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Influence of different storage temperatures on sprouting characteristics of potato microtubers

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Abstract

Experiment was carried out to assess the effect of different storage temperatures (5, 15 and 20 ºC) on sprouting efficiency of harvested potato microtubers before transplantation. The varied response was noticed in the sprouting parameters at different storage temperatures for different cultivars. The high temperature (15 and 20 ºC) gave the better values in sprouting performances than the low one (5 ºC). No significant differences were found between 15 and 20 ºC storage temperatures in sprouting traits except sprout numbers/microtuber after 4-week storage. The sprout length and sprout numbers/microtuber of different cultivars in response to storage temperature were not significantly different for both intervals whereas the sprout weight significantly differed only at 4-week. Survival percentage of microtuber was also studied at three storage intervals (3, 6 and 12 months) and differed in percentages of stored microtubers. The 3-month storage showed higher survival percentages of microtubers than the longer duration (6 and 12 months). The studied potato cultivars showed varied responses with regard to the survival percentage of microtubers. All Blue was found best in survival ability among the studied cultivars. Regarding microtuber sizes (0.3-0.4cm and 0.5-0.6 cm diameter), bigger ones showed higher survival efficiency than the smaller ones. De-sprouting was also found effective for increasing sprouting numbers/microtuber.

Keywords: Potato microtuber, storage temperature, sprouting.

INTRODUCTION

Good quality seed potato production is dependent on micropropagation of virus-free plant materials. The end product of potato micropropagation is either plantlets or microtubers. The microtubers are more convenient than plantlets in respect to long term storing, handling and transporting. So, microtuber could be an ideal propagation material for seed potato programme, and also suitable for both short and long term germplasm storage (Wang and Hu, 1982, Rosell *et al*., 1987, Tovar *et al.*, 1985).

Tubers are considered dormant if they are unable to sprout at a favourable temperature (Coleman, 1987). Dormancy of potato tuber buds is difficult to control. Immediately after harvest, field-grown potato tuber or *in vitro* microtuber or greenhouse-grown minitubers cannot be induced to sprout even under optimal environmental conditions. The dormancy period in potato tubers has been reviewed by Hemberg (1985). In the socalled rest period, tuber buds fail to grow even under optimum temperature and light conditions, and there appears to be an internal 'clock' which triggers cellular events leading to sprouting (Rappaport and Wolf, 1968, 1969). The optimum temperature for sprout growth is 16-20 ˚C (Beukema and Van der Zaag, 1990).

Potato tuber sprouting has been known for a long time. Sprouted potatoes develop more rapidly to produce higher and earlier yield. Sprouting from tuber eyes starts at the end of tuber dormancy period. First of all, an eye of the upper part gets sprouting. Sprouting of the other shoots contained in the tuber eye is blocked. This is called apical dominant effect. If tubers are planted within the apical dominant period, the potato plant will probably grow just one stem overall resulting in low yield (Van der Zaag and Van Loon 1987; Grigoriadou and Leventakis, 1999).

To produce efficient potato yield, different tuber sprouting methods are applied including the method of temperature influence. This method stimulates enzymic activity in a tuber, encourages more rapid germination of eyes, shortens the sprouting period and accelerates development of a plant. With increasing temperature, however, respiration intensities grow, therefore, energy resources of a plant are lost (Kirnak *et al*., 2001). For *in vitro* microtuber induction, several factors are considered to be influential. The major ones are photoperiod, temperature, sucrose concentration in the culture media, illumination (flux density) and growth regulators in the media. Temperature is an important determinant factor for microtuber induction and subsequent sprouting of harvested microtuber. The intention of the present study is to induce temperature stress in harvested microtubers to obtain sprouting efficiency irrespective to chemical treatment.

