

EFFECT OF GA₃ ON OFF-SEASON SPROUTING AND *in vitro* REGENERATION IN THREE POTATO VARIETIES

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ABSTRACT

Off-season sprouting ability and *in vitro* regeneration potentiality were investigated to establish a rapid multiplication protocol in three potato varieties *viz.* Diamant, Cardinal and Granola. The results revealed that sprout initiation time, sprout length and sprout number were directly related to the concentration of GA₃ applied. Higher concentration (400 ppm) of GA₃ showed better performance for all the parameters studied. Among the three varieties, Granola showed quick response to GA₃ application. MS medium supplemented with 1, 2, 3, 4 and 5 mg/L of GA₃ were employed for *in vitro* regeneration of these three cultivars. Among all the treatments, MS medium + 4.0 mg/L of GA₃ showed the best performance on multiple shooting. The hormone IBA was applied for root induction on the regenerated shoot of the potato varieties. In all the varieties 2 mg/L of IBA showed the highest root induction. Variety Granola was the most responsive in both shoots and root development. The regenerated plantlets were acclimatized in natural environment. Among them almost 90% plants ultimately survived.

Key words: Sprouting, regeneration, potato, growth hormone

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the important vegetable crops all over the world. It is usually propagated by tuber. It requires a number of multiplication cycles to get a marketable quantity of seed (Rashid, 1991). The quality of seed tuber is affected due to the exposure to natural infection specially viral and fungal infection. Therefore, rapid multiplication of suitable and quality propagules of potato and their maintenance is very important. Rapid clonal propagation of selected stocks is highly desirable for seed production program. Sprout cutting and stem or shoot cutting techniques are used in many countries (Wang and Ching-yeh, 1982; Uyen and Zaag, 1983). The conventional clonal propagation methods take long time to get sufficient number of seed tuber. Thus, off-season sprouting is essential for year round potato plantlet production. In natural condition, potato sprout initiation starts in the month of September and it is very rare to initiate sprouting just after harvest. During off-season, sprouting is considered as a major source of explant. Therefore, the experiment was designed to initiate sprout in off-season (March to May) for rapid multiplication of potato. *In vitro* regeneration protocol establishment was another objective of this present investigation. Rapid multiplication techniques have been successfully employed for huge propagation in potato. Seed potato production through tissue culture technique is proved to be better than any other method (Naik, 2000). *In vitro* regeneration potentiality varies from genotype to genotype. Hence, it is necessary to identify which variety is more prone to *in vitro* culture. Phytohormones have individual influence on *in vitro* regeneration in potato. Concentration of each hormone has significant effect on micro propagation in potato use reference (Sarker and mustafa, 2002). GA₃, and IBA are different growth regulators which have major effect on sprouting, shoot regeneration and root induction in potato (Farhatullah *et al.*, 2007). Therefore, the experiment was designed to investigate the effect of GA₃ on sprouting and *in vitro* regeneration of potato under combined effect of GA₃ and IBA treatment.

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MATERIALS AND METHODS

The present research work was carried out at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh, during the period from March to December' 2009. The experimental materials were collected from the Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh. Three well known varieties *viz.* Diamant, Cardinal and Granola were used for this work. Disease free healthy tubers were first washed with tap water and then distilled water. Five different concentrations of GA₃ *viz.* 50, 100, 200, 300 and 400 ppm were used to study the sprouting ability in number of sprout per tuber and off-season (March -May) potato production. Ten tubers were treated with a single concentration. Data were recorded on days to sprout initiation, sprout length (cm), node per sprout. One to two week old sprout was used as explant for *in vitro* regeneration. From the regenerated plantlet nodal segments were used as explants.

