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Effect of Growth Regulators on Direct Regeneration of Potato

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Abstract. The experiment was carried out at the Biotechnology Laboratory, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh to find out a suitable growth regulator and its optimum concentration for direct regeneration. Seven different concentrations of 6-benzyleaminopurin (BAP), six different concentrations of Thidiazuron (TDZ) and eight different concentrations of Zeatin riboside (ZR) were tested separately for *in vitro* direct regeneration of potato along with GA_3 (0.2 mg l^{-1}) and IAA (0.01 mg l^{-1}). A total of 49 explants were used in each treatment. Among the different concentrations of BAP, TDZ and ZR, MS medium supplemented with 3 mg l^{-1} of BAP, 0.3 mg l^{-1} of TDZ and 5 mg l^{-1} of ZR showed very good shoot induction. Moreover, among the BAP, TDZ and ZR, ZR showed the very good performance in respect of direct regeneration. Within the different concentration of ZR, MS medium supplemented with 5 mg l^{-1} showed the best performance in respect of shoot induction from both internode and leaf explants.

Key words: Direct regeneration, growth regulator, internode and leaf explants, potato

1. Introduction

Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae are grown worldwide. It is used as most important food for the largest number of peoples in the world. Potato is usually propagated asexually by means of tubers. However, *in vitro* regeneration of potato is easily done from different explants on MS medium supplemented with different auxin and cytokinin for diseases free good quality seeds and pathogen free planting materials [1,2,3]. Both callus induction and plant regeneration from explants require the appropriate combinations and concentrations of plant growth regulators in the culture media [4]. BAP, zeatin or kinetin helps to produce adventitious shoots. Shoot regeneration responses vary with the cultivar but in most cases cytokinin helps to enhance shoot production [5]. Internode explant treated with zeatin showed higher regeneration rate than those treated with BAP [6]. TDZ a substituted N-phenyl urea has been established as an important regulator for morphogenic responses [7]. However, BAP and Thidiazoron (TDZ) are also used as growth regulator to induce shoot from the explants [8] and shows very good response for rapid *in vitro* plant regeneration from stem culture. Recently, use of thidiazuron (TDZ) has shown to enhance shoot organogenesis [9, 10]. ZR is an important and well known growth regulator for direct shoot induction. It reduces callus phage and accelerates bud formation [11]. MS medium supplemented with 3.5 mg l^{-1} IAA and 4 mg l^{-1} ZR produced the most plantlets [12]. Generally a low ratio of auxin to cytokinin is required for adventitious shoot development [14]. Under Bangladesh condition, very few reports are available regarding the effect of BAP, TDZ and ZR on direct regeneration of potato. The present study was therefore, undertaken to find out a suitable growth regulator and its optimum concentration for direct regeneration of potato.

2. Materials and methods

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In vitro raised potato plantlets of 21 days cv. Asterix was used as source materials for this current experiment. For culturing the explants, 4.4 g of MS powder (Duchefa, Netherlands) and 2% sucrose (Biobasic, Canada) were used for one liter media. BAP, TDZ were added as per treatment. GA_3 (0.20 mg l^{-1}) and IAA (0.01 mg l^{-1}) were used based on Cearley and Bolyard [13]. The media was adjusted to pH 5.8, solidify with 2 gl^{-1} gelrite (Biobasic, Canada) and autoclaved at 1.06 kg/cm^2 with 121ºC for 20 min. In case of TDZ and ZR supplemented MS medium, TDZ and ZR were added by filter sterilization and mixed in the media under the laminar air flow cabinet and 20 ml medium was dispensed into each Petri dish when the temperature of media was came down near about 50ºC. *In vitro* plantlets of 18-21 days old was considered for the explants (internodes) collection. For stem explants, only the first 5-6 internodes from the top of the plantlet excluding shoot apex of the each plantlet were excised. Internode segments were excised to avoid the axillary buds and divided into 0.5- 1 cm long segments. For leaf explants, thick, healthy leaves from the upper nodes of the plants were used. The leaf tips and basal portions, including the petiole were discarded and the entire leaf was cut into 5×5 mm pieces. The leaf explants were placed upside down on the medium. Both leaf and internodes explants, seven of each were placed at every Petri dish in the above mentioned medium. The experiment was designed in single factor CRD with 7 replications.

