Plant Tissue Cult. **12**(1) : 69-77, 2002 (June)

PTC

Regeneration and *Agrobacterium*-mediated Genetic Transformation of Two Indigenous Potato Varieties of Bangladesh

R. H. Sarker and Barkat Murtaja Mustafa

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words : Micropropagation, Indigenous, Potato, Genetic transformation, *Agrobacterium*

Abstract

Maximum shoot regeneration in two indigenous potato (*Solanum tuberosum* L.) was observed on MS semi-solid medium supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3. Among the two varieties, namely Lal Pakri and Jam Alu the former showed best response in terms of number of shoots/explant, nodes/shoot and shoot length. Half strength of MS containing 0.1 mg/l IAA was found to be best for root induction from the excised shoots. Two strains of *Agrobacterium* namely, LBA4404 containing pBI121 and EHA105 having plasmid pCAMBIA1301 were used for transformation. Among the two strains, LBA4404 with the vector pBI121 showed better response. In the nodal segment of Lal Pakri, the transformed zone coincided with the regeneration point. Regeneration from transformed tissue was obtained on MS supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3. Histochemical GUS assay showed the expression of GUS gene in the leaf tissues of the transformed shoot.

Introduction

Potato (*Solanum tuberosum* L.) is considered to be the fourth major food crop of the world. In Bangladesh, it ranks third after rice and wheat with respect to land under cultivation. Several modern varieties (HYV) are now cultivated in Bangladesh but the traditional varieties still occupy about 35% of the total potato production area (Ilangantileke et al. 2001). Area under potato cultivation has been increasing over the past several years with an enhanced rate of production (BBS 1999). However, the average yield of potato in Bangladesh is several times lower than that of many European countries.

Although potato is being considered as one of the food crops in Bangladesh its productivity is hampered due to the attack of virus, fungus and bacterial diseases. The total loss caused by these diseases is 30 - 100 % during

cultivation and 2 - 6 months of storage (Anon. 1992). It has been estimated that as high as 57.2 % loss of yield occurs in Bangladesh due to late blight alone (Ali and Dey 1994).

It is now a well-known fact that through meristem culture it is possible to develop virus free potato planting stocks in a mass scale. But it is not yet possible to develop fungal resistant cultivars through *in vitro* culture techniques. In recent years, genetic transformation techniques are being used to develop disease resistant varieties in many crop plants. Although there are reports on the genetic transformation of potato (Sheenman and Bevan 1988, Visser *et al.* 1989, Wenzler *et al*. 1989, Vayda and Belknap 1992) no work has been carried out on locally available Bangladeshi varieties. Therefore, attempts have been made to optimize various factors influencing reproducible transformation protocols in two indigenous potato varieties using two strains of *Agrobacterium* containing different vectors. This paper describes regeneration as well as transformation protocols for two indigenous potato varieties, Lal Pakri and Jam Alu.

Materials and Methods

Two indigenous potato (*Solanum tuberosum* L.) varieties, namely Lal Pakri and Jam Alu were used in the present study. The seed tubers of these two varieties were collected from the local growers of Bogra and Mymensingh, respectively. The tubers were kept in an incubator at 18_C for sprouting. About 4.0 - 5.0 cm long sprouts were cut and washed in distilled water. They were surfaced sterilized with 0.1 % $HgCl_2$ solution (w/v) for 15 min followed by several washes with sterilized distilled water. About 1.0 cm long sprout segments containing one to two nodes were used as explants and cultured on agar solidified MS medium supplemented with various combination of BAP, Kn and GA_3 for induction and proliferation of multiple shoots. pH of the medium was adjusted to 5.8 before autoclaving. The culture vessels containing the explants were incubated in the growth room under 16/8 hrs light/dark cycle at 25 ± 2_C. For induction of roots, regenerated shoots (3 - 4 cm long) were excised and transferred to half strength of MS medium supplemented with different concentrations of IAA, IBA and NAA.

