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PERFORMANCE OF DIFFERENT PROTOCOLS ON *IN VITRO* TUBERIZATION IN POTATO (*Solanum tuberosum*)

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Abstract

Five protocols of micro tuberization were used to induce large size microtuber in three recommended potato varieties, namely Cardinal, Diamant, and Heera under complete dark condition. Tuberization was the earliest (11.8 days) in the protocol P_2 (MS + 5 mg/l BAP + 500 mg/l CCC + 8% sucrose), which was closely followed by that in P_1 (12.7 days) (MS + 5 mg/l BAP + 50 mg/l coumarin + 8% sucrose). Maximum number of microtubers/flask (12.8) was obtained from the protocol P_1 followed by that of P_2 (11.6) that contained growth retardant; but higher average weight of microtuber was obtained in the protocols P_5 (30 days old plantlet + MS media containing 40 meq K + 10 mg/l BA + 9% sucrose), P_4 (MS + 10 mg/l BA + 8% sucrose), and P_3 (MS + 5.0 mg/l BAP + 6% sucrose) which contained BA in absence of growth retardant. The average weight of microtuber was the highest (329.0 mg) in protocol P_5, followed by that in P_4 (280.7 mg), while it was the lowest in protocol P_1. The variety Diamant produced maximum average weight of microtuber (246.3 mg), while Heera produced minimum (226.1 mg), which was statistically similar to Cardinal (228.7 mg). The highest percentage (52.2) of >300 mg size and lowest percentage (19.3) of <150 mg size microtuber was produced in P_5 protocol in the variety Diamant. On overall consideration, all the varieties performed best with the protocol P_5.

Keywords: Protocol, microtuber, potato.

Introduction

Micropropagation systems through node and shoot culture and *in vitro* tuberization have enabled maintenance and propagation of disease-free planting materials in the laboratory for the potato breeder and farmer. Both shoot and *in vitro* tuberization systems are advantageous because of maintenance of disease-free materials. Moreover, *in vitro* tuberization of potato in the laboratory helps produce planting materials which are convenient to handle in transport, storage, and field planting. There are several published protocols for *in vitro* tuberization in potato (Kim, 1982; Wang and Hu, 1982; Hussey and Stacey, 1984; Tovar *et al.*, 1985). In general, there are two basic stages in all protocols. The first stage

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aims to produce vegetative growth and the second stage to induce tuberization and allow enlargement of the tuberlets. Different authors recommended different media for vegetative multiplication and tuberization. Tovar *et al.* (1985) recommended MS + 0.5 mg/l BAP + 0.4 mg/l GA+ 0.01 mg/l NAA + 2% sucrose for the propagation phase and MS + 5.0 mg/l BAP + 500 mg/l CCC + 8% sucrose for the tuberization phase, which was useful for over 50 different genotypes covering a wide genetic base and ploidy levels and found 10 microtubers per flask having a weight of 49.8 to 143.5 mg. Wang and Hu (1982) obtained microtubers having an average weight of 250 mg in MS +10.0 mg/l BAP + 8.0% sucrose while Hussey and Stacey (1984) found microtubers having a weight of 60 to 120 mg by using MS + 2.0 mg/l BAP + 6.0% sucrose. Kim (1982) induced 1-2 microtubers per node in MS + 5.0 mg/l BAP + 6% sucrose. It is clear that microtuber number and average weight varied among protocols. The discrepancies between these findings are probably due to the use of different genotypes (Coleman and Coleman, 2000; Zakaria *et al*., 2008a) different mixtures of nutrient salts, and/or different concentrations of the growth regulators added (Zakaria *et al*., 2008b), together with possible variation in the size of vessel, light intensity and temperature. Therefore, the present study was undertaken to evaluate the performance of different protocols on *in vitro* tuberization in different potato varieties and to find out variety specific protocols.

