

***Agrobacterium*-MEDIATED GENETIC TRANSFORMATION OF POTATO FOR ABIOTIC STRESS TOLERANCE**

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ABSTRACT

Two potato varieties, Cardinal and Heera, were used to optimize the *Agrobacterium*-mediated genetic transformation system in potato for abiotic stress tolerance. MS medium supplemented with different concentrations and combinations of NAA and BAP were used to obtain callus induction from leaf explants of potato cv Cardinal and Heera. Maximum percentage of callus induction (95.83%) was observed in MS medium supplemented with 4 mg/L NAA and 2 mg/L BAP. The highest callusing was found in Cardinal and the lowest in Heera. Calluses were inoculated with *Agrobacterium tumefaciens* strain LBA4404 (pB1121) carrying CIPK sense gene, selectable marker gene *nptII* gene conferring resistance to kanamycin and the GUS reporter gene. Both the varieties were found GUS positive after inoculation and selection. Cardinal had maximum percentage of survived callus (25%) on selection media. Stable integration and expression of the transgenes were confirmed by GUS histochemical assay.

Key words : Potato, *Agrobacterium* mediated transformation, Abiotic stress

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae. It is one of the most agronomically important plants in the world, notably due to its high productivity and its high starch, vitamin and protein content. In the recent years, there has been an increasing trend in both area and production of potato in Bangladesh. But the yield per unit area is not satisfactory. In Bangladesh, 4856 thousand tons of potatoes were produced in 2005 (BBS, 2005). Biotic and abiotic factors are responsible for the low yield of potato which may exert a deleterious effect on yield, marketable quality, storability, germplasm conservation, distribution and international exchange. Abiotic stresses include drought, salinity, extreme temperatures, chemical toxicity and oxidative stress and are serious threats to agriculture and the natural states of environment (Wang *et al.*, 2003). The population of our country is increasing day by day but the land is decreasing. Therefore, we need to utilize the lands which are now not under cultivation, such as, coastal zone which have high saline properties. Furthermore, the crop is damaged by a series of environmental stresses like drought during the development of roots or tubers. Abiotic stress limits plant growth and significantly increases the negative effects on both quantity and quality of crop production (Mohsin, 2004). Crop varieties resistant to abiotic stresses

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need to be developed by using molecular tools of biotechnology. *Agrobacterium*-mediated genetic transformation may be the best option for potato as traditional breeding is not possible in this vegetatively propagated crop. Genetic transformation needs a reliable regeneration protocol. The present research work has been undertaken to optimize the *Agrobacterium*-mediated genetic transformation system for abiotic stress tolerance.

MATERIALS AND METHODS

The present investigation was carried out in the Biotechnology Laboratory of the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh. Two varieties of potato (Cardinal and Heera) were used in the experiment. Leaf explants were used for callus induction. The induced calli were then used for *Agrobacterium*-mediated genetic transformation. Genetically engineered *Agrobacterium tumefaciens* strain LBA4404 (pB1121) containing the *uidA* gene encoding *GUS* (β -glucuronidase), the *nptII* gene and the CIPK sense gene encoding calcineurin B-like protein conferring abiotic stress tolerance driven by CaMV promoter and NOS terminator within the right border (RB) and left border (LB) region of the construct was used. For the induction and maintenance of callus from leaf, MS (Murashige and Skoog, 1962) medium containing different concentrations and combinations of BAP (0, 2, 4 and 6 mg/L) and NAA (0, 1, 2 and 4 mg/L) was used. YMB medium was used for *Agrobacterium* culture maintenance and LB medium was used as *Agrobacterium* working culture medium for transformation. Calli were infected in liquid bacterial strain and co-cultivated for 3 days on the MS medium without growth regulators. After co-cultivation, explants were washed with sterile water with added cefotaxime (500 mg/l) and transferred onto MS medium supplemented with 3 mg/L NAA, 4 mg/L BAP and 200 mg/L cefotaxime. For low selection, MS medium supplemented with, 3 mg/L NAA and 4 mg/L BAP, 20 mg/L kanamycin and 100 mg/L cefotaxime was used. For high selection and regeneration, MS medium supplemented with 3 mg/L NAA and 4 mg/L BAP, 40 mg/L kanamycin and 100 mg/L cefotaxime was used. Transformation ability was assessed through histochemical assay of *GUS* reporter gene in callus tissue. The experiments were laid in Completely Randomized Design (CRD) with three replications. The analyses of variances for different parameters were performed and means were compared by the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The ultimate goal of this experiment was to establish a suitable system for *Agrobacterium*-mediated genetic transformation in potato and also determine the effect of varieties, antibiotics, inoculation time and co-cultivation periods on transformation.

