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IN VITRO TUBERIZATION OF POTATO INFLUENCED BY BENZYL ADENINE AND CHLORO CHOLINE CHLORIDE

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Abstract

In an experiment, six levels of benzyl adenine (0.2.5,5.0,7.5,lO.0,12.5 and 15.0 mg/I) in combination with three levels of (CCC) chloro choline chloride (0,125,250 and 500 mg/I) were evaluated against control treatment (0) in each case to find out their optimum levels for microtuberization in potato variety Diarnant. Microtuberization was the earliest by I 3 days at 10 mg/I BA. The number and average weight of microtuber per flask vas increased with increasing rate of BA and reached maximum of 12.9 and 252.1 mg respectively at 10 mg/I BA and decreased onwards with further increase of BA concentration. The increase rate of CCC increased the number of microtuber but decreased average weight. The maximum number and average weight of microtuber were recorded at 500 mg/I CCC and in absence of CCC respectively.

Key Words: BA, CCC, potato, microtuber.

Introduction

Microtubers have become an important mode of rapid multiplication for pre-basic stock in seed tuber multiplication as well as gerrnplasrn exchange. The number and size of microtubers produced *in vitro* depend on many factors, such as optimum concentration of sugar, growth regulators and anti-gibberellin compounds in the culture medium (Tovar *et al.,* 1985; Dodds *et al.,* 1988). Among the substances used to induce microtubers, cournarin, CCC and cytokinins have received adequate attention (Wang and Hu, 1985; Chandra *ci a!.*. 1988). Cytokinins are believed to have strong promotive effects on tuberization, and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (Palmer and Smith, 1969; Pelacho and Mingo-Castel, 1991). Growth retardant stimulates tuberization of plants under unfavourable environmental conditions (Menzel. 1980) and CCC (Chioro choline chloride) is widely used in tissue culture media to promote microtuher formation (Tovar *et al.,* 1985 and Rosell *et al.,* 1987). It induces microtuber formation by a wide range of *Solanum* genotypes of diverse genetic backgrounds (Estrada *et al.,* 1986). Although CCC stimulates tuber initiation by recalcitrant genotypes, it can

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inhibit microtuher growth in *Solanum tuberosum* cultivars that form tubers readily in its absence. Vecchio *et al.* (1994) reported that *in vitro* tuberization is linked with the genotype and the culture substratum. According to them, the potato variety Desiree tuberized readily than other varieties with a low CCC concentration. Very little information is available about the influence of BA and CCC in microtuberization on the recommended variety Diamant. Therefore, the present investigation was undertaken to determine the optimum level ol BA and CCC for getting higher proportion of large size microtubers of Diamant.

Materials and Method

In vitro ready stocks of plantlets of potato variety Diamant were used and the number of plantlets was increased by subculturing via nodal cutting at 3 weeks interval. The multiplication medium contained mineral salts and vitamins (Murashige and Skoog. 962) plus 0.1 mg/I GA_3, 0.01 mg/I NAA, 4 mg/I calcium pantathonate and 30g/l sucrose. The medium was solidified with 8 g/l agar. Temperature in the growth chamber was 23 ± 1°C with 16 hours photoperiod and light was supplied by fluorescent tubes at an intensity of 3000 lux.

Eight stem segments (each with 3 nodes) of subcultured *in vitro* plantlets were cultured in 250 ml Erlenmeyer flasks containing 40 ml microtuber induction medium, which was based on MS medium (Murashige and Skoog. 1962) supplemented with 80 g/l sucrose. Six levels of Benzyl adenine (BA) and three levels Chloro choline chloride (CCC) having control treatment (0) in each case were added in the culture medium, which in combination made 28 treatment combinations. The experiment was laid out in a Completely Randomized Design (CRD) with four replications. The number of flask per replication was 72. The induced microtubers were harvested aseptically after 70 days of incubation period. The collected data were analyzed with the help of computer using MSTAT-5 program and the mean separation was done by Duncan's new multiple range test.

Results and Discussion

Effect of Benzyl Adenine: Microtuberization was the earliest (13.3 days) at 10 mg/I BA (Table 1). Either higher or lower concentration of this level greatly delayed tuherization. Plants grown in the medium without BA took the longest time for tuberization (20.1 days). The number of microtuber per flask increased with increasing rate of BA upto 10 mg/I, and then gradually decreased with further increase of BA concentration. The results are similar to the findings of Wang and Hu (1982) who reported that at a higher concentrations of BA above 10 mg/I in the medium decreased the number of microtubers. The concentration

of BA at 10 mg/I produced the highest weight of microtuber (252.1 mg) as compared to other concentrations. This agreed well with the results of similar works by other workers (Wang and Hu, 1982; Teixeira and Pinto, 1991). The highest percentage of >300mg size microtuher was produced with 10 mg/I BA (28.7), while no microtuber of >300 mg size was found vith 2.5 and 5.0 mg/I BA (Table 1).

