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Standardization of Sucrose and 6-Benzyl Aminopurine for *in vitro* Micro Tuberization of Potato

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Abstract: *In vitro* micro tuberization from regenerated plantlets of potato varieties was observed in Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University. Tubers of potato cultivars Diamant and Cardinal were used as initial experimental materials for meristem culture. The experiment was consisted of three factors; variety (Diamant, Cardinal), sucrose concentration (3%, 6%, 9%, 12%) and 6-benzyl aminopurine (BAP) levels (2.5, 5.0, 7.5 mg L^{-1}). As a whole, 24 treatments were laid out in complete randomized design with three replications. Among the varieties, Diamant required minimum days (6-17) for micro-tuber initiation, produced more number of micro-tubers (4.97) and produced more average weight of micro-tuber (120.39mg) but there had no significant difference. Among the sucrose levels, quickest (6-15 days) micro-tuber initiation, the highest number of micro-tubers $vial^{-1}$ (5.06) and the highest average weight of micro-tuber (137.31 mg) were found in 9% sucrose level. For different BAP levels, quickest (6-15 days) micro-tuber initiation, the highest number of micro-tubers $vial^{-1}$ (5.38) and the highest average weight of micro-tuber (126.31 mg) were found at 5.0 mg L^{-1}. The best combination for minimum duration (6-8 days) of micro-tuber initiation, the highest number of micro-tubers $vial^{-1}$ (6.00) and the highest average weight of micro-tuber (152.01 mg) was in Diamant with 9% sucrose at 5 mg L^{-1}. Concomitantly, the lowest number micro-tubers $vial^{-1}$ (2.00) and the lowest average weight of micro-tuber (89.98 mg) were found in Cardinal cultured with 3% sucrose media where at 7.5 mg L^{-1} BAP and at 2.5 mg L^{-1} BAP, respectively.

Keywords: 6-Benzyl Aminopurine, Microtuberization, Plantlets, Potato

1. Introduction

Potato (*Solanum tuberosum* L.) is an important vegetables crop and is grown in winter only in Bangladesh [15]. Microtubers have become an important mode of rapid multiplication for seed tuber of potato [21]. These micro-tubers are utilized for minitubers production in greenhouse or screen house. Wherever microtuber and minituber production technologies have been implemented, they have halved the field time necessary for conventional method to supply to the commercial growers. Microtubers from meristem grown and/or regenerated microplants are now produced and used in Australia, Brazil, Chile, China, Ecuador, India, Indonesia, Kenya, Korea, Peru, Philippines, Taiwan, UK, Vietnam and even in Bangladesh as disease free seed [7]. However, the technique is controlled by various physical (light, temperature etc.) and chemical (growth regulators) factors. Among the media components, sucrose played an important role in the induction and development of potato microtubers on in vitro [10] and BAP promoted initiation and growth of micro-tubers [19]. Various sucrose concentrations were tested in the microtuber induction medium where the best results were obtained with 8% [12], with 10% [3], with 12% [20], with 9% and 12% [13] and with 8% and 12% [18]. Tuber induction medium combined with 9% sucrose promoted tuberization and increased microtuber weight more than the

tuber induction medium combined with 6% sucrose, BAP and CCC [11]. Better micro-tuber yield was obtained with 6% sucrose and 5 mg L^{-1} BAP under complete dark condition [1]. Microtuber number and fresh weight were greatest with 10 mg L^{-1} BAP in presence of 6% sucrose [16]. Best response to both initiation and production of microtubers were observed in modified MS media containing 5.0 mg L^{-1} BAP with 500 mg L^{-1} CCC [6] and by using 500 mg L^{-1} CCC together with 5.0 mg L^{-1} BAP[9]. Therefore, the present investigation was undertaken to find out the appropriate doses of sucrose and growth regulators and their combination(s) with potato varieties for rapid microtuberization.

