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Role of incubation temperatures on axillary bud conversion during microtuberization in potato (*Solanum tuberosum* L.)

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Abstract

The present study investigated the effect of incubation temperatures (15, 20 and 25°C) on conversion of axillary buds from the *in vitro* grown plantlets during microtuberization of five potato cultivars (Diamant, Atlanta, All Blue, Shepody and Shilbilaty). Cultures for microtuberization was established in MS (Murashige and Skoog, 1962) liquid media having 8% sucrose along with the above temperature treatments to investigate the axillary bud conversion during microtuberization. Among the tested cultivars, the highest number of sessile microtubers (8.16 microtubers per vessel) was obtained in All Blue followed by Diamant (7.66 microtubers per vessel). On the other hand, Diamant plantlets produced maximum stoloniferus shoots (8.08 shoots/vessel) while that of All Blue yielded the highest tuberized stolon (8.66). Atlanta showed the lower values at all types of axillary bud conversion than other cultivars. Significant differences were noticed among the temperature treatments in forming sessile microtubers. At 15°C, highest number of sessile microtubers (8.50) was formed. On the other hand, tuberized stolon (8.00) and stoloniferus shoots (8.25) were found best at 20 and 25°C respectively.

Keywords: Microtuberization, temperature, axillary bud conversion, sessile microtubers.

INTRODUCTION

Potato microtuber production is strongly affected by temperature (Wang and Hu, 1982; Akita and Takayama, 1994) but its effect on axillary bud conversion into tuber formation has not been studied in great detail. Although much works have been done on the effect of temperatures on the developmental physiology and growth of the conventional potato tubers but relatively little is known about the effects of temperature on potato microtuber production *in vitro.* At lower temperatures, microtubers initiated more rapidly than the microtubers grown under higher temperatures though the growth of micropropagated plantlets (prior to microtuberization) was the optimum at 25±1°C. At low temperatures, the concentration of soluble carbohydrates at the stolon tip is higher and tuber initiation is accelerated (Borah and Milthorpe, 1962) but at higher temperatures, the process of starch assimilation at the stolon tip is retarded. Struik *et al*. (1989) indicated that tuber initiation can be delayed or even inhibited by high temperatures whereas the distribution of dry matter between tubers and haulm can be affected by temperature, particularly at night temperature. The high temperatures during microtuberization not only delay the tuber initiation but ongoing tuber formation can even be arrested temporarily (Struik and Ewing, 1995). According to Wang and Hu (1982) the average number of microtubers was lower at higher *in vitro* temperature (28°C day/night temperature) than at lower temperature (20°C day/night temperature).

These differences in temperature optima can be related to the light intensity (high light intensity increasing the temperature inside the vessels compared to the growth room temperature) and to the use of growth regulators (in the absence of cytokinin, the low temperature may be needed to trigger tuberization) (Wang and Hu, 1985). Akita and Takayama (1994) claimed that the optimum temperature for *in vitro* tuberization is 20°C. There seems to be a controversy in the literature on the minimum and optimum temperatures for efficient microtuber production in potato.

Axillary buds of *in vitro* potato plantlets may offer an interesting system to study the organ development. The type of organ development can easily be manipulated by varying a single factor in the medium. Almost every bud on a potato plant is capable of forming a tuber but generally it is not observed due to complex nature of multinodal interactions of a plantlet, *in vitro* cultural practices, physio-chemical complexities of the cultured explants etc. Under the *in vitro* system each of axillary bud can take the *in vitro* stimuli, commencing their growth as a leafy shoot or as a stolon or as a tuber. Stolon development, tuber induction and tuberization are the key developmental events in the potato plants. A series of complex processes are involved in the developmental switch from stolon elonagation to tuber development which are the most important research topics in potato science. Transmission of the shoot derived inductive signals *in vitro* to the axillary buds is the important gateway to develop microtuberization. The potential switch to axillary bud conversion has made a significant contribution to understanding of tuber growth regulation. In this experiment, the *in vitro* grown potato plantlets was subjected to study axillary bud conversion with three incubation temperatures cultured in MS liquid medium having 8% sucrose.