Agrobacterium tumefaciens strain LBA4404 (Ooms et al. 1982) harboring the binary plasmid pBI121 and strain EHA105 (Hood et al. 1993) containing the plasmid pCAMBIA1301 were used in the present study. Both the plasmids contain the GUS reporter gene. The plasmid pBI121 contains the plant selectable marker gene *nptll* conferring resistance to kanamycin and the

plasmid pCAMBIA1301 contains the plant selectable marker gene conferring resistance to hygromycin, *hptII.*

Leaf, internodal and nodal segments from *in vitro* grown planlets were used in the genetic transformation. The *Agrobacterium* suspension was centrifuged at 10000 rpm for 10 min and the precipitate was resuspended in appropriate volume of MS medium so that the density of bacterial suspension becomes 0.8 - 1.0 (at 600 nm). The cut explants were then immersed in the resuspended *Agrobacterium* suspension for different periods of time, blotted dry and cocultured for three days in the dark on MS medium with 1 mg/l BAP and 0.1 mg/l GA_3. After coculture the explants were washed in cefotaxime (500 mg/l) to kill the *Agrobacterium*. They were then cultured on regeneration medium.

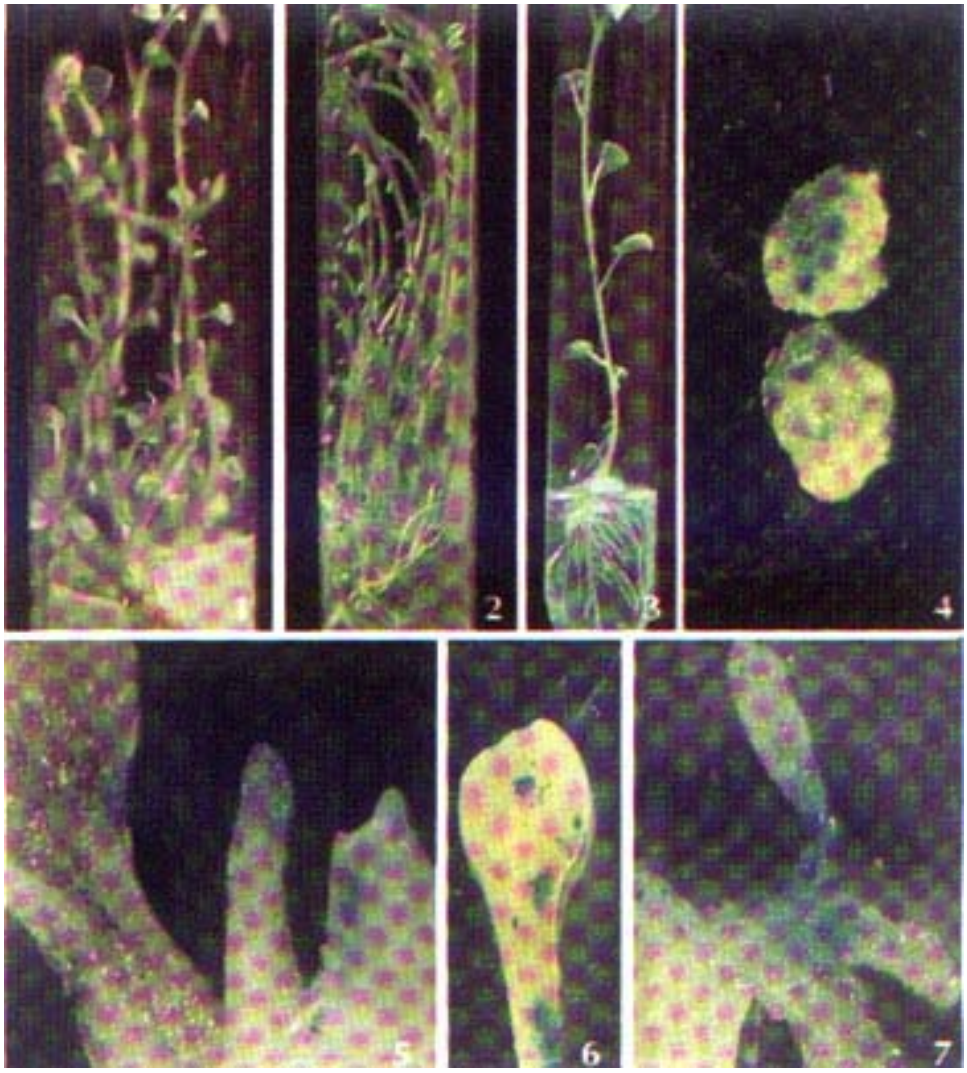
Following cocultivation the explants were subjected to transient GUS histochemical assay according to Jefferson (1987) to monitor the efficiency of infection. The cocultivated explants were incubated for 24 - 48 h at 37_C in the substrate X-gluc (5-bromo, 4-chloro, 3-indolyl-glucuronide) and subsequently bleached with 70% ethanol before scoring for transient GUS expression.

