ISSN 0258-7122
Bangladesh J. Agril. Res. 34(3) : 449-456, September 2009

IN VITRO CALLUS INITIATION AND REGENERATION OF POTATO

A. U. HAQUE[1], M. A. SAMAD[2] AND T. L. SHAPLA[3]

Abstract

The first experiment involving different explants and concentrations of 2,4-D and kinetin showed highly significant differences for length and weight of callus formed except interaction of callus weight. Leaf explant appeared to be best of all for callus length and weight when 1.0 mg/L 2,4-D + 0.25 mg/L kinetin concentration was used. Similarly, different explants versus different concentrations of BAP/GA_3/IAA showed significant differences for shoot length and leaf number per plantlet and also for root length. However, interaction term confirmed node and node/internode explants produced better results in shoot length and number of leaves per plantlet when concentrations 1 .0 mg/L BAP + 0.1 mg/L GA_3 and 1.0 mg/L BAP + 0.2 mg/L GA_3, 1.0 mg/L BAP + 0.4 mg/L GA_3, respectively, were used. Similarly, internode explants produced better results for root length after 21 days plantlet^{-1} when concentration of 1.0 mg/L IAA + 0.25 mg/L GA_3 was used. Shoot tip explants also produced better results in root length after 28 days plantlet^{-1} when concentrations 1.0 mg/L IAA + 0.25 mg/L GA_3 were used.

Key Words: *In vitro,* callus initiation, regeneration, potato.

Introduction

Potato *(Solanum tuberosum* L.) is one of the most important food crops worldwide and is consumed as staple food in more than forty countries in the world. This crop ranks fourth amongst all food crops (Solomon and Barker, 2001), while ranking first both in area and production among the vegetable crops grown in Bangladesh. In Bangladesh, potato represents about 53% of the total edible vegetables although yield is still lower than potential level obtained in advanced countries (FAO/CIP, 1995). Demand for potato is rapidly increasing. In Bangladesh, 2.99 million tons of potatoes are produced from 376 thousand acres of land during 2001-2002 (BBS, 2004). However, production has to be increased even with the current rate of demand.

The research was conducted to investigate the *in vitro* callus initiation ability of potato with optimum concentration of plant growth regulator from the different plant parts. Callus induction is important due to produced genetic variability, which is very important in breeding programme. In addition, it is easy to produce huge amount of *in vitro* plantlet via callus. The present piece of work

[1]Scientific Officer, Tuber Crops Research Centre, BARI, Joydebpur, Gazipur, [2]Senior Scientific Officer, Bangladesh Institute of Nuclear Agriculture, Mymensingh, [3]Assistant Professor, Sher-e-Bangla Agricultural University, Dhaka-1206, Bangladesh.

was undertaken to develop a callus induction and regeneration protocol cv. Diamant from leaf discs, nodes, internodes, and shoot tips and to identify the suitable explants for large scale utilization in tissue culture technique.

Materials and Method

In vitro plant materials of potato *(Solanum tuberosum)* cv. Diamant, developed using leaf, node, internode, and shoot tip were grown on medium consisting of Murashige and Skoog basal medium (Murashige and Skoog, 1962). For callus formation, fresh MS medium was supplemented 2,4-D (0.0, 1.0, 2.0, 3.0 and 4.0 mg/L) and kinetin was 0.25 mg/L in each case. The solid MS medium was prepared having the pH of 5.8 and was sterilized at 121°C for 20 minutes at 15 psi pressure. Glassware and other instruments were also sterilized in an autoclave at the same temperature and pressure. Aseptic conditions were maintained throughout the experimental period. The sprouts of potato were rapidly washed in 70% alcohol and then immersed in 0.1% $HgC1_2$ solution for 15 minutes and then washed in sterilized water for several times and they are placed in MS medium. Leaf, node, internode, and shoot tip of one month old microplant (4-5 cm in height) were cut into small pieces (2-5 mm) and placed in MS media containing different concentrations of 2,4-D and kinetin. Culture was kept in a growth room at 25±2 °C having 1.83 m fluorescent tubes and was illuminated 16 h daily with a light intensity of 1500 lux. Callus initiated after 10-14 days of incubation. Four weeks after inoculation, explants were removed aseptically from the Petridish on a sterilized glass plate inside the laminar air flow cabinet and were placed again on freshly prepared sterilized medium containing fixed concentrations of BAP (1.0 mg/L) and GA_3 (0.0, 0.05, 0.1, 0.2, 0.4 mg/L) for shoot initiation. The subcultured plants were then incubated at 22±2°C with 16 h photoperiod. After shoot initiation, more light intensity was given for shoot elongation. When these shoots grew about 3-4 cm in length, rescued aseptically, separated from each other and again subcultured with freshly prepared root induction medium supplemented with IAA (1.0 mg/L) and GA_3 (0.0, 0.25, 0.5, 1 .0, 2.0 mg/L) to induced root. The experiment was laid down in the CRD with 5 replications.

