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Evaluation of Factors Affecting European Plum (*Prunus domestica* L.) Genetic Transformation

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1. Introduction

With the advancement of genomics research, many genes have been identified and cloned from various plants. Transfer of these genes into plants for gene function studies and for plant improvement is important in the post-genomics era. At this time, the lack of efficient, effective, and high throughput genetic transformation systems in many crops and varieties is a major barrier and a challenge in functional genomics research and for plant trait improvement via biotechnology (Petri and Burgos 2005). Studies and understanding of different aspects and factors in plant transformation are important and are a prerequisite in the development of effective and efficient transformation technologies for various crops and varieties (Gill et al. 2004; Petri and Burgos 2005).

European plum (*Prunus domestica* L.) is an economically important fruit crop and is widely grown across the world (Hartmann 1994; Okie and Ramming 1999; Kaufmane et al. 2002). There have been a number of technology reports of European plum genetic transformation via *Agrobacterium tumefaciens* using hypocotyls as explants (Mante et al. 1991; Padilla et al. 2003; Petri et al. 2008). These studies were actually all conducted in a single research laboratory. Several studies have reported use of transgenic plum for disease resistance (Scorza et al. 1994; 2001; Ravelonandro et al. 2000; Hily et al. 2004; Malimowski et al. 1998, 2006; Capote et al. 2008), however, transgenic plum plants used in these studies were apparently generated in the same research laboratory indicated before. Several other laboratories have reported plum transformation but only putative transformants were reported and Southern blot and other related analyses, which were essential for confirmation of transformation, were not provided (Da Camara-Machado et al. 1994; Yancheva et al. 2002). Use of leaves as explants for plum transformation was described recently but again Southern blot analysis was not provided to confirm transformation

(Kikhailov et al., 2008). Indeed, European plum genetic transformation has only been successful in a few laboratories. Tian et al. (2006) evaluated *in vitro* regeneration of European plum germplasms and varieties adapted to high latitude and developed genetic transformation technology for these types of plum plants via hypocotyl regeneration (Tian et al. 2009). Nevertheless, at this time, wide and practical use of plum genetic transformation technology in many other laboratories and studies is still not feasible and is difficult. In addition, transformation of European plum has been reported in only a few varieties and the efficiency is low. Development of transformation technologies for many commercial plum varieties and improvement of transformation technology for high efficiency are still major tasks and also important for the germplasm improvement of European plum via biotechnology.

Plant transformation is a complicated process which involves various factors, such as plant genotype and variety, regeneration efficiency, culture medium and condition, selectable marker, infection condition, gene construct and *Agrobacterium* strain. Any of these factors can be important in the success of transformation. Studying, understanding and optimizing various factors are important for the development of transformation technologies for different germplasms and varieties and for technology improvement (Gill et al. 2004; Petri and Burgos 2005).

The objective of this research was to study important aspects of *Agrobacterium*-mediated genetic transformation in European plum via hypocotyl regeneration system. The research results contribute to the knowledge advancement of plum transformation and are useful for the development and improvement of transformation technologies for different varieties for European plum improvement, especially for the genotypes and germplasms adapted to high latitude.

2. Materials and methods

2.1 Plant materials

Plum (*Prunus domestica* L.) plants adapted to high latitude were used in this study. These types of plum germplasms and varieties have been developed by Canadian *Prunus* breeding program over the past years. These genotypes and varieties are more resistant to cold weather, the fruit development and maturation of these genotypes are relative slow, and the fruit ripening is also relatively late in the season (Dr. J. Submaranian, *Prunus* tree breeding program, person. communication). Our previous studies indicate that these plums have low response to *in vitro* regeneration (Tian et al. 2006) and the genetic transformation efficiency is also low (Tian et al. 2009).

Plum fruits, two weeks prior to maturation, were collected from plum trees in Vineland, Ontario, Canada. Endocarps were cracked open and the seeds were sterilized in 10% commercial bleach solution. The seeds were then rinsed three times with sterile distilled water in the laminar flow hood and were imbibed in final rinsing water overnight. After a removal of the seed coat, the embryonic axis was excised from the cotyledons. Embryonic axis was cut into five sections with one radicle, three hypocotyls and one epicotyl. Hypocotyl and epicotyl segments were employed in transient gene expression studies while the radicle was discarded. For stable transformation studies, only hypocotyl slices were used in the experiments. Transformation was conducted using hypocotyls as explants as described by Mante et al. (1991) and Padilla et al. (2003) with modifications by Tian et al. (2009) and in this study.

