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Assessment of Regeneration and Transient Expression Factors for *Agrobacterium*-mediated Transformation of *Prunus salicina* Lindl.

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Summary

High levels of regeneration and optimized transient expression factors are critical for increasing transformation rates in plant species. An efficient regeneration protocol was developed and transient expression experiments were carried out to provide information for the improvement of *Agrobacterium tumefaciens* mediated transformation system in Japanese plum (*Prunus salicina* Lindl.). Five different varieties of Japanese plum ('Shiro', 'Early Golden', 'Gladstone', 'Red Heart' and 'Bruce'), four different basal salt mixtures (QL, WPM, B5 and MS), light regimes and TDZ pretreatments were evaluated to optimize adventitious shoot regeneration. Regeneration ability was highly variety dependent. Basal salt composition significantly affected the regeneration frequency. Best regeneration frequencies were obtained from 'Shiro' (56.4 %) on QL salts and 'Early Golden' (42.8 %) on B5 medium. Subsequently transient expression experiments were carried out using three *Agrobacterium tumefaciens* strains; LBA4404, EHA105 and GV3101, each containing binary vector pCAMBIA2301. There were significant differences observed among *Agrobacterium* strains tested. *Agrobacterium* strains LBA4404 and EHA105 were much more effective than GV3101 strain and the presence of acetosyringone in the co-cultivation medium resulted in increased GUS expression

Key words. Japanese plum – GUS – hypocotyls – thidiazuron – regeneration – media

Introduction

As genetic transformation technology becomes more common, the opportunity to transfer otherwise unavailable traits into plant species increases (Hokanson and Pooler 2000). However, genetic improvement by transformation is less successful in woody species than in herbaceous species due mainly to their low regeneration ability (Birch 1997; Hokanson and Pooler 2000). Development of an efficient genetic transformation technology for a species depends largely on availability of an efficient and reliable regeneration protocol and optimized transient expression factors.

Japanese plum (*Prunus salicina* Lindl.) is a widely grown important *Prunus* fruit species across the world (Okie and Ramming 1999). Regeneration protocols have been reported for most of the *Prunus* species such as sweet cherry (*P. avium* L.) (Tang et al. 2002; Bahgwat and Lane 2004; Matt and Jehle 2005; Canli and Tian 2008), sour cherry (*P. cerasus* L.) (Mante et al. 1989; Tang et al. 2002; Song and Sink 2005), almond (*P. dulcis* Mill.) (Miguel et al. 1996), apricot (*P. armeniaca*) (Lane and Cossio 1986) and peach (*P. persica*) (Mante et al. 1989; Pooler and Scorza 1995; Yan and Zhou 2002).

Regeneration protocols have also been reported for European plum (*Prunus domestica* L.) (Mante et al. 1989; Bassi and Cossio 1991; Nowak and Micznskiho 1997) and several publications report on successful recovery of transgenic plants in this close relative of Japanese plum (Mante et al. 1991; Camara Machado et al. 1994; Yankheva et al. 2002). Although there are two reports on regeneration of *P. salicina* using hypocotyls explants, regeneration frequencies of *P. salicina* is much lower than that of *P. domestica* (Tian et al. 2007; Urtibia et al. 2008). Therefore, the recently reported transformation rates of *P. salicina* are very low in comparison with that of *P. domestica* (Urtibia et al. 2008). Improvement of the transformation efficiency in this species requires optimization of multiple factors, among the most important is an improvement of regeneration (Urtibia et al. 2008).

Many factors influence transient expression including plasmid types, *Agrobacterium* strains, co-cultivation time and/or time of inoculation, and environmental factors (Song and Sink 2005; Tian et al. 2008; Urtibia et al. 2008). Studying, understanding and optimizing these factors may possibly help increasing the efficiency of current protocols for the species of interest (Gill et al. 2004). *Agrobacterium* strains used for transformation experiments significantly effects transformation frequencies in different fruit species. About 34 different strains of *Agrobacterium* have been used in transformation of different fruit species so far. Among these strains, EHA 101, EHA 105, LBA 4404, C58 and GV3101 are the most extensively tested strain in fruit crops. The efficiencies of transforma-

tion vary significantly among different strains (Gill et al. 2004).

