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Effect of Nitrogen and Potassium on *In vitro* Tuberization of Potato

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Abstract

Effects of nitrogen @ 20, 40, 60 and 80 meq in combination with potassium @ 10, 20, 30, 40 and 50 meq on *in vitro* tuberization of potato were evaluated to find out the optimum concentration of nitrogen and potassium for developing a standard protocol for *in vitro* tuberization of a popular variety Diamant. Microtuberization was delayed with increasing rate of nitrogen. Number of microtubers were reduced with an increase in total nitrogen but the average weight of microtubers increased with an increase in nitrogen level up to 60 meq. The number and size of microtubers increased with an increase in potassium up to 40 meq. Concentration of nitrogen at 60 meq and potassium at 40 meq in MS medium gave rise to microtubers of large size

Introduction

The potato plant tuberizes *in vitro* as a result of the changing balance of endogenous growth regulators brought about by manipulating chemical and physical culture conditions. Combined with meristem culture, this technology has been used for disease-free seed production in many countries (Wang and Hu 1982, Estrada et al. 1986). One of the major limitations of the technology is the small size of the microtubers. Small microtubers are more vulnerable to storage damage (Naik and Sarker 1997) and difficult for field planting (Jones 1988). *In vitro* tuberization in potato has been studied by many authors (Wang and Hu 1982, Pelacho and Mingo-Castel 1991, Chandra et al. 1992). However, much of the published research on the induction of potato microtubers *in vitro* has been focused on the use of growth regulators. Very little attention has been paid to the effect of mineral nutrition that also plays a major role in the induction and development of potato microtubers *in vitro* (Wang and Hu 1985). Of the essential

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elements, the concentration of nitrogen and potassium significantly affects the *in vitro* tuberization process. Stallknecht and Farnsworth (1979) and Wattimena (1983) found that low nitrogen in both the explant and the tuberization media was best for coumarin induced tuberization in potato. Reducing the total nitrogen supply increases the number but decreases the size of microtubers in cytokinin-induced microtuberization (Sarker and Naik 1998). Potassium shows its promoting effect on microtuber number and size, and it is cultivar specific (Naik and Sarker 1998). However, their work was far from being complete. It is in this background that the present study has been undertaken to find out the optimum concentration of nitrogen and potassium for developing a standard protocol for *in vitro* tuberization of some popular varieties of potato.

Materials and Methods

In vitro plantlets of potato variety Diamant were multiplied as per routine by subculturing of single stem nodes at three weeks interval. The multiplication medium contained mineral salts and vitamins plus 0.1 mg/l GA_3, 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 hr photoperiod and light by fluorescent tubes at an intensity of 3000 lux was used throughout the entire period of experiments.

Eight stem segments (each with three nodes) of subcultured *in vitro* plantlets were cultured in 250 ml Erlenmeyer flasks containing 40 ml microtuber induction medium which was based on MS supplemented with 10 mg/l benzyl adenine (BA) and 80 g/l sucrose. Alterations in MS macronutrients were made to obtain four levels of total nitrogen (20, 40, 60 and 80 meq) and five levels of potassium (10, 20, 30, 40 and 50 meq) as shown in Table 1. The microtuber induction cultures were incubated in the dark at 20° C (Naik and Sarker 1997). The experiment containing 20 treatment combinations was laid out in 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 DMRT.

Results and Discussion

Effect of nitrogen: Microtuberization was delayed with an increasing rate of nitrogen (Table 2). Reducing rate of total nitrogen in the medium increased the number of tubers per flask. The number of tubers per flask was the highest (12.6) at the lowest level of nitrogen (20 meq). The present findings are similar to those reported by Sarker and Naik (1998) who noticed a higher number of microtubers at reducing levels of total nitrogen. Average microtuber weight significantly increased with increasing levels of nitrogen up to 60 meq. Further increase of

nitrogen (80 meq) reduced tuber weights. The present findings are in agreement with those of Sarker and Naik (1998) who reported increase in size of microtubers with increasing levels of nitrogen up to 60 meq. Reduced total availability of inorganic nitrogen in the medium caused a decline in the average

Table 1. Composition of media used in nitrogen and potassium nutrition experiment.

