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Agrobacterium-mediated *GUS* Expression in Bitter Gourd (*Momordica charantia* L.)

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Abstract: Multiple shoots from the immature cotyledonary nodes of two genotypes of bitter gourd were induced in MS media with GA_3, IAA, IBA, NAA, KIN and BAP. The shoots continued to increase in number for 5-6 weeks. On the medium, BAP 2 mg L^{-1} in combination with 0.1 mg L^{-1} IAA + 2 mg L^{-1} GA_3 was the most effective medium for adventitious shoot proliferation from immature cotyledonary node. BGGB1 and BGGB14 genotypes produced over 84 and 80% shoots, respectively on this medium in 6 weeks from the time at inoculation of primary explants. BGGB1 was found to be better in response than BGGB14 in most of the treatments. Rapid and reproducible transformation system for bitter gourd using *Agrobacterium tumefaciens* mediated gene delivery was developed. Immature cotyledonary node from green immature fruits that has been allowed to imbibe was used as explant and regeneration was achieved. Cotyledonary node was co-cultured in MS media with 2.0 mg L^{-1} BAP + 0.1 mg L^{-1} IAA + 2.0 mg L^{-1} GA_3. Histochemical *GUS* assay of the explants and shoots (newly formed) revealed that they were *GUS* positive.

Key words: *Agrobacterium*, *GUS*, cotyledonary nodes, bitter gourd

INTRODUCTION

The most potent biotechnological approach is the transfer of specifically constructed gene assemblies through various transformation techniques; this is constitutes genetic engineering. The plants obtained through genetic engineering contain a gene or genes usually from an unrelated organism are known as transgenic plants. Bitter gourd belongs to the Cucurbitaceae family and eaten throughout the world, specially for its medicinal properties[1]. It is used for stomach pain, diabetes, fevers, colds, headaches, menstrual disorders, hypertension and defend HIV. It also plays an important role in the agro-economy of our country and costlier compared to the other vegetable available in the season. Bitter gourd is susceptible to many diseases and insect pests that affected squash, cucumber and muskmelon. It is a host for watermelon mosaic virus and may be susceptible to other cucurbit viruses. Powdery mildew is a destructive pathogen of bitter gourd. Taking these facts under consideration disease resistant and pest resistant traits of bitter gourd should be developed by using transformation techniques. Therefore, the objective of the present study was to establish a protocol on *Agrobacterium*-mediated transformation in bitter gourd by using immature cotyledonary nodes.

MATERIALS AND METHODS

The successful transformation to plants depends on the availability of a suitable protocol which compatible with *in vitro* plant regeneration methods of the target plant species. Cotyledonary nodes of two genotypes of bitter gourd were transformed with *Agrobacterium tumefaciens* strain LBA4404 (pBxpBI121). Expression of neomycin phosphotransferase (*npt* II) encoding resistance to kanamycin was used as a selectable marker. *Agrobacterium tumefaciens* cultures were grown overnight in YMB medium at 28°C on a shaker at 200 rmp and then diluted in liquid MS medium to varying concentration. The explants were then incubated in the bacterial suspension for 30 min and placed on the co-cultivation medium which consisted of MS medium supplemented with sucrose 3% (w/v), agar 0.6% (w/v), GA_3 (2.0 mg L^{-1}), BAP or KIN (0.5-3.0 mg L^{-1}) and IAA or IBA or NAA (0.1 mg L^{-1}). The duration of co-cultivation varied from 2-5 days. After the co-cultivation time, the inoculated explants were rinsed in MS liquid medium containing cefatoxin 100 mg L^{-1}, blotted dry with sterile filter paper and cultured on selective medium which consisted of 100 mg L^{-1} cefatoxin and 0.05 mg mL^{-1} kanamycin per selection and incubated at 26±1°C with a 16 h photoperiod. After 7-8 weeks, the explants showed regeneration. The green shoots were cultured in rooting medium containing MS with IAA or

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IBA or NAA (0.2, 0.4, 0.6 and 0.8 mg L^{-1}). The expression of β-glucuronidase gene in the transformed plants were analysed. The plant was dipped into histochemical reagent, *X*-gluc and incubated for 24-48 h at 37°C. After *X*-gluc treatment, plant was transformed to 70% ethanol for de-green. Following de-green, plant was observed under stereo microscope which was confirmed the transformation of *GUS* gene.