Table 1. Effect of BA on induction and development of potato microtuber.

BA (mg/I)	Days to tuber initiation	No. of microtubers/ flask	Av. wt of microtuber (mg)	Grade of microtuber by number (%)		
				<150gm	150-300mg	>300mg
0	20.1 d	6.5 e	83,6 f	100	0	0
2.5	17.6 c	7.3 de	117.6 e	91.3	8.7	0
5.0	16.2b	7.8d	160.Od	48.4	51.6	0
7.5	*15.4* b	9.3 c	185.8 c	38.6	53.2	8.2
10.0	13.3 a	12.9 a	252.1 a	27.0	44.3	28.7
12.5	16.0 b	10.7 b	199.6 b	40.6	50.0	9.4
15.0	18.4c	9.2c	168.1 d	43.1	54.0	2.9
Level of significance	**	**	**			

Commonly, cytokinins are included in the media for *in vitro* tuberization of potatoes (Wang and Hu, 1982). Cytokinins promote *in vitro* tuberization of potato by altering GA balance in non-induced stems (Lentini and Earle, 1991), inhibiting root formation and transferring the upright leafy shoots into horizontal stolons (Shibli *et al.,* 2001). At 2% sucrose, cytokinin fails to exert stimulating effect of tuherization at any concentration (Koda and Okazawa, 1983). They further noted that sucrose concentration at above 4%, cytokinins exhibited a promoting effect on tuberization. Requirement of high concentration of sucrose by cytokinins for *in vitro* tuberization was also reported by Palmer and Smith (1969) and Wang and Hu (1982). For several reasons, cytokinin has often been considered to be an important factor for tuberization process. Firstly, cytokinin is known to stimulate cell division (Skoog and Miller, 1957); secondly, there are indications that it inhibits cell elongation (Vanderhoef and Key, 1968), and promote cell expansion (Scott and Liverman. 1956). These phenomena are required for tuber formation and development. Several workers have, therefore, suggested that the unknown tuberization stimulus could be a cytokinin like substance (Madec, 1963; Courduroux, 1966). Although cytokinin is not directly responsible for tuberization as reported by many workers, without doubt, it plays a key role in cell division and thus creating sink activity of the developing tuber.

Effect of chioro choline chloride (CCC): The time required for tuber initiation was reduced with increasing concentration of CCC (Table 2). Tuberization was the most early with CCC concentration at 500 mg/I (15.9 days), while it was the most delayed in absence of CCC (17.8 days). These findings were in agreement with Hossain and Sultana (1998) who reported most early tuberization with 500 mg/I CCC. The number of microtubers was increased with increasing concentration of CCC. The maximum number of microtubers was produced with CCC concentration at 500 mg/I (9.9), while the minimum was 8.1 in absence of CCC. The results were also similar with Hossain and Sultana (1998) who found maximum number of microtubers per plant with 500 mg/I CCC. Weight of microtuber was decreased with increasing rate of CCC concentration. The highest tuber weight was recorded in absence of CCC (190.1 mg), while the minimum at 500 mg/I CCC (145.7 mg). These findings disagreed with those of Hossain and Sultana (1998) in respect of weight but agreed with Harvey *et al.* (1991), Leclerc *et al.* (1994), and Lian-Yong *et al.* (1996). The highest percentage (13.7) of >300 mg size microtuber was produced in absence of CCC which was closely followed by low concentration (125 mg/I) of CCC (8.0) (Table 2).

