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EFFECT OF PLANTLET AGE AND PHOTOPERIOD ON *IN VITRO* PRODUCTION OF POTATO MICROTUBER

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Abstract

In vitro plantlets of five different ages (10, 20, 30, 40 and 50 days) were cultured in three levels of photoperiod (dark, 8h and 16h) for the production of microtuber of potato. Microtuberization was the earliest (16.7 days) in 30 days old plantlets. Plantlets of 30 days old produced maximum number (10.0) and average weight (302.7 mg) of microtubers compared to others. Increasing the photoperiod delayed microtuberization. Number and average weight of microtubers increased with decreasing photoperiod. Plantlets of 30 days old showed the best performance on number and average weight of microtubers under continuous dark.

Key words: In vitro plantlet, photoperiod, microtuber

Introduction

Potato tuberization is a complex developmental process known to be influenced by genetic, environmental and physiological variables. Available evidences indicate that the physiological age (PA) of the mother tuber, photoperiod, temperature, irradiation and nitrogen fertilization act on tuberization, either directly or indirectly mediating changes in hormone concentrations (Vander Zaag and Van Loon 1987, Ewing and Struik 1992). It is stated that tuberization influenced by both the top of the plant and by the mother tuber and that the effect of both parts was additive (Madec and Perennec 1995). The rate of tuberization increases with increasing physiological age of tubers (Koda and Okazawa 1983). This information is in line with the fact that, older tubers tuberize earlier under field conditions (Vander Zaag and Van Loon 1987). Tuberization bioassay using *in vitro* stolons in the dark is excellent in

terms of specificity and sensitivity but, unfortunately, it greatly depends on the availability of tubers of a defined PA. Tubers of various PAs may produce misleading results. It would, therefore, be desirable to develop a tuberization system less dependent on the PA of mother tubers. One possibility would be to use plant material previously multiplied *in vitro* under long days. Now a days, *in vitro* plantlet is being extensively used in microtuberization. Information about the influence of age of in vitro plantlet on microtuberization is scanty. The potato genotypes from different maturity groups exhibit varying numbers of microtubers and times to tuberize in response to photoperiod. Wattimena (1983) stated that the longer the photoperiod, the better the tuberization while Nowak and Asiedu (1992) reported that the variety Desiree produced significantly larger microtubers in the dark. There are little discrepancies in the requirement of photoperiod for microtuberization. Therefore, the present study was undertaken to find out the optimum age of plantlet and photoperiod to maximize microtuber production in potato.

Materials and Method

In vitro plantlets of potato variety Diamant were multiplied via nodal cuttings at three weeks interval. The multiplication medium contained mineral salts and vitamins (Murashige and Skoog 1962) plus 0.1 mg/l GA3, 0.01 mg/l NAA, 4 mg/l D-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. The different age of sub cultured plantlets was maintained with a schedule of date of transfer of plantlet into the tuberization media.

Eight stem segments (each with 3 nodes) 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 10 mg/l benzyl adenine (BA) and 80 g/l sucrose. The experiment containing plantlets of five different ages (10, 20, 30, 40 and 50 days) and three levels of photoperiod (dark, 8h and 16h light), which in combination made 15 treatment combinations. The experiment was laid out in Completely Randomized Design (CRD) with four replications. The induced microtubers were harvested aseptically after 70 days of incubation period. The collected data were analyzed with the help of computer using MSTAT program and the mean separation was done by Duncan's new multiple range test.

Results and Discussion

Effect of plantlet age: Micro-tuberization was the earliest (16.7 days) in 30 days old plantlets and most delayed in 10 days old plantlets (Table 1). Tuberization was the earliest in 30 days old plantlets might be due to the presence of higher tuber inducing activity in vigorous leaves as stated by Koda and Okazawa 1988. The highest numbers of microtuber per flask were observed in 30 days old plantlet (10.0) which was closely followed by 40 days old plantlets (8.7). The results are similar to that of Akita and Takayama (1988) who found the maximum number of microtuber by using *in vitro* plantlets of 4 weeks old. The average weight of microtuber was the highest (302.7mg) with 30 days old plantlets which were closely followed by the plantlet of 20 days old (235.8mg) and the minimum with the plantlet of 10 days old (103.7mg). The present finding is consistent with the physiological age of normal tubers in the field where young tubers were most productive (Iritani 1968). The highest percentage of >300 mg size microtuber (47.5%) was produced by 30 days old plantlets, while 10 and 50 days old plantlets did not produce any tuber of