To date, there is only one report of successful transformation of Japanese plum, but the reported efficiency is very low. This necessitates further regeneration and transient expression studies for Japanese Plum. Five different varieties of Japanese plum ('Shiro', 'Early Golden', 'Gladstone', 'Red Heart' and 'Bruce'), four different culture media, dark incubation and TDZ pretreatments were evaluated for the improvement of adventitious shoot regeneration. Then, transient expression experiments were conducted to provide guidelines for high efficiency transformations of Japanese plum.

Materials and Methods

Abbreviations

AS: acetosyringone; BAP: 6–benzylaminopurine; GUS: β–glucuronidase; IBA: indole-3-butyric acid; MS: Murashige and Skoog; *npt*II: neomycin phosphotransferase gene; QL: Quoiron and Lepoivre; TDZ: thidiazuron; WPM: Lloyd and WcCown; B5: Gamborg

Plant material and explant preparation

Mature plum fruits were harvested from trees of Europan plum cv. 'Shiro', 'Early Golden', 'Gladstone', 'Red Heart' and 'Bruce' in Vineland Station, Ontario, Canada. The fruit flesh was removed, and the endocarps were washed under running tap water. Then, the endocarps were disinfected with a 0.05 % sodium hypochlorite solution and rinsed 3 times with water. The seeds were dried on a bench for 3 d at room temperature and stored at 4 °C in plastic mesh bags for 3 months before use.

To prepare explants for experiments, the endocarps were cracked open and seeds were sterilized using 0.5 % sodium hypochlorite as described in Tian et al. (2007). Following sterilization, the seeds were imbibed in final rinsing water overnight. After the removal of the seed coat, embryonic axes were excised from the cotyledons and embryonic axes were cut into four equal sections as one radicle, two hypocotyls and one epicotyl (Fig. 1A). Radicles and epicotyls were discharged; hpocotyls segments were used in regeneration and transformation experiments. Petri dishes, 10 cm in diameter, containing 30 ml of medium were used to carry out experiments.

Shoot induction medium and growth conditions

As different treatments, shoot induction medium consisted of one of the following four different basal salt mixtures; QL (Quoirin and Lepoivre 1977), WPM (Lloyd and McCown 1980), B5 (Gamborg et al. 1968) or MS (Murashige and Skoog 1962), each supplemented with 2.5 μM indolebutyric acid (IBA), 555 μM myo-inositol, 1.2 μM thiamine HCl, 1.4 μM nicotinic acid, 2.4 μM pyridoxine HCl, 25 g l^{-1} sucrose, and 7 g l^{-1} Bactogar. The pH of the medium was adjusted to 5.6 prior to autoclaving. Thidiazuron (TDZ) (7.5 μM) was filter sterilized and added to the medium after autoclaving at 121 °C and 106 kPa for 30 min.

The explants were placed on one of these induction media containing the different basal salt mixtures as different treatments (Fig. 1B) and incubated at 24 ± 1 °C under 16/8-h (light/dark) photoperiod provided by fluorescent Sylvania "Cool White" light with a Photosynthetic Photon Flux of about 50 μmol m^{-2} s^{-1}. For each treatment, 15 explants were used and data were recorded 28 d after plating.