2.2 *Agrobacterium* and vectors

Three *Agrobacterium tumefaciens* strains, namely EHA105 (Hood et al. 1993), LBA4404 (Hoekema et al. 1983) and GV3101 (Holster et al. 1980) and five vectors were included in the research. The vector pCAMBIA2301 (pC2301) has the GUS (*uidA*) reporter gene coding for β -glucuronidase and the *nptII* gene coding for neomycin phosphotransferase under the control of the 35S promoters in the following order: 35S-*uidA*-35S-*nptII*. The plasmid pCAMBIA1301 (pC1301) carries the GUS reporter gene and the *hpt* gene coding for hygromycin B phosphotransferase under the control of the 35S promoters in the following order: 35S-*uidA*-35S-*hpt*. The two vectors are the same except for the selectable marker. The GUS gene in these pCAMBIA vectors contains a plant specific intron which can only be recognized in plant cells and thus cannot express in *Agrobacterium*. The constructs pPV1, pPV2, and pPV3 carry the genes of interest (not shown, unpublished constructs) other than the *nptII* marker gene. These genes were cloned using proper restriction enzymes into the pCaMter X vector and the constructs were introduced in *Agrobacterium* strains LBA4404 and GV3101, respectively.

2.3 *Agrobacterium* infection and plant transformation

Agrobacterium was grown in LB medium with appropriate antibiotics to optimal OD₆₀₀ reading. Explants were immersed in *Agrobacterium* solution for 30 minutes and were blotted dry on sterile Whatman filter paper. The explants were then transferred on co-culture MS medium. The co-culture medium consisted of MS salts (Murashige and Skoog 1962) supplemented with 2.5 μ M indolebutyric acid (IBA), 555 μ M myo-inositol, 1.2 μ M thiamine HCl, 1.4 μ M nicotinic acid, 2.4 μ M pyridoxine HCl, 25 g L⁻¹ sucrose, and 7 g L⁻¹ Bactogar. The pH of the medium was adjusted to 5.9 prior to autoclaving. Thidiazuron (TDZ, 7.5 μ M) was added to the medium after autoclaving. Different antibiotics and other chemicals were added to the culture medium as needed. The culture was maintained at 25 \pm 1°C with 16 hour photoperiod supplied by fluorescent Sylvania "Cool White" light with a Photosynthetic Photon Flux of about 50 μ mol·m⁻²·s⁻¹. The explants were collected five days after the infection for transient expression studies. For stable transformation, explants were maintained on co-cultivation MS medium for one week. After co-cultivation, the explants were transferred onto the shoot induction medium. Shoot induction media was the same as the co-cultivation medium but contained 75 mg L⁻¹ kanamycin or 5 mg L⁻¹ hygromycin depending on the transformation vector used and 300 mg L⁻¹ timentin was added to the media. The explants were sub-cultured on fresh induction medium every three weeks. For evaluation of medium type on transformation, B5 medium (Gamborg et al. 1968) was included in the research and other chemicals were added in to B5 medium as in MS medium.

Regenerated shoots at about 0.5 -1 cm in length from antibiotic selections were excised from the explants and transferred to fresh shoot induction medium containing the same antibiotics as well as timentin. After another 2-3 subcultures, well established and developed shoots in antibiotic-containing medium were placed in Magenta boxes containing rooting medium. The rooting medium consisted of 1/2-strength MS salts (Murashige and Skoog 1962), 5 μ M naphthalene acetic acid (NAA), 0.01 μ M kinetin, vitamins (555 μ M myo-inositol, 1.2 μ M thiamine HCl, 1.4 μ M nicotinic acid, 2.4 μ M pyridoxine HCl), 10 g L⁻¹ sucrose, and 7 g L⁻¹ Bactoagar. Plants developed in magenta containers were transferred to soil and plants were established in a greenhouse. Plants were analyzed for transformation using different approaches as described in previous studies (Tian et al. 2009).

2.4 Histochemical and Fluorogenic GUS expression assay

Five days after *Agrobacterium* infection, explants were collected from co-cultivation media and incubated in 5-bromo-4-chloro-indolyl β -D-glucuronide (X-Glu) in 100 mM sodium phosphate buffer, pH 7.0 overnight at 37°C. Histochemical GUS analysis followed the procedure described in Jefferson et al. (1987). The GUS expression was scaled from 1 - 3 depending on the intensity of GUS staining, with 1 the minimum and 3 the maximum.

For fluorometric GUS expression, plant tissues five days after *Agrobacterium* infection were ground in liquid nitrogen with a pestle and a mortar. A volume of 50 μ L of the crude extract was incubated at 37°C with 1 mM 4-methylumbelliferyl glucuronide (MUG) in 0.3 mL of GUS assay buffer (50 mM NaPO₄, pH 7.0, 10 mM EDTA, 0.1% [v/v] Triton X-100, 10 mM β -mercaptoethanol). At different time periods of incubation, 0.1 mL aliquot was removed and added to 1.9 mL of 0.2 M Na₂CO₃ to terminate the reaction. Protein standard curve was made by Bradford protein assay and GUS activity was expressed as picomoles of 4-methylumbelliferone (MU) per milligram of protein per hour.