Table 1. Effect of plantlets age on *in vitro* production of microtuber of potato var. Diamant

Plantlet age (Days)	Days to tuber initiation	Number of microtubers/ flask	Av. weight of microtuber (mg)	Grade of microtubers by number (%)		
				<150mg	150-300 mg	>300 mg
10	27.8 c	5.2 b	103.7 e	100	0	0
20	24.7 b	6.8 b	235.8 b	29.8	48.8	21.4
30	16.7 a	10.0 a	302.7 a	22.6	29.9	47.5
40	17.7 a	8.7 a	207.7 c	32.3	49.5	18.2
50	18.3 a	5.9 b	149.0 d	51.9	48.1	0
Level of significance	**	*	*	NA	NA	NA
CV (%)	2.5	3.6	4.2			

Means bearing same letter (s) do not differ significantly at 1 or 5% level of probability
NA, Not analyzed

>300 mg size (Table 1). The initial availability of reserves in the plantlets determines the production potential of microtubers *in vitro* (Garner and Blake 1989). In the present study, healthy and vigorous young plantlets of 30 days old having more reserves produced bigger microtubers than others.

Effect of photoperiod: Micro-tuberization was delayed with the increase in the length of photoperiod (Table 2). The maximum time was needed for tuberization in 16h photoperiod (25.7 days) followed by 8h (21.7 days). The tuberization was most rapid in complete darkness (12.7 days). The present findings are in agreement with Islam (1995), Lentini and Earle (1991), Pelacho and Mingo-Castel (1991) and Janet *et al.* (1993) but disagrees with Hossain and Sultana (1998) who reported that tuberization was earlier in 16h photoperiod than complete dark. Earlier tuberization in dark and shorter days might be due to the presence of tuber inducing activity in leaves which increased gradually in shorter days but remained constant at long days (Koda and Okazawa 1988). The number of microtubers per flask was the highest in total darkness (8.6) followed by 8h (7.3) and 16h (6.0) of photoperiods. These findings are similar to the results of other workers (Nowak and Asiedu 1992, Hossain and Sultana 1998) who found the maximum number of tubers in dark than light. The average weight of microtuber decreased with increasing photoperiod. It was the highest in complete darkness

Table 2. Effect of photoperiod on induction and development of microtuber of potato var. Diamant

Photoperiod	Days to tuber initiation	Number of microtubers/ flask	Av. weight of microtuber (mg)	Grade of microtubers by number (%)		
				<150mg	150-300 mg	>300 mg
Dark	12.7 a	8.6 a	203.5 a	45.7	35.5	18.8
8h	21.7 b	7.3 ab	198.8 ab	48.2	35.0	16.8
16h	25.7 c	6.0 b	196.7 b	48.0	35.3	16.7
Level of significance	*	**	*	NA	NA	NA
CV (%)	2.5	3.6	4.2			

Means bearing same letter (s) do not differ significantly at 1 or 5% level of probability
NA, Not analyzed

(203.5mg) which was statistically similar to that in 8h (198.8) photoperiod. The reduction of average tuber weight in presence of light might be due to late tuber initiation availing less time for bulking of microtuber. The maximum percentage of large size (>300 mg) microtuber (18.8%) was found with continuous dark and the minimum with 16h photoperiod (Table 2).

Interaction effect of plantlet age and photoperiod: The earliest tuberization

Table 3. Interaction effect of plantlet age and photoperiod on induction and development of microtuber of potato var. Diamant