The sprouts were surface sterilized by immersing in 0.2 HgCl₂ solution for 2-3 minutes and washed several times with sterile distilled water. The explants were cultured in culture tubes containing MS semi-solid media (pH 5.8) autoclaved at 121°C with 1.16 kgcm⁻² pressure for 25 minutes. The phyto hormone GA₃ was applied in different concentrations (1, 2, 3, 4 and 5 mg/L) to investigate the *in vitro* shoot induction potentiality of three potato varieties. The cultures were incubated at 25±2°C under white light intensity (2500-3000 lux). The regenerated shoots were sub cultured for root induction. Different concentrations of IBA *viz.* 0.5, 1.0, 1.5, 2.0, 2.5 mg/L were supplemented with MS media for multiple root formation. The experiment was conducted in CRD with three replication. Data were recorded on days to shoot regeneration, number of shoot per explant, shoot length (cm), days to root initiation and number of root per explant. The healthy plantlets were transferred to pots containing sterilized vermiculite for hardening. The pots were covered with aluminum foil to restrict excess evaporation. After 5-7 days, the aluminum foils were removed from the pots and the seedlings were kept under net house. A total of 50 plantlets from each cultivar were transferred to evaluate acclimatization efficiency of the potato varieties. Hardening efficiency was calculated as survival percentage under natural condition.

RESULTS

Three potato varieties *viz.* Diamant, Cardinal and Granola were evaluated for sprouting efficiency through GA₃ treatment. The newly emerged sprouts were used for initial regeneration through *in vitro* culture. Subsequently, nodal segments of regenerated plantlets were used as explants for further study. MS media supplemented with different concentrations of GA₃ and IBA were used for shoot and root induction. The experimental findings are presented below.

Off –season sprouting ability of potato varieties

Off-season sprouting ability of different varieties was tested during the period March to May, 2009 under laboratory condition. The results are presented in Table 1. The potato cultivars responded differently with different combination of GA₃ concentration. It was revealed that, the variety Granola responded very quickly for sprouting. Days to sprout initiation were minimum in the case of 400 ppm of GA₃ application for all the three varieties. Within this concentration, Granola showed minimum days (3 days) for sprouting and it was maximum (5 days) in Diamant. Maximum days for sprout initiation were observed with 50 ppm of GA₃ in all the varieties, where Diamant took the highest (12 days) and the least (9 days) was in Granola. It reveals that, days to sprout initiation gradually decreases with the increased concentration of GA₃. Total sprouting time was also minimum in 400 ppm of GA₃ treatment. Granola took only 7 days for total sprouting and it was maximum (12 days) in Diamant. Average sprout length varied from 2.31 to 5.12 cm for all the treatments and varieties. Number of sprout per tuber was minimum 1 and it was maximum 6 [Fig. 1(a), (b), (c)]. It was observed that lower concentration of GA₃ produced minimum sprout for all the varieties under study and it increased generally with higher concentration. Maximum sprouting was found in 400 ppm of GA₃ application.

Table 1. Off-season sprouting potentiality of three varieties of potato under GA₃ treatment

Treatment	Variety	Parameters				
		Days to sprout initiation	Total sprouting time (days)	Mean sprout length (cm)	Number of sprout per tuber	Node per sprout
Control	Diamant	Sprouting was not occurred in off-season				
	Cardinal					
	Granola					
50 ppm GA ₃	Diamant	12	25	2.31	1	2
	Cardinal	11	25	2.75	2	2
	Granola	9	15	3.0	1	2
100 ppm GA ₃	Diamant	12	22	3.5	1	2
	Cardinal	10	14	4.5	2	2
	Granola	9	16	4.5	4	3
200 ppm GA ₃	Diamant	7	15	3.20	3	3
	Cardinal	7	10	3.99	3	3
	Granola	8	10	4.41	5	3
300 ppm GA ₃	Diamant	7	12	4.51	3	4
	Cardinal	6	10	4.61	4	4
	Granola	5	10	5.0	5	4
400 ppm GA ₃	Diamant	4	12	4.7	5	4
	Cardinal	5	9	4.81	5	4
	Granola	3	7	5.12	6	4

Number of node per sprout ranged from 2-4 for all the treatments and varieties (Table 1 and Fig. 1). The overall experimental findings reveal that, the variety Granola was the most responsive to GA₃ application for sprouting. It was moderate for Cardinal and minimum for Diamant. Higher concentration of GA₃ showed better performance to sprouting for all the materials under study. The second part of the experiment was designed to evaluate the *in vitro* regeneration potentiality of the three varieties on MS medium supplemented with different concentration of GA₃ and IBA. *In vitro* regeneration parameters were studied separately for each of the variety and it is presented as follow.