Among the varieties Cardinal showed the higher percentage of callus (47.66%) than Heera (39.58%) (Table 1). It required more time for callus induction (11.50 days) than Heera (11.46 days) (Table 1). The effect of different concentrations of NAA and BAP on per cent callus induction indicated that MS medium supplemented with 4 mg/L NAA showed the highest percentage (57.81%) of callus induction. Among the BAP

concentrations 2 mg/L BAP gave the highest percentage (60.42%) of callus induction. Similar result was observed by Juan *et al.* (2004). The minimum number of days required for callus initiation (10.16 day) was observed in 4 mg/L NAA. Among the BAP concentrations, 2 mg/L BAP required minimum number of days for callus initiation (13.47 day) (Table 1).

Table 1. Effect of variety on number of calli/vial, per cent callus induction and days to callus induction

Variety/Hormone (mg/l)		Number of calli/vial	Per cent callus induction	Days to callus induction
Variety	Cardinal	3.25 a	47.66 a	11.50 a
	Heera	2.75 b	39.58 b	11.46 b
Hormone NAA	0	2.22 d	36.46 c	13.35 a
	1	3.06 b	39.06 bc	10.44 c
	2	2.47 c	41.15 b	11.97 b
	4	4.25 a	57.81 a	10.16 c
BAP	0	1.50 c	0.00 c	0.00 d
	2	3.63 a	60.42 a	13.47 c
	4	3.50 ab	57.81 ab	50.30 b
	6	3.38 b	56.25 b	17.31 a

Six calli were cultured vial. Figures within the same column bearing different letters are significantly different at 0.05% level according to DMRT.

Combined effect of variety and different concentrations of NAA and BAP was highly significant. The highest percentage of callus (95.83%) was observed in Cardinal with MS medium containing 4 mg/L NAA and 2 mg/L BAP and lowest percentage of callus (41.67%) was observed in Heera with MS medium containing 2 mg/L NAA and 2 mg/L BAP (Table 2). Minimum number of days (8.50 days) was required for Cardinal at 4 mg/L NAA and 2 mg/L BAP compared to any other combination and maximum number of days (20.00 days) was required medium without NAA and with 6 mg/L BAP. In case of Heera, minimum number of days (11.25 day) was required in medium supplemented with 1 mg/L NAA and 4 mg/L BAP and maximum number of days (22.50 day) was required in medium containing 6 mg/L BAP and without NAA (Table 2).

Higher survival potentiality was observed in 20 mg/L (20.83%) than 40 mg/L kanamycin. (Table 3). In case of *Agrobacterium*-mediated transformation experiments inoculation time and duration are important factors. Calli showed higher response (61.11%) to the assay in case of 7 minutes inoculation time (Table 4). The calli co-cultivated for 5 days duration gave higher (58.33%) response to the GUS assay (Table 4). Both the two varieties showed positive response towards transformation. Cardinal showed higher (55.56%) and Heera showed lower response (35.19%) to GUS assay (Table 4). Sultan *et al.* (2000) also reported that Cardinal showed better response to transformation. Yen *et al.* (2000) mentioned that shorter

co-cultivation times resulted in higher number of explants survival. The combined effect of variety, inoculation times and co-cultivation periods were statistically non significant. Cardinal with 7 min inoculation time and 5 days co-cultivation periods gave the highest GUS positive assay (100%) and the lowest (16.67%) was observed in Heera with 3 min inoculation time and 3 days co-cultivation periods that were identical with 3 min inoculation time and 3 days co-cultivation periods (Table 5).