Results and Discussion

Research were conducted to assess the performance of potato variety Diamant on (I) *in vitro* callus formation with the following associated traits, and (2) shoot and root formation with the following characters.

Callus length after 28 days

Callus length as affected by different explants and concentrations, 2,4-D and kinetin, had shown significant differences. Leaf explants had produced significantly the highest callus length (7.34 mm), while in contrast, the shoot tip

the least of all (6.29 mm). The later although held equal statistical rank with the other remainders (Table I). The different concentrations of 2,4-D and Kinetin showed the significant differences. The interaction effect between explants and concentrations of growth regulators also had shown significant differences on callus length. This result suggested with Dobranaszki *et al.* (l999) and Fomenko *et al.* (1998) who also observed significant effects of explants on callus length.

Table 1. The interaction effects between explants and concentrations of 2,4-D and Kinetin.

Explants	Concentrations (mg/L)	Callus length (mm)	Callus weight (g)
Leaf	0.0 2,4D + 0.25 Kn	-	-
	l.0 2,4-D + 0.25 Kn	9.78AB	1.15 A
	2.0 2,4-D + 0.25Kn	10.22A	1.11 A
	3.0 2,4-D + 0.25 Kn	8.50 CDE	0.97 A
	4.0 2,4-D + 0.25 Kn	8.20 DEF	I .04 A
	Mean	7.34 A	0.86 A
Node	0.0 2,4-D+ 0.25 Kn	-	-
	1.0 2,4-D + 0.25 Kn	8.68 BCDE	0.98 A
	2.0 2,4-D + 0.25 Kn	9.14 BCD	0.94 A
	3.0 2,4-D + 0.25 Kn	9.02 BCDE	I .0 A
	4.0 2,4-D + 0.25 Kn	6.62 CH	0.96 A
	Mean	6.69 B	0.77 AB
Internode	0.0 2,4-D+ 0.25 Kn	-	-
	l.02,4-D+ 0.25 Kn	8.22 DEF	1.14 A
	2.0 2,4-D + 0.25 Kn	8.14 DEF	0.97 A
	3.0 2,4-D + 0.25 Kn	9.04 BCDE	0.97 A
	4.0 2,4-D + 0.25 Kn	7.96 EF	1 .0 A
	Mean	6.67 B	0.82 A
Shoot tip	0.0 2,4-D+ 0.25 Kn	-	-
	1.0 2,4-D + 0.25 Kn	8.68 BCDE	0.98 A
	2.0 2,4-D + 0.25 Kn	9.60 ABC	0.66 B
	3.0 2,4-D + 0.25 Kn	7.16 FG	1.0 A
	4.0 2,4-D + 0.25 Kn	6.02 H	0.97 A
	Mean	6.29 B	0.72 B

Means followed by similar letters in each column are not significantly different at 5% level according to DMRT.

Callus weight after 28 days

Callus weight appeared highest (0.86 g) with leaf explants, followed by 0.82g and 0.77g from internode and nodal explants, respectively. The lowest callus weight in contrast was observed with shoot tip explant (0.72g). The effect of different concentrations on callus weight showed significant differences (Table 1). The interaction effects between explants and concentrations did not show

significant differences on the callus weight. This suggests all explants used have similar and equal effect with any/all concentrations used.

