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REGENERATION POTENTIALITY OF POTATO IN *IN VITRO* CONDITION UNDER DIFFERENT HORMONAL COMBINATIONS

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Abstract

The present investigation was conducted to study the effects of different concentrations and combinations of growth regulators on the callus induction and plant regeneration of potato cvs. Diamant, Cardinal and Granula.The internodal segments of three virus tested potato cultivars were used for the establishment of culture.The interaction effects between cultivars and growth regulators showed significant differences for all the parameters used in the experiment except only root length. The *in vitro* callus proliferation was the best in Diamant when MS medium was fortified with 3.0 mgL^{-1} 2,4-D + 0.25 mgL^{-1} KIN in terms of the highest percentage (100) of callus, the maximum callus size (11.04 mm), the maximum fresh weight (1.126 g) of callus and the maximum dry weight (0.1044 g) of callus. In case of shoot regeneration, MS medium fortified with 1.0 mgL^{-1} BAP+ 0.5 mgL^{-1} GA_3 in Granula was best in terms of the highest no. of shoots/callus (2.920), no. of nodes/shoot (6.50) and shoot length (6.760 cm) with the minimum days (14.40). The root regeneration showed the highest results in terms of no. of roots/plantlets (10.80) in Cardinal when MS medium was fortified with 1.0 mgL^{-1} IAA + 0.25 mgL^{-1} GA_3. It was found that the *in vitro* regeneration and multiplication potentiality was the highest in the variety Granula followed by Cardinal and Diamont.

Key words: growth regulators, *in vitro* regeneration,MS media, plantlets, potato

Introduction

Potato is an important tuberous food crop. It is also the most productive economically important and widely grown vegetable crop cultivated and consumed in Bangladesh. In 2008, several international organizations highlighted the role of potato in world food production, in the face of developing economic problems. Thus the United Nations officially declared the year 2008 as the International Year of the potato, to raise its profile in developing nations, calling the crop a “hidden treasure”. This crop ranks fourth amongst allglobal food crops after wheat, rice and maize (Moeinil*et al.,* 2011), while ranks first both in area and production among the vegetable crops grown in Bangladesh. In Bangladesh, about 8.6 million tons of potato were produced from nearly 0.444 million ha with an average yield of 19 tons/haduring 2015-2016(BBS, 2017). Demand for potato is rapidly increasing. However, production has to be increased even with the current rate of demand.

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Although potato is being considered as one of the main food crop in Bangladesh, its productivity is hampered due to infection of virus, fungus and bacterial diseases. The total loss caused by these diseases is 30-100% during cultivation and storage. Potato varieties with higher yield, better agronomic quantities, and resistance to disease are immense importance for increased production. To overcome these impediments, both conventional and biotechnological breeding programmes need to be applied. Disease free and genetically uniform plantlets may be produced by meristem culture through micro-propagation techniques (Hoque*et al*., 2010).

Micro-propagation offers an efficient and accepted method for rapid propagation and production of pathogen-free seed tubers. Micro-propagation or tissue culture techniques have several advantages over traditional propagation methods. High frequency regeneration of plants from in vitro culture tissues is a pre-requisite for crop improvement and for engineering of this crop to supplement conventional breeding. Thus improvementin the growth of such cultures could be benefit in both basic and applied plant biotechnology. Although successful in vitro potato regeneration protocol was established in many laboratoriesfrom different explants like leaf and stem in the world but still there are very less information regarding the comparative studies among the different varieties of potato. Which genotypes are more efficient for rapid multiplication is need to identify. There is necessity to assess the regeneration potentiality of available released varieties of potato for better utilization in commercial purpose. Growth regulators play an important role in potato regeneration.Individual hormone has its own effect on regeneration. Combine effect of rooting and shooting growth hormoneis needed to study for better regeneration of potato. The present study was therefore, designed to establish an effective protocol for rapid callus induction and plantlet regeneration of three potato cultivars from internodes explants under different concentrations and combinations of plant growth regulators.

Materials and Methods

The present investigation was carried out at the Plant Biotechnology Lab, Department of Horticulture, Patuakhali Science and Technology University, Dumki, Patuakhali. The experiment was laid out in a Completely Randomized Design (CRD) with five replications and each replication contains three samples. The virus tested three potatocultiverscvs. Diamant, Cardinal and Granula were obtained from the University of Rajshahi, Rajshahi-6205, Bangladesh. The internodal segments were used as explants for the establishment of culture.

Murashige and Skoog (1962) medium were used with different hormone supplements as the culture medium.