The objective of the present experiment was to investigate how the incubation temperatures influence the axillary buds of *in vitro* grown plantlets and to convert them into microtuber development.

MATERIALS AND METHODS

Plant Materials: The potato cultivars studied in this experiment were Diamant, Atlanta, All Blue, Shepody and Shilbilaty. All these plant materials were collected from potato tissue culture bank at the Plant Breeding and Gene Engineering Lab, Department of Botany, University of Rajshahi, Bangladesh.

***In vitro* propagation**

Nodal cuttings with one leaf node were prepared from 3 weeks old *in vitro* grown potato plantlets and cultured on a standard MS liquid medium (Murashige and Skoog, 1962) with standard vitamins and 3% sucrose. The pH of the medium was adjusted to 5.8 before autoclaving. No growth regulator was added during the development of stock plants. Ten ml of liquid MS media was dispensed into the culture vessels. 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. Subculture was maintained at every 3 weeks intervals and it was repeated until the required number of stock *in vitro* plantlets for the experiment was achieved. At final subculture, the media were refreshed by same media and added with 8% sucrose for microtubrization. The culture vessels were randomly placed in the allocated temperature regimes for 16 weeks in dark.

Temperature treatments

The culture vessels were kept into incubator under total darkness employed with three temperature (15, 20 and 25°C) regimes for 16 weeks followed by 2 weeks incubation at growth room (8 h photoperiod (200-300 lux) and temperature (25 ±1°C).

Observations and analysis

The types of the nodal axillary buds to form tubers was measured as 1. stoloniferus shoots, 2. tuberized stolon and 3. sessile tuber. The experiment was conducted using complete randomized design (CRD) and replicated 4 times. Each replicate consisted of one culture vessel (having twelve nodal 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 axillary bud conversion types and mean square values were calculated.

RESULTS

The frequency of three types of responses of axillary buds as affected by cultivars and incubation temperatures are presented in Table1. The mean square values from ANOVA demonstrated that no significant differences existed among the cultivars in producing stoloniferous shoots and tuberized stolon but significant differences were obtained in number of sessile tubers. The incubation temperatures produced significant differences in stoloniferous shoot and sessile tuber formation. But the

interaction effect between cultivar and incubation temperature (C × T) showed non-significant results at all the axillary bud conversion types (Table 1).

Table 1. Effect of incubation temperatures on axillary bud conversion from *in vitro* grown plantlets during microtuberization of five potato cultivars. Each value is an average of 4 replicates.

Cultivars	Temp. treatment (°C)	Types of axillary bud conversions (numbers)		
		Stoloniferous shoots/vessel	Tuberized stolons/vessel	Sessile tubers/vessel
Atlanta	15	3.25	6.50	7.50
	20	6.75	7.25	6.00
	25	7.25	5.00	4.50
Shepody	15	5.25	6.50	8.00
	20	7.50	6.50	8.25
	25	8.25	6.75	4.75
Shilbilaty	15	6.75	7.25	7.75
	20	7.50	8.75	6.50
	25	8.25	6.75	4.50
AllBlue	15	7.25	8.50	9.75
	20	7.50	9.25	9.25
	25	8.75	8.25	5.50
Diamant	15	7.25	8.00	9.50
	20	8.25	8.25	8.75
	25	8.75	6.25	4.75

Source	df	Mean square values		
Cultivar (C)	4	10.22ns	10.80ns	10.10**
Temperature (T)	2	27.51*	9.81ns	76.51*
C × T	8	2.10ns	1.42ns	1.53ns
Error	45	4.17	4.23	2.91

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

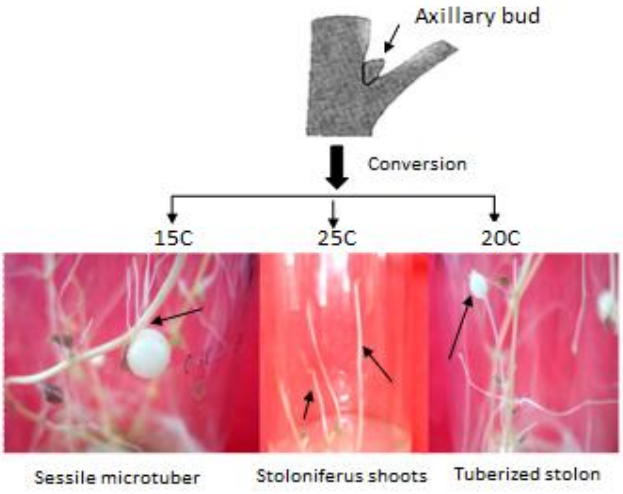

Plate 1. Axillary bud conversion from *in vitro* grown plantlets during microtuberization.

