Efficient genetic transformation of *Momordica charantia* L. by microprojectile bombardment

Muralikrishna Narra¹ · Raghu Ellendula¹ · Srinivas Kota¹ · Bharathkumar Kalva¹ · Yashodhara Velivela¹ · Sadanandam Abbagani¹

Received: 21 September 2017 / Accepted: 26 October 2017 / Published online: 22 November 2017
© Springer-Verlag GmbH Germany 2017

Abstract Here, we report the optimized conditions for biolistic particle delivery-mediated genetic transformation of bitter melon using petiole segments. In this study, DNA-coated gold particles of 0.6 μm were used for optimizing the parameters of transformation and eventually regeneration of bitter melon putative transgenics. Initially, biolistic parameters namely helium pressure and macrocarrier to target tissue distance, were optimized using binary vector pBI121 carrying both β -glucuronidase gene (*GUS*) and neomycin phosphotransferase II gene (*npt II*) as a reporter and as a selectable marker gene, respectively. The effect of optimized physical parameters on the frequency of transient ($79.2 \pm 1.52\%$) and stable (41.9%) expressions has been investigated. The optimized biolistic parameters for petiole segments of *Momordica charantia* L. were determined as follows: 650 psi helium pressure and 6 cm target distance. Using the optimized parameters, transformation of bitter melon was carried out for generation of putative transformants from bombarded tissues on SRM-K medium, with a mean number of 50.3 explants surviving at the end of the final selection (50 mg l⁻¹ kanamycin) round. Finally, the transformants produced were subjected to *GUS* histochemical assay, and integration of the transgenes (*GUS* and *npt II*) into the nuclear genome was confirmed by PCR analysis. DNA blot analysis confirmed the transgene integration in the transformed plantlet genomes. The present study may be used for developing transplastomic technology in this valuable medicinal plant for enhanced

metabolic engineering pathways and production of biopharmaceuticals.

Keywords Biolistic transformation · *GUS* assay · Flight distance · Stable expression

Abbreviations

SRM	Shoot regeneration medium
SRM-K SRM	Medium supplemented with kanamycin
RM-K	Rooting medium
MS	Murashige and Skoog

Introduction

Momordica charantia L., of the Cucurbitaceae family, is commonly known as bitter melon. It is a medicinal and economically important horticultural crop plant species, grown majorly in the tropical and subtropical areas of Asia, East Africa and South America (Grover and Yadav 2004). The available rich contents of vitamins C, E, carotenoids, lycopenes and flavonoids from fruits of bitter melon are helpful in preventing free radical damages (Semiz and Sen 2007). A series of biologically active proteins are found to have highly efficient anti-HIV, anti-tumor, anti-diabetic and anti-rheumatic properties (Bourinbaiar and Lee-Huang 1996). The active components of cucurbitane-type triterpenoids have been reported to exhibit analgesic, anti-obesity, anti-inflammatory, hypotensive, anti-fertility, anti-feedant and anti-oviposition activities (Raman and Lau 1996; Grover and Yadav 2004; Beloin et al. 2005; Ng et al. 1992).

✉ Sadanandam Abbagani
nandamas@rediffmail.com

¹ Department of Biotechnology, Kakatiya University, Warangal 506009, India

To date, the potential applications of genetic engineering in fields of medicine, agriculture and industries have been improvised more quickly and efficiently. In agriculture, the development of transgenic varieties through gene manipulation opens a new avenue for carrying desirable traits such as resistance to disease, insect and herbicide. Taking these facts into consideration, crop improvement of bitter melon will fulfill the increased demand for obtaining the desired agronomic traits. A simple and efficient plant regeneration system from cultured cells is required for developing most of the plant transformation procedures (Thiruvengadam and Yang 2009). Efficient regeneration protocols have been previously reported for *M. charantia* from different explants such as cotyledons (Islam et al. 1994), nodal and shoot tips (Wang et al. 2001; Malik et al. 2007), nodal and internodal explants (Agarwal and Kamal 2004), petiole explants (Yashodhara et al. 2016), and somatic embryogenesis (Thiruvengadam et al. 2006). *Agrobacterium*-mediated gene transfer is considered to be most efficient for the stable integration of genes among the available gene transfer systems. Few reports are available on gene transfer studies from explants of immature cotyledonary nodes (Sikdar et al. 2005) and leaf disc (Thiruvengadam et al. 2012) in *M. charantia*. In view of the demand and potentiality of this plant system in genetic engineering, it is necessary to develop a physical means of transformation via particle bombardment. It is another fine and most effective method of DNA delivery into the plant cell. The feasibility of this technique demonstrates that chloroplast transformation may be attained, which brings exciting possibilities for metabolic engineering and expression of novel genes in the transplastomes for various agronomic and pharmaceutical traits that cannot be achieved by *Agrobacterium* spp. (Altpeter et al. 2005). However, to our knowledge, no reports exist to date for the production of a transgenic bitter melon via the microprojectile bombardment technique.