Meterials and Method

In vitro grown microplants of three recommended potato cultivars viz., Cardinal, Diamant and Heera were used in the study. The microplants were multiplied through subculture of single stem nodes at every three weeks interval in Tissue culture laboratory of Horticulture Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University. The experiment was carried out during the period from July to September 2008. The propagation media used for different protocols are mentioned in the Table 1. All the propagation media were solidified with 0.8% agar. Temperature of the growth chamber was maintained at 23 $\pm 1^{o}$C with 16 hours photoperiod at 3000 lux intensity from fluorescent tubes. At first phase, microplants were multiplied in solid media and then these were transferred into liquid media for microtuberization. Eight stem segments (Each with 3 nodes) of 30 days old *in vitro* microplants of Cardinal, Diamant, and Heera were cultured in 250 ml Erlenmeyer flasks containing 40 ml microtuber induction liquid medium, which was based on MS medium supplemented as per different protocols as shown in Table 2. The cultures were incubated in the dark at 20^{o} C in continuous dark. The experiment included five protocols and three recommended potato varieties which in combination made 15 treatment combinations. The experiment was laid out in a factorial Completely Randomized Design (CRD) with three replications. The induced microtubers were harvested aseptically after 70 days of incubation. The collected data were

analyzed with the help of computer using MSTAT-C programme and the mean separation was done by Duncan's New Multiple Range Test.

Table 1. Propagation media for *in vitro* multiplication of plantlet in potato.

Media code	Media composition	Reference
DOD	MS (Murashige and Skoog, 1962) + GA 0.4 mg/l + BAP 0.5 mg/l + NAA 0.01 mg/l + 2.5% sucrose	Dodds, *et al*., 1988
CIP	MS + GA 0.4 mg/l + BAP 0.5 mg/l + NAA 0.01 mg/l + 2% sucrose	Tovar *et al.,* 1985
KIM	MS + 0.1 mg/l GA + 0.5 mg/l Kinetin + 2% sucrose.	Kim, 1982
SAR	MS + 0.1 mg/l GA + 0.01 mg/l NAA + 4 mg/l Calcium pantathonate + 3% sucrose.	Sarkar *et al.,* 1997

Table 2. Different tuberization protocols of potato.

Protocol code	Propagation medium code	Tuberization media
P_1	DOD	MS + 5 mg/l BAP + 50 mg/l coumarin + 8% sucrose (Dodds *et al*., 1988).
P_2	CIP	MS + 5 mg/l BAP + 500 mg/l CCC + 8% sucrose (Tovar *et al.,* 1985).
P_3	KIM	MS + 5.0 mg/l BAP + 6% sucrose, (Kim, 1982).
P_4	SAR	MS + 10 mg/l BA + 8% sucrose (Naik *et al.,* 1998)
P_5	SAR	MS with 60 meq N & 40 meq K + 10 mg/l BA + 9% sucrose (Zakaria, 2003)

Results and Discussion

Performance of protocols: Tuberization was the earliest (11.8 days) with P_2 (CIP) protocol closely followed by P_1 (Table 3). Tuberization was most delayed (15.9 days) with P_3 protocol. Rapid tuberization with P_2 protocol might be due to the presence of optimum concentrations of BA and CCC in the medium. Microtuberization promoted in presence of CCC (Hossain and Sultana, 1998; Zakaria *et al*., 2008b) and BA (Islam, 1995 and Zakaria *et al*., 2008b). Protocol P_5 showed earlier tuberization than P_3 which may be due to the presence of high concentration of BA and sucrose. Such a result was also reported by Palmer and Smith (1969) and Wang and Hu (1982). The maximum number of microtubers/flask (12.8) was with protocol P_1 closely followed by P_2 (11.6). The minimum number of tubers/flask was with P_3 (9.9). The protocols P_1 and P_2 showed higher number of microtubers per flask because of the presence of 50 mg/l coumarin and 500 mg/l CCC, respectively. More number of microtubers was reported with 50 mg/l coumarin (Dodds *et al.,* 1988) and with 500 mg/l CCC