Fig. 1. Off-season sprouting potentiality of (a) Granola (b) Cardinal and (c) Diamant varieties

Effect of GA₃ and IBA on *in vitro* regeneration in Diamant

The effect of GA₃ and IBA on various parameters for shoot and root induction are presented in Table 2. Days to shoot regeneration ranged from 2.5 to 10.3. It was the highest in simple MS medium followed by MS + 1 mg/L of GA₃ application (8.6 days). Minimum time (2.5 days) was observed in MS + 4

treatment. Days to root initiation were found the highest (17.0) in simple MS medium and it was the lowest (7.6) in MS + 2.0 mg/L IBA concentration. Number of root per explant varied from 2.3 to 7.3. Highest number of root (7.9) was recorded in MS + 2.5 mg/L of IBA application [Table 2 and Fig. 3 (a)]. It was revealed that, the phytohormone GA₃ and IBA had positive effect on *in vitro* regeneration of potato and the concentration of these hormones had significant effects on the various parameter under study. It is interesting to note that, more than 4.0 mg/L of GA₃ had slight negative effect on *in vitro* regeneration in the variety Diamant.

Effect of GA₃ and IBA on *in vitro* regeneration in Cardinal

The experimental finding is presented in Table 2 where days to shoot regeneration ranged from 3.1 to 9.6. Simple MS media took more time for regeneration and it gradually decreased with higher concentration of GA₃. But MS + 5 mg/L of GA₃ treatment showed slight reverse relationship on days to shoot initiation. It took little more time (4.5 days) as compared to MS + 4 mg/L of GA₃ application. Number of shoot per explant was the highest (8.7) in MS + 5 mg/L of GA₃ treatment as it was the lowest (1.3) in simple MS medium [Fig. 2 (b)]. Shoot length was also maximum (6.2 cm) in MS + 5 mg/L of GA₃ treatment and it was the least (1.9 cm) in simple MS medium. The parameters number of shoot per explant and shoot length followed the general equation of hormone application, i.e. it showed positive effect of GA₃ application. Days to root initiation ranged from 5.5 to 16.0. Minimum time was required in the treatment MS + 2.0 mg/L of IBA, where as it was maximum in simple MS medium. Number of root per explant was more in MS + 2.5 mg/L of IBA and the value gradually decreased in lower concentration [Fig. 3 (b)].

Effect of GA₃ and IBA on *in vitro* regeneration in Granola

In vitro regeneration response in Granola variety is presented in Table 2. Days to shoot initiation was reported to be minimum (2.5 days) in MS + 4 mg/L of GA₃ treatment and it was maximum (13.0 days) in simple MS medium. Number of shoot per explant and shoot length also showed similar trend in the case of MS + 4 mg/L and MS + 5 mg/L of GA₃ applications [Fig. 2 (a)]. In both the cases, MS + 4 mg/L of GA₃ showed better response to regeneration. Higher concentration (5 mg/L) of GA₃ had minor negative effect on shoot regeneration. Days to root initiation was the highest (17.3 days) on simple MS media and it was the lowest (7.2) in MS media supplemented with 2.0 mg/L of IBA treatment. Number of root per explant was the highest (9.0) more in MS + 2 mg/L IBA [Fig. 3 (c)] and it was the lowest (1.1) in simple MS medium.



Fig. 3. Multiple root development of three potato varieties (a) Diamant (b) Cardinal and (c) Granola

Hardening potentiality of potato varieties

The survival ability of regenerated plantlets studied are presented in Table 3. Equal number of plantlet (50) was used to study the establishment capacity of different cultivars under field condition. The survival percentage of plantlets varied from 72-90% where 90% of the plantlets showed healthy morphological appearance in Granola (Table 3 and Fig. 4). The lowest performance was observed in the case of Cardinal variety. Diamant variety showed 86% survival rate in field condition.

The overall finding of GA₃ and IBA application reveals that, higher concentration has significant positive effect on *in vitro* regeneration ability in all the three varieties of potato. The concentration 5 mg/L of GA₃ has little negative effect on shoot regeneration. Slight variation was noticed in some of the parameters studied. The phenomena is common for all the three cultivars. Among them the variety Granola was found to be the most potential in all aspects under study.