RESULTS AND DISCUSSION

The physiological state of explants appears to be the key point for the success of regenerating plants. In this study, regeneration system of bitter gourd has been established from immature cotyledonary node, the main advantage of immature cotyledonary node explants being their availability through the whole year. The cotyledonary nodes were cultured on MS media with BAP, KIN, GA_3, IAA, IBA and NAA for induction of multiple shoots. The shoots continued to increase in number for 5-6 weeks. MS medium supplemented with 2.0 mg L^{-1} BAP, 0.1 mg L^{-1} IAA with 2.0 mg L^{-1} GA_3 was the most effective medium for shoot proliferation of bitter gourd. BGGB1 and BGGB14 genotypes of bitter gourd produced over 84 and 80% shoots, respectively on the medium six weeks from the time of inoculation of primary explants and was followed by 80 and 78% in 2.0 mg L^{-1} BAP + 0.1 mg L^{-1} IBA + 2.0 mg L^{-1} GA_3, respectively (Table 1). To have a continuous supply of shoots sub-culturing on fresh medium was reported at 2 weeks intervals. However, the addition of low concentration of auxin (IBA and IAA) to BAP supplemented media enhanced shoot regeneration efficiency and IAA was found superior to IBA. BGGB1 was found to be better in response than BGGB14 for most of the treatments. The average total number of shoots formed from one explant was about 12.7±0.38 and 10.7±0.27 in BGGB1 and BGGB14, respectively (Table 1). Similar results were observed by Ahad *et al.*[2] in watermelon, in their experiment they observed that the MS medium with BA (0.5-1 mg L^{-1}), 0.1 mg L^{-1} IAA was suitable for adventitious shoot regeneration and with addition of 100 mg L^{-1} CH improved shoot regeneration efficiency. Islam *et al.*[3] reported that maximum regeneration occurred on 1.0 mg L^{-1} BA and 0.1 mg L^{-1} IAA in *Aegle marmelons* Corr. Sarker *et al.*[4] reported that the MS medium supplied with 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} KIN and 0.1 mg L^{-1} GA_3 and 5.5 mg L^{-1} tyrosine was the best combination for multiple shoot regeneration from cotyledonary nodes in lentil. The immature cotyledonary nodes are also good source for initiating shoot formation as has been shown by Amin and Akhtar[5], Maity *et al.*[6] and Banerjee *et al.*[7].

Aseptically cotyledonary node explants of bitter gourd were transformed with *Agrobacterium tumefaciens* strain LBA4404 (pBI121). Expression of neomycin phosphotransferase (*npt*-II) encoding resistance to kanamycin was used as a selectable marker. The best growth of cotyledonary node was observed in MS with

Table 1: Effects of different concentrations and combinations of growth regulators on multiple shoot regeneration from immature cotyledonary node

