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REGENERATION EFFICIENCY OF FIVE HIGH YIELDING POTATO VARIETIES OF BANGLADESH

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Abstract

Experiments were conducted to develop an efficient and reliable protocol for *in vitro* direct regeneration of potato for future transformation. Five varieties of potato *viz.* BARI Alu-7 (Diamant), BARI Alu-21 (Provento), BARI Alu-24 (Dura), BARI Alu-25 (Asterix) and BARI Alu-26 (Felsina); 3 levels of Zeatin riboside *viz.* 3, 4 and 5 mg/l ; 2 types of explants *viz.* internode and leaf from 19-22 days old micro plants were used in this study. Internode explants of BARI Alu-25 produced the highest number of shoots (40.17) within16 days without callus phase in MS medium supplemented with 5 mg/l ZR + 0.2 mg/l GA_3 + 0.01 mg/l IAA followed by BARI Alu-21 (38.13), BARI Alu-7 (37.49), respectively. Leaf explants also produced shoots directly without callus phase in the same medium. In this case, the highest number (7.87) of shoots was found from the variety BARI Alu-25 followed by BARI Alu-21(7.42). ½MS medium supplemented with 0.5 – 1.0mg/l IBA resulted the best performance as to the rooting from ZR treated regenerated shoots having 100% plant survival at *ex vitro* conditions. This protocol may be used for rapid shoot proliferation and genetic transformation in potato.

Introduction

Potato (*Solanum tuberosum*) is a good staple source of carbohydrate in many countries including Bangladesh. In Bangladesh, potato is used mainly as vegetables and contributes about 73% of the total edible vegetables (BBS 2019-2020). A total of 9.68 million Mt potato was produced from 0.468 million ha of lands having an average yield 20.68 ton/ha (BBS 2019-2020). Till date, Tuber Crops Research Centre (TCRC), BARI developed 91 high yielding potato varieties (Rahman *et al.* 2019).

Potato is usually propagated asexually by means of tubers. Vegetative propagules produced by conventional method and planting materials are often prone to pathogen such as fungi, bacteria and viruses, thereby resulting in poor quality and yields. Plant tissue culture techniques have been developed as a modern and worldwide accepted means to improve the quality and quantity of vegetative propagated plants. Disease free good quality seeds and pathogen free planting materials are possible to be produced through tissue culture (Ehsanp our and Jones 2000, Molla *et al.* 2011a). It is well known fact that tissue culture technique produces virus free planting material in a mass scale (Zaman *et al.* 2001, Nagib *et al*. 2003) although the technique contributed a very little in the production of disease and pest resistant plants (Wang *et al*. 2020). Therefore, different conventional breeding and biotechnological approaches are being applied in various parts of the world. Insertion of R-gene into elite cultivars through conventional breeding needs 10-15 years and dictated by genetic complexity having inbreeding depression (Barrell *et al.* 2013). To overcome such problems, genetic transformation of crop plants has been evolved, which offers the

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ability to introduce single new character into a plant cultivar with no or very minimal disturbances to their genetic background within a short time (Barrell *et al.* 2013, Wang *et al.* 2020). This is virtually impossible via traditional breeding due to the high heterozygosity in the tetraploid potato. As a consequence, potato transformation represents the only way to produce isogeneic lines of specific cultivars to supplement traditional crop improvement and accelerate the development of new variety against diseases, pests and environmental stresses (Banerjee *et al.* 2006, Barrell *et al.* 2013). Before doing this, *in vitro* regeneration protocol is essential as this differs from genotype to genotype (Morteza *et al.* 2015). Genotype-independent *in vitro* plant regeneration is difficult in potato (Saker *et al.* 2012). Internode-based two steps somatic embryogenesis (SE) protocol showed wide genotypic differences in regenerative capacity (Saker *et al.* 2012). Most of the reported protocols are cultivar-specific (Mani *et al.* 2014, Al-Hussaini *et al.* 2015). The potato regeneration system has been optimized using the cv. Desiree which is known to be highly responsive to cell and tissue culture manipulations *in vitro* (Anon. 2011). In Bangladesh, regeneration efficiency of high yielding tetraploid potato varieties for direct regeneration without callus phases is still lacking so far. The present study was conducted to find out the regeneration efficiency of popular high yielding potato varieties developed by BARI from inter node and leaf explants using zeatin riboside to develop reproducible protocol for future genetic transformation.