Table 1. Experimental treatments

Experiment	Treatment combinations (mgL^{-1})						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Callus initiation	2,4-D+KIN 0.0 + 0.25	2,4-D+KIN 0.5 + 0.25	2,4-D+KIN 1.0 + 0.25	2,4-D+KIN 1.5 + 0.25	2,4-D+KIN 2.0 + 0.25	2,4-D+KIN 2.5 + 0.25	2,4-D+KIN 3.0 + 0.25
Shoot regeneration	GA_3 + BAP 0.0 + 1.0	GA_3 + BAP 0.5 + 1.0	GA_3 + BAP 1.0 + 1.0	GA_3 + BAP 1.5 + 1.0	GA_3 + BAP 2.0 + 1.0	-	-
Root regeneration	GA_3 + IAA 0.0 + 1.0	GA_3 + IAA 0.25 + 1.0	GA_3 + IAA 0.5 + 1.0	GA_3 + IAA 1.5 + 1.0	GA_3 + IAA 2.0 + 1.0	-	-

T_1: 2,4-D 0.0 mg/L + KIN 0.25 mg/L; T_2: 2,4-D 0.5 mg/L + KIN 0.25 mg/L; T_3: 2,4-D 1.0 mg/L + KIN 0.25 mg/L;
T_4: 2,4-D 1.5 mg/L + KIN 0.25 mg/L; T_5: 2,4-D 2.0 mg/L + KIN 0.25 mg/L; T_6: 2,4-D 2.5 mg/L + KIN 0.25 mg/L;
T_7: 2,4-D 3.0 mg/L + KIN 0.25 mg/L.

The abovementioned treatment combinations were used with different stock solutions for the effective callus proliferations. Separate stock solutions for macronutrients, micronutrients, iron source, vitamins/ organics and growth regulators etc. were prepared prior to media preparation and stored in a refrigerator at 4ºC for subsequent use. For efficient cultures the semi-solid MS medium having pH 5.8 were prepared from the stock solutions during the studies carefully.The vial containing prepared media were autoclaved at 15 psi and 121ºC for 20 minutes. Metal instruments, glass wares and other instruments were sterilized for the purpose of aseptic conditions. Collected explants were taken in beaker and were thoroughly washed in running tap water for 2-3 minutes and then washed with sterilized water. The explants were transferred to the laminar airflow cabinet in sterilized petridish. They were surface sterilized first with 70% ethyl alcohol for 30 seconds then with 0.1% mercuric chloride ($HgCl_2$) with two drops/100 ml of Tween-20 for 5 minutes. The surface sterilized explants were then rinsed 4-5 times with sterile distilled water inside the clean bench to remove all traces of $HgCl_2$. The excised explants were then inoculated into each culture bottle containing MS medium with various concentrations of hormonal supplements for in vitro callus initiation. The culture vessels with inoculated explants were incubated in both dark and light in a temperature controlled growth room (25 ± 1ºC) under 16 hours photoperiod with a light intensity 1500 lux and relative humidity 60-70%. Subcultures were repeated at least once in a fortnight into a fresh medium and cultured under same culture condition as mentioned previously for calli and organogenesis. Visual observation of cultures was made every week and the data were recorded after different days. Data were taken on, for callus (days required for calli initiation, percentage for calli initiation, callus size, fresh weight of callus, dry weight of callus after 28 DOC (days of culture), for shoots (days required for shoot initiation, percentage of shoot initiation, no. of shoots/explants, no. of nodes/shoot, shoot length (cm), for root (days required for root initiation, no. of roots/plantlet, root length (cm).

The analyses of variances of collected datawere performed and the means were compared by Least Significant Difference (LSD) test for interpretation of results. The significance of the difference between the pair of means was evaluated at 1% level of probability using MSTAT-C computer package programs (Gomez and Gomez, 1984).

Results and Discussion

Callus proliferation

The different cultivars showed significant differences for the days required for callus initiation. Cardinal took the significantly highest number of days (14.27). In contrast, Granula took least number of days (13.36) and was statistically similar to Diamant (13.44) (Table 2 and Plate 1). Different levels of 2, 4-D and KIN concentrations significantly influenced the days required for callus initiation. Concentration 2, 4-D 0.5 mgL^{-1} + KIN 0.25 mgL^{-1} took the significantly highest days (17.97) followed by 17.07 days at concentration 2, 4-D 1.0mgL^{-1} + KIN 0.25 mgL^{-1} but statistically similar with each other while concentrations 2, 4-D 3.0 mg/L + KIN 0.25 mgL^{-1} took the shortest days (13.93). The maximum days (18.50) required was in Diamant with concentration 0.5 mgL^{-1} 2, -D + 0.25 mgL^{-1} KIN followed by (18.22 days) in Cardinal with same concentration but both are statistically similar with each other and so on (Figure 1). In contrast, the minimum days (13.10) required was observed in Granula with concentration 2.5 mgL^{-1} 2, -D + 0.25 mgL^{-1} KIN followed by (13.70 days) Diamant cultivar with concentration 3.0 mgL^{-1} 2, -D + 0.25 mgL^{-1} KIN. These effective behaviors of 2, 4-D in callus induction were reported in previous studies on potato (Khatun*et al*., 2003; Vargas *et al*., 2005). Cytokinins (Kinetin) added to the medium are very important during tissue culture of plants as those induce cell division and organogenesis, and affect other physiological and developmental processes.

The significant variation was observed among the cultivars regarding percentage of callus initiation. Cardinal produced significantly the highest percentage (80.00) followed by Diamant (78.57) but statistically similar with each other while Granula did produced lowest percentage (72.86) of callus (Table 2). The concentrations 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN and 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN produced significantly the highest percentage (100) of callus followed by (96.67%) at concentration 2.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, but those were statistically similar. In contrast, the concentration 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN produced the lowest percentage of callus (73.33%). The highest percentage of callus initiation (100) was observed in Diamant with 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, 2.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN and 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN concentration, in Cardinal with concentration 1.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN and 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN and Granula with concentration 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, 2.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN and 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN followed by (90%) in Diamant × concentration 1.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, 1.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, in cv. Cardinal × 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN, 2.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN and these are statistically similar. In contrast, the lowest (60%) were found in Granula with 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN concentrations (Figure 2).