Results and Discussion

Different concentrations of BAP (0.5 - 4.0 mg/l) and Kn (0.5 - 4.0 mg/l) were used separately to see their effect on multiple shoot regeneration. Among the two cytokinins used here BAP showed better response in terms of number shoots per explant, shoot length, number of nodes and leaves in both the varieties. Comparative response of both BAP and Kn is shown in Table 1. It was observed that 1.0 mg/l BAP was most effective for induction of multiple shoots and the variety Lal Pakri produced maximum number of shoots per explant. However, in Jam Alu maximum number of shoots developed on MS medium containing 1.5 mg/l BAP. Results of the present investigation indicate that the number of shoots and nodes increased with the increase of BAP or Kn up to (1.0 - 1.5 mg/l) and decreased with higher concentrations (2.0 - 4.0 mg/l). It was also observed that either very low (0.5 mg/l) or very high (3.0 - 4.0 mg/l) concentrations of BAP or Kn decreased shoot length and number of leaves per shoot in both the varieties. Similar findings were obtained by Hoque et al. (1996a), Mila (1991) and Hoque et al. (1996 b) in some other potato varieties.

Table 1. Effects of various combinations of BAP, Kn and GA_3 on multiple shoot regeneration from nodal segments of potato.

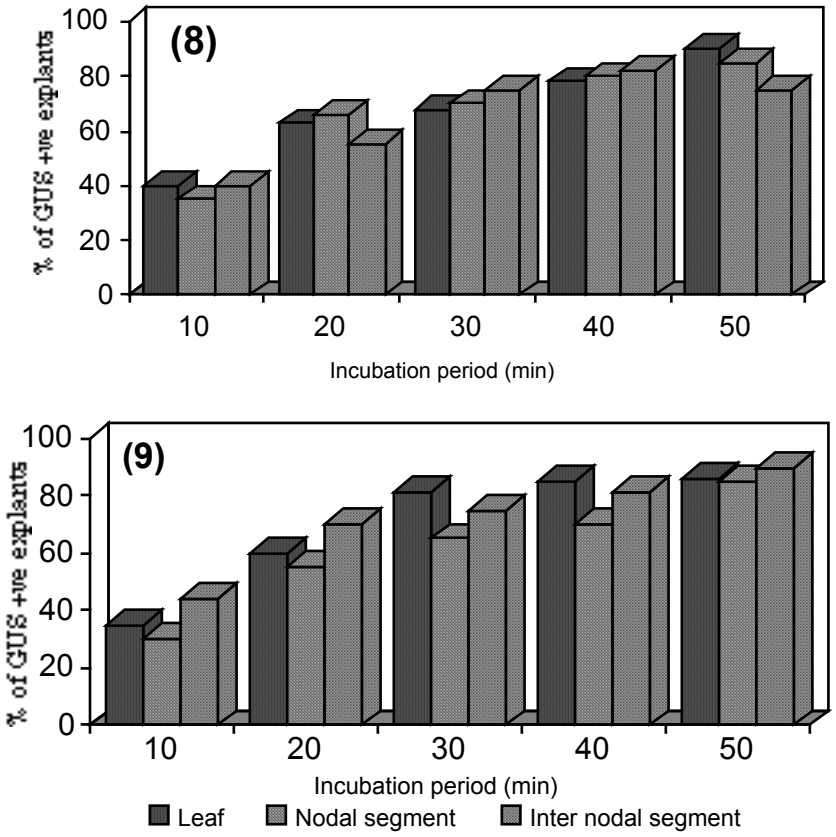
	Hormones		No. of	Mean No.	Av. shoot	Av. No. of	Av. No.
Lal	BAP	0.5	38	4	5.5	6.0	7.0
Pakri		1.0	40	7.5	6.5	10	11.0
		1.5	40	7	6.0	8.0	9.0
		2.0	41	6	5.0	8.0	8.0
		2.5	40	5	5.5	7.0	8.0
		3.0	36	4	6.0	6.0	7.0
		3.5	40	4	5.0	5.0	7.0
		4.0	40	3.5	5.5	4.5	6.0
	BAP + GA_3	1.0 + 0.5	40	9.5	6.5	8	10
	BAP + GA_3	1.0 + 0.1	36	11.0	7.0	12	12
	Kn	0.5	36	4.0	5.5	5	6
		1.0	36	7	7.5	7	8
		1.5	40	6	6.5	6	7
		2.0	37	6	5.0	5	5
		2.5	20	5	5.5	4	6
		3.0	40	4.5	5.5	3	5
		3.5	38	4.5	4.0	4	5
		4.0	36	4.0	4.0	3	4
	Kn + GA_3	1.0 + 0.05	36	8.5	6	8	9
	Kn + GA_3	1.0 + 0.1	40	10	6.5	11	10