Materials and Methods

In vitro raised micro plants var. BARI Alu-7, 21, 24, 25 and 26; three levels of Zeatin riboside (ZR) *viz.* 3, 4 and 5mg/l plus GA_3 (0.2 mg/l) and IAA (0.01 mg/l) concentration in Murahsige and Skoog (MS) media (Murashige and Skoog 1962) based on the authors previous study and two type of explants *viz.* internode and leaf (first 5-6 internodes from the top of the 16-19 days microplants excluding shoot apex and thick, healthy leaves from the upper nodes) were used in the present study. Effects of BAP, TDZ and ZR on direct regeneration of potato were described as per previous study (Molla *et al*. 2011b). The best responsive cytokine in ZR and explants was received from this paper. ZR ($C_{15}H_{21}N_5O_5$) a cytokine is used as a wide range of crops for greater number of shoots per explants (García-Fortea *et al*. 2020). Moreover, ½MS medium supplemented with 0.0, 0.1, 0.5, 1.0, 1.5 and 2.0 mg/l IBA was tested on elongated (3-4 cm in length) shoots derived from ZR treated explants. They were cut at the base and transferred on IBA supplemented ½MS medium for well-developed feeder roots. The experiment was laid out in a CRD with five replications having six culture tubes per treatment. The regenerated healthy rooted plantlets at 6-8 leaf stage were transferred from culture room and kept in room temperature (30ºC) for 5 days. The plantlets were removed from the culture vessels and culture media sticking to *in vitro* roots were washed carefully. The washed plantlets were planted into trays containing sand for 5-7 days for harden the plantlets at green house and watered once or twice a week as needed. The healthy plants were transplanted into small plastic pots containing sterile soil, sand and decomposed cow dung at the ratio of 1:1:1. Data on days to shoot appear (day), number of shoots/explants, length of shoot (mm), number of leaves/plant, days to root initiation (day), percentage of shoots inducing roots (%), number of roots per shoot, percentage of *ex vitro* plant survival (%) etc. were recorded and analyzed statistically using R x 64-program v4.2.0 (R core team 2013). The experiment was conducted at Tissue Culture Lab, Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute, Gazipur-1701.

Results and Discussion

Days required for shoot appearance was significantly affected due to the combined effect of variety, ZR concentrations and type of explants. The maximum days required for shoot appearance

(33.17 days) was recorded in leaf explant of variety BARI Alu-21 with 3 mg/l ZR followed by same variety and explant with 4.0 mg/l ZR (30.48 days). On the contrary, early shoot appearance (15.78 days) was observed in internode explant of BARI Alu-25 with 5 mg/l ZR (Table 1). Profuse shoot was produced from internode explants in ZR supplemented MS medium after 15-20 days (Dhaka and Nailwal 2015). Ghosh *et al.* (2015) obtained adventitious shoots from leaf discs of potato genotypes within 12.6-23.6 days in ZR based MS medium. Number of shoots per explant was also significantly affected due to the combined effect of variety, levels of ZR and type of explants which varied from 5.82 to 40.17 (Figs 1 and 2).The maximum number of shoots (40.17) were found in internode explant of BARI Alu-25 with 5 mg/l ZR followed by internode explant of BARI Alu-21 (38.13)and BARI Alu-7 (37.49) with the same concentration of ZR.