The size of callus had significant differences among the cultivars. Diamant produced significantly the highest size (7.69 mm) followed by Cardinal (7.41 mm) but ranked equal,

while Granula produced the least size (7.00 mm) of callus (Table 2 & Plate 1). The growth regulators of 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN showed the highest callus size (9.98 mm) while the lowest (7.33 mm) was observed with 1.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN (Table 3). The highest callus size (11.04 mm) was observed with Diamant cultivar on 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN while the lowest (6.90 mm) was found at 1.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} KIN in Granula cultivar followed by (7.20 mm) in same cultivar with concentration 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (Plate 1). This result agrees with that of Forooghian*et al.* (2013) who also observed the significant interaction effects among cultivars and concentrations of 2, 4-D and KIN but used 5 mgL^{-1} 2, 4-D and 2 mgL^{-1} Kinetin.

The effect of different cultivars on callus fresh weight varied significantly. Diamant produced the highest fresh weight (0.84 g) followed by 0.82 g in Cardinal but statistically similar with each other. The lowest callus fresh weight was observed in Granula (0.76 g) (Table 2). The growth regulators of 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin gave the highest fresh weight of callus (1.02 g), followed by 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (0.99 g) but ranked equal. The lowest weight of callus (0.86 g) was observed with 1.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin followed by 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (0.89 g) (Table 2). The highest callus fresh weight (1.13 g) was observed in Diamant at 3.0 mgL^{-1} + 0.25 mgL^{-1} Kinetin followed by 1.03 g in Cardinal with concentration 2.0 mgL^{-1} 2, 4-D +0.25 mgL^{-1} Kinetin. In contrast, the lowest callus fresh weight (0.80 g) was found at 0.5 mgL^{-1} 2, 4-D + 0.25mgL^{-1} Kinetin from Granula cultivar followed by (0.81g) in same cultivar on concentration 1.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (Table 3).

Remarkable variations were found among the different cultivars in respect of callus dry weight. Diamant produced the highest dry weight (0.075 g) followed by Cardinal 0.073 g but statistically similar with each other. The lowest callus dry weight (0.063g) was found in Granula (Table 2). The growth regulators 3.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin gave the highest dry weight of callus (0.089 g), followed by 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (0.088 g) but both were of equal rank. The lowest dry weight of callus (0.076 g) with 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin followed by 1.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (0.079 g) and 1.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (0.080 g) but all are ranked equally (Table 3). The highest callus dry weight (0.104 g) was observed with Diamant on 3.0 mgL^{-1} + 0.25 mgL^{-1} Kinetin followed by Cardinal (0.099 g) with concentration 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin but was of equal rank with each other. In contrast, the lowest callus dry weight was found (0.068 g) at 0.5 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin from Granula cultivar followed by (0.071 g) in same cultivar on concentration 2.0 mgL^{-1} 2, 4-D + 0.25 mgL^{-1} Kinetin (Table 3).

Table 2. Effects of different cultivars on the days to callus initiation, percentage of callus initiation, callus size, callus fresh weight and callus dry weight after 28 days of inoculation

Varieties	Fresh weight of explants inoculated (g)	Days to callus initiation	Percentage of callus formation	Callus size (mm)	Callus fresh weight (g)	Callus dry weight (g)
V_1 (Diamant)	0.004	13.44b	78.57a	7.69a	0.840a	0.075a
V_2 (Cardinal)	0.004	14.27a	80.00a	7.41a	0.818a	0.073a
V_3 (Granula)	0.004	13.36b	72.86b	7.01b	0.76b	0.063b
Level of significance		**	**	**	**	**

In a column values having different letter (s) differ significantly at 1% level of probability; ** Significant at 1% level of probability

Table 3. Effects of different concentrations and combinations of 2,4-D and Kinetin on the days to callus initiation, percentage of callus initiation, callus size, callus fresh weight and callus dry weight after 28 days of inoculation

Concentrations and combinations of PGRs (mgL^{-1})	Fresh weight of explants inoculated (g)	Days to callus initiation	Percentage of callus formation	Callus size (mm)	Callus fresh weight (g)	Callus dry weight (g)
T_1 (0.0 2,4-D +0.25 KIN)	0.004	-	-	-	-	-
T_2 (0.5 2,4-D +0.25 KIN)	0.004	17.97a	73.33c	7.99c	0.89cd	0.076c
T_3 (1.0 2,4-D +0.25 KIN)	0.004	17.07ab	83.33b	7.33d	0.86d	0.080c
T_4 (1.5 2,4-D +0.25 KIN)	0.004	16.13bc	86.67b	8.57bc	0.93bc	0.079c
T_5 (2.0 2,4-D +0.25 KIN)	0.004	15.80cd	100.0a	8.83b	0.98ab	0.088ab
T_6 (2.5 2,4-D +0.25 KIN)	0.004	14.93d	96.67a	8.86b	0.95ab	0.089bc
T_7 (3.0 2,4-D +0.25 KIN)	0.004	13.93e	100.0a	9.98a	1.01a	0.089a
CV (%)		7.19	7.21	8.41	7.45	10.10
Level of significance		**	**	**	**	**