	BAP	0.5	40	3.5	4.5	6.0	6
Jam Alu		1.0	38	7.0	5.5	8	8
		1.5	40	7.5	5.0	8	9
		2.0	40	5.0	5.0	6	7
		2.5	40	4.5	4.5	5	6.5
		3.0	41	4.5	4.5	5	5.5
		3.5	35	4.0	4.0	4.5	5.0
		4.0	40	4.0	4.0	4.5	5.0
	BAP + GA_3	1.0 + 0.05	40	8	6	7	8
	BAP + GA_3	1.0 + 0.1	40	10	7	9	9
	Kn	0.5	40	4	5	6	6
		1.0	39	6.5	6.5	7	7
		1.5	36	5.5	6	6	6
		2.0	40	5	5.2	6	7
		2.5	32	5	5	6	6.5
		3.0	36	4.5	4.5	5	5.5
		3.5	40	4	4.5	5	5
		4.0	38	4	4	5	5.0
	Kn + GA_3	1.0 + 0.05	39	7.5	7	7	7
	Kn + GA_3	1.0 + 0.1	40	9.5	6.5	8	8.5



Figs. 1-3. Fully developed multiple shoots in Lal Pakri (Fig. 1) and Jam Alu (Fig. 2) in MS with 1.0 mg/l BAP + 0.1 mg/l GA3. Root induction on micropropagated shoot in half strength MS + 0.1 mg/l IAA in Lal Pakri (Fig. 3). Figs. 4-7. Histochemical assay showing the expression of GUS resporter gene in Lal Pakri. Transient expression in basal callus (Fig. 4), nodal segment with conspicuous blue spots in the axillary shoot bud (Fig. 5). Gus expression in regenerated leaf (Fig. 6) and shoot bud (Fig. 7) developing from cocultured nodal segment explant.

Different concentrations of GA_3 were used in combination with BAP or Kn to see their combined effect on growth and development of the regenerated shoots. Maximum number of healthy shoots with well expanded leaves per explant were observed when the medium was supplemented with 1.0 mg/l BAP

+ 0.1 mg/l GA_3 in both varieties (Figs. 1, 2). It was possible to obtain an average of 11 shoots per explant within three to four weeks. Hoque et al. (1996 b) and Mila (1991) obtained 9.2 and 9.3 shoots per explant, respectively. In the present study it was observed that the growth of the microshoots was much better when



Figs. 8-9. Effects of different incubation periods on transformation of various explants of potato using *Agrobacterium* strain LBA4404 containing the binary plasmid pBI121 in varieties : Lal Pakri (Fig. 8) and Jam Alu (Fig. 9).