2. Materials and Methods

2.1. Design and Treatments of the Experiment

The experiment was carried out in Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University. The experiment was consisted of three factors; variety (Diamant, Cardinal), sucrose levels (3%, 6%, 9%, 12%) and BAP levels (2.5, 5.0, 7.5 mg L^{-1}). As a whole, 24 were laid out in complete randomized design with three replications.

2.2. Explant, Culture Media and Regeneration of Plantlets

Meristem was used to regenerate for virus free plantlet production. From the regenerated virus free plantlets, plantlets again multiplied through rapid multiplication technique [8]. Required amount of MS medium [14] was prepared and supplemented with mentioned sucrose and BAP levels. Thus, prepared all groups of media were taken to separate test tubes and/or vials according to the number of treatments and replications. Then the test tubes/vials containing the media were sterilized by autoclaving. Stem segments having 1-5 nodes from plantlets of one month old which were collected from previous experiment were cultured for microtuberization. Single stem segments were placed to each test tube containing 10 ml medium and 4-5 stem segments were placed to each vial containing 40 ml medium. When placed in test tube, five test tubes were considered as one replication.

2.3. Incubation

The cultures were incubated at a temperature of 25±2^{o}C at 16 hrs photoperiod. Days to microtuber initiation were recorded. After two months of explantation, number of microtubers $vial^{-1}$ was calculated on the basis of total number of vials per treatment and average weight of microtuber was calculated on the basis of total number of microtubers produced.

2.4. Data Collection and Statistical Analysis

After two months of explantation, data were recorded on 1) Days to microtuber initiation, ii) Number of microtubers $vial^{-1}$, iii) Average weight of microtuber. Data were analyzed by statistical software MSTATC and means were adjudged by the Duncan's Multiple Range Test.

3. Results and Discussions

3.1. Main Effect of Variety, Sucrose and BAP on Days to Micro-Tuberization, Number and Weight of Microtubers

Between the varieties, Diamant required comparatively minimum days for microtuber initiation, which started at 6 days and continued up to 17 days, than that of Cardinal where the range was from 8 to 21 days. These findings were supported by Hossain and Sultana (1998) who reported that the response of variety to microtuberization was highly dependent on genetic factors. Microtuber number $vial^{-1}$ did not vary significantly and the result showed that Diamant produced more number of microtubers (4.97) $vial^{-1}$ than that of Cardinal (4.19). The differences of average weight of microtuber were found non-significant where the average weight of microtubers were 120.39 mg and 114.89 mg in case of Diamant and Cardinal, respectively. Considering different concentrations of sucrose, the duration required for microtuber initiation varied with the variation of sucrose levels used in the culture media. Microtuber initiation was quickest (6-15 days) when 9% sucrose was used and this duration gradually increased with both increasing and decreasing the level of sucrose. The present findings were directly in agreement with Jeoung-Lai *et al.* (1996) who reported that tuber induction medium combined with 9% sucrose promoted tuberization. Microtuber numbers $vial^{-1}$ increased with the increasing of sucrose level up to 9% and then again decreased. The highest number of microtubers $vial^{-1}$ was found in 9% sucrose (5.06) followed by 6% sucrose (4.78), whereas, the lowest was found with 3% sucrose (4.06). The highest average weight of microtuber (137.31 mg) was found in 9% sucrose followed by 12% (119.17 mg) while the lowest (99.01 mg) was in 3% sucrose. The findings were similar to the findings of Jeoung-Lai *et al*. (1996) who found the heaviest microtubers with 9% sucrose. Among the three concentrations of BAP, duration required for microtuber initiation was 6-21 days at 2.5 mg L^{-1} BAP, 6-15 days at 5.0 mg L^{-1} BAP and 7-20 days at 7.5 mg L^{-1} BAP. The highest number of microtubers $vial^{-1}$ (5.38) was obtained at 5 mg L^{-1} BAP, whereas 2.5 and 7.5 mg L^{-1} BAP produced about similar number of microtubers (4.25 and 4.13 respectively) $vial^{-1}$. The present finding was supported by Yong *et al*. (1996) who reported that BAP promoted initiation and growth of microtubers. The highest average weight of microtuber (126.31 mg) was found in 5 mg L^{-1} BAP followed by 7.5 mg L^{-1} BAP (115.29 mg) and the lowest (111.33 mg) was in 2.5 mg L^{-1} BAP. (Table 1)

