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Sprouting Behaviour in Response to Gibberellic Acid in Potato Microtuber

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSH designed the study, wrote the protocol, wrote the first draft of the manuscript and performed the statistical analysis. Authors Md. Mahabubul Haque, MZ and M. Mofazzal Hossain reviewed the study design and all drafts of the manuscript. Author MDS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present work was carried out to break dormancy of microtuber from storage environment for seed potato multiplication as well as regulating their future use.
Study Design: The experiment was laid out in a completely randomized design with three replications.
Place and Duration of Study: The experiment was conducted in the Tissue Culture Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh during the period of September 2010 to February 2011.
Methodology: The experiment having two factors. First factor; microtuber weight which are graded as S_1 = >500 mg, S_2 = 250-500 mg, S_3 = <250 mg and second factor; seven levels of GA_3 *viz*. G_1= 0.0 mg L^{-1}, G_2 = 25 mg L^{-1}, G_3 = 50 mg L^{-1}, G_4 =100 mg L^{-1}, G_5 =150 mg L^{-1}, G_6 =250 mg L^{-1}, and

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G_7 = 500 mg L^{-1}. To determine the most effective level of GA_3 were applied on graded fresh microtuber that had been cold-stored at 4-5℃ for 6 w eek. Sprouting was monitored every 2 days' interval. Microtubers per treatment were soaked in different concentration of GA_3 for 24 h in the light, and then placed for incubation under 16/8 h d/n cycle.

Results: The lowest number of days (13) was taken to induce sprout in case of heavier microtuber and highest number of days (20.44) required for sprouting of smaller microtuber when 500 mg L^{-1} of GA_3 was used. Microtubers exposed to lower concentrations of GA_3 exhibited short length sprout. The trend of fresh weights of sprouts decreasing with the decrease of microtuber weight. The microtubers of >500 mg grade produced more sprout mass $unit^{-1}$ length of sprouts than other two grades.

Conclusion: Microtuber <250 mg has longer periods of dormancy than larger microtubers and breaking their dormancy, 500 mg L^{-1} GA_3 showed superior performance.

Keywords: Dormancy; GA_3; physiological state; tuber grades.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a member of the family Solanaceae. It is the 3rd most important food crop of Bangladesh next to rice and wheat in terms of production. Bangladesh producing 9.0 million tons of potato from 0.5 million hectares of land with an average yield 14.74 t ha^{-1} [1] which is much lower compare to many potato growing countries of the world. Seed potato quality can be measured by the ability to produce sprouts and daughter tubers and is affected by production and storage conditions [2]. Quality seed tuber play significance role as seed in case of potato cultivation. At present, Bangladesh Agricultural Development Corporation able to meet only 8% of quality seed for the country [3]. Minitubers are progeny tubers produced on *in vitro* plantlets and much smaller than traditional seed tubers, usually ranging from 5 to 25 mm in diameter, coinciding with a weight range of 0.1-10 g or more [4]. To meet up this demand, innovative methods like micro-propagation is urgent needed for producing quality microtuber. Microtubers should be stored carefully while they are dormant because the storability of microtubers regulates their future use. Dormancy period is influenced by the age of tuber and environmental conditions that prevail during the tuber development on the mother plant and after harvest [5] and [6]. Immediately after harvest, field-grown potato tubers or *in vitro* microtubers or greenhouse-grown minitubers cannot be induced to sprout even under optimal environmental conditions. To break the tuber bud dormancy or to shorten the resting period, GA_3 is reported as hormone and widely used to break potato dormancy as well as stimulating the sprouting of potato seed tuber [7] and [8]. Sprouting can be stimulated by applying chemicals and by manipulating the composition of the atmosphere of the storage environment [6]. GA_3 to break dormancy of potato minitubers is very common but it is cultivar dependent [9]. It is important to break tuber dormancy for seed potato multiplication, rapid post-harvest disease testing, and early production in the field. Considering above facts, the study was undertaken to determine the effective concentration of GA_3 for breaking dormancy of microtubers.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in the Tissue Culture Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur, Bangladesh during the period of September 2010 to February 2011.

2.2 Seed Source

Diseases free *in vitro* plantlets of potato variety-Asterix was collected from tissue culture laboratory of Bangladesh Agricultural Research Institute and Bangladesh Agricultural Development Corporation which prepared through meristem culture earlier.