Shoots incubated at 15°C temperature produced more number of sessile tubers than those incubated at 20 and 25°C. At 20°C, developed tubers at the tip of stolon while stoloniferous shoots were more prominant at 25°C (Table 2).

Table 2. The effect of incubation temperatures on axillary bud conversion types. Each value is an average of 5 cultivars × 4 replications.

Incubation temp.(°C)	Types of axillary bud conversions (numbers)		
	Stoloniferus shoots/vessel	Tuberized stolons/vessel	Sessile tubers/vessel
15	5.95a	7.35a	8.50a
20	7.50a	8.00a	7.75a
25	8.25a	6.60a	4.80b
LSD (P≤0.05)	**NS**	**NS**	**2.176**

In a column mean values followed by different letters are significantly different by DMRT (P≤0.05).

Table 3. Cultivar performances on axillary bud conversion types. Values are the average of 3 temp. treatments × 4 replications.

Cultivars	Types of axillary bud conversions (numbers)		
	Stoloniferous shoots/vessel	Tuberized stolons/vessel	Sessile tubers/vessel
Atlanta	5.75a	6.25a	6.00a
Shepody	7.00a	6.58a	7.00a
Shilbilaty	7.50a	7.58a	6.25a
All Blue	7.83a	8.66a	8.16a
Diamant	8.08a	7.50a	7.66a
LSD (P≤0.05)	**NS**	**NS**	**NS**

In a column mean values followed by same letters are not significantly different (P≤0.05).

Although the differences observed for stoloniferous shoots and tuberized stolon were not statistically significant at three incubation temperatures, a temperature of 25°C gave significantly fewer sessile microtubers than 15 and 20°C (Table 2). Among the cultivars, the highest sessile tubers was induced by All Blue (8.16 tubers per vessel) and the lowest was noted in Atlanta (6 tubers per vessel). No significant differences were noticed among the cultivars with response to temperature treatments in axillary bud conversion types (Table 3). Diamant tended to produce more stoloniferous shoots but also produced sufficient sessile tubers which were comparable to All Blue. Beside the maximum

production of sessile tubers per culture vessel, All Blue showed the highest values in producing tuberized stolons.

DISCUSSION

The 8% sucrose level in microtuber media was an important factors for microtuberization with optimum temperature of 18±2°C (Alsadon *et al*., 2008; Rahman *et al*., 2010). The stimulation of tuber formation by elevated sucrose levels in the medium has also been reported by Garner and Blake (1989), Hussey and Stacey (1984), Vreugdenhil and Helder (1992) but little is known about the effect of high sucrose level with different incubating temperatures on axillary bud conversion from *in vitro* grown plantlets during microtuberization. High temperature (25°C) stimulated the formation of non-sessile tubers or stolon-like shoots, having a hook at the tip. When the cultures were incubated at 20°C, the axillary buds of a plantlet developed mostly into tuberized stolons. At 15°C, the axillary buds tended to produce more sessile tubers. A linear increase of stoloniferus shoots with the increase of incubation temperature was noted whereas a linear decrease of sessile tuber was obtained with the increase of the temperature.

The results clearly demonstrated that the incubation temperature greatly affected in producing sessile tubers at all the cultivars. At low temperature incubation, the axillary buds were more active to proliferate sessile tubers. The media with high sucrose content in microtuber media may attribute to accelerate the process.

In conclusion, the results showed that the 15°C incubation with high sucrose containing microtuberization media was an effective treatment to enhance sessile microtuber production in potato plantlets under *in vitro* conditions. Hence, the present investigation may provide an important clue to produce more sessile microtubers from *in vitro* grown plantlets in order to 'effective field utilization of microtubers' towards seed potato production.

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