0.05 - 0.1 mg/l GA_3 was used in combination with 1.0 mg/l BAP or Kn. GA_3 was found to be the most effective in increasing shoot length and leaf blade area. These observations are in agreement with those of Goodwin et al. (1980), Hoque et al. (1996a), Mila (1991) and Novak and Zadina (1987) in different varieties of potato.

It may be mentioned that among the two potato varieties used in the present investigation Lal Pakri showed better response for regeneration than Jam Alu. Variable responses of different potato varieties due to genetic make up

towards *in vitro* shoot multiplication and their development were also reported by previous workers (Hussey and Stacey 1981, Millar *et al.* 1985, Bajaj 1981).

In some cases several roots developed spontaneously from the *in vitro* grown shoots. But they were found to be inadequate for their transplantation and therefore adequate root induction was necesary. Among the various auxins (IAA, IBA, NAA) used in the present study for induction of roots 0.1 mg/l IAA showed the best response and healthy roots developed within two - three weeks of culture (Fig. 3). The rooted plantlets were successfully transplanted to soil.

Table 2. Influence of optical density (at 600 nm) of *Agrobacterium* suspension on transformation of different explants of potato analyzed by transient GUS histochemical assay (var. Lal Pakri and Jam Alu).

Variety	OD_{600}	Explant	No. of explants infected	No. of explants assayed for GUS	No. of explants +ve for GUS	% of explants +ve for GUS
		L	50	18	10	55.5
	0.604	NS	48	15	10	66.6
Lal Pakri		IS	50	15	9	60.0
		L	50	16	11	73.3
	0.784	NS	49	15	10	66.6
		IS	50	16	11	73.3
		L	51	15	13	86.6
	1.0	NS	50	15	12	80.0
		IS	50	15	12	80.0
		L	53	15	9	60.00
	0.604	NS	40	16	9	56.25
Jam Alu		IS	50	15	8	53.30
		L	45	15	11	73.30
	0.784	NS	51	15	10	66.60
		IS	50	15	12	80.00
		L	53	15	12	80.00
	1.0	NS	50	15	10	66.60
		IS	46	15	12	80.00

L= Leaf, NS = Nodal segment, IS = Internodal segment.

Three different explants, namely leaf, nodal segment and internodal segments of two potato varieties used in the present investigation were tested to see their transformation ability. For this purpose two strains of *Agrobacterium* (LBA4404 pBI121 and EHA105 pCAMBIA1301) were used. Although both the strains of *Agrobacterium* responded positively, LBA4404 pBI121 showed better response than EHA105 pCAMBIA1301 in all the

explants. Factors influencing successful transformation, namely optical density of the bacterial suspension and infection period were optimized during the present study. An infection period of 50 minutes (Figs. 8 , 9) and a bacterial suspention of 0.8 - 1.0 O.D. was found to be optimum for the explants in both the varieties (Table 2).

Transformed tissue were obtained from leaf, nodal and internodal segments. In both the potato varieties leaf explants showed best transformation ability followed by internodal and nodal segments. Histochemical GUS assay revealed the expression of GUS reporter gene in the leaf, basal callus (Fig. 4) and nodal segments (Fig. 5) in Lal Pakri. It was evident that the transformed sectors of the axillary buds coincided with the area of regeneration (Fig. 5). Regenerated shoots from the transformed tissues showed numerous blue sectors in the leaf (Fig. 6) and newly developed shoot bud (Fig. 7). However, future experiments are required to regenerate fully transformed plantlets showing stable integration of GUS and *nptII* gene. The results of the present investigation demonstrated the development of efficient *in vitro* regeneration systems in two local potato varieties, namely Lal Pakri and Jam Alu. Encouraging results of transformation experiments have shown that it may be develop disease resistant varieties of potato through genetic transformation.

References

Ali MS and **Dey** (1994) Pathological Research on Tuber Crops in Bangladesh. Proc. Workshop Trans. Tech. CDP crop under Res-Extn. Linkage programme BARI, Gazipur, Oct. 22-27, 1994, pp. 159 - 165.