Table 2. Combined effect of variety, different concentrations of NAA and BAP on number of calli/vial, per cent callus induction and days to callus induction

Variety	Treatment combinations		Number of calli/vial	Per cent callus induction	Days to callus induction
	NAA (mg/L)	BAP (mg/L)			
Cardinal	0	0	0.00 f	0.00 f	0.00 i
		2	3.25 d	54.17 d	15.50 de
		4	2.75 de	41.67 e	18.75 b
		6	3.25 d	54.17 d	20.00 b
		0	2.75 de	0.00 f	0.00 i
	1	2	2.75 de	45.83 de	11.50 fg
		4	4.50 bc	75.00 bc	13.50 defg
		6	3.25 d	54.17 d	18.00 bc
		0	0.00 f	0.00 f	0.00 i
		2	4.50 bc	75.00 bc	15.50 de
	2	4	4.00 c	66.67 c	15.25 de
		6	3.25 d	54.17 d	18.25 b
		0	3.25 d	0.00 f	0.00 i
		2	5.75 a	95.83 a	8.50 h
		4	4	4.25 bc	70.83 bc
Heera	0	6	4.50 bc	75.00 bc	16.00 cd
		0	0.00 f	0.00 f	0.00 i
		2	3.00 de	50.00 de	14.50 de
		4	2.75 de	45.83 de	15.55 de
		6	2.75 de	45.83 de	22.50 a
	1	0	3.00 de	0.00 f	0.00 i
		2	2.50 e	41.67 e	14.75 de
		4	3.00 de	50.00 de	11.25 g
		6	2.75 de	45.83 de	14.50 de
		0	0.00 f	0.00 f	0.00 i
	2	2	2.50 e	41.67 e	13.75 def
		4	2.50 e	41.67 e	18.75 d
		6	3.00 de	50.00 de	14.25 de
		0	3.00 de	0.00 f	0.00 i
		2	4.75 b	79.17 b	13.75 def
4	4	4.25 bc	70.83 bc	14.75 de	
	6	4.25 bc	70.83 bc	15.00 de	

Six calli were cultured/vial. Figures within the same column bearing different letters are significantly different at 0.05% level according to DMRT

Table 3. Main effect of kanamycin on number of survived calli/vial and per cent survived calli

Kanamycin	Number of survived calli/Vial	% survived calli
20 mg/L	1.25 a	20.83 a
40 mg/L	0.88 b	14.58 b

Six calli were cultured vial. Figures within the same column bearing different letters are significantly different at 0.05% level according to DMRT.

Table 4. Main effect of variety on % GUS positive explants

Variety	% GUS positive explants
Cardinal	55.56 a
Heera	35.19 b
Inoculation time (minute)	
3	22.22 c
5	52.78 b
7	61.11 a
Co-cultivation period (days)	
3	30.56 c
4	47.22 b
5	58.33 a

Six calli were cultured vial. Figures within the same column bearing different letters are significantly different at 0.05% level according to DMRT.

Table 5. Combined effect of inoculation time, co-cultivation periods on % GUS positive assay

Variety	Inoculation time (minute)	Co-cultivation period (days)	Number of explants positive for GUS	% GUS positive explants
Cardinal	3	3	1.00 f	16.67 f
		4	2.00 de	33.33 de
		5	3.00 d	50.00 d
	5	3	3.00 d	50.00 d
		4	3.00 d	50.00 d
		5	4.00 c	66.67 c
	7	3	3.00 cd	50.00 cd
		4	5.00 b	83.33 b
		5	6.00 a	100.00 a
Heera	3	3	0.00 g	0.00 g
		4	1.00 f	16.67 f
		5	1.00 f	16.67 f
	5	3	2.00 de	33.33 de
		4	3.00 d	50.00 d
		5	4.00 c	66.67 c
	7	3	2.00 e	33.33 e
		4	3.00 d	50.00 d
		5	3.00 d	50.00 cd

Six calli were cultured vial. Figures within the same column bearing different letters are significantly different at 0.05% level according to DMRT.