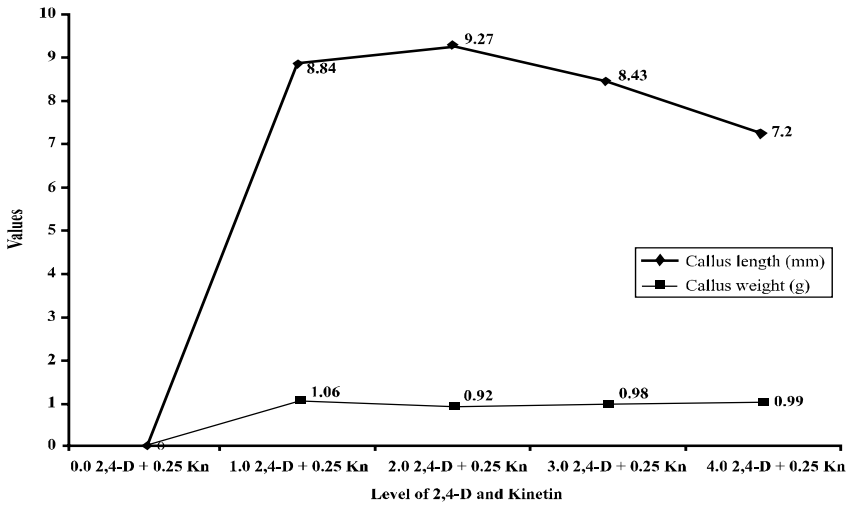

Fig. 1. Mean effect of different concentrations of 2, 4-D and Kinetin.

Shoot length after 28 days

Shoot length appeared highest (3.64cm) with leaf explants, followed by shoot tip (3.46cm) and node explants (3.32cm). Node explants produced the least of all (2.85cm). Different concentrations of growth regulators showed significant differences in shoot length. The interaction effects between explants x concentrations on shoot length had shown also significant differences. The longest shoot (5.28cm) was produced by the treatment combination of 1.0 mg/L BAP + 0.1 mg/L GA_3 (Table 2, Fig. 2). This agrees with Sarker and Mustafa (2002) and Asma *et al.* (2001) who also observed the organogenesis of potato with the BAP and GA_3. Martel *et al.* (1992) reported that both BAP and GA_3 with higher concentrations were necessary for shoot formation.

Number of leaves per plantlet

Explants showed significant differences on number of leaves. Shoot tip explants had the highest number of leaves (2.48) followed by internode (2.28 leaves/plantlet) and node explant (2.08 leaves/plantlet). Different concentrations of BAP and GA_3 on number of leaves also had shown statistically significant differences. The highest number of leaves (2.45) was found at 1 .0 mg/L BAP + 0.1 mg/L GA_3 (Fig. 2). In contrast, the lowest number of leaves (1.95) was found at 1.0 mg/L BAP + 0.05 mg/L GA_3. The interaction effects between explants and concentrations of growth regulators on leaf number had shown significant differences. The highest number of leaves (3.0) was produced from node explants

by combination of 1.0 mg/L BAP + 0.2 mg/L GA_3, while the lowest number of leaves (1.20) was produced by the combination of 1.0 mg/L BAP + 0.4 mg/L GA_3 (Table 2). Sarker and Mustafa (2002) and Zel *et al.* (1999) also observed the multiple shoot regeneration from the nodal segments of' potato, which are similar to the present study.

Table 2. The interaction effects between explants and concentrations of BAP and GA_3.

Explants	Concentrations (mg/L)	Callus length (mm)	Callus weight (g)
Leaf	1.0 BAP + 0.0 GA_3	2.92 E	2.00 CD
	1.0 BAP + 0.05GA_3	3.00 E	1.80 DE
	1.0 BAP + 0.IGA_3	4.48 B	1.80 DE
	1.0 BAP + 0.2GA_3	3.50 C	2.00 CD
	1.0 BAP + 0.4GA_3	4.32 B	2.20 BCD
	Mean	3.64 A	1.96 C
Node	1.0 BAP + 0.0 GA_3	3.18 DE	1.80 DE
	1.0 BAP + 0.05 GA_3	3.40CD	2.00 CD
	1.0 BAP + 0.1GA_3	5.28 A	2.40 ABCD
	1.0 BAP + 0.2GA_3	2.32 F	3.00 A
	1.0 BAP + 0.4GA_3	2.42 F	1.20 E
	Mean	3.32 C	2.08 BC
Internode	1.0 BAP + 0.0 GA_3	l.32G	2.00 CD
	1.0 BAP + 0.05GA_3	2.36 F	1.80 DE
	1.0 BAP + 0.1 GA_3	4.50 B	2.80 AB
	1.0 BAP + 0.2GA_3	2.46 F	1.80 DE
	1.0 BAP + 0.4GA_3	3.62 C	3.00 A
	Mean	2.85 D	2.28 AB
Shoot tip	1.0 BAP + 0.0 GA_3	3.56 C	2.60 ABC
	1.0 BAP + 0.05GA_3	3.52 C	2.20 BCD
	1.0 BAP+0.1GA_3	4.46 B	2.80 AB
	1.0 BAP + 0.2GA_3	3 .42CD	2.60 ABC
	1.0 BAP + 0.4GA_3	2.36 F	2.20 BCD
	Mean	3.46 B	2.48 A

Means followed by similar letters in each column are not significantly different at 5% level according to DMRT.

