for the selection of transgenic lines and the establishment of stable transformants. Because this system can be applied to a variety of plant species (tobacco, tomato, *Arabidopsis* etc.), it will be straight-forward to perform bioassays with insect pests that cannot be reared on artificial diets, and the anti-insect performance of transgenic plants can be measured using a variety of indices (larval weights, mortalities, consumption rates etc.).

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References

Bendahmane A, Querci M, Kanyuka K, Baulcombe DC (2000) *Agrobacterium* transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato. *Plant J* 21: 73–81

Foissac X, Thi Loc N, Christou P, Gatehouse AMR, Gatehouse JA (2000) Resistance to green leafhopper (*Nephotettix virescens*)

and brown planthopper (*Nilaparvata lugens*) in transgenic rice expressing snowdrop lectin (*Galanthus nivalis* agglutinin; GNA). *J Insect Physiol* 46: 573–583

Fowler JH, Narváez-Vásquez J, Aromdee DN, Pautot V, Holzer FM, Walling LL (2009) Leucine aminopeptidase regulates defense and wound signaling in tomato downstream of jasmonic acid. *Plant Cell* 21: 1239–1251

Gatehouse JA (2008) Biotechnological prospects for engineering insect-resistant plants. *Plant Physiol* 146: 881–887

Janmaat AF, Myers J (2003) Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. *Proc Biol Sci* 270: 2263–2270

Kapila J, De Rycke R, Van Montagu M, Angenon G (1997) An *Agrobacterium*-mediated transient gene expression system for intact leaves. *Plant Sci* 122: 101–108

Kobayashi M, Seo S, Hirai K, Yamamoto-Katou A, Katou S, Seto H, Meshi T, Mitsuhara I, Ohashi Y (2010) Silencing of WIPK and SIPK mitogen-activated protein kinases reduces tobacco mosaic virus accumulation but permits systemic viral movement in tobacco possessing the *N* resistance gene. *Mol Plant Microbe Interact* 23: 1032–1041

Kobayashi M, Yamamoto-Katou A, Katou S, Hirai K, Meshi T, Ohashi Y, Mitsuhara I (2011) Identification of an amino acid residue required for differential recognition of a viral movement protein by the *Tomato mosaic virus* resistance gene *Tm-2*(*2*). *J Plant Physiol* 168: 1142–1145

Mochizuki A, Nishizawa Y, Onodera H, Tabei Y, Toki S, Habu Y, Ugaki M, Ohashi Y (1999) Transgenic rice plants expressing a trypsin inhibitor are resistant against rice stem bores, *Chilo suppressalis*. *Entomol Exp Appl* 93: 173–178

Ohta S, Mita S, Hattori T, Nakamura K (1990) Construction and expression in tobacco of β-glucuronidase (GUS) reporter gene containing an intron within the coding sequence. *Plant Cell Physiol* 31: 805–813

Ohtsubo N, Mitsuhara I, Koga M, Seo S, Ohashi Y (1999) Ethylene promotes the necrotic lesion formation and basic PR gene expression in TMV-infected tobacco. *Plant Cell Physiol* 40: 808–817

Sawers RJH, Farmer PR, Moffett P, Brutnell TP (2006) In planta transient expression as a system for genetic and biochemical analyses of chlorophyll biosynthesis. *Plant Methods* 2: 15

Shinoyama H, Mochizuki A, Komano M, Nomura Y, Nagai T (2003) Insect resistance in transgenic Chrysanthemum [*Dendranthema grandiflorum* (Ramat.) Kitamura] by the introduction of a modified δ-endotoxin gene of *Bacillus thuringiensis*. *Breed Sci* 53: 359–367

Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York, pp 1–937

Tabashnik BE, Gassmann AJ, Crowder DW, Carriére Y (2008) Insect resistance to Bt crops: evidence versus theory. *Nat Biotechnol* 26: 199–202

Vaquero C, Sack M, Chandler J, Drossard J, Schuster F, Monecke M, Schillberg S, Fischer R (1999) Transient expression of a tumor-specific single-chain fragment and a chimeric antibody in tobacco leaves. *Proc Natl Acad Sci USA* 96: 11128–11133

Van der Hoorn RA, Laurent F, Roth R, De Wit PJ (2000) Agroinfiltration is a versatile tool that facilitates comparative analyses of *Avr9/Cf-9*-induced and *Avr4/Cf-4*-induced necrosis. *Mol Plant Microbe Interact* 13: 439–446

Wang J, Boets A, Van Rie J, Ren G (2003) Characterization of *cry1*, *cry2*, and *cry9* genes in *Bacillus thuringiensis* isolates from China. *J Invertebr Pathol* 82: 63–71

Wang J, Constabel CP (2004) Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). *Planta* 220: 87–96

Wang Z, Zhang K, Sun X, Tang K, Zhang J (2005) Enhancement of resistance to aphids by introducing the snowdrop lectin gene *gna* into maize plants. *J Biosci* 30: 627–638

Wasano N, Konno K, Nakamura M, Hirayama C, Hattori M, Tateishi K (2009) A unique latex protein, MLX56, defends mulberry trees from insects. *Phytochemistry* 70: 880–888

Wroblewski T, Tomczak A, Michelmore R (2005) Optimization of *Agrobacterium*-mediated transient assays of gene expression in lettuce, tomato and *Arabidopsis*. *Plant Biotechnol J* 3: 259–273

Yang Y, Li R, Qi M (2000) *In vivo* analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. *Plant J* 22: 543–551

Yarasi B, Sadumpati V, Immanni CP, Vudem DR, Khareedu VR (2008) Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. *BMC Plant Biol* 8: 102