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Involvement of jasmonic acid and light periods on potato microtuber growth response

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Abstract

The present study was conducted to determine the effects of Jasmonic acid (JA) and light periods on performance of microtuber growth response in five potato cultivars (Diamant, Atlanta, All Blue, Shepody and Shilbilaty). Microtubers were produced from *in vitro* grown plantlets treated with or without 5µM JA on micropropagation and microtuberization media under 0 and 8 h light periods. Microtuberization was established in MS liquid media having 8% sucrose along with the above treatments. Among the investigated cultivars, the highest number of microtubers (12.97 microtubers per vessel) was obtained in All Blue. On the other hand, Diamant microtubers gave maximum fresh weight (106.7 mg) and Shilbilaty yielded the highest percentage (57.72%) and number of larger microtubers (8.25 larger microtubers out of total 12.78 microtubers per vessel) followed by Diamant and All Blue. Atlanta showed lower values at all investigated microtuber growth traits. Microtuber growth was better in 5µM JA supplemented microtuberization media with 8 h photoperiodic incubation than those were in micropropagation media with the same treatment. The 0 h period was found significantly inferior than 8 h at all growth traits.

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Introduction

Most of the commercial production of potato nuclear seed is still based on tissue culture plantlets, limited information has been gained on the production of microtubers at commercial scale. Over the last few years, attention has been paid to explore the use of microtubers in seed potato production as an alternative propagation materials to plantlets (Struik and Wiersema, 1999; Yu *et al*., 2000; Pruski *et al*., 2002, 2003,). Microtubers have three important advantages over plantlets: they are less delicate than plantlets, can be stored for longer period, handled and transported easier than plantlets.

The main constraints of microtuber utilization in seed potato production are incomplete dormancy, small size and low yield potential which might restrict their use as propagules of choice (Struik and Lommen, 1990). Keeping the drawback in mind, the researchers are being trying to overcome these obstacles by manipulating the *in vitro* tuberizing conditions (Struik and Wiersema, 1999). The explant source, photoperiod, temperature and growth regulators in culture medium were the common influential factors to achieve *in vitro* tuberization.

Several researchers observed that jasmonic acid (JA), a growth regulator produced by plants which exposed to stress and under senescence (Koda, 1997; Biondi *et al.,* 2000) are highly effective inducers of tuberization (Van den Berg and Ewing, 1991; Pelacho and Mingo-Castel, 1991; Ravinkar *et al*., 1992). Pruski *et al*. (2001, 2003) reported the potential use of JA in the development of commercial production of microtubers of potato cultivars: Amisk, Russet Burbank, Sangre, Umatilla Russet, Shepody and Atlantic. They reported that JA effects were cultivar specific. In the dark, Amisk, Umatilla Russet and Atlantic tuberized better in JA treated microtuberization media than in JA treated micropropagation media. These cultivars did not benefit from JA treatments in the light. Pruski *et al*. (2002) studied nodal explants taken from JA conditioned plantlets which tuberized earlier and gave higher yield of microtubers than control. It was found that JA induced plantlets in the tuberization media enhanced tuber size and increase the number of microtubers and benefits of this treatment depended on light and media conditions.

Suitability of cultivar-specific microtuberization methods and their field performances in net house/green house comparison to plantlets is probably the most important part of microtuber utilization. Adaptation of some newly developed methodologies to mass production of microtubers, may prove that microtubers can be accepted as an alternative to plantlets. With a view of the previous reports, the involvement of JA and light periods either treated in micropropagtion and microtuberization media or not, seemed to be important stimulatory events of potato *in vitro* tuberization.

The present experiment was conducted to find out the suitability of five potato cultivars to JA supplemented media with two light periods on microtuber growth performances in order to selection of suitable cultivars for future utilization of microtubers towards commercial seed potato program in Bangladesh.