Table 1. *Main effect of variety, sucrose and BAP on days to micro-tuberization, number and weight of microtubers at two months of ex-plantation.*

Treatments		Days to micro tuberization	No. microtubers vial^{-1} of 5 plants	Average weight of microtuber (mg)
Variety	Diamant	6-17	4.97 a	120.39 a
	Cardinal	8-21	4.19 a	114.89 a
Sucrose (%)	3	9-17	4.06 b	99.01 d
	6	9-15	4.78 ab	115.10 c
	9	6-15	5.06 a	137.31 a
	12	10-21	4.44 ab	119.17 b
BAP (mg L^{-1})	2.5	6-21	4.25 b	111.33 c
	5.0	6-15	5.38 a	126.31 a
	7.5	7-20	4.13 b	115.29 b
CV (%)		-	16.86	2.13

Figures followed by same letter(s) are statistically similar as per DMRT

3.2. Combined Effect of Variety and Sucrose on Days to Microtuber Initiation and Number and Weight of Microtubers

Considering the variety and sucrose combinations, Diamant with 9% sucrose required comparatively minimum time (6-11 days) for microtuber initiation and maximum (12-17 days) with 12% sucrose (Table 2). Accordingly, Cardinal with 9% sucrose required 8-15 days and with 12% sucrose required 10-21 days for microtuber initiation. The highest number of microtubers vial^{-1} (5.56) was found with Diamant at 9% sucrose followed by Diamant with 6% sucrose (5.33), whereas the lowest number (3.33) was obtained from Cardinal with 3% sucrose (Table 2). Varietal difference on microtubers number with various concentrations of sucrose was observed by Warren and Shirlyn (2000) where number of microtubers increased with the increasing concentration of sucrose up to 16% in the variety Shepody. On the other hand, the highest average weight of microtuber (140.15 mg) was found in Diamant with 9% sucrose, whereas the lowest (95.56 mg) was in Cardinal with 3% sucrose.

3.3. Combined Effect of Variety and BAP on Days to Microtuber Initiation and Number and Weight of Microtubers

In case of variety and BAP combinations (Table 3), Diamant with 5.0 mg L^{-1} BAP required comparatively minimum (6-15 days) and Cardinal with 2.5 mg L^{-1} BAP required maximum (9-21 days) time for microtuber initiation. The maximum number of microtubers vial^{-1} (5.58) was obtained from Diamant at 5 mg L^{-1} BAP, whereas the lowest number (3.67) was obtained from Cardinal at 7.5 mg L^{-1} BAP. Thus, the highest average weight of microtuber (127.96 mg) was found in Diamant with 5 mg L^{-1} BAP followed by Cardinal with 5 mg L^{-1} BAP (124.67 mg) while the lowest weight (107.97 mg) was found in Cardinal with 2.5 mg L^{-1} BAP.