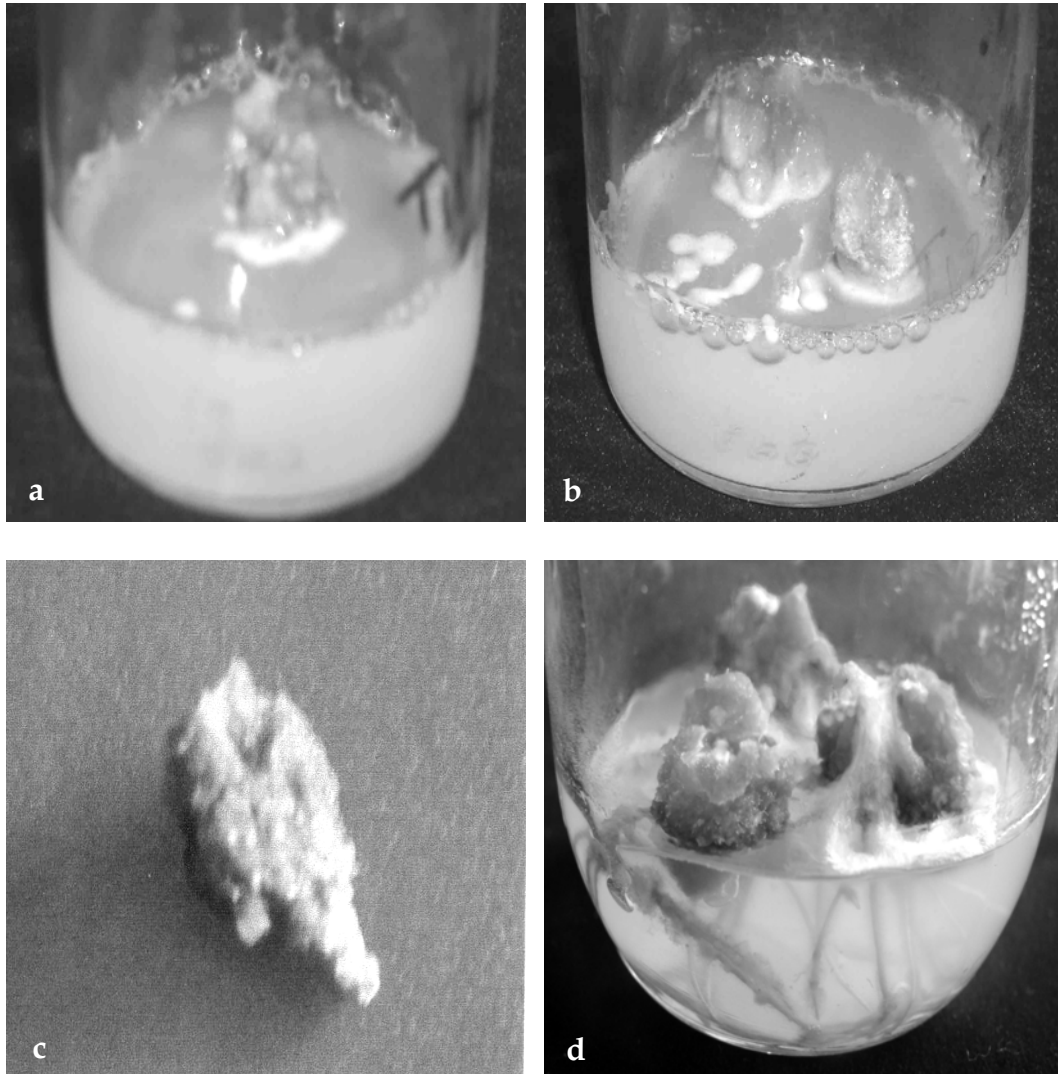


Plate 1. (a) Calli of potato cv. Cardinal treated with kanamycin (40 mg/L), (b) Selection of kanamycin resistance calli of potato cv. Heera, (c) GUS positive tissue of potato cv. Cardinal and (d) Roots obtained from cv. Cardinal on regeneration medium.

After antibiotic treatment the survived calli were transferred to regeneration medium. The regeneration medium comprised with MS medium containing 3 mg/L NAA and 4 mg/L BAP, 100 mg/L cefotaxime and 40 mg/L kanamycin and root was obtained from three Cardinal and two Heera calli (Plate 1). The system developed here would be used for the introduction of agronomically important trait in potato and regeneration of transgenic plants. However, more studies should be conducted including molecular analysis of transgenic plants.

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