2.3 *In vitro* Multiplication of Plantlets

In vitro plantlets multiplied as per routine by sub culturing of single stem nodes at every three weeks interval for growing the explants upto 6-8 nodes stage to get desired number of plantlets for experimentation. The multiplication medium contained minerals salts and vitamins [10] which was 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 pH was adjusted 5.7 prior to autoclaving. Temperature in the growth chamber was 20±1℃ with 16 hours photoperiod and light was supplied by fluorescent tubes at an intensity of 3000 lux.

2.4 *In vitro* Production of Microtuber

2.4.1 Step i

Eight stem segments (each with 3 nodes) of sub cultured *in vitro* plantlets were again cultured in liquid medium in 250 ml Erlenmeyer flasks contained mineral salts and vitamins [10] supplemented with 0. 01 mg L^{-1} GA_3, 0.01 mg L^{-1} NAA, 4 mg L^{-1} D-calcium pantathonate and 30 g L^{-1} sucrose for 28 days.

2.4.2 Step ii

After 28 days, the liquid media were decanted off and 40 ml microtuber induction medium based on MS medium [10] supplemented with 10 mg L^{-1} benzyl adenine (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℃ [11]. All cultures in Erlenmeyer flask were closed with cotton cap.

2.5 Treatments and Design of the Experiment

The experiment was laid out in a completely randomized design with three replications having two factors. First factor; microtuber weight which are graded as S_1 = >500 mg, S_2 = 250-500 mg, S_3 = <250 mg and second factor; seven levels of GA_3 *viz*. G_1= 0.0 mg L^{-1}, G_2 = 25 mg L^{-1}, G_3 = 50 mg L^{-1}, G_4 =100 mg L^{-1}, G_5 =150 mg L^{-1}, G_6 =250 mg L^{-1}, and G_7 = 500 mg L^{-1}. Concentration of GA_3 was prepared on the basis of strength viz, zero, x, 2x, 4x, 6x, 10x and 20x. To determine the most effective level of GA_3 were applied on graded fresh microtuber that had been cold-stored at 4-5℃ for 6 week. Sprouting was monitored every 2 days' interval. For the experiment, a sample size of 10 microtubers of each grade was used for each treatment. Microtubers per treatment were soaked in different concentration of GA_3 for 24 h in the light, and then placed for incubation under 16/8 h d/n cycle.

2.6 Data Collection

The data were collected on dormancy period, sprout length, fresh weight of sprout and sprout mass $unit^{-1}$ length.

2.7 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 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.

2.8 Analysis of Data

All the collected data were analyzed by analysis of variance and the means were compared according to Duncan's Multiple Range Test at P = .05 level of probability.

3. RESULTS AND DISCUSSION

3.1 Dormancy Period

Dormancy period decreased with an increase in concentration of GA_3. GA_3 at a concentration of 500 mg L^{-1} was found to be the most effective in breaking dormancy. All grades of microtuber produced active sprout within short period of time in presence of 500 mg L^{-1} of GA_3 (Fig. 1). The lowest number of days (13) was taken to induce sprout in case of heavier microtuber and highest number of days (20.44) required for sprouting of smaller microtuber when 500 mg L^{-1} of GA_3 was used. The length of the dormancy period decreased with increasing microtuber weights, confirming results of [12]. [13] suggested that the longer dormancy periods of small microtubers might reflect differences in tuber age at the time of harvest.GA_3 might have the most active compound for early as well as multiple sprouting. The microtuber with or without variable periods of cold storage, 500 mg L^{-1} GA_3 was the most efficient in breaking-dormancy and inducing precocious sprouting. GA_3 was the only agent that was able to break dormancy of minitubers that had not been cold stored. It is shown that increasing the concentration of GA_3 resulted in a decrease in number of days to sprouting. This results are in agreement with the findings of [7, 8,14,15] where they stated that minimum days required for sprouting of potato seed minitubers

using gibberellic acid and it is suggested that GA_3 should be used to terminate the breaking dormancy and to promote the sprouting of potato seed tubers.

3.2 Length of Sprouts

Irrespective of microtuber size, the length of sprouts following treatment with GA_3 was significantly higher than those of the controls (Fig. 2). Microtubers exposed to lower concentrations of GA_3 exhibited short length sprout. The maximum length of sprout was produced by the concentration of 500 ppm of GA_3 in all grade of microtuber, again 150 ppm concentration also produced longest sprout in medium grade. [8] and [16]. reported that the length of sprouts was significantly enhanced by treating minitubers with GA_3. The results are also in agreement with the findings of [15] and [17] where they reported that increase in GA_3 lead to increases number of sprout per tuber, sprout length and sprout vigor.