Table 2. Effect of CCC on induction and development of potato microtuber

CCC (mg/I)	Days to tuber initiation	No. of microtubers/ flask	Av. wt of microtuber (mg)	Grade of microtuber by number (%)		
				<150gm	150-300mg	>300mg
0	17.8	8.1 b	190.1 a	48.6	37.7	13.7
125	16.9ab	8.9ab	173.3 b	53.6	38.4	8.0
250	16.3	9.5 a	157.5 c	59.0	37.6	3.4
500	15.9a	9.9a	145.7d	61.0	35.9	3.1
Level of significance	**	**	**			

Interaction effect of BA and CCC: Tuberization was the earliest (12.7 days) with 10 mg/l BA in combination with 500 mg/I CCC (Table 3). This might be due to the combined beneficial effect of BA (Teixeira and Pinto, 1991) and CCC (Hossain and Sultana, 1998). The minimum number of microtubers per flask was *5.0* in absence of both BA and CCC, while it was highest with 10mg/I BA in combination with 500 mg/I *CCC* (13.7). This might be due to the positive effect of both BA (Wang and Hu, 1982) and CCC (Lian-Yong *et al.*, 1996) in the medium. The highest average weight of microtuber was recorded with 10 mg/I BA in absence of CCC (292.3 mg), which was closely followed by the same concentration of BA (10 mg/I BA) in combination with low concentration (125 mg/I) of CCC. The maximum weight of microtuber with 10 mg/I BA without CCC might be due to the presence of beneficial effect of BA (Teixeira and Pinto,

1991) and absence of harmful effect of CCC (Leclerc *et al.,* 1994 and Lian-Yong *et al.,* 1996).

Table 3. Interaction effect of BA and CCC on induction and development of potato microtuber.

Treatment Combination BA (mg/I) × CCC (mg/I)			Days to tuber initiation	No. of microtubers/ flask	Av. wt of microtuber (mg)	Grade of microtuber by number (%)		
						<150mg	150-300mg	>300mg
0	×	0	22.3 k	5.0 i	89.2 o	100.0	0	0
		125	20.0 j	6.3 ij	85.3 o	100.0	0	0
		250	19.3 ij	7.0 g-j	83.8 o	100.0	0	0
		500	18.7 ghi		76.2 o	100.0	0	0
2.5	×	0	19.0 hij	6.3 ij	126.4 mn	85.1	14.9	0
		125	17.7 f-j	7.0 g-i	119.3 n	88.3	11.7	0
		250	17.0 e-i	7.7 f-i	115.6 n	91.9	8.1	0
		500	16.7 e-i	8.0 e-i	109.1 n	100.0	0	0
5.0	×	0	17.0 e-i	6.7 h-j	175.2 hi	43.6	56.4	0
		125	16.3 d-g	7.3 g-j	162.4 i-j	47.4	52.6	0
		250	16.3 d-g	8.3 e-i	153.1 jkl	50.1	49.9	0
		500	15.0 b-e	9.0 e-i	149.32 kl	52.3	47.7	0
7.5	X	0	16.3 d-g	8.0 e-i	210.1 efg	30.2	49.1	20.7
		125	15.7 c-f	9.3 d-i	191.3 gh	34.3	53.4	12.3
		250	15.0 d-e	9.7 d-h	176.4 hg	44.8	55.2	0
		500	14.7 a-e	10.0 c-g	176.4 hi	45.1	54.9	0
10.0	×	0	14.0 a-d	12.0 a-b	292.3 a	23.1	36.7	40.2
		125	13.7 abc	12.7 abc	261.4 b	26.7	44.2	29.1
		250	13.0 ab	13.3 ab	240.6 c	28.7	47.4	23.9
		500	12.7 a	13.7 a	214.1 ef	29.3	48.9	21.8
12.5	×	0	16.6 e-h	10.0 c-g	238.2 cd	28.1	48.4	23.5
		125	16.0 c-f	10.7 b-f	220.3 de	36.4	49.5	15.1
		250	15.7 c-f	10.0 a-e	175.7 hi	48.7	51.3	0
		500	15.7 c-f	11.0 a-e	164.1 ijk	49.1	50.9	0
15.0	×	0	19.0 hji	8.7 e-i	199.5 fg	30.1	58.2	11.7
		125	18.7 g-j	9.0 e-i	173.2 hij	42.3	57.3	0
		250	18.0 f-j	9.3 d-i	157.4 i-j	48.7	51.3	0
		500	18.0 f-j	9.7 d-h	142.3 lm	51.3	48.7	0
Level of significance			*	*	**			

Chloro choline chloride (CCC) at all concentrations produced cent percent small microtuber (<150 mg) in absence of BA and it did not produce any microtuber of >300 mg size in presence of BA concentration upto 5.0 mg/I. Benzyl Adenine (BA) at 10.0 mg/I produced the highest percentage (40.2%) of >300 mg size microtuber in absence of CCC, which was closely followed by same concentration of BA in presence of low concentration (125 mg/I) of CCC.

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