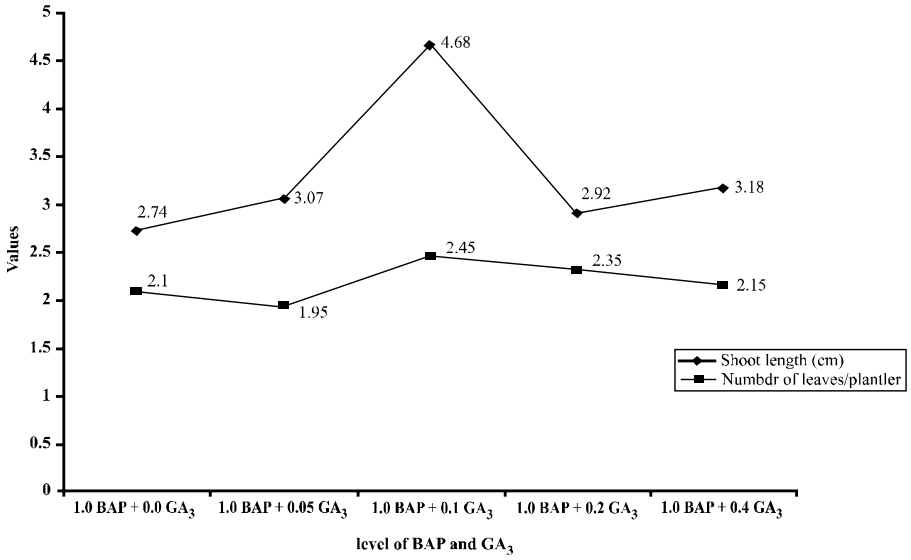

Fig. 2. Mean effect of different concentrations of BAP and GA_3.

Length of roots after 21 days

The highest length of root after 21 days (5.72cm) appeared with internode explants, followed by shoot tip (5.46 cm) and node explants (4.93cm). The lowest length (4.18cm) of root was with leaf' explants. Explants x growth regulators showed significant difference. The highest length (7.3 8cm) of root was observed after 21 days from internode explant x 1.0 mg/L IAA + 0.25 mg/L GA_3 concentration (Fig. 3). The lowest length (3.26 cm), in contrast, was observed from leaf explant with 1.0 mg/L IAA + 0.0 mg/L GA_3 (Table 3).

Length of roots after 28 days

Different explants also showed highly significant differences for root length. The highest length of root after 28 days (7.62cm) was observed at internode explant, followed by 6.43cm with node and 6.39cm with shoot tip explants. Different concentrations of growth regulators on the root length after 28 days also showed significant differences. The highest length of root (7.04cm) appeared with 1.0 mg/L IAA + 0.25 mg/L GA_3 concentration, while observed with the lowest length (5.57 cm) 1.0 mg/L IAA + 1.0 mg/L GA_3 concentrations (Table 3, Fig. 3). The other concentrations produced intermediate forms of root length, between the highest and lowest extremes.

Table 3. The interaction effects between explants and concentrations of IAA and GA_3.

Explants	Concentrations (mg/L)	Callus length (mm)	Callus weight (g)
Leaf	1.0 IAA + 0.0 GA_3	3.26 J	5.16 DE
	1.0 IAA + 0.25 GA_3	4.44 HI	4.20 F
	1.0 IAA + 0.5GA_3	5.10 EFGH	6.14 C
	1.0 IAA + 1.0 GA_3	3.60 J	3.64 F
	1.0 IAA + 2.0 GA_3	4.52 HI	6.28 C
	Mean	4.18C	5.08C
Node	1.0 IAA + 0.0 GA_3	4.48 HI	5.74 CD
	1.0 IAA + 0.25 GA_3	5.80 CD	7.44 B
	1.0 IAA + 0.5GA_3	5.42 DEF	7.46 B
	1.0 IAA + 1.0 GA_3	4.64 GHI	6.08 C
	1.0 IAA + 2.0 GA_3	4.34 1	5.44 D
	Mean	4.93 B	6.43 B
Internode	1.0 IAA + 0.0 GA_3	4.56 HI	7.52 B
	1.0 IAA + 0.25 GA_3	7.38 A	8.26 A
	1.0 IAA + 0.5GA_3	6.36 BC	8.22 A
	1.0 IAA ± 1.0 GA_3	5.02 FGHI	7.80 AB
	1.0 IAA + 2.0 GA_3	5.32 DEF	6.34 C
	Mean	5.72 A	7.62 A
Shoot tip	1.0 IAA + 0.0 GA_3	6.20 BC	7.44 B
	1.0 IAA + 0.25 GA_3	6.62 B	8.28 A
	1.0 IAA+0.5GA_3	3.52J	5.16 DE
	1.0 IAA + 1.0 GA_3	5.28 DEFG	4.78 F
	1.0IAA±2.OGA_3	5.72CDE	6.32C
	Mean	5.46 A	6.39 B

Means followed by similar letters in each column are not significantly different at 5% level according to DMRT.