TDZ pre-treatments

For this experiment, dissected hypocotyls were imbibed for 24 h in sterile distilled water containing different lev-

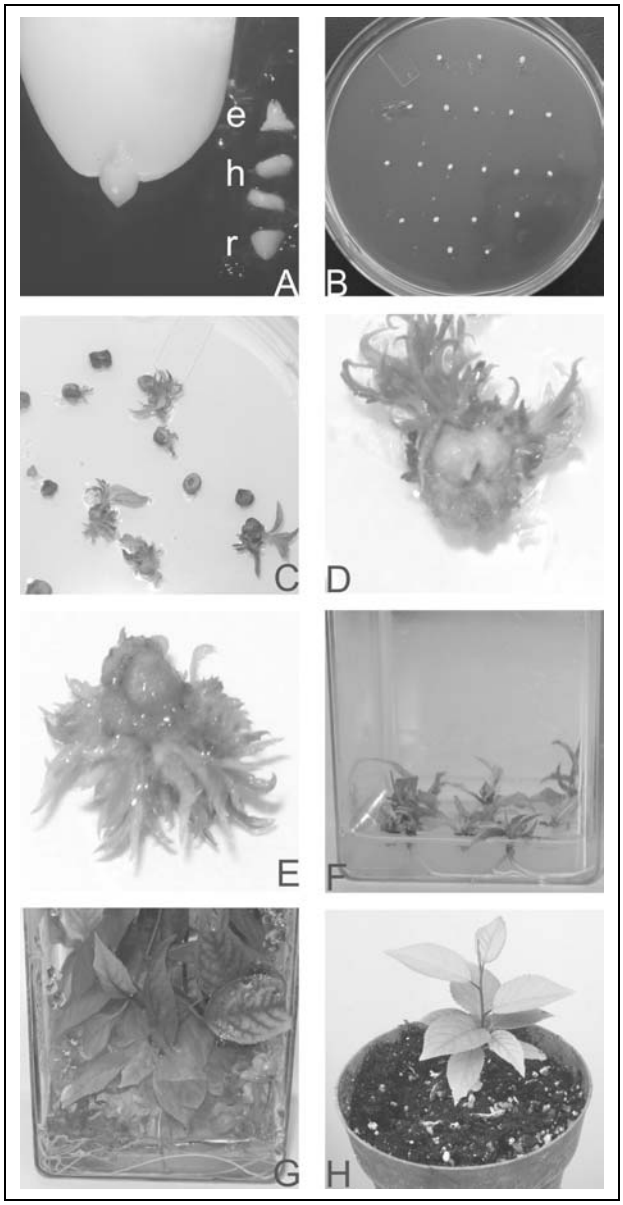


Fig. 1. Adventitious shoot formation from hypocotyls explants of *Prunus salicina* Lindl. *in vitro*. (A) Excision of hypocotyls from an embryo. (B) Hypocotyls explanted on petri plates. (C) Initiation of adventitious shoots from hypocotyls of 'Early Golden' seeds on B5 medium. (D–E) Adventitious shoot formation from hypocotyl explants of cv. 'Early Golden' seedlings. (F) Rooting of adventitious shoots excised from hypocotyls of cv. 'Early Golden'. (G) *In vitro* plantlets of seedlings of cv. 'Early Golden' growing in a Magenta. (H) A tissue culture derived seedlings of cv. 'Early Golden' in the greenhouse.

els of TDZ (0, 1.15, 2.3 or 4.6 μM TDZ) as different treatments, then they were explanted onto shoot induction medium consisted of QL basal salts (prepared using the same ingredients as described above) supplemented with 7.5 μM TDZ and 2.5 μM IBA. Each treatment was replicated three times and each replicate contained 15 hypocotyl explants.

Dark treatment

For dark incubation treatment, first hypocotyls explants were imbibed in sterile distilled water (0 μM TDZ) for 24 h, then explanted onto shoot induction medium consisted of QL basal salts (prepared using the same ingredients as described above) supplemented with 7.5 μM TDZ and 2.5 μM IBA. Then, Petri dishes were maintained in darkness in the culture room for the first ten days of the culture by wrapping with aluminium foil. Dark treatment was replicated three times (as Petri dishes) and each replicate contained 15 hypocotyl explants. Control plates were not incubated in the darkness and placed in normal culture room condition as described above.

Shoot growth medium

Individual shoots or clusters of shoots were excised from the hypocotyls and were transferred to a shoot growth medium consisted of QL basal salts containing 0.5 μM indolebutyric acid (IBA), 4.4 μM benzyl adenine (BA), 30 g l^{-1} sucrose and vitamins as described above. Before addition of Bactoagar (7 g l^{-1}), the pH of the media was adjusted to 5.6 and autoclaved as described above.