The present investigation, describes an efficient protocol for biolistic-mediated genetic transformation of bitter melon as a possible alternative approach using petiole explants. This versatile and efficient transformation system with optimized physical factors, viz., acceleration pressure and flight distance effecting transient and stable expression levels in *M. charantia*, is helpful for its rapid genetic improvement.

Materials and methods

Plant material and culture conditions

Petiole explants (1 month old) from in vitro-grown shoots, which were maintained under controlled laboratory

conditions at 25 ± 2 °C for a 16/8 h photoperiod on MS (Murashige and Skoog 1962) media containing 3% sucrose (w/v) and 0.8% agar as solidifying agent, were selected for the experiment. Explants of 5–6 mm size were arranged aseptically at the center of a 9 cm Petri dish of 3 cm radius (20 explants per dish) and pre-cultured for 3 days before bombardment on optimized solid MS media, supplemented with 8.9 μ M BAP along with 1.14 μ M IAA and 0.34 μ M GA₃ (Yashodhara et al. 2016).

Plasmid DNA

Plasmid pBI121 was used as a vector system (Chen et al. 2003) to optimize various parameters of particle gun mediated transformation harboring the neomycin phosphotransferase II (*nptII*) gene which is driven by the nopaline synthase (NOS) promoter and terminator sequences, as a selectable marker that provides resistance to kanamycin and the β -glucuronidase (*GUS*) gene as reporter gene with cauliflower mosaic virus (CaMV) 35S promoter. The plasmid DNA that was maintained in *E. coli*-DH5 α was isolated from overnight bacterial culture and used for the bombardment experiments.

Preparation of microcarriers

Microcarriers were coated with DNA using the CaCl₂/spermidine precipitation method. Under continuous vortexing, 5 μ l of plasmid DNA (1 μ g μ l⁻¹), 50 μ l of CaCl₂ (2.5 M) and 20 μ l of spermidine (0.1 M) was added to 50 μ l of gold particles (60 mg ml⁻¹; 0.6 μ m diameter), followed by centrifugation of the contents at 20 s pulse. The supernatant was removed and the pellet resuspended in 250 μ l of absolute ethanol. Centrifugation was repeated three times, followed by washing in ethanol, and finally the pellet was suspended in 75 μ l of absolute ethanol and kept on ice until bombardment. Following vortexing, 10 μ l of the mixture was applied to microcarriers for each bombardment event (Srinivas et al. 2016).

Microprojectile bombardment parameters

The Biolistics PDS-1000/He device (Bio-Rad) was used under a vacuum of 25 in. of Hg and distance of 25 mm from the rupture disc to the macrocarrier for all the bombardment events. The variables that are to be optimized include rupture disc pressures (650, 900 and 1100 psi) and microprojectile travel distances (3, 6, 9 and 12 cm) with 0.6 μ m size microcarriers. The experiments were repeated thrice, along with control. The percent frequency of transient *GUS* expression was used for evaluating the physical biolistic parameters.

