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MORPHOGENIC POTENTIAL OF THREE POTATO (*SOLANUM TUBEROSUM*) CULTIVARS FROM DIVERSE EXPLANTS, A PREREQUISITE IN GENETIC MANIPULATION

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Abstract

In vitro response and its relationship with different varieties, explants and media were investigated in potato (*Solanum tuberosum*). Direct *In vitro* regeneration protocol from diverse explant source is a prerequisite for transformation studies. Three potato cultivars viz., Cardinal, Altamash and Diamont were selected for *in vitro* responses. High regeneration and morphogenic potential of different explants i.e., shoot tips, leaf discs, nodes and internodes have been tested for direct regeneration. Basal media was Murashige & Skoog and different hormonal combinations of benzyl adenine and indoleacetic acid were supplemented. Statistical analysis showed that explant source had significant effect on direct regeneration and the nodal explants had maximum regeneration. The number of shoots obtained from node was 17.6 from Cardinal followed by Diamont 14.3 and Altamash 9.0. Shoot apices also resulted in shoot regeneration comparatively better than leaf discs and internodal explants but lesser than from nodes. Most suitable medium was MS with 2.0 mg/l BAP and IAA @ 0.5 mg/l giving maximum regeneration. It was also observed that interaction of cultivars with explant and media is highly significant at P 1.0%.

Introduction

Potato is an important cash crop widely cultivated through out the world. In Pakistan it is cultivated over an area of 10,5000 hectare with an annual production of 1678 thousand tones. Potato is prone to several fungal, viral and bacterial pathogens and causes heavy economic loss every year. Recent advances in plant biotechnology have made it possible to produce resistant varieties by introducing desired genes from many different organisms into plants. It is possible to modify agricultural and horticultural crops now, which was otherwise difficult by conventional breeding techniques. A successful and reproducible plant transformation system requires a responsive *in vitro* regeneration system. Regeneration response *in vitro* is generally species and often genotype specific (Ritchie & Hodges, 1993). Therefore, regeneration conditions and characteristics may vary among genotypes and need to be determined prior to transformation. In *Solanum* different approaches so for have been adapted to obtain efficient *in vitro* regeneration system either from petioles with intact leaflets (Shirley *et al*., 2001), leaves (Ooms *et al.,* 1987, Cearley & Bolyard, 1997; Trujillo *et al.,* 2001; Sarker & Mustafa, 2002; Anderson *et al*., 2003), tuber discs (Sheerman & Beaven, 1988; Vasquez & Clarence, 2002), and from stem (Visser *et al.,* 1989; Chang *et al*., 2002) after passing through callus phase. Recently Osusky *et al*., (2005) reported regeneration of plants from leaf disc tissues during genetic modification of potato.

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It is generally difficult to separate genotypic effects on somaclonal variation from the differences caused by *in vitro* regeneration response, since both characteristics are genetically controlled (Karp, 1995). However, Bebeli *et al*., (1988) demonstrated that genotype can influence somacIona1 variation irrespective of regeneration response in rye. In potato, increasing evidence is available showing genotypic effects on *in vitro* regeneration and variation of plants derived from leaf discs (Fleming *et al.*, 1992; Trujillo *et al*., 2001), stem segments (Cardi *et al.,* 1992), anthers (Mix, 1983; Vernneau *et al.*, 1992) and protoplasts (Sree-Ramulu *et al.*, 1983; Coleman *et al.*, 1990).

The objective of this study was to a) establish direct *in vitro* regeneration protocol from diverse explants, b) study the morphogenic potential of explants and their relationship to genotype and characteristics of regeneration c) examine the effect of media combination on *in vitro* response.

Materials and Methods

Plant material: Field grown potato tubers (*Solanum tuberosum,* L) of Cardinal, Altamash and Diamant were acquired from Potato programme, NARC, Islamabad, Pakistan.