In a column values having different letter (s) differ significantly at 1% level of probability; ** Significant at 1% level of probability

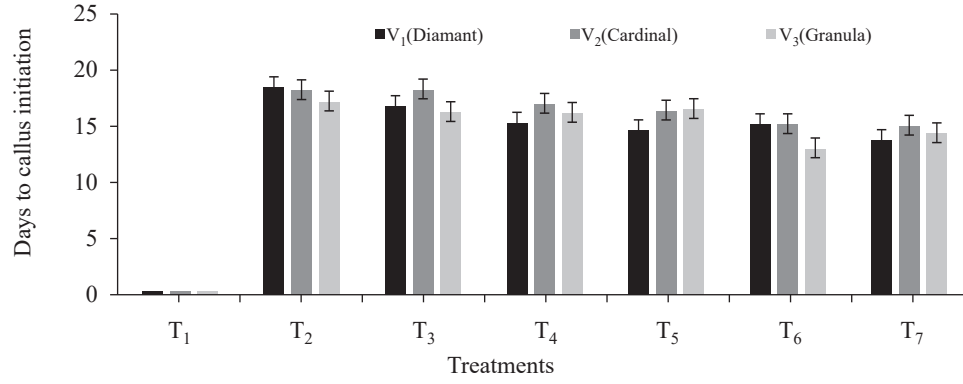

Fig. 1. Effects of cultivars on the days to callus initiation under different concentrations and combinations of 2,4-D and KIN(Vertical bars represent the standard error)

Shoot regeneration

There were no significant differences among different cultivars on days required for shoot initiation. Out of three cultivars, Cardinal took the highest number of days (16.26) for shoot initiation. In contrast, Granula took the lowest number of days (15.94) (Table 4). This result agrees with that of Minar (2005). The interaction effects among different cultivars and concentrations of growth regulators showed significant differences on days required for shoot initiation. Granula with 1.0 mgL^{-1}BAP + 2.0 $mgL^{-1}GA_3$ took the highest days (18.20) for shoot initiation followed by 14.80 days in Cardinal x 1.0 mgL^{-1}BAP + 1.5 $mgL^{-1}GA_3$. In contrast, lowest number of days was required (14.40 days) for shoot initiation in Granula with concentration 1.0 mgL^{-1}BAP + 0.5 $mgL^{-1}GA_3$ followed by 14.80 days with Diamant x 1.0 mgL^{-1}BAP + 0.5 $mgL^{-1}GA_3$ (Table 5 and Plate 2). The minimum days (14.40) to shoot initiation, the maximum no. of shoots (2.92) per callus, the maximum no. of node (6.50) per callus and the tallest shoot (6.76 cm) were noted in Granulaat GA_3 0.5 mgL^{-1}+ BAP 1.0 mgL^{-1}treatment combinations. Remarkable variations were found among different cultivars in respect of percentage of shoot initiation. Granula cultivar produced significantly the highest percentage (90.00) of shoot while Diamant produce the lowest percentage (72.00) of shoots (Table 4). The percentage of shoot initiation was also influenced by the supplementation of different concentration and combinations of BAP and GA_3 (Table 5). The interaction effects between different cultivars and concentrations of growth regulators showed significant effects

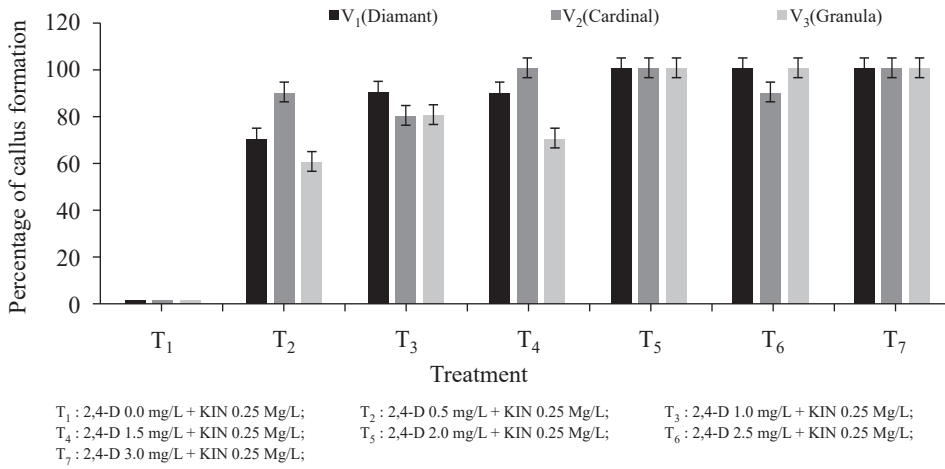

Fig. 2: Effects of cultivars on the percentage of callus formation under different concentrations and combinations of 2,4-D and KIN(Vertical bars represent the standard error)