Dormancy breaking of microtuber before plantation is one of the most important constraint of microtubers. So, it is very important to obtain microtubers with predictable dormancy (Garner and Blake, 1989). The dormancy period of microtubers is very long (Struik and Lommen, 1990) and can be affected by size of microtubers but it depends on the *in vitro* tuber production system (Leclerc *et al.*, 1995). They also demonstrated that microtuber dormancy and sprouting varies with *in vitro* cultural practices, post-harvest storage conditions and the genetic nature of cultivars. These variabilities play a significant role to obtain efficient microtuber production that can be utilized for minituber or nuclear seed production in net house (Jones, 1988, Garner and Blake, 1989). Coleman and Coleman (2000) found that when sucrose concentration increased from 4 to 16% in the induction media and provided with 8 h photoperiod the resulted microtubers gained shorter dormancy and enhanced sprouting.

After harvest, microtubers are generally very dormant and need efficient sprouting before planting them to greenhouse or net house for seed tuber production (Struik and Wiersema, 1999; Taborri *et al.*, 1999). Dormancy release and sprouting of conventional potato tubers have received considerable interest but little attention has been paid to microtubers (Coleman, 1987; Wiltshire and Cobb, 1996).

The present experiment studied the potato microtuber induction to investigate the sprouting nature and survival percentage of the harvested microtubers in response to three different storage temperatures.

During the experiment, the following objectives were planned 1) to determine the effects of three storage temperatures (5, 15 and 20 °C) on sprouting nature of harvested microtubers, 2) survival percentages of microtubers during the cold (4 °C) storage and 3) de-sprouting effect on number of sprouts emergence per microtuber.

MATERIALS AND METHODS

Plant materials: The potato (*Solanum tuberosum* L.) cultivars studied in this experiment were Diamant, Atlanta, All Blue, Shepody and Shilbilaty. All these plant stocks were obtained from potato tissue culture bank at the Plant Breeding and Gene Engineering Lab., Department of Botany, University of Rajshahi, Bangladesh**.**

Preparation of harvested microtubers

Freshly harvested microtubers of five potato cultivars were subjected to conduct this experiment. The microtubers were produced under *in vitro* cultural process. Microtubers were propagated in MS (Murashige and Skoog, 1962) basal salt solution with standard vitamins and 8% sucrose, but no growth regulators were added during the production of microtubers. For initial development of microtubers, the culture vessels were kept in orbital shaker at 70 rpm for 4 weeks. After that the culture vessels were kept in culture rack and maintained under complete darkness at 18±2 °C growth room temperature for 16 weeks. After harvesting, the microtubers were rinsed with Clorox (Sodium hypochloride 5.25%) for 2-3 minutes and washed with distilled water. Before storage, microtubers were placed onto Petri dishes and left on for 24 h at room temperature, and then the microtubers were stored in three regimes : 1) A part of microtubers incubated in three storage temperatures at 5, 15 and 20 °C for 12 weeks under complete darkness to evaluate the sprouting nature. In

this case, microtubers of ≥150 mg fresh weight were used and sprout length of ≥2 mm was considered as starting point for measuring sprouting. Samples of 40 microtubers with four replicates were planned for each temperature treatment. Each replicate consisted of one Petri dish containing ten microtubers, 2) To study the survival percentage of microtubers, a part of microtubers with different sizes (0.3-0.4 cm and 0.5-0.6 cm diameter) were stored in a refrigerator at 4 ˚C in the dark and their survival percentage was evaluated at 3, 6 and 12 months storage. Three replicates were planned per storage month and microtuber sizes. Each replicate consisted of 10 microtubers of two sizes (0.3-0.4 cm and 0.5-0.6 cm diameter), 3) To study the de-sprouting effect on sprouted microtubers, microtubers were stored in refrigerator at 4 ˚C in the dark for 3 months. At this time, the dominant sprout(s) were removed. Microtubers were then incubated at 20 ˚C in dark for 8 weeks. A saturated solution of $MgSO_4.7H_2O$ was used to maintain the relative humidity at 90% inside the incubator as described by Leclerc (1993). The number of sprouts was then counted.