Selection and regeneration of transformants

The explants were kept in dark for 48 h after the bombardment and shifted onto shoot regeneration medium (SRM), i.e., MS medium supplemented with 3% sucrose and plant growth regulators and 8.9 μM BAP + 1.14 μM IAA solidified with 0.8% agar. After 15 days, the explants were transferred to selection medium (SRM-K) containing a lethal dose concentration of kanamycin (SRM-K; SRM medium supplemented with 50 mg l⁻¹ kanamycin). After effective cycles of selection (thrice), putative transformed shoots were transferred to shoot elongation medium (SEM-K; 8.9 μM BAP + 1.14 μM IAA + 0.34 μM GA₃) without any change in the selection agent. For rooting, elongated shoots were shifted to the rooting medium (RM-K; MS basal salts + 0.49 μM IBA) without any change in the lethal dose concentration of kanamycin. After 4 weeks, the rooted plantlets were transplanted into pots and gradually exposed to greenhouse conditions. Fully established plantlets were hardened in field soils. The transformation efficiency was determined by calculating the percent of number of responding petiole explants on kanamycin selection with the number of bombarded explants.

GUS histochemical analysis

Randomly, 20 transformed petioles per shot per plate were selected for transient *GUS* expression analysis after 24 h of bombardment. The constitutive expression of the *GUS* gene in the responding cultures on the selection medium at the initial stages and the whole plantlets of transgenic lines raised after 3–4 months of bombardment were also analyzed histochemically. *GUS* assay was done by incubating the tissues in buffer containing 1 g/L X-Gluc—5-bromo-4-chloro-3-indolyl- β -glucuronide-cyclohexylammonium salt, with 0.05 M Na₂HPO₄, 10 mM EDTA and 0.1% (v/v) Triton X-100 for 24 h at 37 °C. Thereafter, the tissue samples were destained with 70% alcohol and fixed in a solution of glacial acetic acid:alcohol (1:3; v/v). The explants with discrete blue foci were scored for *GUS*-positive expression and along with non-bombarded explants that were treated as control (Jefferson et al. 1987).

Molecular analysis

PCR amplification

The total genomic DNA of transformed lines was isolated using the CTAB method (Doyle and Doyle 1987). PCR was performed (C1000TM programmable thermal cycler, Bio-Rad) for verification of gene integration into the host plant genomes using primers specific for the reporter gene (*GUS*) (F:5' CGACGGCCTGTGGGCATTCA 3'; R:5'

TGGTCGTGCACCATCAGCAC 3') and primers specific for kanamycin selection marker gene (*nptII*) (F: 5' TGCGCTGCGAATCGGGAGCG 3'; R: 5' GAGGC-TATTCGGCTATGACT 3'). The PCR reaction mixture of volume 25 μl contained 50 ng DNA, 10 $\times$ PCR buffer, 25 mM MgCl₂, 2.5 mM dNTP, 10 pmol of each specific primer and 1 U *Taq* DNA polymerase. The PCR conditions were set up as follows: 94 °C for 5 min as initial denaturation, subsequently followed by 30 cycles at 94 °C for 30 s, 55 °C for 45 s for primer annealing of *GUS* gene and 58 °C for *nptII* with primer extension at 72 °C for 2.3 min, and a final extension at 72 °C for 7 min.

Southern blot analysis

The transgene integration corresponding to *nptII* in PCR-positive lines was analyzed using Southern blot analysis. The total genomic DNA (20 μg) from the transgenic and non-transformed lines was subjected to digestion with *EcoRI* and subsequently transferred on to a Hybond + nylon membrane by the capillary method. The blot was hybridized with PCR-generated DIG-labeled probe for the *nptII* gene region, which is complementary to 750 bp. Standard protocol was performed for labeling and chemiluminescent detection.

Statistical data

The mean comparison for all the data was analyzed statistically by ANOVA and DMRT. Each treatment consisted of at least two plates and was repeated thrice. The frequency percent of *GUS* activity was calculated in terms of the number of petiole explants showing transient *GUS* expression (with blue foci) to the total number of explants stained after bombardment.