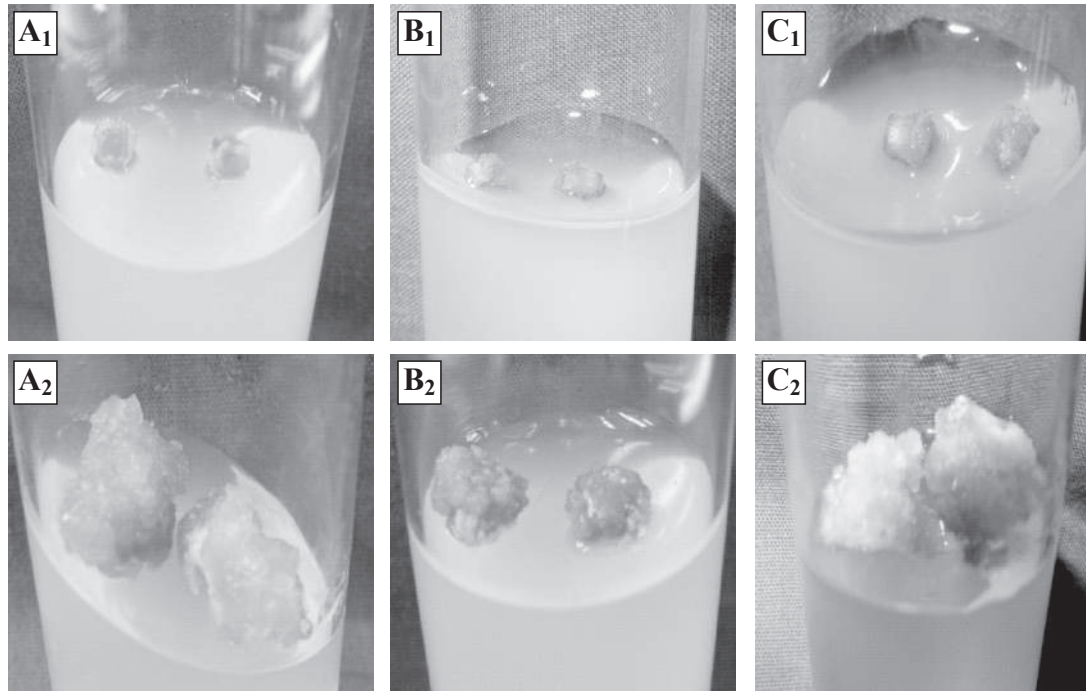

Plate 1. The callus initiation and the maximum callus size (mm) in cultivarDiamant with 2,4-D 3.0 mg L^{-1} + Kinetin 0.25 mg L^{-1} (A_1-A_2), cultivar Cardinal with 2,4-D 3.0 mg L^{-1} + Kinetin 0.25 mg L^{-1} (B_1-B_2), cultivar Granula with 2,4-D 3.0 mg L^{-1} + Kinetin 0.25 mg L^{-1} (C_1-C_2)

on the percentage of shoot initiation. The highest percentage of shoot initiation (100) was observed in Cardinal and Granula cultivars with 1.0 mgL^{-1} BAB + 1.0 mgL^{-1} GA_3 treatment concentration followed by (90%) with concentrationof 1.0 mgL^{-1} BAB + 0.5 mgL^{-1} GA_3 in cvs. Diamant and Cardinal, concentrations 1.0 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 and 1.0 mgL^{-1} BAP + 2.0 mgL^{-1} GA_3 in Granular cultivar but ranked equal. In contrast, the lowest (60%) was found in Diamant cultivar with 1.0 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 and 1.0 mgL^{-1} BAP + 2.0 mgL^{-1} GA_3 concentrations. That variable response in regeneration potential might be dependent on cultivar-specific effect.

Remarkable variations were found among different cultivars in respect of number of shoots per plantlet. Among different cultivars, Granula produced the highest number of shoots (2.49), while the Cardinal had the lowest number (1.56) of shoots per explants (Table 4).The maximum number of shoots (2.407) was produced by 1.0 mgL^{-1} BAP + 0.5 mgL^{-1} GA_3 treatment combination followed by 2.320 at 1.0 mgL^{-1} BAP + 1.0 mgL^{-1} GA_3 but both are ranked equal. In contrast, the minimum number (1.561) of shoot was produced with 1.0 mgL^{-1} BAP + 2.0 mgL^{-1} GA_3 treatment combination followed by 1.895 at 1.0 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 treatment combination (Table 5).Distinct variations were found in respect of number of shoots per callus due to the interaction effect of cultivars and different concentrations of growth regulators. Granula cultivar × 1.0

mgL^{-1} BAP + 0.5 mgL^{-1} GA_3 produced highest number (2.92) of shoot followed by 2.76 shoots with same cultivar at 1.0 mgL^{-1} BAP + 1.0 mgL^{-1} GA_3 treatment combination while ranked equal. In contrast, lowest number of shoots was (1.40) produced in cv. Cardinal with 1.0 mgL^{-1} BAP + 0.0 mgL^{-1} GA_3 and 1.0 mgL^{-1} BAP + 2.0 mgL^{-1} GA_3 (Figure 3).