Materials and methods

Plant materials

The potato cultivars studied in this experiment were Diamant, Atlanta, All Blue, Shepody and Shilbilaty. The plant materials were derived from potato tissue culture bank at the Plant Breeding and Gene Engineering Lab, Department of Botany, University of Rajshahi, Bangladesh. Multiplication of plantlets and preparation of stock plants for microtuberization are as follows under two heads:

Micropropagation

The plantlets were multiplied as single-node explants taken from 3 week-old plantlets and cultured on 10 ml hormone-free MS liquid medium (Murashige and Skoog, 1962). Sucrose was added at 3% and media were adjusted to pH 5.8 before autoclaving. Twelve microcuttings were placed in each culture vessel and

cultures were incubated for 3 weeks in growth room at 25±1°C with 16 h photoperiod at 600-800 lux, illumination and subcultured was maintained at every 3 weeks intervals. The process was repeated until the required number of plantlets for *in vitro* tuberization was achieved. During the final subculture, the culture vessels of single node explants of all cultivars were divided into two groups: 1) the same MS liquid media as above and 2) the same MS media as above added with 5µM JA. Both groups of cultures were grown for 3 weeks again under the growth conditions as described above.

Table 1. The treatments used for the experiment.

Treatments	JA (5 µM) Micropropaga-tion media	JA (5 µM) Microtuberizat-ion media	Light periods (hrs)
T1	Absent	Absent	0
T2	Absent	Absent	8
T3	Absent	Present	0
T4	Absent	Present	8
T5	Present	Absent	0
T6	Present	Absent	8
T7	Present	Present	0
T8	Present	Present	8

Microtuberization

Plantlets grown after 3 weeks during the micropropagation steps the residual propagation medium was drained and replaced by 20 ml microtuber media. The microtuber media comprised with 1) the same MS liquid media as above and supplemented with 8% sucrose and 2) the same MS media as above and added with 8% sucrose plus 5µM JA . The pH of the media for both the treatments was adjusted to 5.8 and JA was filter sterilized before adding to the media. The plantlets grown from twelve nodal cuttings in each culture vessel containing 20 ml tuberization medium. Cultures were incubated at 18±2°C and provided with 0 or 8 h photoperiod at 200-300 lux for 16 weeks. After 16 weeks microtubers were harvested and data were recorded.

Four parameters were considered for this experiment: 1) number of total microtubes per vessel, 2) fresh weight of microtuber, 3) % of larger microtubers (>100 mg fresh weight) and 4) number of larger (>100 mg fresh weight) microtubers per vessel. The experiment was conducted using complete randomized design (CRD) and replicated 4 times. Each replicate consisted of one culture vessel (having twelve nodal microcuttings grown plantlets). Means of all treatments and cultivars were compared by Duncan's Multiple Range test at 5% level of significance ($P \leq 0.05$). Analysis of variance was performed for all the microtuber traits and mean square values are presented in Table 3.

Results and discussion

The JA in MS media and incubated light periods were significantly affected the microtuber growth traits. The results showed that all the variables (cultivar, treatment and cultivar × treatment) were significantly different in microtuber traits except the cultivar × treatment interaction was non-significant in microtuber fresh weight (Table 3). The results obtained from different potato cultivars at different treatments on microtuber growth traits are presented in Table 2. It was observed that Shilbilaty produced more microtubers (20.25 microtubers per vessel) on microtuberization media comprising 5µM JA and 8 h light period (T4 treatment) followed by Diamant (19.00 microtubers per vessel) at the same treatment. The culture with T4 treatment, Diamant microtuber produced the highest fresh weight (156.25 mg) among the other cultivars and treatments used. The percentages of larger microtubers (>100 mg fresh weight) ranged from 8.67-83.92% as also shown in Table 2. In this case, Shilbilaty yielded the highest percentages (83.92%) of larger microtubers on microtuberization media having T4 treatment. The cultivars produced significantly larger microtubers (>100 mg fresh weight) in 8 h light than in dark exposure. The differences between the two light periods were significant at all microtuber growth traits (Table 2).