Rooting and acclimatization

After 3 to 4 weeks on the growth medium, individual shoots at approximately 1–2 cm in length were excised and transferred to a rooting medium consisting of $^1/_2$ strength QL salts, vitamins (555 μM myo-inositol, 1.2 μM thiamine HCl, 1.4 μM nicotinic acid, 2.4 μM pyridoxine HCl), 5 μM NAA, 25 g l^{-1} sucrose, and 7 g l^{-1} Bactoagar. The pH of the medium was adjusted to 5.9 and autoclaved at 121 °C and 106 kPa.

After rooting, plantlets were transferred to the magenta boxes containing the same rooting medium. Plantlets about 4–5 cm in size were transferred from Magenta containers to pots (8 x 8 cm) containing a commercial potting mix (PRO-MIX BX, Premier Horticulture Inc., Quakertown, PA, 18951, USA). Then, they were placed in a culture room maintained at 24 ± 1 °C under 16/8-h (light/dark) photoperiod and were acclimatized by being covered with a transparent lid to provide a humid environment for the first week. After a month of growth in the culture room, acclimatized plantlets were transferred to a greenhouse.

Transient expression experiments

Agrobacterium strains, binary vectors and co-cultivation. *Agrobacterium* strains EHA105 (Hood et al. 1993), LBA4404 (Hoekema et al. 1983), and GV3101, each containing binary vector pCAMBIA2301 were used for transient expression experiments. The plasmid pC2301 has the β-*glucuronidase* (GUS) reporter gene and *nptII* marker gene conferring resistance to kanamycin under the control of 35S promoters in the following order: 35S-GUS-35S-*nptII*.

Bacterial suspensions were grown overnight in an orbital shaker, at 27 °C and 100 rpm, in LB medium (strains EHA105 and GV3101) or YM medium (LBA4404 strain) with appropriate antibiotics until they reached the stationary phase and then diluted to OD_{600} = 0.2 just before inoculation using either liquid LB or YM. Plum seed explants were inoculated by being dipped in the *Agrobacterium* cultures for 30 min. Each treatment was replicated three times and each replicate had 15 explants. Then, the explants were blotted on sterile Whatman filter papers and transferred to the shoot induction medium (QL, prepared as described above) containing 20 mg l^{-1} acetosyringone and co-cultivated at 24 ± 1 °C for 5 d.

Histochemical GUS assay. After co-cultivation, histochemical GUS assay of explants was performed as described in Jefferson et al. (1987). Explants were incubated in 1.5 mM X-glucuronide (X-Gluc) overnight at 37 °C (Fig. 2A). After incubation, explants were washed using 70 % ethanol to visualize blue staining. Since the explants were heavily occupied by blue spots, the staining intensities of hypocotyls were divided into five classes visually (Fig. 2B-C). For each replication, the staining intensity value was calculated by multiplying the number of explants falling into a class with the class value for each class, and then adding up all the class values together for each replication and dividing the sum by the total number of explants.