3. Results and discussion

Efficient infection of *Agrobacterium* to plant cells and the subsequent transfer of T-DNA from *Agrobacterium* into plant cells are the first and also essential steps in the stable transformation process. Transient reporter gene expression can be used to evaluate *Agrobacterium* infection and gene transfer into plants cells. The relationship between transient and stable transformation is complicated and varies among species. Studies must be conducted for a particular species to understand how these two aspects are related. If a positive relation can be found and established for a plant species, study of transient reporter gene expression can be very useful for evaluation of various factors for development and optimization of genetic transformation technologies (Chen et al. 1998; Petri et al. 2004).

The plum explants were infected with different *Agrobacterium* strains containing construct pC2301 or pC1301. These constructs carry the GUS-intron design and GUS expression is only activated in plant cells and the GUS expression cannot be due to the presence of *Agrobacterium* cells. Histochemical assay was first conducted to evaluate transient GUS expression in explants infected by different *Agrobacterium* strains and plum varieties Stanley and Vanette were used in the experiments. Results showed that GUS expression was significantly higher when *Agrobacterium* strains LBA4404 and EHA105 were used (Fig. 1, Fig. 2). Enzymatic assay was then conducted in plum Stanley and the results showed that GUS expression in explants was significantly higher using *Agrobacterium* strains LBA4404 and EHA105 than using GV3101 (Fig.3). Research was also conducted to evaluate transient GUS expression using some additional plum varieties including V72511, Veeblue, and Italian. Results showed that explants infected with *Agrobacterium* strains LBA4404 and EHA105 in overall exhibited higher levels of GUS enzyme activities than GV3101 (not shown).

Stable transformation were conducted with either kanamycin selection or hygromycin selection depending on the vectors used. It appeared that transient reporter gene expression was well related to the effectiveness of stable transformation in plum (Table 1, Fig. 2&3). Specifically, higher levels of transient GUS expression after EHA105 and LBA4404 infection led to the effectiveness of stable transformation and consistently generated transgenic lines (Table 1). On the other hand, lower transient GUS expression using strain GV3101 resulted in ineffectiveness of stable transformation (Table 1, Fig. 2&3). Such relation is consistent

using different constructs and with either the kanamycin selection or the hygromycin selection (Table 1).












<i>Agrobacterium</i> Strain	Plant Variety and Vector Type		
	Stanley-2301	Stanley-1301	Vanette-1301
EHA105			
LBA4404			
GV3101			
Control			

Fig. 1. Transient GUS expression in European plum (*Prunus domestica* L.) after infection by *Agrobacterium* strains LBA4404, EHA105 and GV3101 containing either pCAMBIA2301 or pCAMBIA1301 plasmids.

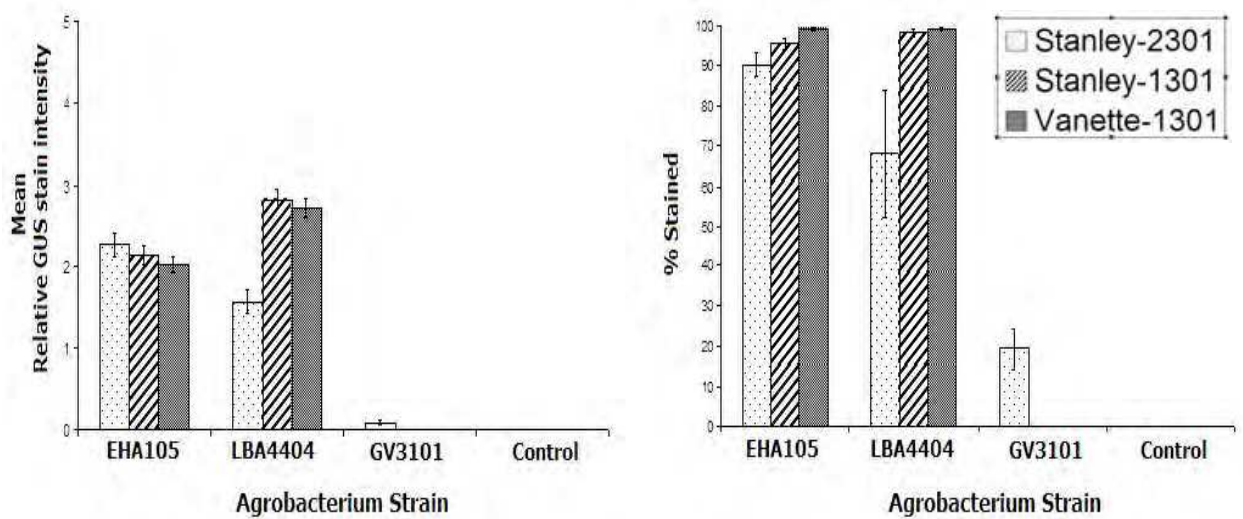


Fig. 2. Histochemical analysis of GUS gene expression in Stanley and Vanette varieties infected by different *Agrobacterium* strains containing either pCAMBIA2301 or pCAMBIA1301 vector.