Each petri dish was considered as single replication which contained 7 explants. Results were analyzed using MSTAT-C statistical package. The percentage data were subjected to appropriate transformation like arcsine and squire root system.

3. Results and discussions

The present investigation was conducted to find out the effects of growth regulators on direct regeneration of potato. Data on explants producing shoots (%), days required for shoot appearance, number of shoot per explant, length of visible shoot at 21 days (cm), number of visible leaves at 21 days and diameter of visible shoot (mm) were recorded. The results and discussions of the study have been presented below:

Percentage of explants producing shoot significantly varied due to the different concentration of BAP, TDZ and ZR. 100% explants survived and produced shoots on BAP @ 2 and 3 mg l^{-1}, TDZ 0.3 mg l^{-1} and ZR 2, 3, 4 & 5 mg l^{-1} where minimum was BAP @ 1 mg l^{-1} (31.79%), TDZ 0.7 mg l^{-1} (88.12%) and ZR 6 mg l^{-1} 91.39% and 96.16% (Table 1,2,3 and 4).Days required for shoot appearance significantly influenced due to the different levels of BAP, TDZ and ZR. The explants treated with BAP @ 1 mg l^{-1} required maximum days for shoot appearance (85.23 days) followed by 5 mg l^{-1} (78.10 days). However, BAP @ 3 mg l^{-1} treated explants took minimum days (47.60 days) for shooting (Table 1) The explants treated with TDZ @ 1 mg l^{-1} needed maximum days for shoot appearance (45.31) followed by TDZ @ 0.7 mg l^{-1} (37.10 days) which was statistically significant. But the explants treated with TDZ @ 0.3 mg l^{-1} required minimum days for shoot appearance (24.37 days) (Table 2). The explants treated with ZR @ 5 mg l^{-1} required minimum days for shoot appearance (14.67 and 18.32 days) in internode and leaf explant, respectively (Table 3 and 4). No. of shoot/explant influenced significantly due to the effect of BAP, TDZ and ZR. The maximum shoots per explant (3.67) were recorded with BAP @ 3 mg l^{-1} followed by 2 mg l^{-1} (3.12) which was statistically significant. The minimum number of shoots per explant (0.31) was recorded in BAP @ 5 mg l^{-1} (Fig. 1a). The highest number of shoots per explant (9.50) was recorded in 0.3 mg l^{-1} TDZ followed by 0.5 mg l^{-1} TDZ (4.37) but it was the lowest (3.67) in 0.7 mg l^{-1} TDZ supplemented medium (Fig. 1b). On the other hand, 1 mg l^{-1} TDZ treated explants produced shoots which were did not survived. This might be due to adverse effect of higher concentration of TDZ.