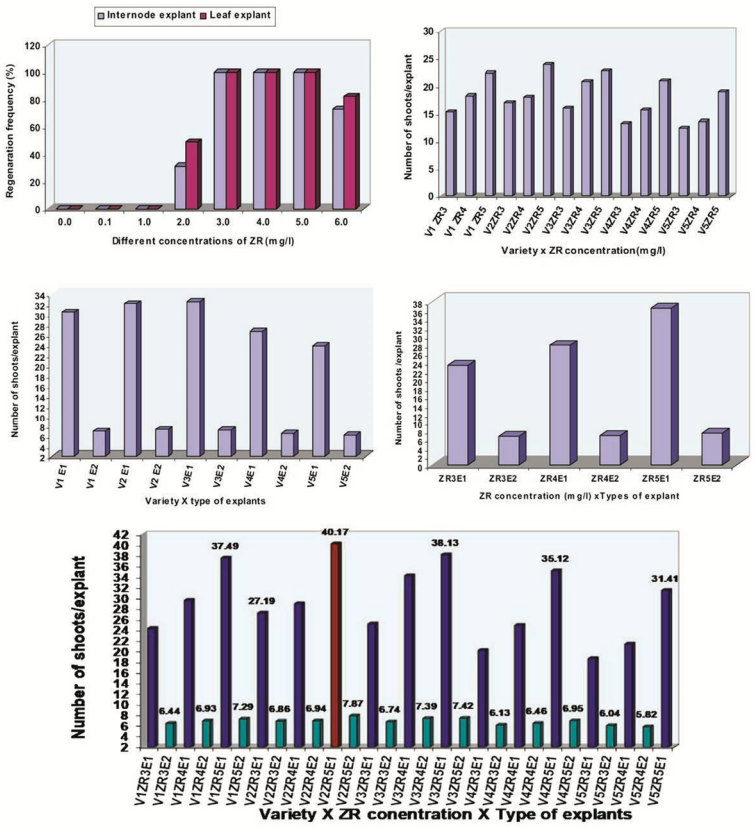

Fig. 1. *In vitro* regeneration efficiency of potato as influenced by variety, explant and zeatin riboside.

In case of leaf explant, highest number of shoots 7.87 was found in BARI Alu-25 followed by BARI Alu-21 (7.42). It was the lowest (6.13) in leaf explant of BARI Alu-24 with 5mg/l ZR (Fig. 1 and 3). Internode explants generally gave a better result than leaves of potato (Dhital *et al.* 2010, Kaur *et al*. 2017). These studies also stated that significant differences were found in regeneration frequencies from leaf vs. internode explants within the genotype. Internode of potato cultivars showed the highest efficiency in number of shoot production per explant than leaf and petiole in shoot induction medium. This observation supported the findings of the present study to some

extent (Kamrani *et al.* 2015). Nodal tissue (base of the leaves or internode on twigs) contains intercalary meristem. Meristem is a type of stem cell with actively dividing nature. Moreover, more vascular tissues are available in the internode. Therefore, internode explants produced more shots than the leaves. The results of the investigation demonstrated that shoot length was increased with the increasing ZR levels irrespective of explants (Table 1). However, it ranged from 2.52 to 3.30 cm. The combination of variety, different levels of ZR and types of explants had remarkable influenced on length of shoots. The maximum shoot length (3.51 cm) was measured in leaf explant of BARI Alu-25 with 5 mg/l ZR followed by internode explant (3.41 cm) of same variety and ZR concentration. Leaf explant of BARI Alu-21 and BARI Alu-7 with same level of ZR produced 3.40 and 3.38 cm length shoots, respectively which was statistically identical (Table 1). Dhaka and Nailwal (2015) reported the highest average number of shoots, nodes and leaves per explant found in the MS medium supplemented with Zeatin +IAA+GA_3 without callus phase.