Medium[a] (meq)		Salts (mg/l)			
Nitrogen	Potassium	KNO_3	NH_4NO_3	K_2SO_4	KC1
20	10	884	450	-	-
20	20	1900	50	-	-
20	30	1900	50	435	373
20	40	1900	50	870	746
20	50	1900	50	1305	1119
40	10	884	1250	-	-
40	20	1900	850	-	-
40	30	1900	850	435	373
40	40	1900	850	870	746
40	50	1900	850	1305	1119
60	10	884	2050	-	-
60	20	1900	1650	-	-
60	30	1900	1650	435	373
60	40	1900	1650	870	746
60	50	1900	1650	1305	1119
80	10	884	2850	-	-
80	20	1900	2450	-	-
80	30	1900	2450	435	373
80	40	1900	2450	870	746
80	50	1900	2450	1305	1119

[a]Other nutrient salts are as in MS medium.

Table 2. Effect of nitrogen on induction and development of potato microtubers.

Nitrogen level (meq)	Days to tuber initiation	Number of microtubers/ flask	Av. wt. of microtubers (mg)	% of grade of microtubers by number (mg)		
				<150	150-300	>300
20	11.2 c	12.6 a	144.0 d	63.1	36.9	0
40	12.7 bc	11.7 b	205.1 c	33.8	53.1	13.1
60	13.5 b	11.0 b	287.4 a	23.0	39.7	37.3
80	18.4 a	7.2 c	237.3 b	26.4	51.3	22.3

Values within a column followed by separate letters are significantly different at $p < 0.05$ level.

microtuber weight or size of the microtubers, suggesting that the mineral nitrogen is a major limiting factor in the control of their size. The inhibitory effect of reduced nitrogen level on microtuber size was also reported in potato when the microtubers were induced on media free of growth regulating substances (Garner and Blake 1989). The lowest concentration of nitrogen (20 meq) produced no microtubers greater than 300 mg size (Table 2). The high

concentration of nitrogen (60 meq) produced maximum percentage (37.3) of > 300 mg size microtubers, while the lowest concentration of nitrogen (20 meq) yielded maximum number of smallest sized (< 150 mg) microtubers. The beneficial effect of mineral nitrogen on microtuber induction observed in this study might be well related to the endogenous-exogenous balance of regulators in the tuberization process.

Effect of potassium: Tuber initiation time decreased with increasing rate of potassium up to the level of 40 meq, while it was enhanced again with 50 meq potassium in the medium (Table 3). Number of microtuber**s** gradually decreased with increasing levels of potassium. The results are similar to those of Naik and Sarker (1998), who reported that microtuber number declined with increasing potassium concentration in cv. Kufri Sindhuri. Weight of microtubers significantly increased with an increasing potassium level up to 40 meq, but further increase of potassium, decreased the weight of microtubers. The highest average weight (253.3 mg) was observed with 40 meq potassium, while it was the minimum (170.8 mg) with 10 meq potassium concentration in the medium. Naik and Sarker (1998) reported that microtuber weight increased significantly at 40 mM potassium concentration in Kufri Ashoka that corroborates the present findings. The highest percentage (26.8) of microtubers of > 300 mg size was found with 40 meq potassium (Table 3).

Table 3. Effect of potassium on induction and development of potato microtubers.

Potassium level (meq)	Days to tuber initiation	Number of microtubers / flask	Av. wt. of microtubers (mg)	% of grade of microtubers by number (mg)		
				<150	150-300	>300
10	15.0 a	11.1 cd	170.8 e	49.1	43.8	7.1
20	14.2 ab	10.9 bc	211.5 d	37.9	45.7	16.4
30	13.4 ab	10.5 ab	234.1 b	30.9	44.2	24.9
40	13.2 b	10.4 ab	253.3 a	28.2	45.0	26.8
50	13.9 ab	9.8 d	222.6 c	36.8	47.6	15.6

Values within a column followed by separate letters are significantly different at $p < 0.05$ level.