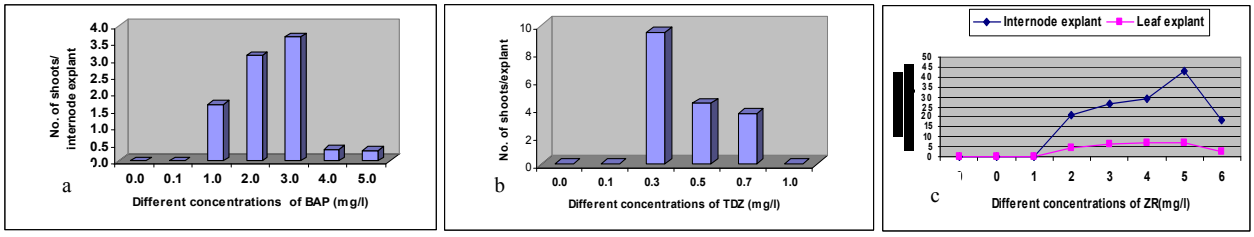


Fig. 1(a-c): Effects of BAP, TDZ and ZR on number of shoots per explant

The results of the study reflected that number of shoots per explant increased with the increasing ZR concentration up to 5 mg l^{-1} and then decreased. The maximum number of shoots per explant 43.20 and 7.25 was recorded in ZR @ 5 mg l^{-1} from internode and leaf explant, respectively (Fig. 1c) where minimum number of shoots per explant 18.41 and 2.43 was counted in ZR @ 6 mg l^{-1} both from internode and leaf, respectively (plate 1).

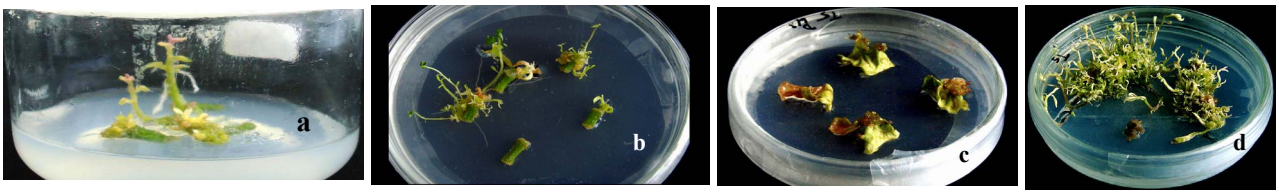


Plate 1: Shoot induction of potato cv. Asterix as influenced by:
(a) BAP 3 mg l^{-1} (b) TDZ 0.3 mg l^{-1} (c) ZR 0.5 mg l^{-1}(leaf) and (d) ZR 5 mg l^{-1} (internode)

Length of shoot increased with increasing BAP concentration up to 3 mg l^{-1} and than decreased (Table 1). The longest length (3.79 cm) was measured from BAP @ 3 mg l^{-1} followed by 2 mg l^{-1} (3.31 cm) which was statistically significant. The shortest length (1.19 cm) was recorded in BAP @ 5 mg l^{-1}. Different levels of TDZ had significant effect on length of shoot. The longest length of shoot (2.97 cm) was measured in TDZ @ 0.3 mg l^{-1} followed by 0.7 mg l^{-1} (2.37 cm) which was statistically significant. The lowest shoot length (2.10 cm) was noted in TDZ @ 0.5 mg l^{-1} (Table 2). The maximum shoot length 3.20 and 3.43 cm was measured from internode and leaf explants, respectively with 5 mg l^{-1} ZR containing MS medium followed by 4 mg l^{-1} (2.86 cm) and 2 mg l^{-1} (2.91 cm) (Table 3 and 4). Similar trend was also found in respect of leaves as evident in length of shoots. It ranged from 0.37 to 2.75. The maximum number of leaves (2.75) was recorded under BAP @ 3 mg l^{-1} while it was the lowest (0.37) in BAP 5 mg l^{-1} (Table 1). TDZ @ 0.3 mg l^{-1} also produced the maximum number of leaves (3.79) while it was the minimum (2.69) with TDZ @ 0.7 mg l^{-1} (Table 2). Distinct variation was observed in number of leaves per shoot due to the different levels of ZR concentration both in internode and leaf explants. The result reflected that longest shoot produced maximum number of leaves. The maximum number of leaves 3.18 and 3.11 was noted from ZR 5 mg l^{-1} both in internode and leaf explants, respectively followed by ZR 2 mg l^{-1} (2.73 and 2.68) (Table 3 and 4).The results of the investigation reflected that diameter of shoot increased with the increasing BAP concentration up to 3 mg l^{-1} and than decreased. It was narrow as 7.19 mm in BAP @ 5 mg l^{-1} and as wide as 15.60 mm in BAP @ 3 mg l^{-1} followed by BAP @ 2 mg l^{-1} (13.14 mm) and BAP @ 1 mg l^{-1} (11.20 mm) (Table 1). There was no significant effect ofTDZ on shoot diameter. However, shoot diameter significantly influenced due to the different levels of ZR both in internode and leaf explants. The widest shoot diameter 14.12 mm and 12.39 mm were recorded from 5 mg l^{-1} ZR supplemented MS medium in internode and leaf explants, respectively (Table 3 and 4). Significant variation was observed as to the regeneration frequency rate due to the different levels of BAP, TDZ and ZR. It was as low as 20% in the MS medium supplemented with 5 mg l^{-1} BAP and was high as 99.99% in 2 and 3 mg l^{-1} BAP followed by 1 mg l^{-1} (41.54%) (Fig. 2a).