Table 4. Effects of different cultivars on the days to shoot initiation, percentage of shoot initiation, no. of shoots/callus, no. of nodes/shoot and shoot length (cm) after 28 days of inoculation

Varieties	Days to shoot initiation	Percentage of shoot initiation	No. of shoot per callus	No. of node per shoot	Shoot length (cm)
V_1 (Diamant)	16.06a	72.00c	2.16b	4.20b	3.87b
V_2 (Cardinal)	16.26a	82.00b	1.56c	4.94a	4.72a
V_3 (Granula)	15.94a	90.00a	2.49a	5.02a	4.82a
Level of significance	ns	**	**	**	**

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** Significant at 1% level of probability

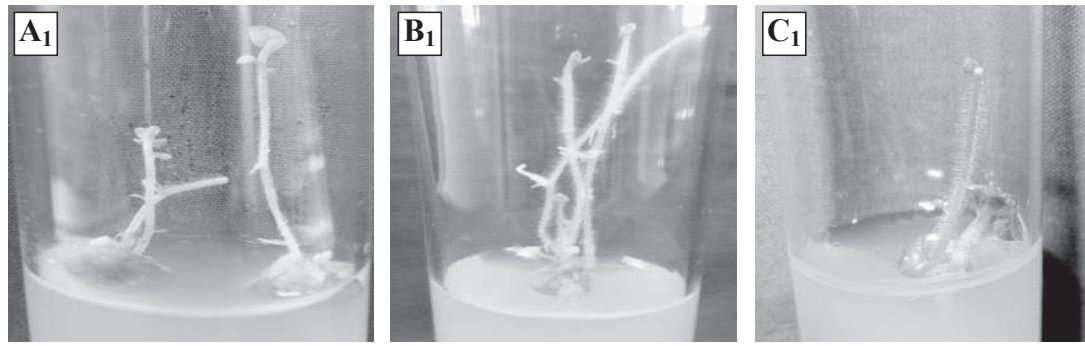

Plate 2. The shoot initiation and the highest shoot length in cv. Diamant with GA_3 0.5 mg/L + BAP 1.0 mgL^{-1}(A_1), cv. Cardinal with GA_3 1.0 mgL^{-1}+ BAP 1.0 mgL^{-1}(B_1), cv. Granula with GA_3 0.5 mgL^{-1}+ BAP 1.0 mgL^{-1}(C_1)

Different cultivars showed significant differences in shoot length. Granula produced the tallest shoot (4.82 cm) followed by Cardinal (4.72 cm) but both were ranked equal while Diamant produced the shortest shoot (3.87 cm) (Table 4 and Plate 2). Different concentrations of growth regulators showed significant differences in shoot length 1.0 mgL^{-1} BAP + 0.5 mgL^{-1} GA_3 combinations produced significantly the longest shoot (5.607 cm). In contrast, the shortest shoot (3.702 cm) was with 1.0 mgL^{-1} BAP + 0.0 mgL^{-1} GA_3 followed by 4.101 cm with 1.0 mgL^{-1} BAP + 2.0 mgL^{-1} GA_3 (Table 5).

The interaction effects between cultivars and the concentrations of growth regulators on shoot length also showed significant differences. The longest shoot (6.76 cm) was produced by the treatment combination of 1.0 mgL^{-1}BAP + 0.5 $mgL^{-1}$$GA_3$ with Granula but the shortest one (3.23 cm) was in the treatment combination of 1.0 mgL^{-1}BAP + 0.0 $mgL^{-1}$$GA_3$ with Diamant followed by 1.0 mgL^{-1}BAP + 1.5 $mgL^{-1}$$GA_3$ with same variety (Figure 4).

Table 5. Effects of different concentrations and combinations of BAP and GA_3 on the days to shoot initiation, percentage of shoot initiation, no. of shoots/callus, no. of nodes/shoot and shoot length (cm) after 28 days of inoculation

Concentrations and combinations of PGRs (mg/L)	Days to shoot initiation	Percentage of shoot initiation	No. of shoot per callus	No. of node per shoot	Shoot length (cm)
T_1 (GA_3 0.0 + BAP 1.0)	15.80b	73.33b	2.18ab	4.16c	3.70c
T_2 (GA_3 0.5 + BAP 1.0)	14.73c	90.00a	2.41a	5.87a	5.61a
T_3 (GA_3 1.0 + BAP 1.0)	15.90b	93.33a	2.32a	4.93b	4.79b
T_4 (GA_3 1.5 + BAP 1.0)	16.50b	76.67b	1.90bc	4.37c	4.15c
T_5 (GA_3 2.0 + BAP 1.0)	17.50a	73.33b	1.56c	4.27c	4.10c
Level of significance	**	**	**	**	**

In a column values having different letter (s) differ significantly at 1% level of probability
** Significant at 1% level of probability