Explants: To obtain virus free *in vitro* explants source cultures of above mentioned varieties were established after thermotherapy treatment from meristems. ELISA was conducted for virus indexing. Virus free *In vitro* stock was maintained on basic (Murashige & Skoog, 1962) liquid medium containing 1mg/l GA_3 and 100 mg/l Silver thiosulphate. When the virus free *in vitro* plantlets were of two weeks old, explants were excised under aseptic condition. Leaf discs, internodes, shoot tips and nodes with leaflets attached were taken from *in vitro* grown plantlets of Cardinal, Altamash and Diamont. To maintain the uniformity, the leaf discs were excised with the help of sterilized cork borer. Inter nodes 6 mm in size and nodes 2 mm internodal region on each side from the point of nodes with attached single leaflet, and shoot apices of 4 mm in size were excised under aseptic conditions.

Regeneration media: The basic culture media contained MS (1962) plus vitamins supplemented with different combinations of BA and IAA and 30g/l sucrose as given on Table 1.

pH of the all media was maintained at 5.8 prior to autoclaving. Explants were shifted on fresh media after every 15 days.

Culture condition: The cultures were kept under 16 hrs photoperiod at 25 ± 2^{o}C for all experiments.

Statistical analysis

The regeneration potential of three genotypes was investigated and analyzed in completely randomized design (CRD) with three factor factorial arrangement having three replicates containing 10 explants each with 12 treatments, four explants and three varieties. Range test was conducted by LSD for comparison of means. MSTATC program was used for data analysis.

Table 1. MS (1962) Medium supplemented with BAP and IAA.

MS	BAP mg/l					
IAA mg/l	**0.0**	**0.1**	**0.5**	**1.0**	**1.5**	**2.0**
0.5	T1 0.5/0.0	T2 0.5/0.1	T3 0.5/0.5	T4 0.5/1.0	T5 0.5/1.5	T6 0.5/2.0
1.0	T7 1.0/0.0	T8 1.0/0.1	T9 1.0/0.5	T10 1.0/1.0	T11 1.0/1.5	T12 1.0/2.0

Results and Discussion

In vitro plants produced after thermotherapy treatment at 34-37^{o}C for a period of 3 weeks followed by meristem culture were found to be appropriate for elimination of viral pathogens from the plants. ELISA results showed that *in vitro* plants of Cardinal, Diamont and Altamash are free from PLRV, PVX, PVV, PVA, PVM and PVS.

Effect of explant sources on regeneration: Regeneration response as morphological development of explants to shoots was investigated from diverse explant sources and significant differences were observed among the explants. Means of all explant ranged from 0.324 to 4.231 (Table 2). The morphogenic potential varied alongwith the explants sources on each media regime. The maximum numbers of plantlets (Fig. 1) regenerated from nodal tissues was 13.67 followed by shoot apices (Fig. 2) having 5.00 from all the three varieties and all media compositions collectively. The reason for high morphogenic potential and regeneration from the nodal explants and shoot apices lies in the juvenility and meristematic nature of the tissues. On the other hand leaf discs and internodes showed lowest level of shoot regeneration which had respectively 0.32 and 0.33 mean numbers of shoot collectively from all media combination and all varieties. These lower numbers of mean from leaf discs and internodal tissues are due to the reason that leaf discs and inter nodal tissues of only one variety Cardinal have shown some response (Table 4) while both of these tissue from varieties Diamont and Altamash did not show any response. It was also observed that internodal and leaf disc tissues initially underwent callus-inducing phase first, after that regeneration took place (Fig. 3 and Fig. 4). There are reports of regeneration from single leafy nodes at the frequency ranging from 1 to 5 shoots per fragment (Dobigny *et al.,* 1996) after 30 days of culture and also from leaf explant which was used as one step method regeneration. Trujillo *et al.,* (2001) obtained regeneration of plants after passing through callus phase. Direct regeneration system has an edge over regeneration after passing through callus phase to maintain the true-to-type nature of the regenerated plantlets and to avoid the variation. Sarker & Mustafa (2002) have used three explants viz., leaf, node segments and inter nodal segments of two potato varieties. They regenerated plantlets from leaf explant which was followed by internodes. In the present study, nodal explants resulted in high regeneration potential followed by shoot apices explants. Similarly Philip & Hampson (1995) have also reported high regeneration frequency from internode and leaf tissue explant of potato.