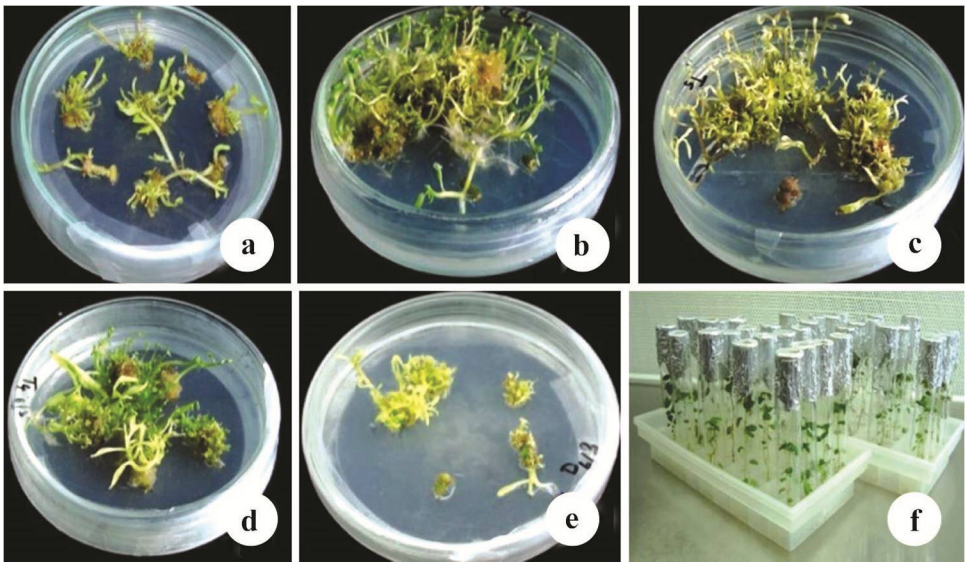

Fig. 2. Shoot induction from internode of five potato varieties as influenced by 5 mg/l ZR: (a) BARI Alu-7, (b) BARI Alu-25, (c) BARI Alu-26, (d) BARI Alu-21 (e) BARI Alu-24 and (f) Established plantlets.

The lowest shoot length (2.24 cm) was recorded in internode explant of variety BARI Alu-24 with 4 mg/l ZR followed by same explant and variety with 3 mg/l ZR (2.38).The highest number of leaves per shoot (3.42) was counted in internode explant of variety BARI Alu-26 with 5 mg/l ZR followed by the variety BARI Alu-24 in same explant and same ZR concentration (3.36), which was statistically at par (Table 1). On the other hand, the lowest number of leaves per shoot (2.38) was recorded in leaf explant of BARI Alu-7 with 3 mg/l ZR. The reason for the higher number of leaves per shoot might be due to higher number of shoots per explant and maximum length of shoot.

Days to root initiation varied due to the different concentrations of IBA which ranged from 5 to 12 days. The explants needed minimum days (5-6 days) treated with 0.5, 1.0, 1.5 and 2.0 mg/l IBA. The maximum days required for root initiation (9-12 days) was noted in the explants grown without IBA (Table 2). Result of the study revealed that all the shoots under different treatments induced roots. A significant difference was observed among the treatment as to the number of

roots per plant. The highest number of roots per plant (22.35) was recorded in ½MS medium supplemented with 1.0 mg/l IBA followed by ½MS plus1.5 mg/l IBA (21.70) which was statistically identical. However, the shoots under the treatment 0.5mg/land 1.0 mg/l IBA produced healthy and vigorous roots followed by 1.5 and 2.0 mg/l (Table 2). Based on the eye estimation, it was observed that ½MS medium without IBA produced very lean roots. On the other hand, the lowest number of roots per plant (4.35) was noted in ½MS medium without IBA whereas, 0.1mg/l IBA produced5.98roots per plant which was statistically identical (Table 2). But0.5mg/l IBA produced 18.31 roots per plant which were healthy and vigorous in growth. However, ½MS medium supplemented with 2.0 mg/l IBA produced 16.13 fibrous roots per plant. A higher concentration of growth regulators is the best for the production of maximum roots per plant up to