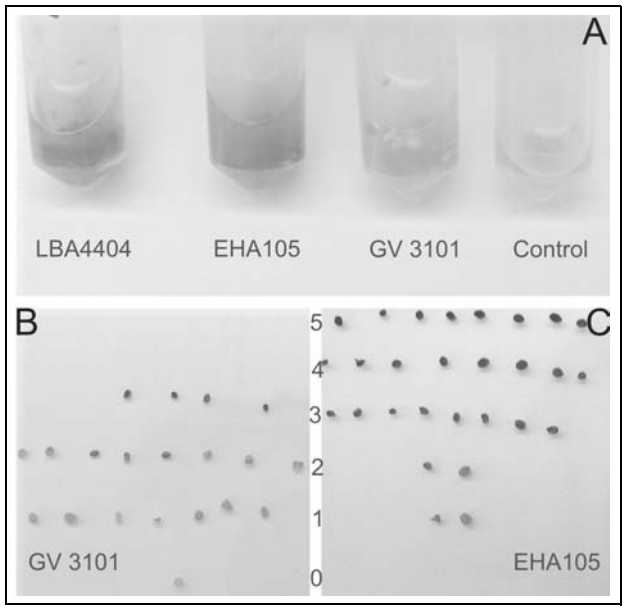


Fig. 2. Transient GUS expression results in Japanese plum (*Prunus salicina* Lindl.) with *Agrobacterium* strains LBA4404, EHA105 and GV3101 each containing pC2301 plasmid. (A) GUS gene expression of different strains in cv. 'Shiro'. (B) Classification of relative GUS staining intensity of hypocotyls of 'Shiro' seeds into five different levels after infection with strain LBA4404 (C) Classification of relative GUS staining intensity of hypocotyls obtained from 'Shiro' seeds into five different levels after infection with strain GV3101.

Statistics

All experiments were conducted in a completely randomized design. Each treatment was replicated three times and each replicate had 15 explants. Following arcsine square root transformation, data were analyzed using ANOVA and means were separated by Duncan's PROCGENMOD (SAS 9.1, SAS institute, Cary, NC).

Results

Regeneration

Hypocotyls placed on shoot induction medium turned green and expanded in a week, and then started to form adventitious shoots about two weeks after the initiation of the cultures (Fig. 1C). The adventitious shoots often formed in clusters along the cut edges of the hypocotyls (Fig. 1D–E).

Basal salt compositions significantly effected regeneration frequencies of the cultivars tested (Table 1). In general, all cultivars performed best on the QL salts, except 'Early Golden'. The highest regeneration frequency for 'Early Golden' was obtained on B5 salts followed by WPM and QL (Table 1). Although using QL salts also resulted in higher regeneration rate in 'Bruce', it was not statistically different from the other treatments (Table 1). The lowest regeneration frequencies were obtained when the medium was supplemented by MS basal salts and some of the cultivars did not regenerate at all on the medium containing MS basal salts (Table 1). In general, B5 and QL medium yielded more shoots per regeneration explant than the WPM and MS medium (Table 1).

Although the dark treatment did not increase the regeneration frequencies, it increased the shoot number per regenerating explant for 'Shiro' and 'Early Golden' (Table 2), and diameter of the hypocotyls (data was not presented) were increased about three times in the dark incubation treatment as compared to that of non-dark incubated ones. There were no significant differences between control and TDZ pre-immersments in regards to regeneration frequencies of 'Early Golden' hypocotyls (Table 2) in any of the TDZ levels tested. TDZ pre-immersment did not improve the regeneration frequencies; in fact, TDZ pretreatment at 2.3 μM reduced the regeneration frequencies of 'Shiro' and 'Gladstone' (Table 2). Shoot number was increased by the intermediate concentrations of TDZ pre-treatment (0.15 and 2.3 μM) in 'Early Golden' hypocotyls only (Table 2). Regeneration ability was appeared to be genotype dependant; 'Shiro' and 'Early Golden' were the best regenerating varieties followed by 'Gladstone'. 'Red Heart' and 'Bruce' had the lowest regeneration frequencies (Table 1).

Following three to four weeks of growth on the shoot growth medium, the shoots were introduced onto rooting medium. The root formation was visible as early as 14 d after transferring shoots to the rooting medium, but most of the rooting occurred after three to four weeks (Fig. 1F). After rooting, the plantlets were transferred to magenta boxes containing the same rooting medium (Fig. 1G) and then they were acclimatized and transferred to the greenhouse (Fig. 1H)

Transient expression

There were significant differences among *Agrobacterium* strains tested in regards to transient GUS gene expression in Japanese plum hypocotyls (Table 3). Explants co-cultivated with *Agrobacterium* strains EHA105 and LBA4404 showed higher observable levels of GUS staining than explants co-cultivated with *Agrobacterium* strain GV3101 (Fig. 2A) regardless of the variety tested.