Table 2. Interaction of treatment and explant.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	Means of explants
Leaf disc	0.00 P	0.00 p	0.33 op	0.67 no	0.67 no	0.67 no	0.00 p	0.00 p	0.00 p	0.33 op	0.56 no	0.67 no	0.324 c
Nodes	1.00 mn	1.00 mn	1.89 jk	0.78 no	11.67 b	13.67 a	1.33 lm	3.78 efg	4.78 c	4.22 de	3.00 h	1.67 kl	4.231 a
Inter nodes	0.00 p	0.00 p	0.33 op	0.44 op	0.56 no	0.67 no	0.00 p	0.00 p	0.33 op	0.33 op	0.67 no	0.67 no	0.333 c
Shoot apices	1.00 mn	2.00 ij	2.33 ij	3.11 h	4.00 ef	5.00 c	1.00 nm	1.67 kl	2.44 i	3.33 gh	3. 67 fg	4.56 cd	2.870 b
Means of treatment	**0.50 G**	**0.83 fg**	**1.22 ef**	**1.75 cd**	**4.22 b**	**5.00 a**	**0.58 g**	**1.36 de**	**1.89 c**	**2.05 c**	**1.97 c**	**1.89 c**	

Table 3. Interaction of treatment and variety.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	Means of explants
Cardinal	0.50 p	1.00 mno	1.50 jkl	2.33 hi	6.17 b	7.00 a	0.50 p	1.00 mno	1.92 ij	3.00 ef	2.42 gh	2.83 fg	**2.51 a**
Diamant	0.50 p	0.75 op	1.00 mno	1.17 lmno	3.50 d	4.67 c	0.75 op	2.25 hi	2.50 gh	1.42 klm	1.1 1.92 ij	1.58 jkl	**1.83 b**
Altamash	0.50 p	0.75 op	1.67 lmno	1.75jk	3.00 ef	3.33 de	0.50 p	0.833 nop	1.25 lmn	1.75 jk	1.58 jkl	1.25 lmn	**1.47 b**
Means of Treatment	**0.50 g**	**0.83 fg**	**1.22 ef**	**1.75 cd**	**4.22 b**	**5.00 a**	**0.58 g**	**1.36 de**	**1.89 c**	**2.05 c**	**1.97 c**	**1.89 c**	

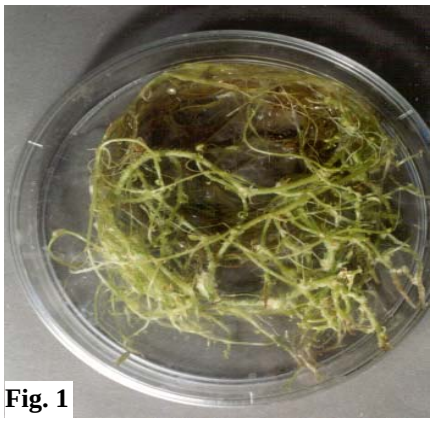
Fig. 1


Fig. 2


Fig. 3


Fig. 4

Fig. 1. Direct regeneration from nodal explants
Fig. 2. *In vitro* response from shoot apices.
Fig. 3. Callus induction from leaf discs.
Fig. 4. Callus induction from inter nodes

Table 4. Interaction of explant and variety.