Treatment Combination Plantlet age X Photoperiod	Days to tuber initiation	No. of microtuber per flask	Average weight of microtuber (mg)	Grade of microtubers by number (%)		
				<150 mg	150-300 mg	>300 mg
10 days X dark	20.3bc	6.3de	105.6i	100.0	0	0
8h	28.6de	5.3de	102.1i	100.0	0	0
16h	34.3f	4.0e	103.5i	100.0	0	0
20 days dark	15.3ab	8.3a-d	245.2c	27.5	47.8	24.7
8h	26.7d	6.7cde	234.7d	29.8	49.4	20.8
16h	32.0ef	5.3de	226.4e	32.1	49.3	18.6
30 days dark	12.7a	11.7a	306.1a	21.2	28.4	50.4
8h	17.3abc	10.3ab	302.4ab	24.5	30.1	45.4
16h	22.0bc	8.0bcd	299.7b	22.1	31.1	46.8
40 days dark	14.0a	10.0abc	210.3f	30.8	50.4	18.8
8h	17.7abc	8.7a-d	205.5g	33.4	48.9	17.7
16h	21.3bc	7.3b-e	207.2fg	32.8	49.1	18.1
50 days dark	16.3abc	6.7cde	150.5h	49.2	50.8	0
8h	18.0abc	5.7de	149.1h	53.4	46.6	0
16h	20.7bc	5.3de	147.3h	53.1	46.9	0
Level of ignificance	*	*	*	NA	NA	NA
CV (%)	2.5	3.6	4.2			

Means bearing same letter (s) do not differ significantly at 5% level of probability
NA, Not analyzed

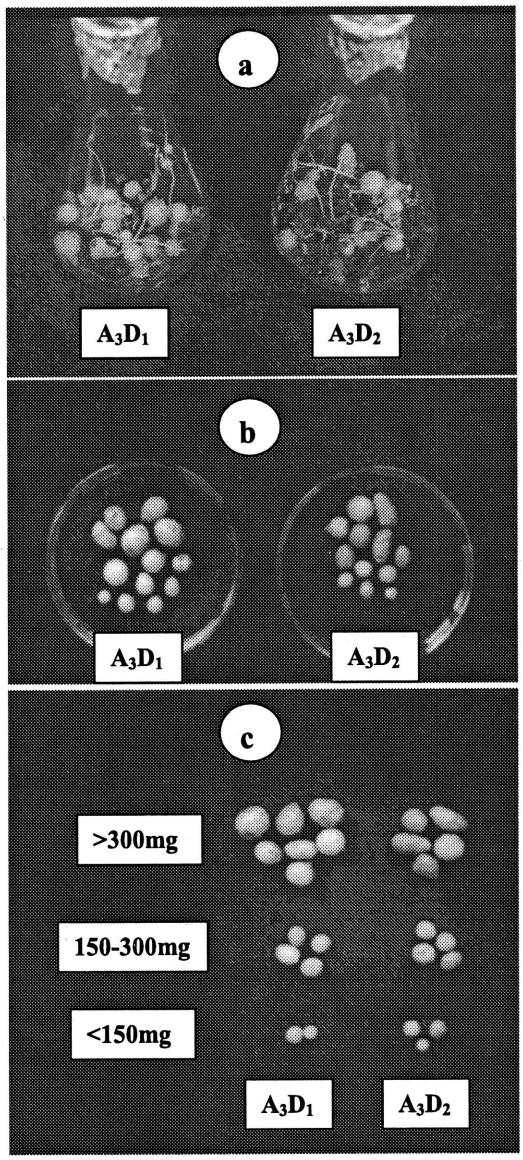

Fig. 1: Effect of plantlet age and photoperiod on microtuberization of potato

a. Production of microtubers, b. Harvested microtubers, c. Graded microtubers

A_3-*In vitro* plantlets of 30 days old, D_1-Complete dark, D_2-8h light

was obtained from 30 days old plantlets at dark (12.7 days), while it was most delayed with 10 days old plantlets at 16h (34.3 days) photoperiod (Table 3). This delay in tuberization was due to the exposure of less number of leaves at long photoperiod resulting lower tuber inducing capacity (Koda and Okazawa 1988). The highest number of microtubers (11.7) per flask was found with 30 days old plantlets in continuous dark (Table 3, Fig.1a & 1b). The average weight of microtuber was the highest (306.1mg) with 30 days old plantlets in dark, which was statistically similar to plantlets of same age in 8h photoperiod. Akita and Takayama (1988) found the greatest microtuber with 28 days old plantlets in continuous dark incubation. This corroborates with the present findings. The highest percentage of >300 mg size microtubers were produced by the 30 days old plantlets in continuous dark (Table 3, Fig.1c).

Conclusion: Plantlet of 30 days old is recommended to culture at continuous dark for maximizing microtuber production in potato.

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