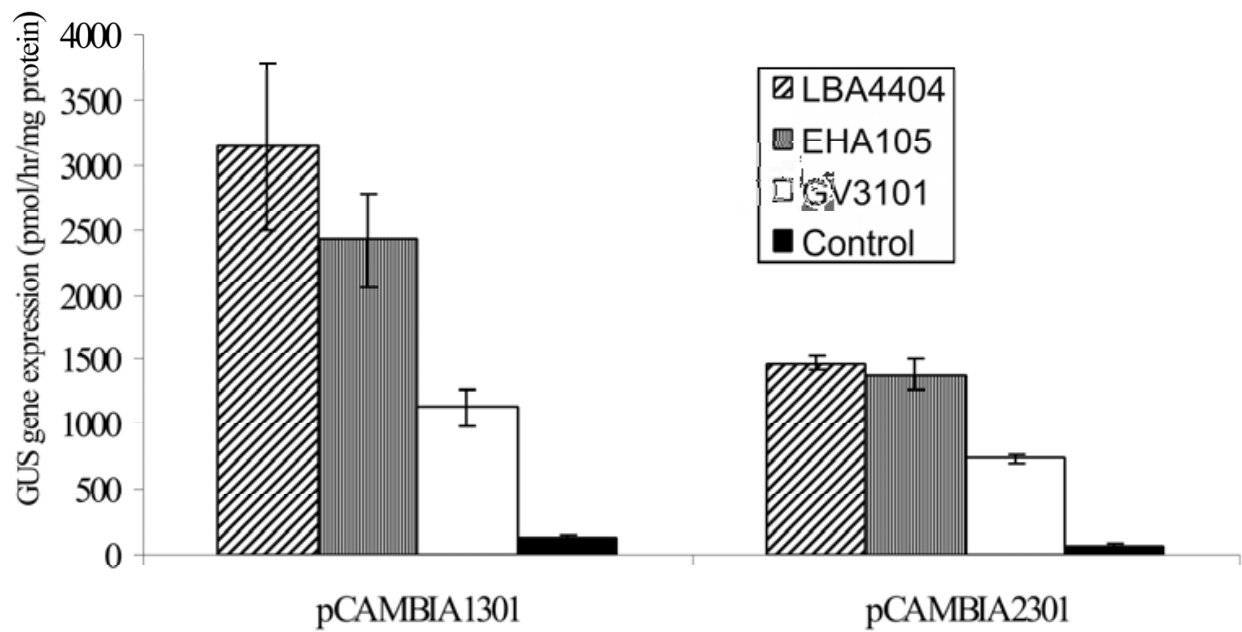


Fig. 3. GUS gene expression via fluorometric assay with Stanley after infection by different *Agrobacterium* strains containing either pCambia2301 or pCambia1301 vector.

Vector	<i>Agrobacterium</i> Strain	Selection scheme	No of Explants	No. of Transformants	Transformation Efficiency
pC2301	EHA105	Kanamycin	272	6	2.2%
	LBA4404	Kanamycin	270	2	0.7%
	GV3101	Kanamycin	272	0	0%
pC1301	EHA105	Hygromycin	272	3	1.1%
	LBA4404	Hygromycin	271	2	0.7%
	GV3101	Hygromycin	272	0	0%

Table 1. Stable transformation of European plum using and *Agrobacterium* strains LBA4404, EHA105 and GV3101 containing either pCambia2301 or pCambia1301 vector.

L-cysteine (mg/L)	Number of explants	% of explants transiently expressing GUS gene	Number of transgenic line	Transformation efficiency
0	78	80.0	2	2.6%
900	78	22.7	0	0

Table 2. Transient GUS expression and stable transformation of European plum using L-cysteine in culture medium.

Our previous studies have indicated that use of culture medium including L-cysteine, which was used in media for transformation improvement (Olhoft et al. 2001), could affect transient GUS gene expression in plum (non-published results). We conducted research to study how transient gene expression was related to stable transformation using medium containing L-cysteine. The results showed that explants cultured in medium with L-cysteine resulted in significantly low levels of transient GUS gene expression (Table 2) as found in previous studies. No transformation was obtained from the explants cultured in the presence of L-cysteine. On the other hand, high level of transient GUS expression was observed in explants without L-cysteine treatment and stable transformation was routinely recovered (Table 2). This study further indicated that transient reporter gene expression was related to stable transformation in European plum. The positive relationship between transient reporter gene expression and stable transformation in plum could be important for studying and evaluating various factors and conditions for transformation, which can be useful in the development and improvement of stable transformation technologies in different plum varieties.