Table 2. Effect of light periods and jasmonic acid (JA) in micropropagation and microtuberization media on growing status of microtubers. Each value is an average of 4 replicates.

Cultivars		JA in micro-propagation Media(5µM)	JA in microtuber media(5 µM)	Light (hrs)	Total number of microtubers per vessel	Fresh weight of microtuber (mg)	% of larger microtubers (>100mg)	Number of larger microtubers per vessel (>100 mg)
Diamant	T1	Absent	Absent	0	13.75	97.50	42.45	6.00
	T2	Absent	Absent	8	11.00	113.75	46.15	5.00
	T3	Absent	Present	0	14.00	114.00	70.12	9.50
	T4	Absent	Present	8	19.00	156.25	70.50	13.25
	T5	Present	Absent	0	8.25	89.50	51.30	4.25
	T6	Present	Absent	8	12.25	102.00	73.57	9.00
	T7	Present	Present	0	8.75	88.25	41.57	3.50
	T8	Present	Present	8	8.50	92.50	38.62	3.50
Atlanta	T1	Absent	Absent	0	13.00	85.50	45.05	6.00
	T2	Absent	Absent	8	14.50	84.25	45.82	6.00
	T3	Absent	Present	0	11.25	88.75	45.45	5.25
	T4	Absent	Present	8	18.00	94.75	43.02	7.50
	T5	Present	Absent	0	4.25	66.75	21.65	1.00
	T6	Present	Absent	8	7.00	88.25	36.27	2.50
	T7	Present	Present	0	1.75	65.00	12.50	0.25
	T8	Present	Present	8	5.25	77.50	15.40	0.75
All Blue	T1	Absent	Absent	0	16.25	96.25	51.87	8.25
	T2	Absent	Absent	8	18.25	107.00	57.20	10.25
	T3	Absent	Present	0	11.50	89.00	48.85	5.50
	T4	Absent	Present	8	15.00	133.50	78.30	11.50
	T5	Present	Absent	0	9.25	66.75	15.90	1.50
	T6	Present	Absent	8	14.00	85.25	29.92	4.75
	T7	Present	Present	0	9.00	69.50	22.62	2.25
	T8	Present	Present	8	10.50	95.75	52.97	5.75
Shepody	T1	Absent	Absent	0	14.00	77.75	29.70	4.25
	T2	Absent	Absent	8	16.75	85.00	45.95	8.25
	T3	Absent	Present	0	11.00	72.75	14.82	1.50
	T4	Absent	Present	8	18.75	113.75	66.10	12.25
	T5	Present	Absent	0	10.00	69.00	8.67	0.75
	T6	Present	Absent	8	13.75	99.25	54.07	7.25
	T7	Present	Present	0	4.50	66.50	13.32	0.50
	T8	Present	Present	8	10.25	87.50	45.27	4.75
Shilbilaty	T1	Absent	Absent	0	12.25	100.25	60.22	7.500
	T2	Absent	Absent	8	16.25	119.25	65.47	10.25
	T3	Absent	Present	0	19.00	117.75	75.95	14.50
	T4	Absent	Present	8	20.25	153.75	83.92	17.00
	T5	Present	Absent	0	8.75	86.25	43.50	4.00
	T6	Present	Absent	8	10.50	107.50	71.95	7.50
	T7	Present	Present	0	5.75	72.25	13.87	0.75
	T8	Present	Present	8	9.50	89.00	46.87	4.50
				t-value	**2.34***	**6.09****	**2.82***	**2.68***

t-value of two photoperiodic (0 and 8h) conditions, *, **, significant, highly significant at 5% level.

Table 3. The mean square values of microtuber growth traits as affected by cultivars and growth treatments after 16 weeks of incubation.