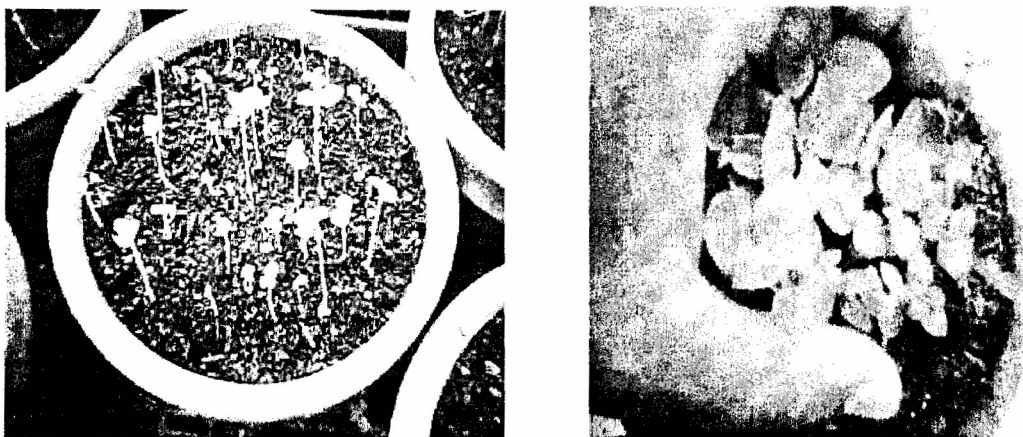


Fig. 4. Acclimatized *in vitro* plantlet of Granola in vermiculite pot mixture

DISCUSSION

The potential value of tissue culture technique in crop improvement has been widely recognized (Yasmin *et al.*, 2003). High frequency regeneration of potato from *in vitro* cultured tissues are prerequisite for successful application in the area of seed tuber production. Hence, the experiment was designed to evaluate the sprouting ability and its subsequent *in vitro* regeneration potentiality under Bangladesh condition. Although remarkable work has been done on plant regeneration with different explants and growth regulators but the combination study for sprouting ability, and hormones like GA₃ and IBA impact is very less. In potato, evidence is available to show genotypic effect on *in vitro* regeneration and variation of plants derived from leaf discs (Fleming *et al.*, 1992; Trujillo *et al.*, 2001). Regeneration response as morphological development of explants to shoots investigated from diverse sources showed significant variation (Hossain *et al.*, 2005). The effect of GA₃ studied on the morphogenesis of explant of potato revealed that, the dosage of 0.245 mg/L of GA₃ boosted all the characters over control (Farhatullah *et al.*, 2007). GA₃ stimulated development of nodal cutting on MS but at high concentration it produced narrow elongated shoot depending on genotypes (Novak *et al.*, 1980). The finding of the present research is in agreement with these former workers. Webb *et al.* (1983) reported that, the addition of hormones and GA₃ to the MS media enhanced shoot growth. Al-Momani *et al.* (1999) found that, 3 mg/L of GA₃ application had significant effect on increase in number of leaves per plantlet. The present finding also reveals that, sprouting can occur promptly by the application of GA₃. Among the three varieties Granola is more responsive to GA₃ application for sprouting.

The highest regeneration percentage of in potato was achieved with 2.5 mg/L NAA + 2.0 mg/L GA₃ concentrations (Yasmin *et al.*, 2003). Maximum percentage (70.00) of calli-induced shoot was observed in potato cultivar Diamant on MS medium fortified with 5.0 mg/L of GA₃. The regenerated shoots were rooted on MS media containing of different concentrations of IBA indicating maximum rooting response was achievement in 0.5 MS + 1.0 mg/L IBA (Khatun *et al.*, 2003). Sarker and Mustafa (2002) reported that, maximum shoot regeneration in two indigenous potato was observed on MS semi-solid medium supplemented with 1.0 mg/L GA₃, and MS containing 0.1 mg/L of IAA was found to be the best for root induction from the excised shoots.

Huge number of potato seedling is pre-requisite for large scale production of potato. A conventional method of seed tuber production is costly, laborious and time consuming. In respect of that, *in vitro* methods of regeneration will be helpful for production of more seedling to accelerate higher production of potato under Bangladesh condition. GA₃ application can promote the sprouting ability of potato to serve huge amount of initial materials. MS media supplemented with 4 mg/L GA₃ was the best for shoot regeneration in all the three varieties of potato. The regenerated shoots were excised for root induction and it reveals that 2.0 mg/L of IBA was the best for root development among the potato genotypes under study. This method of regeneration can be used for rapid multiplication of potato.

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