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Performance of microtuber derived from *in vitro* plantlets of potato varieties on sprout attributes in relation to its weight

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ABSTRACT

The present work was conducted to evaluate the varietal performance of three potato varieties namely- Asterix, Granola and Diamant with different microtubers weights of >500 mg, 250-500 mg and <250 mg on sprout characters. The variety Granola showed longer dormancy period (30.33 days) in the case of less weight microtuber and it was decreasing the rate with increasing of microtuber weight in all varieties. The variety Diamant produced slightly more sprout per microtuber for all weights while the variety Asterix showed higher number of sprout per microtuber by >500 mg. Asterix had significantly longer sprouts (28.43 mm) than other two varieties and the trend of the length of sprout was decreased with the decrease of microtuber weight. The larger microtubers (>500 mg) of the variety Diamant and Asterix tended to have higher values on fresh weight of sprouts and sprout mass per unit of sprout length than Granola variety.

INTRODUCTION

In Bangladesh, potato (*Solanum tuberosum* L.) mainly is a winter vegetable crop. Microtuber induction and development of Bangladeshi potato cultivars protocols are needed for their growth and yield efficiency study compare to other propagules for seed tuber production. Utilization of microtubers for the production of seed tuber is becoming an important technique (Zakaria et al. 2008). Bangladesh Agricultural Development Corporation providing only 8% of quality seed tuber for the country (Dey 2001). To meet up this gap, quality microtuber through micropropagation can minimize this problem. In recent years, alternative seed production program has been developed in which the first multiplication steps are speeded up by using in vitro plantlets (Roca et al. 1978; Hussey and Stacey 1981 and Wattimena et al. 1983), microtubers (Hussey and Stacey 1984; Rosell et al. 1987 and Forti et al. 1992), and minitubers (Struik and Lommen 1990). Microtubers are particularly convenient for handling, storage, and transportation of germplasm and for the development of disease-free materials (Hussey and Stacey 1984 and Wang and Hu 1982). Storage environment should be considered for microtubers because the storability regulates their future use. Microtubers in different grades have different dormancy requirements and differ widely in relative growth potential and productivity. Dormancy period is influenced by the variety, maturity of tuber and environmental conditions that prevail during the growth and storage conditions (Beukema and Van der Zaag 1990 and Vreugdenhil 2007). During the rest period, field-grown potato tubers or in vitro microtubers or greenhouse-grown minitubers cannot be induced to sprout even under optimal environmental conditions and it is of 5-10 weeks' duration in different cultivars (Cho et al. 1983). Microtubers dormancy also influenced by its size and time of harvest (Leclerc et al. 1995). It is important to break tuber dormancy for seed potato multiplication, rapid post-harvest disease testing,

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and early production in the field or greenhouse. Genotypic diversity, tuber grades, and storage environment might have a potential impact on dormancy of microtuber as well as sprouting beheaviours. Although there are some protocols for in vitro microtuberization, there is also lack of information regarding their storage performance according to size especially for Bangladeshi potato cultivars. Considering above facts, the study was undertaken to evaluate the effective microtuber grades and varietal potentiality to enhance sprouting of microtubers.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Tissue Culture Laboratory in the controlled environments during the period of September 2010 to February 2011.

In vitro multiplication of plantlets

Diseases free *in vitro* plantlets of three potato varieties namely- Asterix, Granola, and Diamant were collected from Bangladesh Agricultural Research Institute and Bangladesh Agricultural Development Corporation Tissue Culture Laboratory which were prepared through meristem culture earlier. *In vitro* plantlets of three potato varieties were multiplied as per routine by subculturing of single stem nodes at every three weeks' interval for growing the explants up to 6-8 nodes stage to get the desired number of plantlets for experimentation. The multiplication medium contained minerals salts and vitamins (Murashige and Skoog 1962) which were supplemented with 0.1 mg L^{-1} gibberellic acid (GA_3), 0.01 mg L^{-1} Napthal acetic acid (NAA), 4 mg l^{-1} D-calcium pantathonate and 30 g l^{-1} sucrose. The medium was solidified with 8 g L^{-1} agar and before autoclaving the pH was adjusted to 5.7 . The temperature in the growth chamber was 20±1^{0}C with 16 hours' photoperiod and the light was supplied by fluorescent tubes at an intensity of 3000 lux.