Relative mean GUS intensity with *Agrobacterium* strains EHA105 and LBA4404 were significantly higher compared to explants co-cultivated with *Agrobacterium* strain GV3101 in all of the varieties tested (Table 3) (Fig. 2B–C). Hypocotyl explants were heavily occupied by blue spots and most of the explants were placed into the staining categories five and four when *Agrobacterium* strains EHA105 and LBA4404 were used (Fig. 2C), whereas the intensity of blue spots were much lower and no hypocotyls had as much blue spots to be placed in to the staining categories five and four when strain GV3101 was used (Fig. 2B). In addition, 100 % of the explants infected with EHA105 and LBA4404 showed transient GUS gene expression, however only 52.3–88.5 % of the explants infected with GV3101 showed GUS expression (Table 3). Transient GUS expression pattern of different *Agrobacterium* strains were similar for all Japanese plum

Table 1. Effects of different basal salt mixtures on shoot induction from hypocotyls of *Prunus salicina*, cv. 'Shiro', 'Early Golden', 'Gladstone', 'Red Heart' and 'Bruce' in the presence of 7.5 μM TDZ and 2.5 μM IBA.

Treatment	'Shiro'		'Early Golden'		'Gladstone'		'Red Heart'		'Bruce'	
	Regeneration[1]	Shoot number[3]	Regeneration	Shoot number	Regeneration	Shoot number	Regeneration	Shoot number	Regeneration	Shoot number
B5	17.0 b[2]	2.6 ± 0.42	42.8 a	2.5 ± 0.59	8.1 b	1.3 ± 0.32	2.6 b	1.0 ± 0.00	7.5 a	2.3 ± 0.87
WPM	19.5 b	1.8 ± 0.31	24.3 ab	2.0 ± 0.45	17.1 ab	1.7 ± 0.28	2.6 b	1.0 ± 0.00	7.1 a	1.0 ± 0.00
MS	0.0 c	0.0 ± 0.00	4.8 b	2.0 ± 0.00	5.0 b	1.5 ± 0.49	0.0 b	0.0 ± 0.00	0.0 a	0.0 ± 0.00
QL	56.4 a	2.6 ± 0.33	13.9 ab	2.8 ± 0.87	30.6 a	2.2 ± 0.22	11.9 a	3.8 ± 1.20	12.8 a	3.0 ± 0.40

n= 45 (3 x 15).
[1]Presented as treatment means. Explant number with at least one shoot/total explants number x 100.
[2]Significant differences by P≤0.05 by Duncan's test within the same column are presented by different letters.
[3]Average number of shoot ± standard error.

Table 2. Effect of TDZ pretreatments and dark incubation on shoot regeneration from hypocothyl explants of *Prunus salicina* Lindl. *in vitro*.

TDZ	'Early Golden'[1]		'Shiro'		'Gladstone'	
Pretreatment	Regeneration[2]	Shoot number	Regeneration	Shoot number	Regeneration	Shoot number
0.00 μM TDZ	39.4 a[3]	2.6 ± 0.68[4]	35.1 a	2.0 ± 0.20	40.0 a	2.5 ± 0.31
0.23 μM TDZ	27.7 a	1.9 ± 0.34	20.0 ab	1.6 ± 0.24	15.4 ab	1.8 ± 0.48
1.15 μM TDZ	43.5 a	3.9 ± 0.64	14.2 ab	1.5 ± 0.22	–[5]	–
2.30 μM TDZ	35.7 a	3.2 ± 0.47	10.0 b	1.5 ± 0.29	5.7 b	1.5 ± 0.50
4.60 μM TDZ	34.2 a	1.5 ± 0.21	23.0 ab	1.7 ± 0.33	23.8 ab	
Dark (0.00 μM TDZ)	22.2 a	3.3 ± 0.64	14.6 ab	2.8 ± 0.66	26.7 ab	2.6 ± 0.41

n= 45 (3 x 15).
[1]Cultivars were tested for the effect of TDZ immersment and dark incubation on the media that they were performed the best; B5 medium is used for 'Early Golden', whereas QL medium is used for 'Shiro' and 'Gladstone'. Both solid media were supplemented with 7.5 μM TDZ.
[2]Presented as treatment means. Explant number with at least one shoot/total explant number x 100.
[3]Significant differences by P≤0.05 by Duncan's test within the same column are presented by different letters.
[4]Average number of shoot ± standard error.
[5]Infected.