Measured parameters

Sprouting nature of microtubers

The parameters evaluated on sprouting nature under 5, 15 and 20 ˚C storage conditions after 4 and 12 weeks were 1) sprout length, 2) sprout weight and 3) number of sprout(s)/ microtuber.

The experimental design was a completely randomized with two factors (cultivar × storage temperature). Data on different parameters were statistically analyzed by MSTAT statistical program. Means were separated using Duncan's Multiple Range Test at 5% level of significance.

Survival percentages of microtubers

Two sizes of microtubers (0.3- 0.4 cm and 0.5- 0.6 cm diameter) stored in refrigerator at 4 ˚C for 3, 6 and 12 months and were tested to evaluate survival percentages (%) of microtubers**.** The entire experiment was executed in 3 replications and included 10 microtubers per replicate. Only the mean data were calculated during this experiment.

De-sprouting effect

De-sprouting effect was measured as the number of sprout(s) emerged per microtuber eye after removing the primary sprouts. Three replicates with 5 microtubers per replicate was planned for the experiment. Only the mean value was measured.

RESULTS AND DISCUSSION

Sprouting nature of microtubers

The storage temperatures significantly affected the sprouting characteristics. The cultivars and temperatures showed varied response in sprouting traits. The cultivars were not significantly affected by the storage temperature in sprouting number at both 4 and 12-week storages (Table 1).

Table 1. The influence of cultivars and storage temperatures on sprouting nature of potato microtubers at 4 and 12 weeks incubation periods. Values are an average of 4 replicates.

Cultivar / Storage temp. (˚C)	Sprouting trait					
	Sprout length (cm)		Sprout weight (mg)		Sprout no./microtuber	
	4 wks	12wks	4 wks	12wks	4 wks	12wks
All Blue						
5	0.225	0.225	0.45	0.45	0.50	1.00
15	0.425	1.525	0.95	3.47	1.25	1.25
20	1.000	1.500	2.27	3.40	1.00	2.00
Shepody						
5	0.275	0.625	0.55	1.32	1.00	1.00
15	0.925	1.525	1.95	3.27	1.25	2.00
20	0.900	1.750	1.90	3.75	1.00	1.75
Diamant						
5	0.225	0.225	0.35	0.35	0.50	1.00
15	0.500	1.400	0.80	2.35	1.25	1.00
20	0.450	1.325	0.72	2.22	1.00	1.50
Shilbilaty						
5	0.200	0.300	0.20	0.37	0.50	1.00
15	0.950	1.600	1.32	2.27	1.25	1.00
20	0.525	1.400	0.70	2.00	1.25	1.25
Atlanta						
5	0.225	0.325	0.25	0.42	1.00	1.25
15	0.775	1.650	1.05	2.30	1.50	1.25
20	0.500	2.100	0.67	2.92	1.25	1.50

Mean square values from ANOVA

Sources of variation	df	Mean square values					
Cultivar (C)	4	0.15*	0.29ns	1.72*	3.40*	0.23ns	0.47ns
Temp. (T)	2	1.45*	10.23*	5.11*	32.71*	1.86*	1.51*
C × T	8	0.17*	0.12ns	0.77*	0.45ns	0.09ns	0.28ns
Error	45	0.02	0.13	0.07	17.26	0.18	0.26

*, Significant at 0.05, 0.01 and 0.001 levels respectively; ns, non-significant.

The main factors, cultivar and storage temperature both differed significantly in sprout weight while the same parameter showed non-significant results at cultivar ×

temperature interaction after 12-week storage (Table 1). The highest sprout length was observed in Atlanta (1.35 cm) followed by Shepody (1.30 cm) and the lowest in Diamant (0.98 cm) at 12-week storage conditions. The sprout weight (2.78 mg) and sprout number (1.58 sprouts / microtuber) were highest in Shepody and the lowest was noted in Shilbilaty (1.55 mg) and sprout number (1.08 sprouts/microtuber) after 12-week period. No significant differences were noticed between the sprout length and sprout number for the studied cultivars after 12-week storage while sprout weight showed significant difference at 4 and 12-week storages (Table 2). Among the cultivars, Shepody showed the highest performances in sprouting characteristics which was comparable to All Blue. Storage of microtubers at low temperature (5 °C) prolonged the rest period considerably (data not shown). Difference was higher between tubers stored at 5 and 15 °C than between tubers stored at 15 and 20 °C (Table 2). In most of the cases it was found that higher storage temperature resulted in higher values for sprouting parameters at the end of storage (12 weeks). On average, 15°C storage temperature enhanced length and number of sprouts (Table 2).