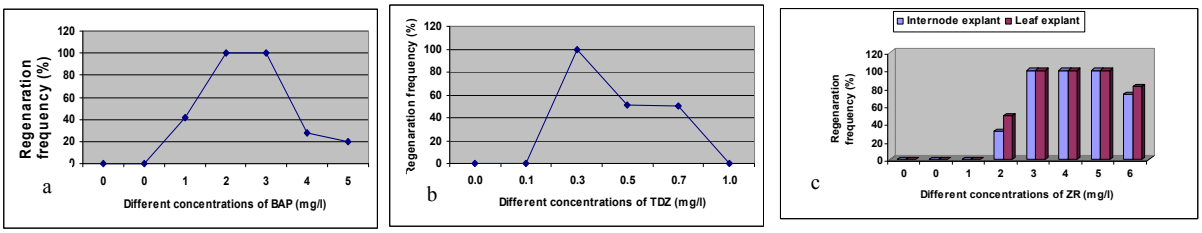
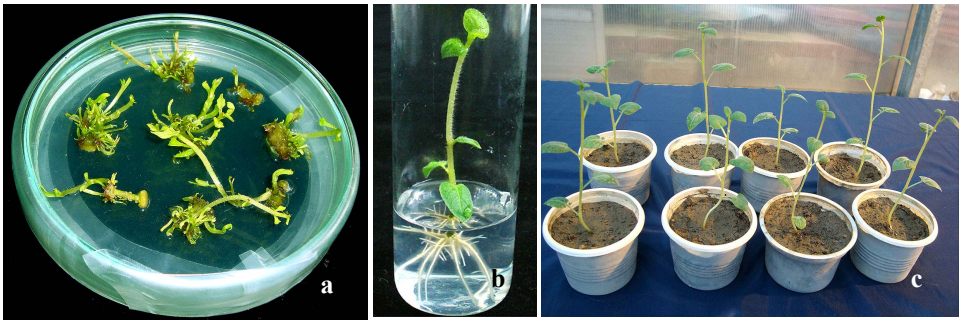


Fig. 2: Effect of different concentration of BAP, TDZ and ZR on regeneration frequency

The highest regeneration frequency 100% was found in TDZ @ 0.3 mg l^{-1} while it was the lowest 50.54% in TDZ @ 0.7 mg l^{-1} (Fig. 2b). Significant difference was also observed on regeneration frequency rate due to the different levels of ZR in internode and leaf explants. The highest regeneration frequency rate 100%

was recorded in ZR @ 3, 4 and 5 mg l^{-1} supplemented MS medium in internode and leaf explants, respectively where the lowest 31.40% was found in 2 mg l^{-1} ZR in internode explants (Fig. 2c). It is easier to work with internodal segments which are less sensitive to injuries during different manipulation steps. Moreover old leaves away from the shoot apex are less reactive to the *in vitro* culture when compared to young leaves. On the other hand, ZR reduced the callus phase and to accelerated shoot regeneration [11]. These statements are in agreement with the findings of the present study.