Table 3. Effect of *Agrobacterium* strains on transient GUS activity in hypocotyls of *Prunus salicina* Lindl.

Agro strain	'Shiro'		'Red Heart'		'Bruce'		'Gladstone'	
	% stained[1]	staining intensity[2]	% stained	staining intensity	% stained	staining intensity	% stained	staining intensity
EHA105	100.0a[3]	3.5 a	100.0a	3.2 a	100.0a	1.8 b	100.0a	3.3 a
LBA4404	100.0a	3.8 a	100.0a	3.1 a	100.0a	2.5 a	100.0a	3.3 a
GV3101	88.5 b	1.6 b	87.8 b	1.2 b	77.0 b	1.2 c	52.3 b	0.7b

n= 45 (3 x 15).
[1]Presented as treatment means. Explant number with at least one blue spot/total explants number x 100.
[2]Since the explants were heavily occupied by blue spots, the staining intensities of hypocotyls were divided into five classes visually. Then, the staining intensity value was calculated by multiplying the number of explants falling into a class with the class value for each class, and then adding up all the class values together for each replication and dividing the sum by the total number of explants.
[3]Significant differences by Duncan's test (P≤0.05) within the same column are presented by different letters.

varieties tested (Table 3). The presence of acetosyringone significantly increased the percentage of explants that showed GUS expression (70 vs. 33 %) and intensity of blue staining (0.89 vs. 0.47 %) in GV3101 infected treatments (Table 4). Although there were no significant differences in respect to the percentages of explants stained when strain EHA105 was used, the intensity of staining highly increased when acetosyringone added to the co-cultivation medium (Table 4).

Discussion

An efficient regeneration protocol was devised and transient expression experiments were carried out to provide guidelines for the *Agrobacterium*-mediated stable transformation of Japanese plum.

Media basal salt composition significantly effected regeneration frequencies and all cultivars performed best on QL salts excluding 'Early Golden' which regenerated best on B5 medium. In general, regeneration ability was significantly lowered when MS basal salt mixture was used. MS is the medium of choice for many species; however, this is not universal for woody plants as *Prunus* species (Tang et al. 2002). In our study, regeneration efficiencies were much higher than the efficiencies reported by Urtibia et al. (2008) for Japanese plum varieties 'Angeleno' and 'Larry Anne'. Regeneration percentages were increased about four times for 'Shiro' by using QL salts and about two times for 'Early Golden' by using B5 medium as compared to regeneration percentages on MS medium reported by Tian et al. (2007). Similarly, QL medium stimulated regeneration more than MS and WPM in sweet cherry leaves (Matt and Jehle 2005). Comparison of the mineral composition of the media used indicates that QL like B5 medium has significantly lower levels of ammonium ion concentrations than remaining of the media tested. Many researchers preferred to use low concentrations of ammonium due to its toxicity (Brand 1993). QL medium, especially developed for *Prunus* species (Quoirin and Lepoivre 1977), contains also medium levels of macro salt content with an increased level of Ca+ ions, which is essen-

Table 4. Effect of acetosyringone on transient GUS expression in hypocotyls of *Prunus salicina* Lindl. cv. 'Gladstone'.