Table 2. Sprouting nature of microtubers as affected by five potato cultivars and three storage temperatures at two incubation periods of 4 and 12 weeks.

Treatments	Sprouting traits					
	Sprout length (cm)		Sprout weight(mg)		Sprout no. /microtuber	
[a]Effect of cultivars	4 wks	12 wks	4 wks	12 wks	4 wks	12 wks
All Blue	0.55ab	1.08a	1.22a	2.44ab	0.91a	1.41a
Shepody	0.70a	1.30a	1.46a	2.78a	1.08a	1.58a
Diamant	0.39b	0.98a	0.62b	1.64b	0.91a	1.16a
Shilbilaty	0.55ab	1.10a	0.74b	1.55b	1.00a	1.08a
Atlanta	0.50ab	1.35a	0.65b	1.88ab	1.25a	1.33a
LSD (p ≤0.05)	**0.232**	**NS**	**0.456**	**1.019**	**NS**	**NS**
[b] Effect of Temp.(°C)						
5	0.23b	0.34b	0.36b	0.58b	0.70b	1.05a
15	0.71a	1.54a	1.21a	2.73a	1.30a	1.30a
20	0.67a	1.61a	1.25a	2.86a	1.10ab	1.60a
LSD (p ≤0.05)	**0.180**	**0.469**	**0.353**	**0.789**	**0.559**	**NS**

[a] Each value is an average of 3 temperatures and 4 replicates, [b] Each value is an average of 5 cultivars and 4 replicates. In a column mean values followed by different letters are significantly different ($p \leq 0.05$).

Sprout length of microtubers increased as storage period increased. The average sprout length was about 0.34 cm at the end of 12-week storage period at 5 °C. However, it exceeded 1.5 cm for microtuber stored at 15 and 20 °C. On the other hand, the microtubers stored with 20 °C produced higher sprout length than those stored with 5 and 15 °C at all the storage periods. Microtubers stored with lower temperature (5 °C) yielded shorter sprout length (Table 2). No significant differences were found in sprout weight between 15 and 20 °C storage temperatures. Sprout weight also progressively decreased with the decrease of storage temperatures at both the storage periods (Table 2). The mean number of sprouts/microtuber was not significantly different among the storage temperatures but at 20 °C storage temperature, highest sprout number was achieved at 12-week period. After 4-week storage, sprout number was significantly different and 15 °C resulted highest number of sprouts (Table 2). The number of sprouts was affected by storage temperature as observed by Wiersema *et al.* (1987).

Survival percentages of microtubers

Cultivars, storage months and microtuber sizes, all had a effect on survival percentage of microtubers during storage (Table 3). The two microtuber sizes differed in their survival ability to the storage months. Microtubers of 0.3-0.4 cm were more susceptible to desiccation or exhaustion to the subsequent storage months than the larger (0.5-0.6 cm) microtubers.

Table 3. Effects of storage months and microtuber sizes on survival percentages of microtubers. Each value is an average of 3 replicates. Each replicate consisted of 10 microtubers.