Anonymous. (1992) Potatoes : Improving disease resistance and quality. Biotechnology & Development Monitor. **12** : 3 - 5.

Bajaj YPS (1981) Regeneration of plants from potato meristems freeze- preserved for 24 months. Euphytica, **30**(1) : 141 - 145.

BBS (1999) Statistical pocketbook of Bangladesh, Bangladesh Bureau of Satistics, Dhaka, Bangladesh. pp. 466.

Goodwin PB, Kim YC and **Andisarwanto T** (1980) Propagation of potato by shoot tip culture. Potato Res. **23** : 9 - 18.

Hood EE, Gelvin SB, Melchers LS and **Hoekema A** (1993) New *Agrobacterium* helper plasmids for gene transfer to plants. Transgenic Res. **2 :** 208 - 218**.**

Hoque MI., Islam MA, Sarker RH and **Islam AS (**1996a) *In vitro* microtuber formation in potato (*Solanum tuberosum* L.). *In:* Plant Tissue Culture (Ed. A.S. Islam), Oxford & IBH Publ. Co., Calcutta/New Delhi, pp. 221-228

Hoque MI., Mila NB, Khan MS and **Sarker RH** (1996b) Shoot regeneration and in vitro microtuber formation in potato (*Solanum tuberosum* L.) Bangladesh J. Bot. **25(**1) : 87 - 93.

Hussey G and **Stacey NJ** (1981) *In vitro* propagation of potato (*Solanum tubersom* L.) Ann. Bot. **48** : 787 - 796.

Ilangantileke SG, Kadian MS, Hossain M, Hossain AE, Jayasinghe U and **Mahmood AA** (2001) Toward alleviating poverty of rural potato farmers by strengthening the potato seed system in Bangladesh: A rapid rural appraisal. CIP Program Report. pp. 259-264.

Jefferson R.A. (1987) Assaying chimeric genes in plants : the GUS fusion system. Plant. Mol. Biol. Reptr. **5** : 387 - 405.

Jefferson RA, Kavanagh, TA and **Bevan MW** (1987) GUS Fusions: -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6** : 3901 - 3907.

Mila NB (1991) Optimization of *in vitro* microtuber formation in potato (*Solanum tuberosum* L.). M. Sc. Thesis, Plant Breeding and Tissue Culture Lab., Department of Botany, University of Dhaka.

Millar PR, Amirouche L, Stuchbury T and **Mathews S** (1985) The use of plant growth regulators in micropropagation of slow-growing potato cultivars. Potato Research. **28** : 479 - 486

Novak FJ and **Zadina J** (1987) *In vitro* micropropagation of potato - Progress in Czechoslovkia. *In:* Biotechnology in Agriculture and Forestry Vol. **3**: potato , Y.P.S. Bajaj (ed.) . Springer-Verlag, berlin/Heidelberg, pp. 23 - 29.

Sheenman, S. and **M.W. Bevan** (1988) A rapid transformation method for *Solanum tuberosum* using binary *Agrobacterium tumefaciens* vectors. Plant Cell Rep. **7** : 13 - 16.

Ooms G, Hooykaas PJJ, Van Veen RJ, Van Beelen P, Regensburg-Tunik and **Schilperoort RA** (1982) Octapine Ti plasmid deletion mutants of *Agrobacterium tumifaciens* with emphasis on the right side of the T-region. Plasmid **7** : 15 - 29.

Vayda ME and **Belknap WR** (1992) The emergence of transgenic potato as commercial products and tools for basic research. Transgenic Res. **1** : 149 - 163.

Visser RGF, Jacobsen E, Witholt B and **Feenstrata WJ** (1989) Efficient transformation of potato using a binary vector in *Agrobacterium rhizogenes*. Theor. Appl. Genet. **78** : 594 - 600.

Wenzler H., Mignery G, May G and **Park W** (1989) A rapid and efficient transformation method for the production of large number of transgenic potato plants. Plant Sci. **63** : 79 - 85.