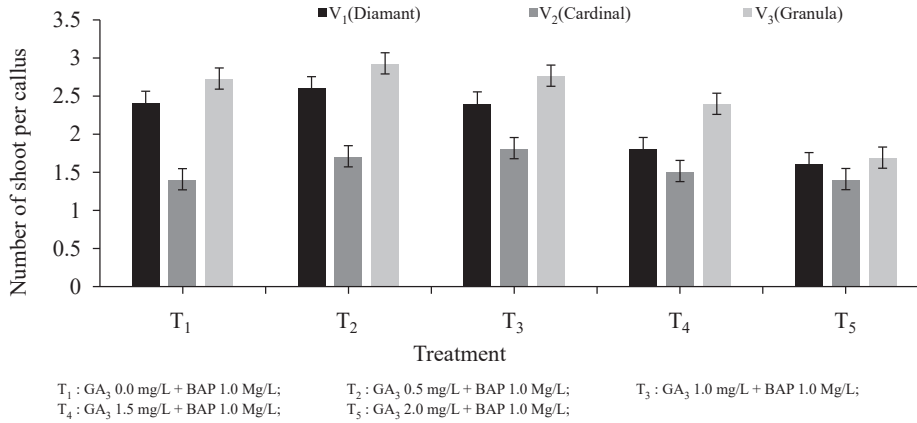

Fig.3. Effects of cultivars on the no. of shoots/callus under different concentrations and combinations of BAP and GA_3(Vertical bars represent the standard error)

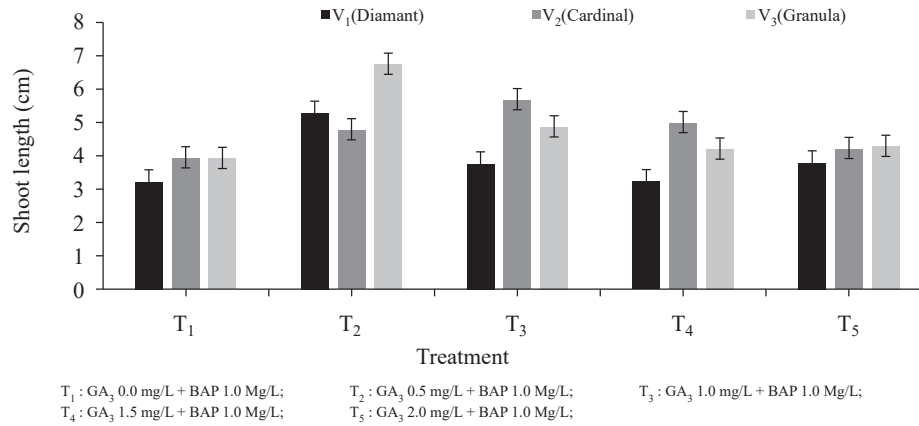

Fig.4. Effects of cultivars on shoot length (cm) under different concentrations and combinations of BAP and GA_3(Vertical bars represent the standard error)

Root regeneration

Distinct variations were found in respects of roots per plantlets due to the interaction effect of cultivars and different concentrations of growth regulators. Granula produced the highest number of roots (10.24) followed by (9.688) in Cardinal and those ranked equal while the lowest number (8.920) was found from Diamant (Table 6 and Plate 3). The *in vitro* regeneration and multiplication potentiality were highest in the cultivarGranula followed by Cardinal and Diamant. This result agrees with that of Hoque (2010).

The effects of different concentrations of IAA (Indole acetic acid) and GA_3(Giberellic acid) on the number of roots showed significant differences. The maximum number of roots (10.03) appeared with 1.0 mgL^{-1} IAA + 0.25 mgL^{-1} GA_3 concentrations followed by 9.780 at 1.0 mgL^{-1} IAA + 0.5 mgL^{-1} GA_3 concentrations and the lowest number of roots (9.033) was observed in 1.0 mgL^{-1} IAA + 0.0 mgL^{-1} GA_3 concentrations (Table 7 and Plate 3).The present study has similar finding with those of Laboney*et al.* (2013), Minar(2005) and Amezqueta*et al.* (1989) who also observed that the highest regenerated plants and fastest growth rates were with IAA and GA_3.

Distinct variations were found in respects of roots per plantlets due to the interaction effect of cultivars and different concentrations of growth regulators. The maximum number of roots (10.80) was observed with Cardinal at 1.0 mgL^{-1} IAA + 0.25 mgL^{-1} (Figure 3 and plate 3). Distinct variations were found in respects of roots per plantlets due to the interaction effect of cultivars and different concentrations of growth regulators. The highest number of roots (10.80) was observed with Cardinal at 1.0 mgL^{-1} IAA + 0.25 mgL^{-1} GA_3 followed by 10.64 on the same cultivar at 1.0 mgL^{-1} IAA + 0.5 mgL^{-1} GA_3 concentrations (Figure 3 and plate 3). But, the lowest number of roots (8.30) was observed with Cardinal × 1.0 mgL^{-1} IAA + 0.0 mgL^{-1} GA_3 followed by 8.700 on Diamant at 1.0 mgL^{-1} IAA + 0.5 mgL^{-1} GA_3 concentrations.