In vitro production of microtuber

Step I: Eight stem segments (each with 3 nodes) of subcultured *in vitro* plantlets were again cultured in liquid medium in 250 mL Erlenmeyer flasks contained mineral salts and vitamins (Murashige and Skoog 1962) which were supplemented with 0.1 mg l^{-1} Gibberellic acid (GA3), 0.01mg l^{-1} naphthalene acetic acid (NAA), 4 mg l^{-1} D-calcium pantothenate, and 30 g l^{-1} sucrose for 28 days.

Step II: After 28 days, the liquid media were decanted off and 40 ml microtuber induction medium based on MS medium (Murashige and Skoog 1962) supplemented with 10 mg l^{-1} benzyladenine (BA) and different concentrations of sucrose (0, 3, 4, 6, 8, 10, 12 and 14%). Then the microtuber induction cultures were incubated in the dark at 20 ^{0}C **(**Naik and Sarker 1997). All cultures in Erlenmeyer flask were closed with a cotton cap.

Treatments of the experiment

The experiment having three potato varieties *viz*. Asterix, Granola and Diamant and, microtuber weighted as >500 mg, 250-500 mg and <250 mg. Graded fresh microtuber that had been cold-stored at 4-5 ^{0}C for 6 weeks. Sprouting was monitored every 2 days' interval. For the experiment, a sample size of 10 microtubers of each grade was used for each treatment.

Data collection

The data were collected on dormancy period, an average number of sprouts microtuber^{-1}, sprout length, fresh weight and sprout mass unit^{-1} length of microtuber. Measuring dormancy period: Microtubers were considered sprouted when a tuber had at least one sprout of at least 2 mm long. The development of sprouts of the microtubers was recorded at two-day interval until all microtubers had sprouted. The dormant period was assessed as a number of days from treatment to sprouting and was considered to have ended when 80% of the microtubers had at least one sprout of at least 2 mm long.

Experimental design and data analysis

The experiment was laid out in a completely randomized design (CRD) with three replications. All the collected data were analyzed by analysis of variance and the means were compared according to Duncan's Multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Dormancy period

The dormancy period tended to increase with a decrease in the size of microtubers and Granola had a longer dormancy period (30.33 days) in <250 mg microtuber than other two varieties (Table 1). This is the normal behaviour in open field condition of potato cultivar Granula required comparatively more time for sprouting. In this experiment, the potato cultivar solely shows their own dormancy behavior which strictly depend on their genetic constituents. The results of our experiment are also consistent with the earlier findings of Lommen (1995) where he reported that, the length of the dormancy period decreased with increasing of

Table 1. Performance of variety on dormancy period (days) as influenced by microtuber weight

Variety	Microtuber weight (mg)		
	>500	250-500	<250
Asterix	12.57 b	14.00 c	19.95 c
Granola	22.00 a	23.38 a	30.33 a
Diamant	12.57 b	18.29 b	22.76 b

Mean followed by same letter(s) in a column are not significantly different by DMRT at 5% level of probability

microtubers weights, confirming results of. Leclerc et al. (1995) suggested that the longer dormancy periods of small microtubers might reflect differences in microtuber age at the time of harvest. Emillson (1949) and Cho et al. (1983) also reported that *In vitro* microtubers cannot be induced to sprout even under optimal environmental conditions and the rest period is 5-10 weeks duration in different cultivars. Moreover, the length of dormancy is cultivar-specific (Ranalli et al. 1994) and it can be affected by environmental conditions during growth and storage (Burton 1989 and Suttle and Hultstrand 1994).

Number of sprouts

The number of sprout microtuber^{-1} was insignificant irrespective of variety and weight (Figure 1). However, the number of sprout microtuber^{-1} was slightly more in Diamant in all grades and in Asterix of higher grade.

Length of sprout

The length of sprouts tended to increase with an increase in the weight of microtubers and Asterix had significantly longer sprouts than other two varieties (Table 2). Salimi et al. (2010) reported that the length of sprout tended to increase with an increase in minituber weight.

Fresh weight of sprout

Larger microtubers showed higher fresh weights of sprouts than smaller ones and Diamant

Table 2. Response of variety on length of sprout (mm) influenced by microtuber weight

Variety	Microtuber weight (mg)		
	>500	250-500	<250
Asterix	28.43 a	24.85 a	17.55 b
Granola	10.58 c	8.81 c	8.03 c
Diamant	27.01 b	23.01 b	19.36 a

Mean followed by same letter(s) in a column are not significantly different by DMRT at 5% level of probability

Table 3. Response of variety on sprout mass (mg mm^{-1}) influenced by microtuber weight

Variety	Microtuber weight (mg)		
	>500	250-500	<250
Asterix	0.42 b	0.32 b	0.21 a
Granola	0.39b	0.23 c	0.19 b
Diamant	0.49 a	0.36 a	0.20 ab

Mean followed by same letter(s) in a column are not significantly different by DMRT at 5% level of probability

and Asterix gave higher fresh yields of sprouts than Granola (Figure 2). Salimi et al. (2010) reported that the fresh weight tended to increase with an increase in minituber weight.