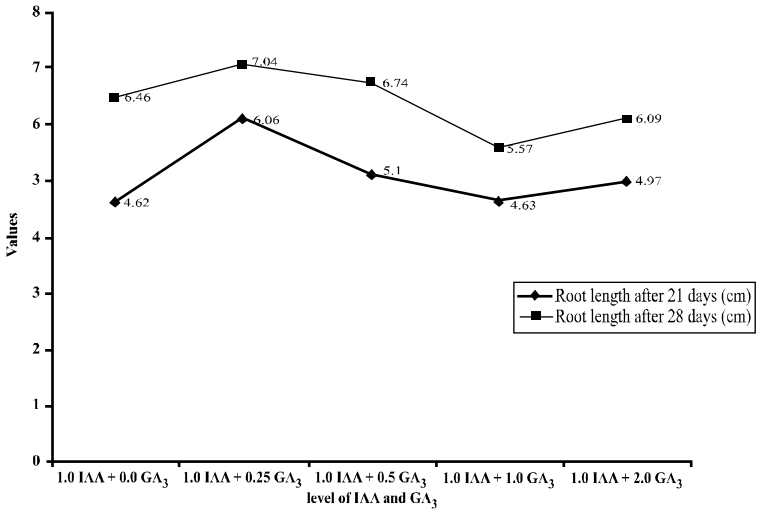

Fig. 3. Mean effect of different concentrations of IAA and GA_3.

From the above discussion, it is revealed that the variation of callus development among the different types of explants were not so wide. In the conclusion, the findings of the present study could be useful to develop protocol to identify the appropriate explants of potato with exact concentration. Furthermore, the results could be used to produce large scale production of healthy and disease free planting materials commercially.

References

Asma R. B. Askari, N. A. Abbasi, M. Bhatti and A, Quraishi, 2001. Effect of growth regulators on *in vitro* multiplication of potato. *International Journal of Agriculture and Biology* **3:** 181-182.

BBS. 2004. Statistical Pocket Book of Bangladesh Agricultural Statistics Wing (April), Bangladesh Bureau of Statistics, Bangladesh Secretariat, Dhaka, pp. 168.

Dobranszki J, H.A Takacs, T, K. Magyar and A. Ferenczy. 1999. Effect of medium on the callus forming capacity of different potato genotypes. *Acta Agronomica Hungarica* **47:** *59-6* 1.

FAO/CIP. 1995. Potatoes in the 1990s: Situation and prospects for the world economy. Rome: FAQ.

Fomenko T.I., V. N. Reshetnikov, M.K. Malyush, I.P. Kondratskaya and I.M. Chumakova. 1998. Conditions of development of callus tissues of potato *in vitro.* Vestsi Akademii Navuk Belarusi. *Seria Biyalagichnykh Navuk* **4:** 97-105.

Martel A. and E. Carcia. 1992. *In vitro* formation of adventitious shoots on discs of potato *(Solanuin tuberosum* L. cv. Sebago) tubers. *Phyton Buenos Aires* **53:** 57-64.

Murashige T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15:** 473-497.

Sarker R.H. and B.M. Mustafa. 2002. Regeneration and *Agrobacterium-mediated* genetic transformation of two indigenous potato varieties of Bangladesh. *Plant Tissue Culture* **12:** 69-77.

Solomon B.R.M. and H. Barker 2001. Breeding virus resistant potatoes *(Solanum tuberosun):* a reviev of traditional and molecular approaches. *Heredity* **86:** 17-35.

Zel J., M.M. Mlakar, B. Vilhar, D. Grill and H. Guttenberger 1999. The efficient regeneration of the potato *(Solanum tuberosum* L.) cv. Igor *in vitro.* Special issue: 2nd Slovenian symposium on plant physiology with international participation, Gozd Martuljek, Slovenia, September 30-October 2. 1998. *Phyton. Horn* **39:** 277-282.