	Leaf disc	Nodes	Inter nodes	Shoot apices	Mean of varieties
Cardinal	0.97 d	4.58a	1.00d	3.50b	2.51a
Diamant	0.00 e	4.77a	0.00e	2.55c	1.83b
Altamash	0.00 e	3.33b	0.00e	2.55c	1.47b
Mean of explant	0.32c	4.23a	0.33c	2.87b	

Effect of media on regeneration: Along with explant source, media has also played a significant role in *in vitro* regeneration response. It was observed that BAP played important role in shoot regeneration. At lower concentration, shoot numbers were 0.83 but it increased gradually with increase in BAP to 5.00 number of shoot collectively from all the varieties and explants as shown in Table 2. As BAP has significant role in cell multiplication therefore number of shoots also increased. On the other hand, IAA reduced the shoot regeneration potential when its concentration was doubled from 0.5 to 1.0 mg/l, the shoot number reduced from 5.00 to 1.89 on the media having same concentration of

BAP i.e., at 2.0 mg/l collectively from all the varieties and explants. When we considered the interaction of specific explant and media, nodal explant and media containing BAP at 2.0 mg/l and IAA at 0.5 mg/l i.e.T6 was highly significant (Table 2) followed by shoot apices explant on the same media. On the other hand on same media the leaf discs and internodal explants showed minimum interaction value of 0.67 for both explants. Over all, the maximum mean (5.00) was highly significant on media containing BAP at 2.0 mg/l and IAA at 0.5 mg/l T6 from the interaction of media and explant from all the three varieties and also from the nodal explants (4.321) collectively from all media regimes tested (Table 2) followed by shoot apices which showed maximum number of shoots (5.00) with mean of 2.87.

Similar results are also reported by Sarker & Mustafa (2002) that the BAP showed better response in terms of shoot per explant, shoot length, number of nodes and leaves in Potato varieties Lal Pari and Jam Alu. Similar behavior was also observed in varieties Daimant, Altamash and Cardinal. The results also coincide with the reports of Hoque *et al.,* (1996a, 1996b) and Mila (1991) for other potato varieties.

Genotypic effect on regeneration: Genotype also played a vital role in shoot regeneration as well in transformation efficiency among potato varieties as reported in the literature (Sheerman & Bevan, 1988; Wenzeler *et al.,* 1989, Phillip & Hampson 1995). It was also observed in this study that Cardinal showed over all highly significant mean value of 2.51 (Table 3 and 4) followed by Diamont and Altamash. But interaction of variety and explant has shown that Diamont and Cardinal regenerated maximum number of shoots i.e., 4.77 and 4.58 respectively from nodal explant alone and followed by Altamash having a mean of 3.33 shoots from the same explant (Table 4). This highly significant difference between varieties viz., Cardinal with a mean of 2.51; Diamont with 1.83 and Altamash with 1.47 (Tables 3, 4) from all the explants as whole was due to the fact that internodal and leaf disc explant of variety Diamont and Altamash did not show any response while same tissues of the variety Cardinal have shown some response i.e., mean number of shoots 0.97 from leaf discs and 1.0 from the internodal explants. So leaf discs and inter nodal tissues are the least responsive explants for direct regeneration. These explants underwent callus induction phase and then resulted in shoot regeneration indirectly and may cause variation. Sarker & Mustafa (2002) also reported that Lal Pari showed better response as compared to Jam Alu. This variable response of different varieties was due to genetic diversity which leads towards *in vitro* response as also reported previously by (Hussey & Stacey, (1981); Bajaj, (1981) and Miller *et al.,* (1985).

Shirley *et al*., (2001) has reported high efficiency of *in vitro* regeneration from potato petioles with intact leaflets on MS medium supplemented with BAP at 3.0 mg/l, GA_3, Silver thiosulphate, thidiazuron and IAA at 1 mg/l. Compared with other regeneration system for potato, the results here appear to yield the highest direct regeneration rates for single step protocols (Keil *et al.,* 1989; Tavazza *et al.,* 1998) and are comparable to or higher than two step and three step protocols (Webb *et al.,* 1983; De Block, 1988; Visser *et al.*, 1989; Hulme *et al.,* 1992, Hansen *et al.,* 1999). Our results are similar to the results of Philip & Hampson (1995) who also used the same explants (leaf discs and internodes) and got the high regeneration frequency from 12 different varieties. From the data obtained in the present study, it can be concluded that the media choice may depend to some extent on the variety to be used. There are many advantages of the nodal tissue as an explant, i.e., a large number of aseptic plants can be obtained quickly and easily, and plants produced may remain true to type because of direct regeneration protocols.