Plate(a-c): *In vitro* rooting and hardening of direct regenerated shoots

Well developed shoots were transferred to MS medium supplemented with 0.5 IBA for rooting. 100% plantlets were survived and established at ex vitro conditions (plate 2).

Table 1: Effect of BAP on in vitro direct regeneration of potato at 21 days

BAP (mg l^{-1})	Explants Producing shoot (%)	Days required for shoot appearance	Length of shoot (cm)	Number of leaves/shoot	Shoot diameter (mm)
1.0	31.79 b (33.11)	85.23 a	2.19 c	1.97 c	11.20 c
2.0	100.0 a (86.27)	56.27 b	3.31 b	2.29 b	13.40 b
3.0	100.0 a (86.27)	47.60 e	3.79 a	2.75 a	15.60 a
4.0	27.42 c (30.41)	63.21 c	1.37 d	0.45 d	7.66 d
5.0	19.98 d (25.61)	78.10 b	1.19 d	0.37 d	7.19 d
CV %	3.83	2.77	9.78	9.50	8.32

Means bearing same letters do not differ significantly at 1 % level of probability. Data within parentheses represent the arcsin transformed values

Table 2: Effect of TDZ on in vitro direct regeneration of potato at 45 days

TDZ (mg l^{-1})	Explants producing shoot (%)	Days required for shoot appearance	Length of shoot (cm)	Number of leaves/shoot	Shoot diameter (mm)
0.3	100.0 a (86.27)	24.37 c	2.97 a	3.79 a	8.24 a
0.5	89.31 b (68.41)	25.60 c	2.10 c	3.11 b	8.13 a
0.7	88.12 bc (67.37)	37.10 b	2.37 b	2.69 b	7.89 a
1.0	86.39 d (65.94)	45.31 a			
CV %	1.88	3.19	11.00	12.94	9.81

Means bearing same letters do not differ significantly at 1% level of probability. Data within parentheses represent the arcsin transformed values.

Table 3: Effect of different concentrations of ZR on direct regeneration of potato from internodes explants at 21 days

ZR (mg l^{-1})	Explants Producing shoot (%)	Days required for shoot appearance	Length of shoot (cm)	Number of leaves/shoot	Shoot diameter (mm)
2.0	100.0 a (86.27)	23.13 b	2.89 d	2.73 b	11.17 c
3.0	100.0 a (86.27)	16.89 c	2.77 c	2.51 b	12.98 d

4.0	100.0 a (86.27)	16.37 c	2.86 b	2.61 b	13.42 ab
5.0	100.0 a (86.27)	14.47 d	3.20 a	3.18 a	14.12 a
6.0	91.39 b (70.35)	27.16 a	1.56 e	0.76 c	8.14 d
CV %	3.34	6.65	9.29	7.69	10.92

Means bearing same letters do not differ significantly at 1 % level of probability. Data within parenthesis represent the arcsin transformed values

Table 4: Effect of different concentrations of ZR on direct regeneration of potato from leaf explants at 21 days

ZR (mg l^{-1})	Explants producing shoot(%)	Days required for shoot appearance	Length of shoot (cm)	Number of leaves/shoot	Shoot Diameter (mm)
2.0	100.0 a (86.27)	24.89 b	2.91 c	2.68 b	10.92 b
3.0	100.0 a (86.27)	23.10 c	2.83 b	2.35 b	11.29 b
4.0	100.0 a (86.27)	20.36 d	2.90 a	2.42 b	12.12 a
5.0	100.0 a (86.27)	18.32 e	3.43 a	3.11 a	12.39 a
6.0	96.16 b (77.66)	30.14 a	1.68 d	0.89 c	7.31 c
CV %	3.55	8.39	11.87	8.52	9.07

Means bearing same letters do not differ significantly at 1 % level of probability. Data within parentheses represent the squire root transformed values.

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