Construct	Agro strain	No. of total explants	Lines recovered	Efficiency
pPV-1	GV3101	444	0	0.0%
	LBA4404	1019	17	1.7%
pPV-2	GV3101	330	1	0.3%
	LBA4404	601	11	1.8%
pPV-3	GV3101	283	0	0.0%
	LBA4404	769	20	2.6%
Summary	GV3101	1057	1	0.09%
	LBA4404	2389	48	2.0%

Table 3. Stable genetic transformation of European plum using *Agrobacterium* strains LBA4404 and GV3101 containing different transformation vectors with the genes of interest

Medium	Number of explants	Number of transgenic lines	Transformation efficiency (%)
MS	300	6	2.0
B5	310	19	6.1

Table 4. Plum genetic transformation using B5 and MS co-cultivation and shoot induction media.

Agrobacterium strain is a major factor in plant transformation. Numerous studies have indicated that the effectiveness of transformation via different strains of *Agrobacterium* can be significantly different (e.g., De Bondt et al. 1994; Le Gall et al. 1994; Bond and Rose 1998; Cervera et al. 1998; Gill et al. 2004; Petri et al. 2004; Joyce et al. 2010). Several *Agrobacterium* strains, including LBA4404, EHA101, EHA105, GV3101, have been used in plum transformation previously (Mante et al. 1991; Padilla et al. 2003; Petri et al. 2008). No transformation efficiency difference was found using EHA105 and LBA4404 (Padilla et al. 2003). Petri et al. (2008) conducted plum transformation using EHA105 and GV3101. The study showed that use of the same *Agrobacterium* strains carrying different transformation constructs resulted in significant difference of transformation efficiency (Petri et al. 2008). This difference in the transformation efficiency could be due to the presence of the different constructs. Till date, the effect of *Agrobacterium* strains on plum transformation is still not well understood. In this research we studied two *Agrobacterium* strains, LBA4404 and GV3101, which have never been directly compared in plum transformation. These two strains, in contrast to the study by Petri et al (2008), carried the identical constructs and transformation was conducted using a large number of explants. No stable transformation was achieved with constructs pPV-1 and pPV-3 when the strain GV3101 was used, whereas with strain LBA4404, the transformation efficiencies with these constructs were 1.7% and 2.6%, respectively (Table 3). Of the three constructs, only one transformant was recovered using GV3101 (Table 3). Combining the results of all three constructs and all experiments, the transformation efficiency with LAB4401 was 22 times higher than that using GV3101 (Table 3). The different transformation efficiencies of *Agrobacterium* strains can also be observed in the study of transient gene expression and stable transformation described before (Fig. 2,3; Table 1). The results showed that *Agrobacterium* strain LBA4404 was much more effective and was more suitable than GV3101 in plum transformation. The study suggests that *Agrobacterium* strains can be an important factor in plum transformation and should be carefully considered for various studies and for transformation of different plum varieties. Effective plum transformation using *Agrobacterium* strain LBA4404 is illustrated in Fig. 4.

Plant genetic transformation is usually conducted *via* tissue culture systems. The culture medium is the platform and the fundamental base of transformation. Medium can be an important factor for plant transformation (Joyce et al. 2010). There is not report regarding the effect of different culture media on plum genetic transformation. We conducted research on this aspect. Two commonly used culture media, B5 medium and MS medium, and construct pCAMBIA2301 were used in the research. Explants, after *Agrobacterium* infection, were cultured on MS and B5 co-cultivation media as well as regeneration media respectively. The transformation efficiency using MS medium was 2.0% and the efficiency with B5 medium was 6.1% (Table 4). Use of B5 medium was three times more efficient in plum transformation. Use of different media apparently had a major impact on plum transformation. The B5 medium might have promoted more transformed cells to develop and regenerate into plants, resulting in higher transformation efficiency. A recent study has showed that adding 2, 4-D to culture medium can significantly increase plum transformation efficiency (Petri et al. 2008). This addition of a plant growth regulator probably increased recovery of more transformed cells as discussed in this study. We would conduct experiments to evaluate the effect of 2, 4-D on the transformation of plum genotypes adapted to high latitude.