Table 6. Effects of different cultivars on the days to root initiation, no. of roots/plantlet and root length (cm) after 28 days of inoculation

Varieties	Days to root initiation	No. of root per plantlet	Root length (cm)
V_1 (Diamant)	14.46b	8.920b	5.584b
V_2 (Cardinal)	15.56a	9.688a	5.444b
V_3 (Granula)	14.18b	10.24a	6.028a
CV (%)	5.15	8.70	7.55
Level of significance	**	**	**

In a column values having different letter (s) differ significantly at 1% level of probability; ** Significant at 1% level of probability

Table 7. Effects of different concentrations of IAA and GA_3 on the days to root initiation, no. of roots/plantlet and root length (cm) after 28 days of inoculation

Concentrations and combinations of PGRs (mgL^{-1})	Days to root initiation	No. of root per plantlet	Root length (cm)
T_1 (GA_3 0.0 + IAA 1.0)	14.57ab	9.03b	5.37c
T_2 (GA_3 0.25 + IAA 1.0)	14.33b	10.03a	6.15a
T_3 (GA_3 0.5 + IAA 1.0)	14.37b	9.78ab	5.92ab
T_4 (GA_3 1.0 + IAA 1.0)	15.07ab	9.70ab	5.40c
T_5 (GA_3 2.0 + IAA 1.0)	15.33a	9.53ab	5.59bc
$LSD_{0.01}$ value	0.74	0.81	0.42
Level of significance	**	*	**

In a column values having different letter(s) differ significantly at 1% and 5% level of probability.
** Significant at 1% level of probability* Significant at 5% level of probability

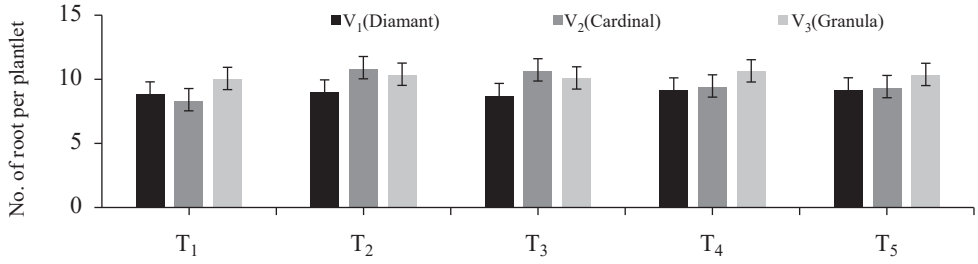

Fig. 5. Effects of cultivars on the no. of roots/plantlet under different concentrations and combinations of IAA and GA_3

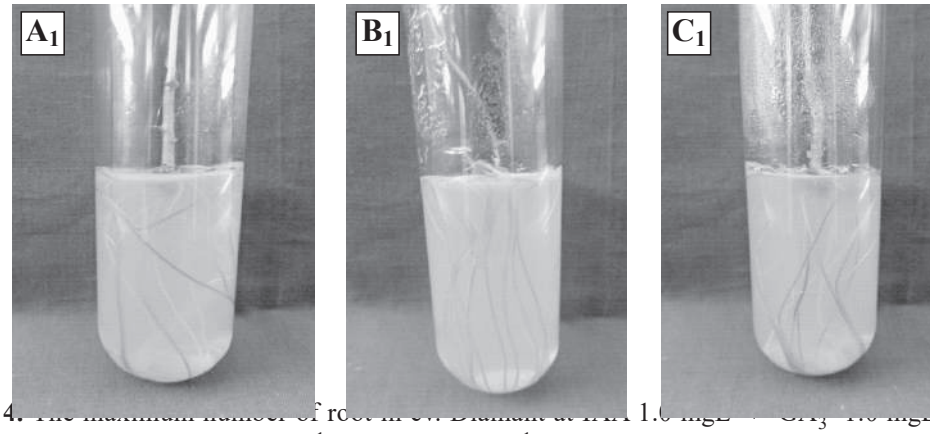

Plate 4. The maximum number of root in cv. Diamant at IAA 1.0 mgL^{-1} + GA_3 1.0 mgL^{-1}(a), cv. Cardinal at IAA 1.0 mgL^{-1} + GA_3 0.25 mgL^{-1}(b) and cv. Granula at IAA 1.0 mgL^{-1} + GA_3 1.0 mgL^{-1}(c)

The highest length (6.028 cm) of root after 28 days was observed in Granula. The lowest length (5.444 cm) of root was found in Cardinal followed by 5.584 cm with Diamant but those ranked equal (Table 6). Different concentrations of growth regulators on the root length after 28 days also showed significant difference. The longest root (6.147 cm) appeared with 1.0 mgL^{-1}IAA + 0.25 $mgL^{-1}GA_3$ concentrations followed by 5.920 cm at 1.0 mgL^{-1}IAA + 0.5 $mgL^{-1}GA_3$ concentrations while the shortest roots (5.373 cm) was observed in 1.0 mgL^{-1}IAA + 0.0 $mgL^{-1}GA_3$ followed by 5.400 cm at 1.0 mgL^{-1}IAA + 1.0 $mgL^{-1}GA_3$ concentrations (Table 7). The other treatments produced intermediate root length. The present study has similar results with those of Laboney*et al.* (2013),Minar(2005) and Amezqueta*et al.* (1989) who also observed that the highest regenerated plants and fastest growth rates were with IAA and GA_3.

The *in vitro* performance of Granula was best followed by Cardinal and Diamant. From the above discussion, it is revealed that different growth regulators with their different concentration and combination provided the best results in *in vitro* plant regeneration of different cultivars of potato. In the conclusion, the findings of the present study could be useful to develop protocol to identify the potentiality of exact concentration of different growth regulators. Furthermore, the results could be used to produce large scale production of healthy and disease free planting materials commercially.

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