(Hossain and Sultana, 1998). Coumarin at the rate of 50 mg/l produced more number of microtubers than 500 mg/l CCC (Dodds *et al.*, 1988) which corroborate with the present findings. The average weight of microtuber was the highest (329.0 mg) with P_5 protocol, which was closely followed by P_4 (280.7 mg), while it was the lowest with P_1 protocol. The protocol P_5 produced the highest average weight of microtuber might be due to the presence of high concentrated BA (10 mg/l) and sucrose (9%). High concentration of BA in presence of high concentration of sucrose showed best performance in micro tuberization (Texeira and Pinto, 1991 and Naik *et al.*, 1998). The heaviest microtuber with 10 mg/l BA (Teixeira and Pinto, 1991) and with 9% sucrose was reported by Jeoung-Lai *et al.* (1996). Production of lower weight of microtubers by protocols P_1 and P_2 might be due to presence of growth retardant (Dodds *et al.*, 1988; Harvey *et al.*, 1991 and Lian-Yong *et al.*, 1996). The highest percentage (44.6) of large size (>300 mg) microtuber was produced with protocol P_5 followed by P_4 (29.3%), while in P_1, no large sized microtubers were produced. The maximum percentage (45.4) of <150 mg size microtubers was found with P_1. Dodds *et al.* (1988) found very small (<100 mg) microtubers with P_1 protocol. Tovar *et al.*(1985) also reported small microtubers (<150 mg) with P_2 protocol. Wang and Hu (1982) found the highest microtuber by using 10 mg/l BA in combination with 8% sucrose.

Table 3. Effect of protocols on induction and development of potato microtuber.

Protocols	Days to tuber initiation	No. of microtubers/ flask	Av. wt of microtuber (mg)	Grade of microtubers by number (%)		
				<150mg	150-300mg	>300 mg
P_1	12.7 c	12.8 a	157.4 e	45.4	54.6	0
P_2	11.8 c	11.6 b	177.5 d	44.3	52.0	3.7
P_3	15.9 a	9.9 d	223.9 c	32.4	48.2	19.5
P_4	14.9 ab	10.4 c	280.7 b	27.0	43.7	29.3
P_5	14.2 b	10.9 c	329.0 a	21.3	34.0	44.6
Level of significance	**	**	**	NA	NA	NA
CV (%)	3.57	3.14	2.16	-	-	-

Means bearing same letter (s) in the same column do not differ significantly at 1% level of probability. NA- Not analyzed

Performance of potato variety: Microtuber initiation was most delayed (15.1 days) in the variety Diamant, while it was most rapid (12.3 days) in Heera (Table 4). This finding is consistent with the normal behaviour of potato cultivar in the field condition where tuberization occurs earlier in Heera than in Cardinal and Diamant (Rashid, 1999). Islam (1995) reported that microtuber initiation time

varied with genotype. He found earlier microtuber initiation with exotic varieties (Cardinal, Kufri Shindhuri) compared to local varieties of potato. Statistically similar numbers of microtuber per flask were observed with Cardinal and Diamant. The minimum number of microtubers was found with Heera. Varietal differences on the number of microtubers were found by Islam (1995) who reported that the local varieties of potato produced poor number of microtubers compared to exotic varieties. Hossain and Sultana (1998) also found that the variety Chamak produced very poor number of microtubers compared to Patrones. Average weight of microtuber was the highest (246.3 mg) in Diamant (Table 4). The minimum average weight of microtuber was shown by Heera, which was statistically similar to Cardinal. Genotypic variation on average weight of microtuber was observed by Islam (1995) who reported that the exotic varieties of potato produced higher average weight of microtuber than local varieties. Hossain and Sultana (1998) found that the variety Patrones produced significantly heavier microtuber compared to Lalsheel and Chamak. The highest percentage (22.0) of >300 mg size microtuber was produced by Diamant, while it was minimum in Heera (17.1%). Cardinal produced maximum percentage (35.7) of <150 mg size microtuber followed by Heera (34.2%) and Diamant (32.4%).

Table 4. Effect of variety on induction and development of potato microtuber.