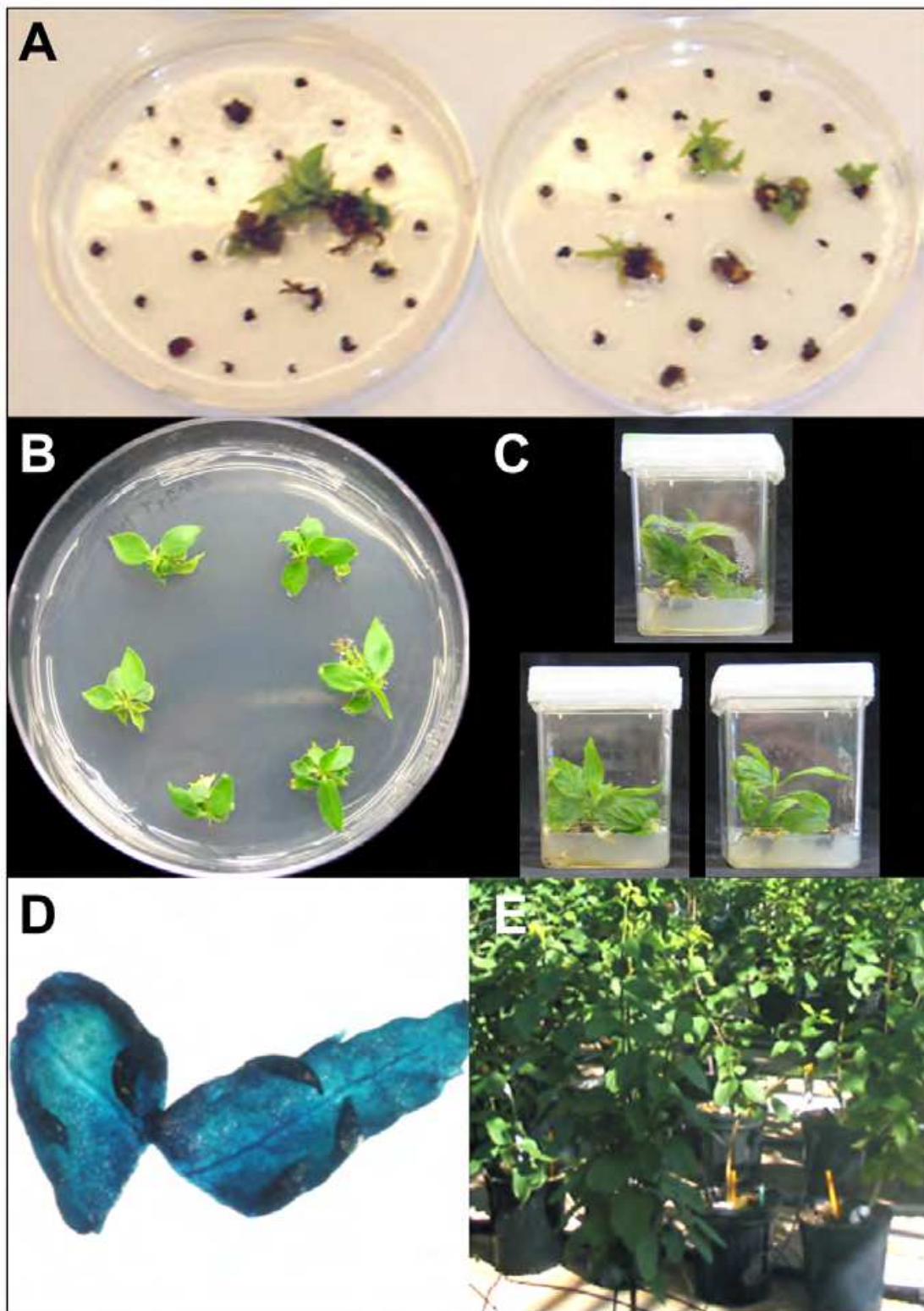


Fig. 4. European plum using *Agrobacterium* LBA4404. (A) Development of transgenic shoots on selection medium containing $75 \text{ mg}\cdot\text{L}^{-1}$ kanamycin. (B) Shoots excised from explants grew vigorously upon subculturing to the same selection medium. (C) Development of transgenic plants on rooting medium in Magenta boxes. (D) GUS expression in leaves of transgenic plum plants. (E) Transgenic plants in the greenhouse.

4. Conclusion

Genetic transformation efficiency in *Prunus* crops is significantly lower and the technology is much less developed compared to some other fruit crops. We have studied some aspects of plum transformation which have not been explored before. The study shows that the transient gene expression is in general well related to stable transformation in plum. This is important for studying and optimizing various conditions and factors for stable transformation of plum varieties. The study also shows that certain *Agrobacterium* strains strongly affect European plum genetic transformation. While *Agrobacterium* strains LBA4404 can lead to successful plum transformation, the strain GV3101 is ineffective in generating transgenic lines. Moreover, use of different types of media can significantly affect stable transformation. The results obtained from this research would contribute to the knowledge advancement and to the development of more efficient and effective transformation technologies for plum fruit crop, especially for germplasms and varieties adapted to high latitude.

5. Acknowledgements

The authors would like to thank Dr. Jay Subramanian for providing plum research materials and for research discussion and Dr. A. Wang for providing some transformation constructs. We also thank Dr. Ralph Scorza for providing some *Agrobacterium* strains and for transformation research discussion. We thank Susan Sibbald, Janny Lac and Kaalak Reddy for technical assistance.