		Mean square values			
Source	df	Number total of microtubers /vessel	Fresh weight of microtuber (mg)	% of larger microtubers (>100 mg)	Number of larger microtubers (>100 mg)
Cultivar (C)	4	68.16*	4499.60*	3951.32*	98.30*
Treatment(T)	7	334.59*	6409.65*	4560.05*	240.73*
C × T	28	20.18*	362.10ns	657.68*	19.85*
Error	120				

*Significant at 5, 1 and 0.1% levels respectively; ns, non-significant.

Table 4. The overall performances of five potato cultivars on microtuber growth traits. Each value is an average of 8 treatments × 4 replications.

Cultivars	Total number of microtubers per vessel	Fresh. weight of microtuber (mg)	% of larger microtubers (>100 mg)	Number of larger microtubers per vessel(>100 mg)
Diamant	11.94ab	106.7a	54.29a	6.75ab
Atlanta	9.37b	81.34b	33.15b	3.66c
All Blue	12.97a	92.88ab	44.71ab	6.21ab
Shepody	12.38ab	83.94b	34.74b	4.93bc
Shilbilaty	12.78ab	105.8a	57.72a	8.25a
LSD (P≤0.05)	3.19	19.86	15.59	2.39

In each column values followed by different letters are statistically significantly different at 5% level of significance by DMRT.

The overall performances of cultivars differed significantly in each of the parameter of microtuber growth traits (Table 4). Of the cultivars, All Blue produced the highest microtubers (12.97 microtubers per vessel) but their fresh weight was lower than Diamant and Shilbilaty. Diamant microtubers scored the highest fresh weight (106.7 mg), on the other hand Shilbilaty yielded the highest percentage (57.72%) of larger microtubers; though there was no significant differences between Diamant and Shilbilaty in both these traits (Table 4). The number of larger microtubers produced by five cultivars differed significantly. In this case, Shilbilaty produced the highest number (8.25 larger microtubers out of total 12.78 microtubers per vessel) of larger microtubers followed by Diamant (6.75 larger microtubers out of total 11.94 microtubers per vessel). Atlanta showed poor performances at all studied parameters (Table 4). Among the 8 treatments employed, T4 treatment was found to be the best for all the microtuber growth characteristics (Fig. 1).

The investigation of JA in media and incubating with two light periods on microtuber growth performances gave varied results. However, in the present investigation the results were contrary to the general trends and somewhere coincided with the findings of Pruski *et al.,* (2002). JA pre-treatment of micropropagated plantlets, prior to microtuber induction was an effective inducer of microtuber formation as was reported in potato cv. Russet Burbank (Pruski *et al*., 2002). But the results from the present findings it was noted that JA pretreated plantlets before microtuberization showed less performances in microtuber growth efficiency than

JA treated during microtuberization. The JA treated micropropagated plantlets produced more roots than plantlets grown in JA induced microtuberization media (data not shown). The 8 h light period during tuberization was found an important factor for microtuber growth efficiency than on dark incubated microtuberization.