3.4. Combined Effect of Sucrose and BAP on Days to Microtuber Initiation and Number and Weight of Microtubers

In case of sucrose and BAP combination (Table 4), 9% sucrose performed minimum time (6-10 days) for microtuber initiation with 5.0 mg L^{-1} BAP followed by 2.5 mg L^{-1} BAP (6-12 days), whereas maximum time (15-20 days) was taken for 12% sucrose with 7.5 mg L^{-1} BAP. The highest number of microtubers vial^{-1} (5.67) was obtained with 6% and 9% sucrose at 5 mg L^{-1} BAP followed by 12% sucrose with 5 mg L^{-1} BAP (5.17), whereas, the lowest number (3.33) was found at 3% sucrose with 7.5 mg L^{-1} BAP. The result was directly supported by Texeira and Pinto (1991). Average weight of microtuber (150.38 mg) found to be the best in combination of 9%sucrose and 5 mg L^{-1} BAP, whereas, the lowest (93.81 mg) was in 3% sucrose and 2.5 mg L^{-1} BAP. Variation in microtuber yield were also recorded by Al-Abdallat and Suwwan (2002) where 0, 3, 6, and 9% sucrose and 5 mg L^{-1} BAP were used and better microtuber yield was obtained with 6% sucrose under complete dark condition. [Table 4]

3.5. Combined Effect of Variety, Sucrose and BAP on Days to Microtuber Initiation and Number and Weight of Microtubers

Considering all the three factors (Table 5), it was observed that the best combination was Diamant with 9% sucrose at 2.5 and at 5.0 mg L^{-1} BAP which required minimum duration (6-8 days), whereas, Cardinal with 12% sucrose at 2.5 mg L^{-1} BAP required maximum duration (16-21 days) for microtuber initiation. On the other hand, the highest number of microtubers vial^{-1} (6.00) was obtained from Diamant when cultured on media with 6% and 9% sucrose at 5 mg L^{-1} BAP. At the same time, the lowest number (2.00) was found in Cardinal cultured on media with 3% sucrose and 7.5 mg L^{-1} BAP (Plate 2). The present result may be considered as similar to the previous study of Estrada *et al.* (1986) where it was observed that MS medium with 5.0 mg L^{-1} BAP, 8% sucrose and 500 mg L^{-1} CCC would able to produce microtubers in a broad range of genotypes. Thus, the highest average weight of microtubers (152.01 mg) was found in Diamant with 9% sucrose and 5 mg L^{-1} BAP followed by Cardinal with 9% sucrose and 5 mg L^{-1} BAP (148.75 mg). Concomitantly, the lowest average weight of microtuber (89.98 mg) was found in Cardinal with 3% sucrose and 2.5 mg L^{-1} BAP. (Table 5)

Table 2. *Combined effect of variety and sucrose on days to microtuber initiation and number and weight of microtubers after two months of inoculation.*

Combination		Days to microtuber initiation	No. microtubers vial^{-1} of 5 plants	Average weight of microtuber (mg)
Variety	**Sucrose (%)**			
Diamant	3	9-15	4.78 ab	102.44 e
	6	9-13	5.33 ab	118.55 c
	9	6-11	5.56 a	140.15 a
	12	12-17	4.22 bc	120.41 c
Cardinal	3	10-17	3.33 c	95.56 f
	6	10-15	4.22 bc	111.64 d
	9	8-15	4.56 ab	134.46 b
	12	10-21	4.67 ab	117.93 c
CV (%)		-	16.86	2.13

Table 3. *Combined effect of variety and BAP on days to microtuber initiation and number and weight of microtubers after two months of inoculation.*

Combination		Days to microtuber initiation	No. microtubers vial^{-1} of 5 plants	Average weight of microtuber (mg)
Variety	**BAP (mg L^{-1})**			
Diamant	2.5	6-15	4.75 ab	114.69 bc
	5.0	6-15	5.58 a	127.96 a
	7.5	7-17	4.58 ab	118.52 b
Cardinal	2.5	9-21	3.75 b	107.97 d
	5.0	8-15	5.17 a	124.67 a
	7.5	10-20	3.67 b	112.06 cd
CV (%)		-	16.86	2.13

Table 4. *Combined effect of sucrose and BAP on days to microtuber initiation and number and weight of microtubers after two months of inoculation.*