Interaction effect of nitrogen and potassium: The time required to start tuber initiation was minimum (10.7 days) with the lowest concentration (20 meq) of nitrogen in combination with higher dose (40 meq) of potassium (Table 4). Quick tuber initiation with lower nitrogen in combination with high potassium might be due to the beneficial effect of low nitrogen and high potassium together on *in vitro* tuber initiation. Tuber initiation was mostly delayed (19.3 days) with the highest dose (80 meq) of nitrogen in combination with the lowest dose (10 meq) of potassium. Late tuberization might be due to the inhibitory effect of high nitrogen and low potassium levels on *in vitro* tuber initiation. The number of microtubers per flask was maximum (12.7) with the lowest concentration of

nitrogen (20 meq) in combination with lower concentration (10 and 20 meq) of potassium, while it was minimum with the highest concentration of nitrogen (80 meq) in combination with highest dose (50 meq) of potassium (Table 4, Fig. 1a,b). Low concentration of nitrogen in combination with low potassium exhibited positive impact on the number of microtubers. Such results might be due to the beneficial effect of low nitrogen (Sarker and Naik 1998) and low potassium concentration (Naik and Sarker 1998). The highest average weight (342.2 mg) of

Table 4. Interaction effect of nitrogen and potassium on induction and development of microtubers.

Treatment combination (meq)		Days to tuber initiation	No. of microtubers /flask	Av. wt. of microtubers (mg)	% of grade of microtubers by number (mg)		
N	K				<150	150-300	>300
20	10	11.7de	12.7	120.3n	87.3	12.7	0
	20	11.3de	12.7	141.2m	66.4	33.6	0
	30	11.0de	12.0	150.4klm	51.2	48.8	0
	40	10.7e	12.3	161.6kl	45.5	54.5	0
	50	11.3de	12.0	146.3lm	65.0	35.0	0
40	10	13.3cde	12.0	162.5k	44.3	55.7	0
	20	13.0cde	12.0	203.4ij	32.8	51.2	16.0
	30	12.7de	11.7	220.3gh	29.6	53.3	17.1
	40	12.0de	11.3	240.4ef	29.1	52.5	18.4
	50	12.3de	11.3	199.1ij	33.1	53.0	13.9
60	10	15.7bc	11.7	210.0h	31.6	53.1	15.3
	20	13.7cd	11.3	273.4d	25.7	46.5	27.8
	30	12.3de	11.0	315.2b	18.5	25.3	56.2
	40	12.3de	11.0	342.2a	15.1	24.4	60.5
	50	13.7cd	10.7	295.6c	24.2	49.1	26.7
80	10	19.3a	7.7	189.8j	33.1	53.8	13.1
	20	18.7a	7.3	228.1f-j	26.7	51.5	21.8
	30	17.7ab	7.0	250.4e	24.3	49.2	26.5
	40	18.0ab	7.3	268.7d	23.1	48.7	28.2
	50	18.3ab	6.7	249.3e	24.9	53.4	21.7

Values within a column followed by separate letters are significantly different at $p < 0.05$ level.

microtuber was found with high concentration (60 meq) of nitrogen in combination with 40 meq of potassium, while it was the lowest with low concentration of nitrogen (20 meq) in combination with low concentration (10 meq) of potassium. The findings are in agreement with those of Naik and Sarker (1998) who reported that average microtuber weight was maximum with 60 meq of nitrogen in combination with 40 meq potassium. The highest percentage (60.5) of >300 mg size microtubers was produced by high rate of nitrogen (60 meq) in combination with high rate of potassium (40 meq), which was more than twice compared to the control (60 × 20 meq nitrogen and potassium). The lowest percentage of smaller (<150 mg) size tuber was produced by the same treatment

(Table 4, Fig. 1c). The highest percentage (60.5) of bigger (>300 mg) size tuber with 60 meq nitrogen in combination with 40 meq potassium may be due to the beneficial effect of high nitrogen (Wang and Hu 1985) and high potassium (Naik and Sarker 1998) on microtuber size in cytokinin induced *in vitro* tuberizaiton.