3.3 Fresh Weight of Sprout

Larger microtubers showed higher fresh weights of sprouts than smaller ones and 500mg GA_3 gave higher fresh yields of sprouts than other treatments (Table 1). [8] reported that the fresh weight tended to increase with an increase in minituber weight.

3.4 Sprout Mass

The larger the microtubers the larger the sprout mass $unit^{-1}$ of sprout length microtubers treated with GA_3 showed significantly lower sprout mass $unit^{-1}$ length than those untreated microtubers (Table 2). In microtubers treated with GA_3, the response depended on the microtuber weight. The microtubers of >500 mg grade produced heavier sprout mass $unit^{-1}$ length of sprouts than other two grades. This result is in agreement with the findings of [8] where he stated that the increase in sprout length is most likely also associated with an increase in cell elongation.

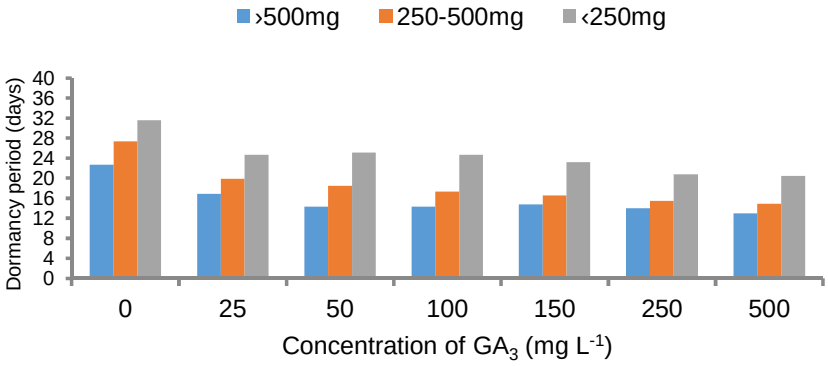

Fig. 1. Effect of GA_3 on dormancy period of different grades of microtuber

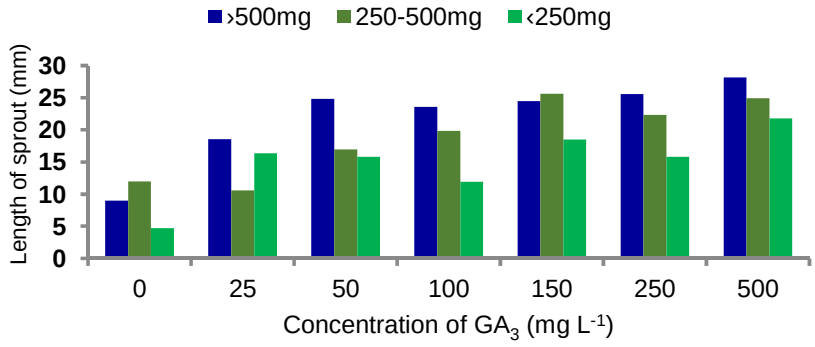

Fig. 2. Response of GA_3 to length of sprout of different grades of microtuber

Table 1. Response of GA_3 to fresh weight of sprout of different grades of microtuber

GA_3 (mg L^{-1})	Grades (mg)		
	>500	250-500	<250
0	4.33 d	4.71 e	1.22 e
25	9.33 c	6.67 c	3.33 b
50	9.96 b	5.55 d	2.26 c
100	10.33 ab	7.11 b	1.70 d
150	9.33 c	5.70 d	3.37 b
250	10.22 b	3.41 f	4.26 a
500	10.81 a	8.07 a	3.33 b

Mean followed by same latter(s) in a column are not significantly different by DMRT at P = .05 level of probability

Table 2. Response of GA_3 to sprout mass $unit^{-1}$ length of different grades of microtuber

GA_3 (mg L^{-1})	Grades (mg)		
	>500	250-500	<250
0	0.57 a	0.36 a	0.29 a
25	0.49 b	0.30 cd	0.26 b
50	0.41 c	0.32 bc	0.15 d
100	0.41 c	0.33 b	0.14 d
150	0.37 c	0.30 cd	0.15 d
250	0.40 c	0.28 d	0.20 c
500	0.38 c	0.21 e	0.20 c

Mean followed by same latter(s) in a column are not significantly different by DMRT at P = .05 level of probability

4. CONCLUSION

GA_3 had a significant effect in breaking dormancy of microtubers. GA_3 effectively shortened the dormancy but it was dependent on microtuber grades. Sprouts from GA_3 treated microtubers tended to be long. Small microtubers (<250 mg) showed longer periods of dormancy than larger microtubers. For breaking microtuber dormancy, 500 mg L^{-1} GA_3 to be the most potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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