Variety	Days to tuber initiation	No. of microtubers/ flask	Av. wt of microtuber (mg)	Grade of microtubers by number (%)		
				<150mg	150-300 mg	>300mg
Cardinal	14.3 b	11.5 a	228.7 b	35.7	45.2	19.1
Diamant	15.1 a	11.9 a	246.3 a	32.4	45.6	22.0
Heera	12.3 c	9.9 b	226.1 b	34.2	48.7	17.1
Level of significance	**	**	**	NA	NA	NA
CV (%)	3.57	3.14	2.16	-	-	-

Means bearing same letter (s) in the same column do not differ significantly at 1% level of probability. NA- Not analyzed.

Interaction effect of protocols and variety: The protocol P_2 followed by P_1 enhanced tuber initiation in all the varieties (Table 5). Tuber initiation was most delayed (17.3 days) with protocol P_3 in Diamant. Hossain and Sultana (1998) found that the tuberization was earlier with 5 mg/l BA + 500 mg/l CCC irrespective of genotypes which corroborates the present findings. The highest number of microtubers (13.7) was produced with P_1 protocol in Diamant. The lowest number of microtubers per flask was produced by P_3 protocol in case of all varieties. Hossain and Sultana (1998) found maximum number of microtubers per plant with 5 mg/l BAP + 500 mg/l CCC, which was similar to the

Table 5. Interaction effect of protocols and potato varieties on induction and development of microtuber.

Treatment combination Protocol × Variety			Days to tuber initiation	No. of microtubers/ flask	Average wt of microtubers (mg)	Grade of microtubers by number (%)		
						<150 mg	150-300 mg	>300 mg
P_1	X	Cardinal	12.7de	13.0ab	145.1j	45.7	54.3	0
		Diamant	13.3d	13.7a	162.1i	46.7	53.3	0
		Heera	12.0ef	11.7cde	165.0i	43.8	56.2	0
P_2		Cardinal	11.3f	12.0cd	160.3i	51.6	48.4	0
		Diamant	12.7de	12.3bc	171.3i	42.1	57.9	0
		Heera	11.3f	10.3gh	201.4h	39.2	49.8	11
P_3		Cardinal	16.7ab	10.3gh	216.2g	32.3	47.1	20.6
		Diamant	17.3a	10.7fg	235.3f	29.4	49.1	21.5
		Heera	13.3d	8.7j	220.2g	35.4	48.3	16.3
P_4		Cardinal	15.7bc	11.0efg	285.4d	28.1	45.2	26.7
		Diamant	16.6ab	11.3def	297.4c	24.2	39.3	36.5
		Heera	12.3def	9.0ij	259.4e	28.7	46.6	24.7
P_5		Cardinal	15.0c	11.3def	336.5b	20.6	31.2	48.2
		Diamant	15.7bc	11.7cde	365.5a	19.3	28.5	52.2
		Heera	12.0ef	9.6i	285.1d	24.1	42.4	33.5
Level of significance			**	**	**	NA	NA	NA
CV (%)			3.57	3.14	2.16	-	-	-

Means bearing same letter (s) in the same column do not differ significantly at 1% level of probability. NA- Not analyzed.

performance of P_2 protocol. Dodds *et al.* (1988) found more number of microtubers by using 50 mg/l coumarin (P_1) instead of 500 mg/l CCC (P_2). The protocol P_5 produced the highest (365.5 mg) average weight of microtuber in the variety Diamant (Table 5). Lower average weight of microtuber was with P_1 and P_2 protocol in all varieties. Lower microtuber weight might be due to the presence of growth retardant which reduced microtuber weight (Harvey *et al.*,1991; Leclerc *et al.*, 1994 and Lian-Yong *et al.*, 1996). Higher microtuber weight with P_5 and P_4 was obviously due to the presence of high concentration of BA and sucrose (Koda and Okazawa, 1983; Wang and Hu, 1982, and Palmar and Smith, 1969). The highest percentage (52.2) of >300 mg size and lowest percentage (19.3) of <150 mg size microtuber was produced with P_5 protocol in Diamant variety of potato (Table 5). In case of all varieties, the protocol P_1 did not produce any microtuber of size >300 mg, while P_2 produced the highest percentage (51.6) of <150 mg size microtuber in Cardinal.

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