Results and discussion

Optimization of bombardment parameters

The particle bombardment-mediated gene integration is the most efficient and consistent physical process with no biological limitation (Altpeter et al. 2005). Microprojectile bombardment is an independent technique employed to any kind of target tissue, and the ability of transformed tissues to regenerate is an additional prerequisite for successful gene delivery and to attain genetically modified plants. Greenish, high regenerative tissues that are capable of sustained cell division over long periods represent the choice of high-quality target tissue for high-frequency transformation (Sailaja et al. 2008). The biolistic approach for the transformation of the *GUS* gene into bitter melon

tissues was influenced by a combination of important physical parameters, including rupture disc pressures and flight distances, which show greater impact on stable transformation efficiencies and subsequently employed to generate transgenic bitter melon plants. Therefore, the optimization of biolistic-mediated genetic transformation in any system primarily depends on the acceleration pressure and flight distance, as they vary in different plant systems (Gharanjik et al. 2008; Ramesh and Gupta 2005; Singh et al. 2010). The effective parameters that are standardized facilitate the even distribution of microcarriers over the target tissue that prevents damage and increases the transformation rates (Tadesse et al. 2003). In the present study, a simple and efficient method for effective penetration is adopted to treat petiole explants of *M. charantia* as a possible alternative approach for transgenic recovery.

Different flight distances and acceleration pressures were found to have significant effect on transient *GUS* expression that initially acts as an indicator to explain the frequency of transformation. The highest mean (79.2 ± 1.52) for transient *GUS* expression was observed in explants bombarded at a flight distance of 6 cm and an acceleration pressure of 650 psi. The low acceleration pressure (650 psi) at which the microcarriers were able to reach the recipient tissue without causing injury indicates its suitability as the most beneficial and right parameter. There was a slight reduction in the percent of transformation (67.4 ± 1.26) at 9 cm flight distance, and at 12 cm only $27.9 \pm 1.13\%$ transformation efficiency was noticed at the same distance. The next highest efficiency was recorded again with an acceleration pressure of 900 psi (48.1 ± 0.90) and 6 cm flight distance, while 22.3 ± 1.40 and $36.2 \pm 2.11\%$ efficiencies were noticed at 3 and 9 cm, respectively. The acceleration pressure 1100 psi was found to be less effective on transient expression frequencies when compared with 650 and 900 psi acceleration pressures. i.e., 10.9 ± 0.78 , 14.3 ± 1.19 and 12.2 ± 1.24 for 3, 6 and 9 cm flight distances, respectively. However, flight distances 3 cm at 650 psi and 12 cm for 900 and 1100 psi did not show any considerable impact on bombardment events, with null transformation efficiency (Fig. 1c₁, c₂, c₃, c₄). Many other reports are consistent with the present results where the frequency of transient *GUS* expression was significantly decreased with increase of travel distance in combination with helium pressures (Ruma et al. 2009; Singh et al. 2010). There was a remarkable decrease in the percentage of expression with rise in acceleration pressures to 900 and 1100 psi under similar flight distances of 3, 6, 9 and 12 cm (Table 1). The variations in helium pressure have significant effect on the transformation frequency in *M. charantia*, where the increased penetration velocities

can cause injury and eventually reduce the efficiency of transformation (Joshi et al. 2011).

Therefore, the highest *GUS* expression efficiencies were observed at 650 psi acceleration pressure and 6 cm flight distance and were found to be optimal for developing an efficient protocol for delivery cum integration of the transgene into the nuclear genome. These standardized effective factors and conditions finally resulted in the efficient production of microprojectile-mediated transgenic *M. charantia*. No blue foci (spots) were found in the control tissues that were bombarded with non-coated gold particles.

Selection of kanamycin-resistant tissues and regeneration of transgenic plants

A suitable lethal concentration of kanamycin (50 mg l^{-1}) was optimized for the recovery of transgenic plants without any escape. The optimal concentration of the selection agent was found to be effective toward cell development and regeneration of putative transformants with slow growth rate during each round of selection, whereas non-transformed tissues gradually bleached with decreased regenerative ability, poor growth and consequent death (Lin et al. 1995). Three days after bombardment, explants were transferred onto SRM-K medium for the first round of selection for 2 weeks. The pre-culturing of explants on MS medium maximizes stable integration, as actively dividing cells of explants have the ability to survive and grow under selection pressure during the bombardment process. The formation of kanamycin-resistant shoot buds from the cut ends of petiole explants was observed after the second round of selection in the SEM-K medium for the next 2 weeks (Fig. 1a, b).