There is evidence that nitrogen is an important limiting factor in the control of tuberization. It is suggested that applied nitrogen triggers tuberization via the levels of endogenous phytohormones. Applied nitrogen affects all endogenous levels of GA, ABA and cytokinin. The shift in ABA: GA ratio is considered one of the key factors controlling tuberization. Any deviation from the optimum level of nitrogen will affect the ABA: GA balance leading to tuberization (Kraus 1985).

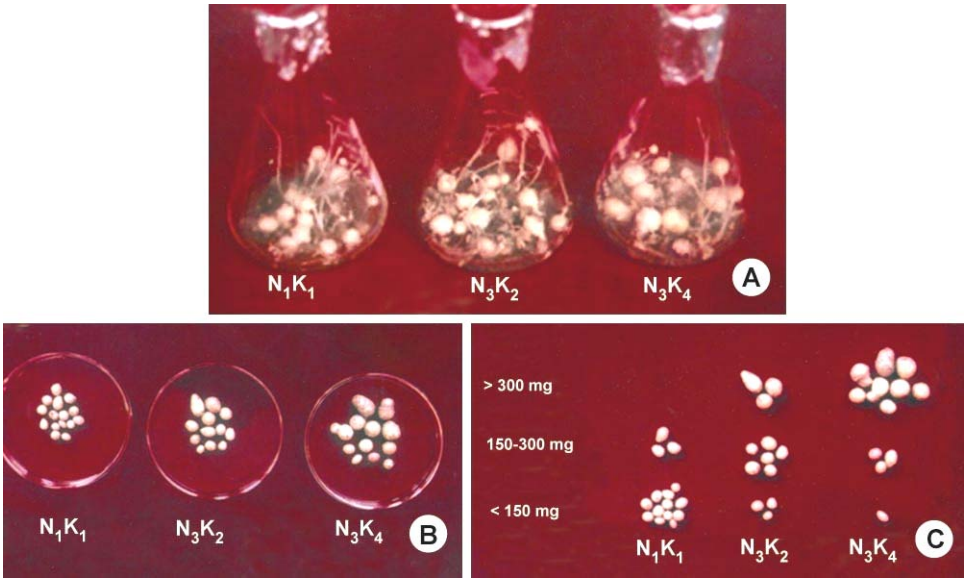

Fig. 1. Effect of nitrogen and potassium on microtuberization of potato. A. Production of microtubers. B. Harvested microtubers. C. Graded microtubers, N_1 20 meq nitrogen, N_3 60 meq nitrogen. K_1 10 meq potassum. K_2 20 meq potassium and K_4 40 meq potassium.

There are no reports available on the effect of mineral nutrition or growth regulators on assimilate-partitioning during microtuberization process. It seems probable that nitrogen in association with potassium and other endogenous tuberization signals regulate source and sink coordination during cytokinin-induced *in vitro* tuberization in potato. Inorganic nitrogen and potassium are the critical factors in the induction and development of potato microtubers by cytokinin-induced *in vitro* tuberization. Higher numbers of microtubers could be obtained from MS supplemented with 20 - 40 meq total nitrogen, 10 - 30 meq potassium but an increase in microtuber number was associated with simultaneous decrease in microtuber size. For commercial seed potato

production, microtuber size is more important than microtuber number because small sized microtubers are more vulnerable to storage loss (Naik and Sarker 1997) and are unsuitable for direct field planting (Jones 1988). The present study demonstrated that a concentration of nitrogen (60 meq) and potassium (40 meq) in MS medium yields microtubers of larger size - a procedure that has potential for commercial exploitaion.

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