Sprout mass

The larger the microtubers the larger the sprout mass unit^{-1} of sprout length and Diamant and Asterix tended to have higher values than Granola (Table 3). Salimi et al. (2010) reported that the sprout mass unit^{-1} sprout length of the longest sprout tended to increase with an increase in microtuber weight. The sugar content at harvest is one of the important parameters determining the maturity and sprouting vigor of seed potato because sucrose, glucose, and fructose are known to play a primary role in the sprouting metabolism (Rees and Morrell 1990). These results are also supported by Gregory (1956) and Ewing (1990) where they

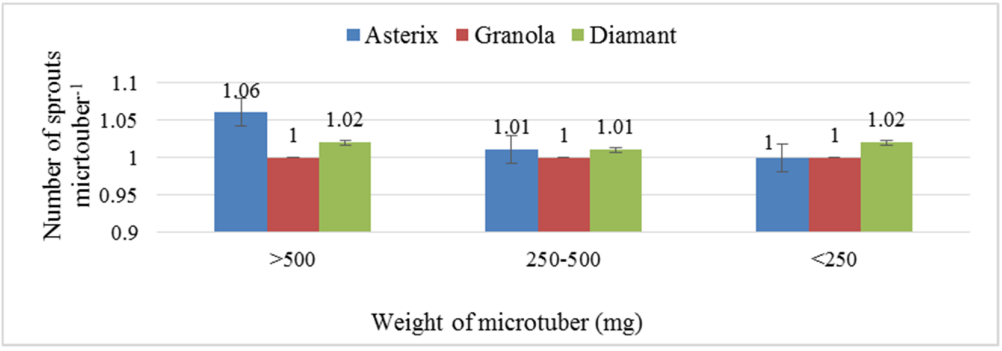

Figure 1. Response of variety on sprout number influenced by microtuber weight

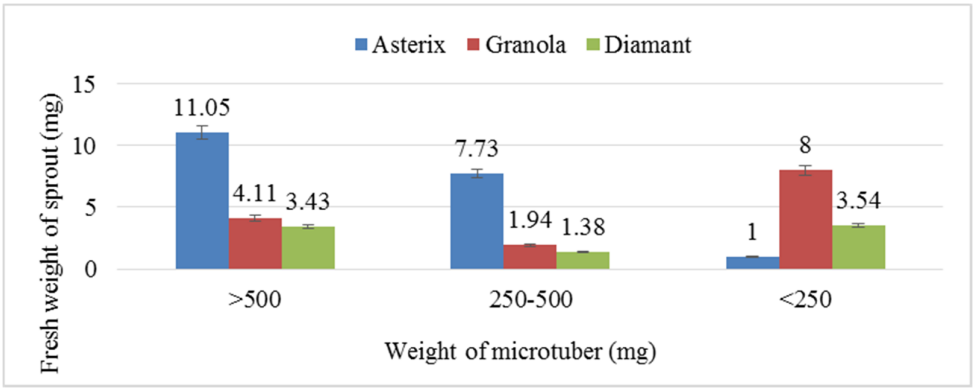

Figure 2. Response of variety on fresh weight of sprout influenced by microtuber weight

suggested that sucrose may be the only compound necessary for induction of microtuber. Sucrose provides a concentration promising for the development of microtubers (Aslam and Iqbal 2010), microtuber induction response on MS medium supplemented with 8% sucrose (Carlson 2004; Sushruti et al. 2004 and Miranda et al. 2005).

CONCLUSION

Microtuber grades influenced positively on sprouting of potato varieties. Increasing rate of microtuber weight significantly takes minimum days to induce sprout, increases their number, fresh weight as well as fresh mass. All these characters are genotype specific. The variety Granola had a longer dormancy period and slightly longer sprouts in Asterix than other two varieties. Besides, Diamant and Asterix gave higher fresh yields of sprouts and sprout mass.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author Md. Sadek Hossain designed the study protocol. The author Md. Dulal Sarkar wrote the manuscript and managed the statistical analyses of the study. Author Md. Mahabubul Haque and Md. Rezaul Karim Talukder reviewed the drafts of the manuscript. Md. Quamruzzaman and Rojobi Nahar Rojoni managed the literature searches. All authors read and approved the final manuscript.

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