Treatment	*Agrobacterium tumefaciens* strain			
	EHA105		GV3101	
	% GUS stained[1]	Staining intensity[2]	% GUS stained	Staining intensity
With acetosyringone	100.0a[3]	3.38 a	70.0 a	0.89 b
Without acetosyringone	100.0a	1.74 b	33.0 b	0.47 b

n= 45 (3 x 15).
[1]Presented as treatment means. Explant number with at least one blue spot/total explants number x 100.
[2]Since the explants were heavily occupied by blue spots, the staining intensities of hypocotyls were divided into five classes visually. Then, the staining intensity value was calculated by multiplying the number of explants falling into a class with the class value for each class, and then adding up all the class values together for each replication and dividing the sum by the total number of explants.
[3]Significant differences by Duncan's test ($P \leq 0.05$) within the same column are presented by different letters.

tial for the cell wall synthesis (Yepes and Aldwinckle 1994). Therefore, the improved regeneration rates in these media might be attributed to its low nitrogen and salt content as demonstrated in *Malus* (Yepes and Aldwinckle 1994) and to relatively increased level of Ca+ ions in QL medium. TDZ pre-treated hypocotyls did not produce superior regeneration frequencies than the controls in this study. Canli and Tian (2008) and Canli (2009) reported that TDZ pre-treated explants did not enhance regeneration frequencies in cherry cotyledons, whereas Song and Sink (2005) obtained better regeneration from sour cherry leaves with TDZ pre-immersments. Therefore, the effect of TDZ pre-treatment might be specific to explant type and species. Although no significant differences observed between controls and dark treated explants in regards to regeneration frequencies, the shoot number per regenerating explant was increased by dark incubation. The effectiveness of dark incubation in promoting shoot formation was reported by several researchers for different *Prunus* species such as *P. armenicana, P. serotina* and *P. avium* (Nedelcheva and Tsoneva 1998; Espinosa et al. 2006; Canli and Tian 2008).

Agrobacterium strains had significant effect on transformation frequencies in different fruit species (Gill et al. 2004). In the current study, transient expression results showed considerable variation among the strains tested and EHA105 and LBA4404 showed higher transient expression than GV3101. Similarly, the strain EHA 105 has also been used successfully in genetic transformation of crops such as grapes, strawberries, pears, bananas and citrus (Gill et al. 2004). EHA105 is observed as being superior in facilitating stable transformation of foreign genes to plant cells in several woody species like cottonwood (Han et al. 2000) or apple (De Bondt et al. 1994). LBA4404 strain was proven to be effective in transformation of grape (Gall et al. 1994; Martinelli and Mandolino 1994; Golles et al. 1997), trifoliate orange (Kaneyoshi et al. 1994) and Troyer Citrange (Gentile et al. 1999). On the other hand, the strain GV 3101was the best and LBA 4404 was inefficient in grape cv. Chardonnay (Berres et al. 1992) and in Washington Novel orange (Bond and Rose 1998). All these results indicate that a particular strain behaves differently in different host genotypes, therefore, virulence of *Agrobacterium* strains appears to be host dependant and unpredictable (Cervera et al. 1998)

There was a consistency between the percentage of explants showing GUS staining and the relative GUS staining intensity in transient experiments in all of the strains and genotypes tested in our study. Specifically, strains having higher percentages of GUS expressions also had higher levels of GUS staining intensity. Petri et al. (2004) stated that they did not find any discrepancies in the effect of different treatments on transient and stable *gfp* expression. In these cases, optimizing transient expression factors may result in increased transformation efficiencies. However, transient expression results may not always be relevant to stable transformation efficiencies in the same plant genotype. While the transfers of T-DNA from *A. tumefaciens* to the plant cell occur in an appropriate manner, as observed by efficient transient expression, stable integration of T-DNA may be limiting. In such cases, improving transient expression efficiencies will not lead to better stable transformation (Maximova et al. 1998). Similar to the previous reports on transformation of various species (Li et al. 1992; Song and Sink 2005), addition of AS to the co-cultivation media increased the GUS expression.

Efficient regeneration protocols and optimized transient expression factors are important pre-requirements for the improvement of genetic transformation efficiencies for plant species. The optimized regeneration protocol and transient expression factors in the current study provide useful and important information towards the development of more efficient transformation systems for the Japanese plum.

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