Cultivar		Survival % of microtubers	
		Microtuber sizes (cm)	
	Storage at 4 °C (months)	0.3 – 0.4	0.5 – 0.6
Atlanta	3	85	96.66
	6	73.33	95
	12	46.66	63.33
Shepody	3	86.66	96.66
	6	65	86.66
	12	45	65
Shilbilaty	3	81.66	98.33
	6	66.66	95
	12	60	75
Diamant	3	88.33	100
	6	68.33	85
	12	51.66	75
All Blue	3	96.66	100
	6	85	98.33
	12	70	90

After 3 months storage, the survival percentage of small microtubers (0.3-0.4 cm) were 85, 86.66, 81.66, 88.33 and 96.66% for Atlanta, Shepody, Shilbilaty, Diamant and All Blue, respectively while that of large microtubers (0.5-0.6 cm), all the cultivars showed more than 95% survivability. The Diamant and All Blue microtubers

showed 100% survival rate after 3-month storage. After 12 months, the survival rate of small microtubers decreased considerably and some tubers were no longer viable, while the large microtubers resulted in 63.33, 65, 75, 75 and 90% survival for Atlanta, Shepody, Shilbilaty, Diamant and All Blue, respectively (Table 3).

It was observed that the survival percentages of microtuber at different storage months were increased with the increased size of microtubers. Earlier works have also reported similar findings (Singh *et al.*, 1994; Tabori *et al*., 1999). Such response can be attributed to the increased susceptibility of smaller microtubers to low temperatures during storage due to their immature state, as well as to the excessive exhaustion of food reserves in small tubers on account of longer storage intervals (Tabori *et al*., 1999).

The survival efficiency of microtubers was differed in their microtuber sizes. Some microtubers of small sizes were a considerable loss which can be a result of their immature state. This loss was present both during the period immediately after harvest and during storage. Tuber losses in the larger size were observed but these losses mainly happened during the storage period, and they were accompanied with turgor loss and brownish discoloration as reported by Lommen (1993).

The 3-month storage, resulted the highest survival percentages of microtubers than the extended months (6 and 12 months). The cultivars showed varied response in survival percentages of microtubers. All Blue was found best in survival capacity than the other four cultivars (Table 3). Microtuber sizes also showed varied response towards different storage months (Table 3).

Table 4. The effect of de-sprouting on number of sprouts compared to intact microtubers after 8 weeks of incubation in dark at 20 °C. Values are an average of 3 replicates. Each replicate consisted of 5 microtubers (0.3-0.6 cm diameter).

Cultivar	Number of sprouts emerged /microtuber	
	Intact	De-sprouted
Atlanta	1.20	1.80
Shepody	1.00	2.20
Shilbilaty	1.20	2.80
Diamant	1.00	2.60
All Blue	1.00	3.00
Mean	**1.08**	**2.48**

De-sprouting effect

The five cultivars showed different responses regarding the number of sprouts before and after de-sprouting. The effect before de-sprouting was most likely associated with cultivar differences in dormancy whereas de-sprouting broke the remaining dormancy and apical dominance causing increasing number of sprout. Removing the primary sprout(s) of the microtubers, increased the number of secondary sprout(s) compared with intact microtubers. After de-sprouting, the number of secondary sprout(s) depended on microtuber sizes and cultivars. All Blue produced more sprouts (3 sprouts/ microtuber eye) than the others. The sprout number increased after de-sprouting than the initial intact microtubers and those were mostly of one sprouted microtubers (Table 4). This observation is in agreement with the findings of Carli *et al.* (2012). The beneficial effect of de-sprouting (increasing the sprout bud/microtuber) was observed only with large microtubers, but a large proportion of small microtubers (0.3-0.4 cm) failed to produce new sprouts once the primary sprout was removed (data not shown).

CONCLUSION

Microtubers are very small tubers and it should be stored while they are dormant. Thus the storability of microtubers can determine their further use especially as a source materials of 'virus-free seed potato' production. From this experiment, it is demonstrated that the dormant period of *in vitro* grown potato microtubers can be predicted by storage temperatures and thus timing of microtuber production and sprouting can be planned. It is an important agronomic step for microtubers before transplanting them to the field. Different number of sprouts per microtuber suggests differences in physiological age of microtubers, which can be related to differences in duration of sprouting. These valuable information will help our potato farmers for tuber seed production using microtubers,

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