Table 5. Interaction of varieties, explants and media.

	Cardinal				Diamaont				Altamash			
	Leaf disc	Nodes	Inter nodes	Shoot apices	Leaf disc	Nodes	Inter nodes	Shoot apices	Leaf disc	Nodes	Inter nodes	Shoot apices
T1	0.0t	1.0s	0.0t	1.0s	0.0t	1.0s	0.0t	1.0s	0.0 t	1.0s	0.0t	1.0s
T2	0.0t	1.0s	0.0t	3.0mn	0.0 t	1.0s	0.0 t	2.0pq	0.0 t	1.0s	0.0t	2.0pq
T3	1.0s	2.0pq	1.0s	2.0pq	0.0t	2.0pq	0.0t	2.0pq	0.0t	1.7r	0.0t	3.0 mn
T4	2.0pq	3.0mn	1.3rs	3.0mn	0.0t	2.7no	0.0t	2.0pq	0.0t	2.7no	0.0t	4.3j
T5	2.0pq	16.0b	1.7qr	5.0i	0.0t	11.0d	0.0t	3.0mn	0.0t	8.0f	0.0t	4.0jk
T6	2.0pq	17.6a	2.0pq	6.3h	0.0t	14.3c	0.0t	4.3j	0.0t	9.0e	0.0	4.3j
T7	0.0t	1.0s	0.0t	1.0s	0.0t	2.0pq	0.0t	1.0s	0.0t	1.0s	0.0t	1.0t
T8	0.0t	2.0pq	0.0t	2.0pq	0.0t	7.0g	0.0t	2.0pq	0.0t	2.3op	0.0t	1.0t
T9	0.0t	3.3lm	1.0s	3.3lm	0.0t	8.0f	0.0t	2.0pq	0.0t	3.0mn	0.0t	2.0pq
T10	1.0s	6.0h	1.0s	4.0jk	0.0t	2.6no	0.0t	3.0mn	0.0t	4.0jk	0.0t	3.0mn
T11	1.6qr	1.0s	2.0pq	5.0i	0.0t	3.7kl	0.0t	4.0jk	0.0t	4.3j	0.0t	2.0pq
T12	2.0pq	1.0s	2.0pq	6.3h	0.0t	2.0pq	0.0t	4.3j	0.0t	2.0pq	0.0t	3.0mn

Interaction of media, explant and varieties in an *in vitro* condition has shown significant difference among the media, variety and explant (Table 5). The variety Cardinal has produced maximum number of shoots (17.6) from nodal explant on MS medium containing 2.0 mg/l BAP and 0.5 mg/l IAA. Variety Diamont has produced 14.3 and variety Altamash has produced 9.0 shoots from the nodal explant on the same media combination. On the other hand shoot apices tissues of Cardinal has produced maximum number of shoots 6.3 on MS medium containing 2.0 mg/l BAP and 0.5 mg/l IAA. Variety Diamont has produced 4.3 and variety Altamash has produced 4.3 shoots on the same media combination. Leaf disc and internodal tissues were least responsive explant for direct regeneration in our study. The number of shoots produced from Cardinal, Diamont and Altamash were 2.0, 0.0, 0.0 from leaf discs and 2.0, 0.0, 0.0 from internodal explants. From these results it was concluded that variety Cardinal has high regeneration potential than Diamont and Altamash and among the explants, nodal tissue is the most responsive tissue for direct regeneration as compared to the shoot apices, leaf discs and internodal explants. BAP @ 2.0 mg/l along with 0.5 mg/l IAA was found to be the most appropriate media for maximum regeneration.

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