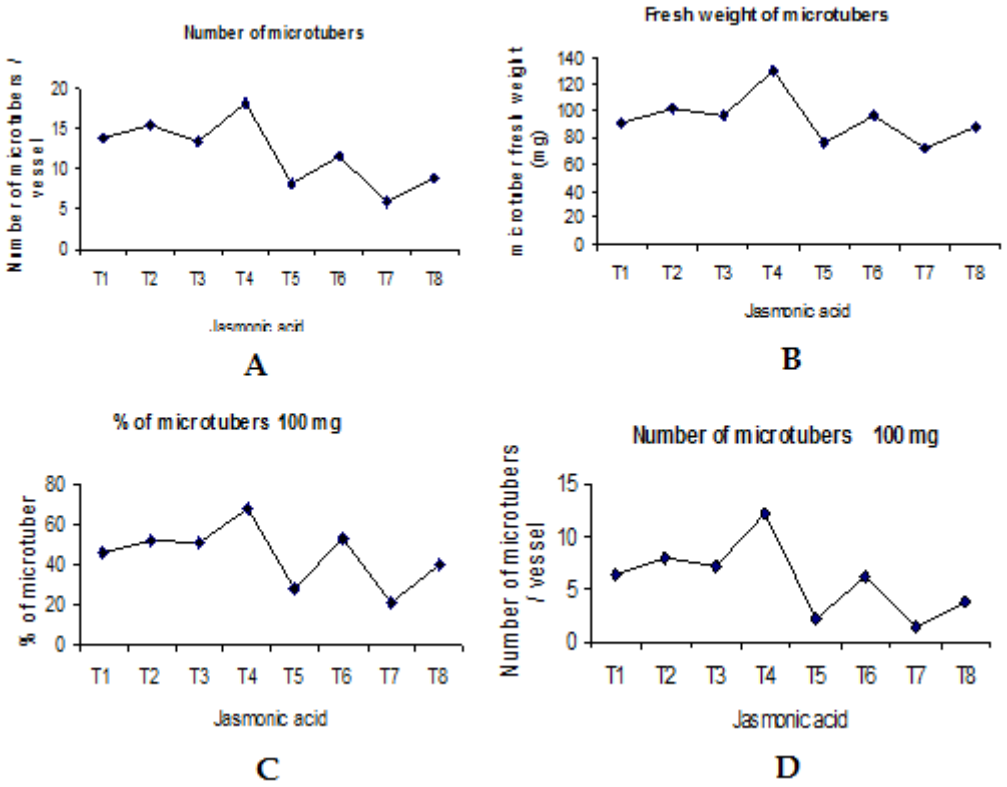

Fig. 1 (A-D). The overall performances of JA-light treatments on microtuber growth characteristics. Each value is an average of 5 cultivars × 4 replications.

In most of the cases, Diamant, All Blue, Shepody and Shilbilaty produced more than one microtubers per plantlet but the Atlanta produced the fewer number of microtubers (on average less than one per plantlet) than the other cultivars (data not shown).

The 0 h period was found inferior than 8 h period for *in vitro* tuberization since the larger microtubers produced in 0 h treatments were much lower than in treatments with 8 h light (Table 2). This results is in agreement with the other research findings. Garner and Blake (1989) found that 8 h light period suitable for the production of larger microtubers than darkness. Khuri and Moorby (1996) reported successful microtuber production of cvs. Estima and Cultra under short days. Ranalli (1997) stated that most cultivars give much better microtuber production in short days (8 h) than in complete darkness.

Stimulatory effects of JA on *in vitro* tuberization and on potato stem node cultivars have been reported by several authors (Koda *et al*., 1991; Palacho and Mingo-Castel, 1991; Ravnikar *et al*., 1992; Pruski *et al*., 1993; Pruski *et al*., 2002). JA is involved in control of leaf senescence in potato which is closely associated with tuber induction (Van den Berg and Ewing, 1991). The JA treated plantlets particularly during *in vitro* tuberization media under 8 h incubation period may have possessed the signal to induce tuberization quickly and to produce higher number of microtubers. Pelacho and Mingo-Castel (1991) reported that JA induction favored tuberization in potato stolons cultured *in vitro*.

Jackson (1999) reported that the JA or other JA-related compounds may be responsible for inducing or promoting *in vitro* tuberization; differences in endogenous levels of JA itself do not control tuberization.

In conclusion, the results showed that the T4 treatment was proposed as an effective treatment to enhance microtuber growth and Shilbilaty, Diamant and All Blue were recommended as suitable potato cultivars since they produced larger microtubers. Production of larger microtubers is the pre-requsite condition for successful utilization of microtubers in seed potato production as reported by several researchers (Ravnikar *et al.*, 1992; Pruski *et al.*, 1993; 2002; 2003). Hence the present authors propose this protocol a simple, economical and highly reproducible to obtain more larger microtubers from the above three potato cultivars under laboratory conditions and it could be attributed for future exploitation of commercial seed potato program from *in vitro* grown microtubers.

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