	BGGB1		BGGB14	
	Immature cotyledonary node		Immature cotyledonary node	
Growth regulators (mg L^{-1})	% of regeneration	No. of shoots/explant	% of regeneration	No. of shoots/explant
2.0 GA_3+0.5 BAP+0.1 IAA	30	5.3±0.52	30	5.3±0.41
2.0 GA_3+0.5 KIN+0.1 IAA	28	5.1±0.27	26	5.1±0.25
2.0 GA_3+0.5 BAP+0.1 IBA	28	5.1±0.18	28	4.7±0.92
2.0 GA_3+0.5 KIN+0.1 IBA	24	4.7±0.90	26	4.5±0.89
2.0 GA_3+0.5 BAP+0.1 NAA	24	4.4±0.78	22	4.5±0.57
2.0 GA_3+0.5 KIN+0.1 NAA	18	4.2±0.92	18	4.1±0.29
2.0 GA_3+1.0 BAP+0.1 IAA	58	7.4±0.23	54	6.8±0.31
2.0 GA_3+1.0 KIN+0.1 IAA	52	7.1±0.11	48	6.5±0.25
2.0 GA_3+1.0 BAP+0.1 IBA	54	6.9±0.37	50	6.4±0.52
2.0 GA_3+1.0 KIN+0.1 IBA	52	6.5±0.41	50	6.1±0.17
2.0 GA_3+1.0 BAP+0.1 NAA	44	6.3±0.21	42	5.7±0.43
2.0 GA_3+1.0 KIN+0.1 NAA	38	6.1±0.71	34	5.2±0.62
2.0 GA_3+2.0 BAP+0.1 IAA	84	12.7±0.38	80	10.7±0.27
2.0 GA_3+2.0 KIN+0.1 IAA	80	12.3±0.17	76	10.1±0.83
2.0 GA_3+2.0 BAP+0.1 IBA	80	12.1±0.33	78	10.5±0.19
2.0 GA_3+2.0 KIN+0.1 IBA	76	11.4±0.52	72	9.5±0.64
2.0 GA_3+2.0 BAP+0.1 NAA	72	10.2±0.11	74	8.6±0.57
2.0 GA_3+2.0 KIN+0.1 NAA	66	9.7±0.19	60	8.1±0.31
2.0 GA_3+3.0 BAP+0.1 IAA	52	7.7±0.15	56	7.1±0.25
2.0 GA_3+3.0 KIN+0.1 IAA	48	7.2±0.24	50	6.7±0.51
2.0 GA_3+3.0 BAP+0.1 IBA	54	7.4±0.22	52	6.6±0.42
2.0 GA_3+3.0 KIN+0.1 IBA	44	6.7±0.71	46	6.1±0.14
2.0 GA_3+3.0 BAP+0.1 NAA	46	6.6±0.35	40	5.7±0.61
2.0 GA_3+3.0 KIN+0.1 NAA	40	5.9±0.12	36	5.1±0.10

Table 2: *GUS* assay and regeneration ability of transformed cotyledonary node of bitter gourd

	Types	
	BGGB1	BGGB14
Characters	Immature cotyledonary node	Immature cotyledonary node
Infected explant number	45.00	45.00
Number of explants assayed for *GUS*	7.00	7.00
Number of explants '+'ve for *GUS*	7.00	7.00
Concentration (OD)	1.47	1.47
Approximate strained area (%)	100.00	100.00
Percent survival after 3 weeks	34.21	23.38
Direct regeneration (%)	31.57	23.68

2.0 mg L^{-1} BAP + 0.1 mg L^{-1} IAA + 2.0 mg L^{-1} GA_3. The optimal procedure involved exposing the explants to a bacterial suspension (1.47 OD) followed by 3 days cocultivation period (Table 2).

After co-cultivation, infected explants were transferred to the selection media for 5-6 weeks after infection and some explants were subjected to histochemical assay to verify the transient expression of *GUS*. The growth of the newly appeared cultures were slow at first but become vigorous within 3-4 weeks. At that time, in some culture media high bacterial growth was detected which was prevented by frequent sub-culturing (3 days) of the explants on the selected media. Most of the explants for cotyledonary node produced a few shoots on selected media (kanamycin containing) were *GUS* positive.

The expression of β-glucuronidase gene in transformed explants and regenerated shoots were analyzed as described by Jefferson *et al.*[8]. Histochemical *GUS* assay of the explants and shoots (newly formed) revealed that they were *GUS* positive. This study also provides a basis for further research to generate transgenic bitter gourd.

Agrobacterium based vectors has been successfully employed to transfer gene into a number of dicotyledonous plants and a few grain and legumes[9,10]. Similarly reported by Nehra *et al.*[11] in strawberry, Sarker and Islam[12] in peanut, Southgate *et al.*[13] in maize, Ahmed *et al.*[14] in tobacco, Lokmanhakim[15] in potato, Peng *et al.*[16] in rice, Cardi[17] in *Solanum commersonii* Dun, Frankiin *et al.*[18] in greenbean and Noorain *et al.*[19] in rice. But Geetha *et al.*[20] failed to successfully transfer of *Agrobacterium* strain LBA4404 (pB x pBI121) in blackgram.