After the third round of selection for the next 2 weeks, the successively transformed subcultures survived with an overall mean frequency of 50.3 explants. During the selection process, the non-transformed lines were gradually bleached, while the putative transformants remained green and exhibited slow growth (Fig. 1d, e, h). The maximum transformation efficiency of 41.9% was observed with 0.6 μM gold, 650 psi acceleration pressure and 6 cm travel distance (Table 2). Transformation efficiency is calculated as the number of positive lines that survived after the third round of selection to the total number of explants bombarded. Subsequently, the putative transformants were successfully shifted to the RM-K medium for rooting induction for approximately 20 days. Finally, the rooted plants were hardened under controlled greenhouse conditions (Fig. 1j). Meanwhile, the transformed tissues at different stages were assayed for the constitutive *GUS* gene expression along with control explants (Fig. 1f, g, i).

Fig. 1 Microprojectile-mediated genetic transformation of *Momordica charantia* L., using petiole explants. **a** Survival of petiole explants after 1 week of culture on selective shoot regeneration medium (SRM-K) after bombardment. **b** Complete inhibition of control explants within a week of culture on SRM-K medium. **c** **c**₁ Transient *GUS* expression shown by bombarded explants at 650 psi with flight distance of 6 cm. **c**₂ Transient *GUS* expression shown by bombarded explants at 900 psi with flight distance of 6 cm. **c**₃ Transient *GUS* expression shown by bombarded explants at 1100 psi with flight distance of 6 cm. **c**₄ Non-bombarded explants (control). **d**, **e** Proliferated explants on selection medium (SRM-K). **f**, **g** Histochemical analysis of proliferated shoots at different stages of growth. **h** Completely developed putative transgenic plants on SEM-K medium. **i** Well-developed shoots with leaves showing blue coloration. **j** Transgenic plant hardened and maintained in the greenhouse

Molecular analysis of transgenic plants

The putative transgenic plants, which were generated with optimized conditions of helium pressure and travel distance

and survived after three rounds of selection with constitutive expression of *GUS*, were selected for confirmation of genetic transformation by PCR amplification. The expected band sizes of 0.5 and 0.75 kb, corresponding to the reporter (*GUS*) and selectable marker (*nptII*) genes, were amplified, respectively (Fig. 2a, b). A few PCR results showed kanamycin-resistant lines that did not express *GUS*, probably due to the position effect of the genome (Baulcombe 2004).

Southern blot analysis was carried out for the same transformants which were positive for both *GUS* and the *nptII* genes. Detectable signals in Southern blot revealed positive integration of the desired genes in the transformants (Fig. 3). In general, multiple site integration of genes was encountered with microprojectile-mediated transformation (Altpeter et al. 2005). However in the present work, the transformation efficacy was confirmed by 50% of single and multiple copy insertions.

Table 1 Effect of helium pressure and target distance on percent transient *GUS* expression in *M. charantia* L.

Helium pressure (psi)	Flight distance (cm)	No. of petiole explants bombarded/Petri plate	Mean $\pm$ SE	
			No. of petiole explants showing <i>GUS</i> expression	Transient <i>GUS</i> expression (%)
650	3	20	0 $\pm$ 0.00 ^a	0 $\pm$ 0.00 ^a
	6	20	15.8 $\pm$ 0.55 ^g	79.2 $\pm$ 1.52 ^g
	9	20	13.4 $\pm$ 0.47 ^f	67.4 $\pm$ 1.26 ^f
	12	20	5.5 $\pm$ 0.24 ^c	27.9 $\pm$ 1.13 ^c
900	3	20	4.4 $\pm$ 0.26 ^c	22.3 $\pm$ 1.40 ^c
	6	20	9.6 $\pm$ 0.33 ^c	48.1 $\pm$ 0.90 ^c
	9	20	7.2 $\pm$ 0.54 ^d	36.2 $\pm$ 2.11 ^d
	12	20	0 $\pm$ 0.00 ^a	0 $\pm$ 0.00 ^a
1100	3	20	2.1 $\pm$ 0.11 ^b	10.9 $\pm$ 0.78 ^b
	6	20	2.8 $\pm$ 0.21 ^b	14.3 $\pm$ 1.19 ^b
	9	20	2.4 $\pm$ 0.25 ^b	12.2 $\pm$ 1.24 ^b
	12	20	0 $\pm$ 0.00 ^a	0 $\pm$ 0.00 ^a