Combination		Days to microtuber initiation	No. microtubers vial^{-1} of 5 plants	Average weight of microtuber (mg)
Sucrose (%)	**BAP (mg L-1)**			
3	2.5	12-16	3.83 cd	93.81 h
	5.0	9-12	5.00 ab	105.10 f
	7.5	11-17	3.33 d	98.10 g
6	2.5	9-14	4.50 bc	106.36 f
	5.0	9-13	5.67 a	123.71 d
	7.5	9-15	4.17 bcd	115.22 e
9	2.5	6-12	4.83 abc	128.93 bc
	5.0	6-10	5.67 a	150.38 a
	7.5	7-15	4.67 abc	132.61 b
12	2.5	13-21	3.83 cd	116.23 e
	5.0	10-15	5.17 ab	126.07 cd
	7.5	15-20	4.33 bcd	115.21 e
CV (%)		-	16.86	2.13

Figures followed by same letter(s) are statistically similar as per DMRT

Table 5. *Combined effect of variety, sucrose and BAP on days to microtuber initiation and number and weight of microtubers after two months of inoculation.*

Treatment combination			Days to micro-tuber initiation	No. microtubers vial^{-1} of 5 plants	Average weight of microtuber (mg)
Variety	**Sucrose (%)**	**BAP (mg L^{-1})**			
Diamant	3	2.5	12-15	4.33 b-e	97.64 ij
		5.0	9-12	5.33 abc	108.50 h
		7.5	11-13	4.67 a-e	101.19 i
	6	2.5	9-13	5.00 a-d	111.53 gh
		5.0	10-13	6.00 a	124.86 d
		7.5	9-11	5.00 a-d	119.26 ef
	9	2.5	6-8	5.67 ab	130.36 c
		5.0	6-8	6.00 a	152.01 a
		7.5	7-11	5.00 a-d	138.09 b
	12	2.5	13-14	4.00 cde	119.23 ef
		5.0	12-15	5.00 a-d	126.48 cd
		7.5	15-17	3.67 de	115.51 fg
Cardinal	3	2.5	13-16	3.33 ef	89.98 k
		5.0	10-12	4.67 a-e	101.70 i
		7.5	15-17	2.00 f	95.01 j
	6	2.5	11-14	4.00 cde	101.19 i
		5.0	9-11	5.33 abc	122.56 de
		7.5	13-15	3.33 ef	111.18 gh
	9	2.5	9-12	4.00 cde	127.51 cd

Treatment combination			Days to micro-tuber initiation	No. microtubers vial^{-1} of 5 plants	Average weight of microtuber (mg)
Variety	Sucrose (%)	BAP (mg L^{-1})			
		5.0	8-10	5.33 abc	148.75 a
		7.5	10-15	4.33 b-e	127.13 cd
	12	2.5	16-21	3.67 de	113.22 gh
		5.0	10-15	5.33 abc	125.65 cd
		7.5	17-20	5.00 a-d	114.91 fg
CV (%)				16.86	2.13

Figures followed by same letter(s) are statistically similar as per DMRT

A. During explantation B. Fifteen days after explantation C. One month after explantation D. Two months after explantation

Plate 1. *Microtuberization stages of potato cv. Diamant.*

A. Diamant with 6% Sucrose and 5 mg L^{-1} BAP B. Cardinal with 3% Sucrose and 7.5 mg L^{-1} BAP

Plate 2. *Number of microtubers at two months of explantation under different treatments.*

A. Cardinal B. Diamant

Plate 3. *Microtubers of two potato varieties after harvest.*

4. Conclusion

Microtuber production was affected by different levels of sucrose and 6-benzyl aminopurine (BAP). Genotypic variation was also found among the two genotypes regarding microtuber production. The best combination for rapid microtuber production of potato varieties was Diamant with 9% sucrose at 5 mg L^{-1} of 6-benzyl aminopurine. Hence the present protocol has the potential for the rapid multiplication of true-to-true type clones without changing the genetic fidelity.

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