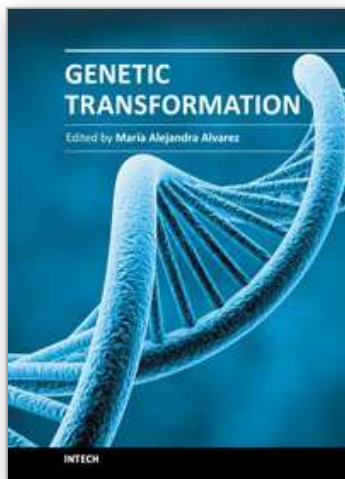
6. References

- Bond, J. E. and Roose, M. L. 1998. *Agrobacterium*-mediated transformation of the commercially important citrus cultivar Washington navel orange. *Plant Cell Rep.* 18:229-234.
- Capote, N., Pérez-Panadés, J., Monzó, C., Carbonell, E., Urbaneja, A., Scorza, R., Ravelonandro, M. and Cambra, M. 2008. Assessment of the diversity and dynamics of Plum pox virus and aphid populations in transgenic European plums under Mediterranean conditions. *Transgenic Res.* 17:367-377.
- Cervera, M., Lopez, M. M., Navarro, L. and Pena, L. 1998. Virulence and supervirulence of *Agrobacterium Tumefaciens* in woody fruit plants. *Physiological and Molecular Plant Pathology* 52:67-78.
- Chen, L., Zhang, S., Beachy, R. N. and Fauquet, C. M. 1998. A protocol for consistent, large-scale production of fertile transgenic rice plants. *Plant Cell Rep.* 18:25-31.
- da Câmara Machado, A., Katinger, H. and Laimer da Câmara Machado, M. 1994. Coat protein-mediated protection against plum pox virus in herbaceous model plants and transformation of apricot and plum. *Euphytica* 77:129-134.
- De Bondt, A., Eggermont, K., Druart, P., De Vil, M., Goderis, I., Vanderleyden, J. and Broekaert, W. F. 1994. *Agrobacterium*-mediated transformation of apple (*Malus x domestica* Borkh.): An assessment of factors affecting gene transfer efficiency during early transformation steps. *Plant Cell Rep.* 13:587-593.
- Gamborg, O. L., Miller, R. A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cell. *Exp. Cell Research* 50:151-158.

- Gill, M. I. S., Zora, S. and Agrez, V. 2004. Factors affecting *Agrobacterium*-mediated genetic transformation in fruit and nut crops - an overview. *J. Food, Agriculture & Environment* 2:327-347.
- Hartmann, W. 1994. Plum production in Germany. *Acta Hort* 359:17-25.
- Hily, J. M., Scorza, R., Malinowski, T., Zawadzka, B. and Ravelonandro, M. 2004. Stability of gene silencing-based resistance to Plum pox virus in transgenic plum (*Prunus domestica* L.) under field conditions. *Transgenic Res.* 13:427-436.
- Hoekema, A., Hirsch, P. R., Hooykaas, P. J. J. and Schilperoort, R. A. 1983. A binary plant vector strategy based on separation of vir- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. *Nature* 303:179-180.
- Holsters, M., Silva, B., Van Vliet, F., Genetello, C., De Block, M., Dhaese, P., Depicker, A., Inzé, D., Engler, G., Villarroel, R. and others. 1980. The functional organization of the nopaline *A. tumefaciens* plasmid pTiC58. *Plasmid* 3:212-230.
- Hood, E. E., Gelvin, S. B., Melchers, L. S. and Hoekema, A. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants. *Transgenic Res.* 2:208-218.
- Jefferson, R. A., Kavanaugh, T. A. and Bevan, M. W. 1987. GUS fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* 6:3901-3907.
- Joyce, P., Kuwahata, M., Turner, N. and Lakshmanan, P. 2010. Selection system and co-cultivation medium are important determinants of *Agrobacterium*-mediated transformation of sugarcane. *Plant Cell Rep.* 29:173-183.
- Kaufmane, E., Ikase, L., Trajkovski, V. and Lacis, G. 2002. Evaluation and characterization of plum genetic resources in Sweden and Latvia. *Acta Hort.* 577:207-213.
- Kikhailov R.V, Shuga O.A., and Dolgov, S. V. 2008. Genetic transformation os somatic tissues and molecular biology approach for improving resistance to sharka disease of plum (*Prunuc domestica* L.) varieties. First International Symposium on Biotechnology of Fruit Species. September 1-5, 2008. Dreston, Germany.
- Le Gall, O., Torregrosa, L., Danglot, Y., Candresse, T. and Bouquet, A. 1994. *Agrobacterium*-mediated genetic transformation of grapevine somatic embryos and regeneration of transgenic plants expressing the coat protein of grapevine chrome mosaic nepovirus (GCMV). *Plant Sci.* 102:161-170.
- Malinowski, T., Zawadzka, B, Ravelonandro, M. and Scorza, R. 1998 Preliminary report on the apparent breaking of resistance of a transgenic plum by chip bud inoculation of *Plum pox virus* PPV-S. *Acta Virol.* 42:241-243
- Malinowski, T., Cambra, M., Capote, N., Zawadzka, B, Gorris, M. T., Scorza, R. and Ravelonandro, M. 2006. Field trials of plum clones transformed with the Plum pox virus coat protein (PPV-CT) gene. *Planr Disease* 90: 1012-1018.
- Mante, S., Morgens, P. H., Scorza, R., Cordts, J. M. and Callahan, A. M. 1991. *Agrobacterium*-mediated transformation of plum (*Prunus domestica* L.) hypocotyl slices and regeneration of transgenic plants. *Bio/Technol* 9:853-857.
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
- Okie, W. R. and Ramming, D. W. 1999. Plum breeding worldwide. *HortTechnology* 9:162-176.
- Olhoft, P. M. and Somers, D. A. 2001. L-Cysteine increases *Agrobacterium*-mediated T-DNA delivery into soybean cotyledonary-node cells. *Plant Cell Rep.* 20:706-711.