The mean number of explants showing *GUS* expression with letters a, b, c, d, e, f and g were different significantly ($P \leq 0.05$)

Table 2 Effect of helium pressure and target distance on transformation efficiency (%)

No. of experiments	No. of explants bombarded	Number of explants surviving on selection medium				No. of transgenic shoots/explants	Transformation efficiency (%)
		Selection I	Selection II	Selection III	<i>GUS</i> +ve (%)		
1.	120	117	74	57	45	3.3 $\pm$ 0.17	47.5 ^b
2.	120	115	69	48	38	2.4 $\pm$ 0.25	40.0 ^b
3.	120	112	68	46	35	2.1 $\pm$ 0.22	38.3 ^a
Mean		114.6	70.3	50.3	39.3	2.6 $\pm$ 0.21	41.9

The mean values with significant difference in transformation efficiencies are shown by letters a and b

Fig. 2 PCR analysis of transgenic lines of *M. charantia* produced via microprojectile-mediated genetic transformation. **a** PCR detection of *GUS* gene in transgenic lines (lanes: 1–4) resulted in a 500 bp amplicon. No such band was detected in wild (W) type; M molecular marker. **b** PCR detection of *npt II* in transgenic lines (lanes: 1–4) resulted in a 750 bp amplicon and the absence of the same in wild (W) type; M molecular marker

Fig. 3 Southern blot analysis of total DNA isolated from transgenic plants digested with *EcoRI* and probed with 0.7 kb PCR fragment of the *nptII* gene. W Wild type, lanes 1, 2 DNA of transgenic lines

Conclusion

An efficient and successful biolistic-mediated genetic transformation in bitter melon was developed using petiole explants. The results of the present investigation adequately exhibited a stable direct gene transfer method with 41.9% transformation frequency. This optimized protocol will be helpful in introducing several characteristic gene traits of agronomic and economic importance in the near future.

Acknowledgements The authors greatly acknowledge the financial assistance provided by the Department of Science and Technology—INSPIRE (DST/INSPIRE Fellowship/2011/426), New Delhi.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Agarwal M, Kamal R (2004) *In vitro* clonal propagation of *Momordica charantia* L. Indian J Biotechnol 3:426–430
- Altpeter F, Baisakh N, Beachy R, Bock R, Capell T, Christou P, Daniell H, Datta K, Datta S, Dix P, Fauquet C, Huang N, Kohli A, Mooibroek H, Nicholson L, Nguyen TT, Nugent G, Raemakers K, Romano A, Somers DA, Stoger E, Taylor N, Visser R (2005) Particle bombardment and the genetic enhancement of crops: myths and realities. Mol Breeding 15:305–327
- Baulcombe D (2004) RNA silencing in plants. Nature 431:356–363
- Beloin N, Gbeassor M, Akpagana K, Hudson J, Soussa KD, Koumaglo K, Arnason JT (2005) Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J Ethnopharmacol 96:49–55
- Bourinbaiar AS, Lee-Huang S (1996) The activity of plant derived antiretroviral proteins MAP30 and GAP31 against herpes simplex virus in vitro. Biochem Biophys Res Commun 219:923–929
- Chen PY, Wang CK, Soong SC, To KY (2003) Complete sequence of the binary vector pBI121 and its application in cloning T-DNA insertion from transgenic plants. Mol Breeding 11:287–293
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem Bull 19:11–15
- Gharanjik S, Moieni A, Mousavi A, Alizadeh H (2008) Optimization of transient expression of *uidA* gene in androgenic embryos of wheat (*Triticum aestivum* L. cv. Falat) via particle bombardment. Iran J Biotechnol 6:207–213
- Grover JK, Yadav SP (2004) Pharmacological actions and potential uses of *Momordica charantia*: a review. J Ethnopharmacol 93:123–132
- Islam R, Sarkar PK, Naderuzzaman ATM, Joarder OI (1994) In vitro regeneration of plants from cotyledons of *Momordica charantia* L. Plant Tissue Cult 4:105–109
- Jefferson RA, Kavanagh TA, Bevan MW (1987) *GUS* fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901
- Joshi M, Mishra A, Jha B (2011) Efficient genetic transformation of *Jatropha curcas* L. by microprojectile bombardment using embryo axes. Ind Crop Prod 33:67–77
- Lin JJ, Assad-Garcia N, Kuo J (1995) Plant hormone effect of antibiotics on the transformation efficiency of plant tissues by *Agrobacterium tumefaciens* cells. Plant Sci 109:171–177
- Malik S, Zia M, Rehman R, Chaudhary F (2007) In vitro plant regeneration from direct and indirect organogenesis of *Momordica charantia*. Pak J Biol Sci 10:4118–4122
- Murashige T, Skoog FA (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497
- Ng TB, Chan WY, Yeung HW (1992) Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from *Cucurbitaceae* plants. Gen Pharmacol 23:579–590
- Raman A, Lau C (1996) Anti-diabetic properties and phytochemistry of *Momordica charantia* L. (Cucurbitaceae). Phytomedicine 2:349–362
- Ramesh M, Gupta AK (2005) Transient expression of β -glucuronidase gene in indica and japonica rice (*Oryza sativa* L.)