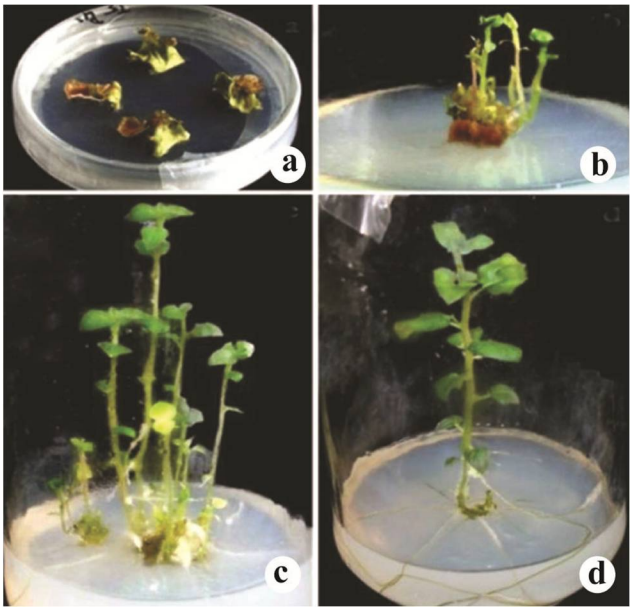

Fig. 3. Shoot induction from leaf explants of potato cv. BARI Alu-25 as influenced by ZR 5 mg/l: (a) Shooting of explants, (b). Shoot induction at 28 days, (c) Shoot induction at 42 days and (d) *In vitro* plant at 28 days.

certain levels. Auxin is generally considered for the accusation of the meristematic competence of the responsive cells. Once this competence has been established, excessive auxin concentration was often found to be inhibitory for further embryonic or adventitious root development (Molla *et al*. 2017). Lower concentration of auxin and cytokine is influenced the formation of both root and shoot but at higher concentrations plantlets showed reversed trends. It was observed that only 1.0mg/l IBA supplemented ½MS medium produced highest secondary roots per root (5.30) followed by 0.5mg/l IBA supplemented ½MS medium (5.13 (Table 2). Optimum concentration of IBA produced vigorous and healthy feeder roots resulting the secondary roots. Growth of the roots was estimated visually and was observed that ½MS medium supplemented with 0.5-1.0 mg/l IBA produced more healthy and vigorous roots than the other treatments. Plantlets grown in ½MS medium supplemented with 0.5 - 2.0 mg/l IBA, survived hundred per cent at hardening and field conditions (Table 2, Fig. 4).

Table 1. Effect of varieties, zeatin riboside (ZR) and explants on *in vitro* direct regeneration of potato at 21 days.

Variety	ZR Conc.	Days required for shoot appearance		Length of shoot (cm)		Shoot diameter (mm)		Number of leaves/shoot	
		Leaf	Internode	Leaf	Internode	Leaf	Internode	Leaf	Internode
BARI Alu-7	3.0	25.30 h	18.46 r	2.69 ij	2.55 jk	11.31 l-o	11.97 hij	2.38 l	2.51 i-l
	4.0	22.72 h	17.41 s	3.33 bc	3.13 def	11.38 k-o	12.94 def	2.59 h-l	2.84 e-h
	5.0	20.46 n	16.39 v	3.38 ab	3.17 d	11.60 j-n	13.71 b	3.00 c-g	3.11 b-e
BARI Alu-21	3.0	25.33 h	19.17 q	2.78 hi	2.61 ijk	11.43 ko	12.89 ef	2.47 i-l	2.51 i-l
	4.0	26.78 f	19.92 o	3.36 ab	3.17 cde	11.59 j-n	13.47 bc	2.52 i-l	2.71 g-k
	5.0	21.3 m	16.84 tu	3.40 ab	3.29 bcd	11.73 j-n	13.89 b	2.98 d-g	3.00 c-g
BARI Alu-24	3.0	33.17 a	25.73 g	2.69 ij	2.38 lm	11.00 o	11.24 mno	2.52 i-l	2.61 h-l
	4.0	30.48 b	23.19 k	2.76 hi	2.24 m	11.26mno	12.00 g-j	2.72 g-j	2.86 e-h
	5.0	28.93 c	21.48 m	3.04efg	2.96 g	11.28 l-o	13.13 cde	3.29 a-c	3.36 ab
BARI Alu-25	3.0	24.19 j	17.16 st	2.71 ij	2.56 jk	12.32 ghi	13.16 c-e	2.40 kl	2.42 j-l
	4.0	21.13 m	16.52 uv	2.96 g	2.90 gh	12.38 ghi	13.61 bc	2.48 i-l	2.73 g-j
	5.0	19.56 p	15.78 w	3.51 a	3.41 ab	12.46 fgh	15.12 a	3.08 b-f	3.21 a-d
BARI Alu-26	3.0	30.41 b	24.63 i	2.57 jk	2.48 kl	11.17 no	11.80 j-l	2.42 j-l	2.58 h-l
	4.0	28.45 d	22.79 l	2.99 fg	2.69 ij	11.89 ijk	12.48 fg	2.78 f-i	2.99 c-g
	5.0	27.31 e	20.17 no	3.15 def	3.00 fg	11.28mno	13.41 bcd	3.17 a-d	3.42 a
CV		3.39		3.60		7.56		3.43	

Means bearing same letters do not differ significantly at 1% level of probability.