- Padilla, I. M. G., Webb, K. and Scorza, R. 2003. Early antibiotic selection and efficient rooting and acclimatization improve the production of transgenic plum plants (*Prunus domestica* L.). *Plant Cell Rep.* 22:38-45.
- Petri, C., Albuquerque, N., Garc a-Castillo, S., Egea, J. and Burgos, L. 2004. Factors affecting gene transfer efficiency to apricot leaves during early *Agrobacterium*-mediated transformation steps. *Journal of Hort. Sci. Biotech.* 79:704-712.
- Petri, C. and Burgos, L. 2005. Transformation of fruit trees. Useful breeding tool or continued future prospect? *Transgenic Res.* 14:15-26.
- Petri, C., Webb, K., Hily, J. M., Dardick, C. and Scorza, R. 2008. High transformation efficiency in plum (*Prunus domestica* L.): A new tool for functional genomics studies in *Prunus* spp. *Mole. Breed.* 22:581-591.
- Ravelonandro, M., Scorza, R., Callahan, A., Levy, L., Jacquet, C., Monsion, M. and Damsteegt, V. 2000. The Use of Transgenic Fruit Trees as a Resistance Strategy for Virus Epidemics: the Plum Pox (Sharka) Model. *Virus Res.* 71:63-69.
- Scorza, R., Ravelonandro, M., Callahan, A. M., Cordts, J. M., Fuchs, M., Dunez, J. and Gonsalves, D. 1994. Transgenic plums (*Prunus domestica* L.) express the plum pox virus coat protein gene. *Plant Cell Rep.* 14:18-22.
- Scorza R, Callahan A, Levy L, Damsteegt V, Webb K, Ravelonandro M 2001 Post-transcriptional gene silencing in Plum pox virus resistant transgenic European plum containing the Plum pox potyvirus coat protein gene. *Transgenic Res.* 10:201-209.
- Tian, L., Sibbald, S., Subramanian, J and Svircev, S. 2006. Characterization of *Prunus domestica* L. *in vitro* regeneration via hypocotyls. *Sci. Hort.* 112:462-466.
- Tian, L., Canli, F. A., Wang, X. and Sibbald, S. 2009. Genetic transformation of *Prunus domestica* L. using the *hpt* gene coding for hygromycin resistance as the selectable marker. *Sci. Hort.* 119:339-343.
- Yancheva, S. D., Druart, P. and Watillon, B. 2002. *Agrobacterium* - mediated transformation of plum (*Prunus domestica* L.). *Acta Hort.* 577:215-217.

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Genetic Transformation

Edited by Prof. MarÃa Alvarez

ISBN 978-953-307-364-4

Hard cover, 328 pages

Publisher InTech

Published online 06, September, 2011

Published in print edition September, 2011

Genetic transformation of plants has revolutionized both basic and applied plant research. Plant molecular biology and physiology benefit from this power tool, as well as biotechnology. This book is a review of some of the most significant achievements that plant transformation has brought to the fields of Agrobacterium biology, crop improvement and, flower, fruit and tree amelioration. Also, it examines their impact on molecular farming, phytoremediation and RNAi tools.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Yuan Song, Fatih Ali Canli, Farida Meerja, Xinhua Wang, Hugh A. L. Henry, Lizhe An and Lining Tian (2011). Evaluation of Factors Affecting European Plum (*Prunus domestica* L.) Genetic Transformation, Genetic Transformation, Prof. MarÃa Alvarez (Ed.), ISBN: 978-953-307-364-4, InTech, Available from: <http://www.intechopen.com/books/genetic-transformation/evaluation-of-factors-affecting-european-plum-prunus-domestica-l-genetic-transformation>

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