- callus cultures after different stages of co-bombardment. *Afr J Biotechnol* 4:596–600
- Ruma D, Dhaliwal M, Kaur A, Gosal S (2009) Transformation of tomato using biolistic gun for transient expression of the β -glucuronidase gene. *Indian J Biotechnol* 8:363–369
- Sailaja M, Tarakeswari M, Sujatha M (2008) Stable genetic transformation of castor (*Ricinus communis* L.) via particle gun-mediated gene transfer using embryo axes from mature seeds. *Plant Cell Rep* 27:1509–1519
- Semiz A, Sen A (2007) Antioxidant and chemoprotective properties of *Momordica charantia* L. (bitter melon) fruit extract. *Afr J Biotechnol* 6:273–277
- Sikdar B, Shafiullah M, Chowdhury AR, Sharmin N, Naha S, Joarder OI (2005) *Agrobacterium*-mediated *GUS* expression in bitter melon (*M. charantia* L.). *Biotechnology* 4:149–152
- Singh N, Mishra A, Joshi M, Jha B (2010) Microprojectile bombardment mediated genetic transformation of embryo axes and plant regeneration in cumin (*Cuminum cyminum* L.). *Plant Cell Tiss Org* 103:1–6
- Srinivas K, Muralikrishna N, Bharath Kumar K, Raghu E, Mahender A, Kiranmayee K, Yashodahara V, Sadanandam A (2016) Biolistic transformation of *Scoparia dulcis* L. *Physiol Mol Biol Plants* 22:61–68
- Tadesse Y, Sagi L, Swennen R, Jacobs M (2003) Optimisation of transformation conditions and production of transgenic sorghum (*Sorghum bicolor*) via microparticle bombardment. *Plant Cell Tiss Org* 75:1–18
- Thiruvengadam M, Yang CH (2009) Ectopic expression of two MADS box genes from orchid (*Oncidium* Gower Ramsey) and lily (*Lilium longiflorum*) alters flower transition and formation in *Eustoma grandiflorum*. *Plant Cell Rep* 28:1463–1473
- Thiruvengadam M, Varisai Mohamed S, Yang CH, Jayabalan N (2006) Development of an embryogenic suspension culture of bitter melon (*Momordica charantia* L.). *Sci Hortic* 109:123–129
- Thiruvengadam M, Praveen N, Chung IM (2012) An efficient *Agrobacterium tumefaciens*-mediated genetic transformation of bitter melon (*Momordica charantia* L.). *AJCS* 6:1094–1100
- Wang S, Tang L, Chen F (2001) In vitro flowering of bitter melon. *Plant Cell Rep* 20:393–397
- Yashodhara V, Muralikrishna N, Raghu E, Bharathkumar K, Rathnaprabha D, Sadanandam A (2016) In vitro plant regeneration from petiole explants and assessment of genetic fidelity using ISSR markers in *Momordica charantia* L. *PCBMB* 17:49–56