Table 2. Response of IBA on root induction behavior *in vitro* and *ex vitro* establishment of plantlet at 28 days.

IBA (mg/l)	Days to root initiation	Shoot inducing root (%)	Number of roots /plantlet	Number of secondary roots /root	Visual growth of roots	Days required for well-developed roots	*ex vitro* establishment (%)
0.0	9 - 12	100 +	4.53 d	1.0e	+	18 - 21	67.98 b (55.39)
0.10	7 - 9	100 +	5.98 d	3.35d	+	16 - 18	75.39 b (60.23)
0.50	5 - 6	100 +++	18.31b	5.13ab	+++	12 - 14	100.00 a (89.96)
1.00	5 - 6	100 +++	22.35 a	5.30a	+++	14 - 16	100.00 a (89.96)
1.50	5 - 6	100 ++	21.70 a	4.90bc	+	14 - 16	100.00 a (89.96)
2.0	5 - 6	100 ++	16.13c	4.77c	+	14 - 16	100.00 a (89.96)
CV %	-	-	5.68	4.80	-	-	3.71

Means bearing same letters do not differ significantly at 1% level of probability; Data within parentheses represent the arcsine transformed values. Where, + = poor, ++ = good, +++ = very good, - = absent.

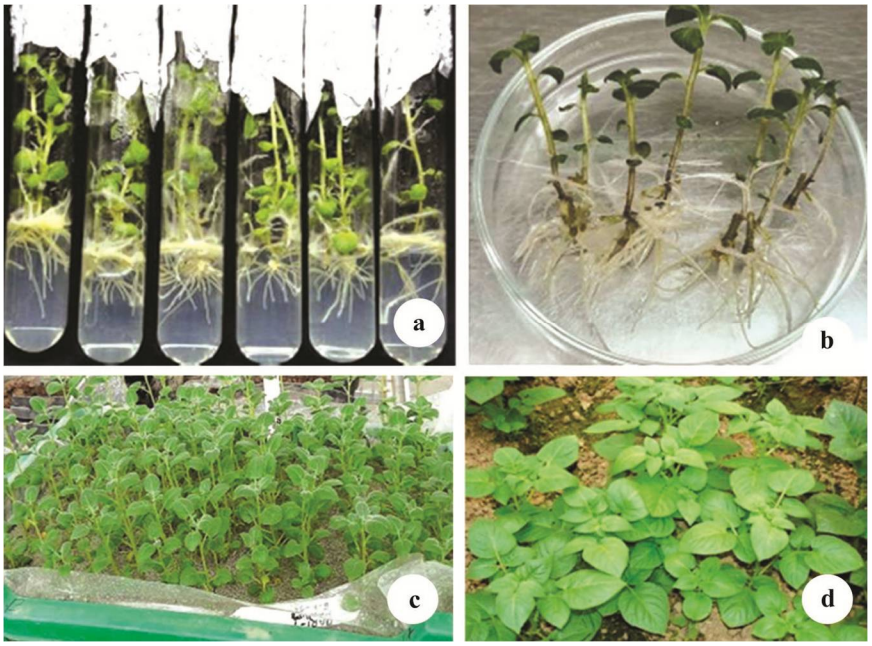

Fig. 4. Effect of IBA on rooting and *ex vitro* establishment of potato cv. BARI Alu-7, (a-b) Rooted plantlets at 21 days and (c-d) *Ex vitro* establishment.

From the above results, it may be concluded that ZR 5.0mg/l supplemented MS medium is suitable for *in vitro* direct shoot regeneration from internode and leaf explants. The internode explant of BARI Alu-25 exhibited the best performance among the varieties. Also, ½MS medium supplemented with 0.5-1.0mg/l IBA produced healthy and vigorous roots at *in vitro* conditions having 100% plant survival at *ex vitro* conditions.

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