REFERENCES

1. Morton, J.F., 1967. The balsam pear an edible medicinal and toxic plant. Econ. Bot., 21: 57-58.
2. Ahad, A., R. Islam, M. Hossain, M. Khalekuzzamman and O.I. Joarder, 1994. Plan regeneration from immature and mature embryo axes of watermelon. Plant Tissue Cult., 4: 39-44.
3. Islam, R., M.R. Karim, A. Haque, M. Hossain and O.I. Joarder, 1993. High frequency plant regeneration from cotyledon cultures of *Aegle marmelous* Corr. Plant Tissue Cult., 3: 107-110.
4. Sarker, R.A., B.M. Mustafa, A. Biswas, S. Mahbub, M. Nahar, R. Hashem and M.I. Hoque, 2003. *In vitro* regeneration in lentil (*Lens culinaris* Medik.). Plant Tissue Cult., 13: 173-177.
5. Amin, M.N. and S. Akhtar, 1993. Regeneration of plants *in vitro* from seedling explants of pummelo (*Citrus grandis* (L.) Osb.). Plant Tissue Cult., 3: 71-79.
6. Maity, S., S. Rey, S. Mandal and N. Banerjee, 2001. Direct and indirect plant regeneration through *in vitro* cotyledonary node culture in *Albiza lebbek* (L.) Benth. Perspective in Cytology and Genetics, 10: 737-742.
7. Banerjee, P., K.C. Ghosh, S.K. Sen and N. Banerjee, 2001. *In vitro* studies of *Arachis hypogaea* L. Var. JL-24. Perspective in Cytology and Genetics, 10: 731-736.
8. Jefferson, R.A., Takavanagh and M.W. Bevan, 1986. *GUS* fusion: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J., 6: 3901-3907.
9. De Kathen, A. and H.J. Jacobsen, 1990. *Agrobacterium tumefaciens* mediated transformation of *Pisum sativum* L. using binary and cointegrate vectors. Plant Cell Rep., 9: 276-279.
10. Lulsdorf, M.M., H. Rempel, J.A. Jackson, D.S. Balisk and S.L.A. Hobbs, 1991. Optimizationg the production of transformed pea (*Pisum sativum* L.) callus using disarmed *Agrobacterium tumefaciens* strains. Plant Cell Rep., 9: 479-483.
11. Nehra, N.S., R.N. Chibbar, K.K. Kartha, S.S. Datta and U.L. Crosby, 1990. Genetic transformation of strawberry by *Agrobacterium tumefaciens* using a leaf disk transformation system. Plant Cell Rep., 9: 293-298.
12. Sarker, R.H. and A. Islam, 1999. *In vitro* regeneration and genetic transformation of peanut (*Arachis hypogaea* L.). Dhaka Univ. J. Biol. Sci., 8: 1-9.
13. Southgate, E.M., R.J. Westcott, J.B. Power and M.R. Davey, 1996. *Agrobacterium*-mediated *GUS* expression in maize tissues with evidence for a Bacteriocidal effect in immature embryos. Plant Tissue Cult., 6: 25-34.

14. Ahmed, G., A.N.K. Mamun and P.K. Ray, 2000. *GUS* gene expression in somatic and reproductive callus of tobacco, *Nicotiana tabacam* Var. W 38. Bangladesh J. Genet. Biotechnol., 1: 71-76.
15. Lockmanhakim, 2000. Agrobacterium mediated transformation in potato without selection pressure. Bangladesh J. Genet. Biotechnol., 1: 65-69.
16. Peng, J.Y., F.J. Wen, R.K. Lister and T.K. Hodges, 1995. Inheritance of *GUS* and neogenic in transgenic rice. Plant Mol. Biol., 27: 91-104.
17. Cardi, T., V. Iannamico, F.D. Ambrosis, E. Filippone and P.F. Lurguin, 1992. *Agrobacterium tum*-mediated genetic transformation of *Solanum commersonii* Dun. Plant Sci., 87: 179-189.
18. Frankiin, C.I., T.N. Trieu, B.G. Cassidy, R.A. Biyon and R.S. Nelson, 1993. Genetic transformation of greenbean callus via *Agrobacterium* mediated DNA transfer. Plant Cell Rept., 12: 74-78.
19. Noorain, M., Rasul, K.M. Ali, M.R. Islam and Z.I. Seraj, 1997. Transformation of an indica rice cultivar binnatoa with *Agrobacterium tumefaciens*. Plant Tissue Cult., 7: 71-80.
20. Geetha, N., P. Vendatachalam and G.R. Rao, 1997. Factors influencing production of *Agrobacterium*-mediated genetically transformed calli and plant regeneration in blackgram (*Vigna mungo* (L.) Hepper). Plant Tissue Cult., 7: 149-152.