Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

In Vitro Studies On Regeneration of Potato (Solanum tuberosum L.) From Various Explants

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Abstract

Potato (*Solanum tuberosum* L.) is one of the world's most economically important crop. An efficient and reproducible tissue culture procedure for the generation of shoots is a pre-requisite for crop improvement. Two step method was employed in shoot regeneration from various explants i.e stem, Leaf and Tuber disc. Where step I or step II media alone did not yield any substantial shoot formation. One step method was adopted for efficient regeneration of tuber discs. Where as Two step method is essential for effective regeneration of Stem and Leaf explants'

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most economically important crop. The origin of this crop is known to be the highland of the Andes in South America. It is now cultivated in many temperate and tropical zones all over the world. North America and European countries are still the largest producers of potatoes, but developing countries are now increasing their share of global production.

Potato is an integral part of the diet of the world's population. In developing world, potato ranks number 11 in food terms, but in monetary terms, it is the forthmost most importance food crop after rice, wheat and maize. The average potato consumption in most developing countries is 20 kg percapita annually, while it is more than 80 kg percaptia in Western Europe. Potatoes supply atleast 12 essential vitamins and minerals including an extremely high density of vitamin `C'. Potatoes also provide significant amounts of protein, carbohydrates and iron (Gray and Hughes, 1978). Due to its exceptional qualities, the potato has become one of the major food crops , and its use is increasing even now for more rapidly than that of any other crop.

Potato is usually vegetatively propagated, which assures the stability of cultivar characteristics over successive plantings, but tubers spread disease. This has given rise to the use of in *vitro* culture techniques for vegetative propagation (Lam, 1975; Espinoza et al, 1986; Martel and Garcia, 1992).

During 80's and 90's potato conventional breeding is extensively used, Tissue culture methods, particularly anther culture and protoplast fusion were found to be useful tools to overcome some problems in conventional breeding. An efficient and reproducible tissue culture procedure for the

generation of shoots is a pre-requisite for crop improvement with the advancing technology of genetic engineering. Creation of noval germplasm through the techniques of tissue culture and gene transfer, holds great potential for improving the quality, resistance to disease and agronomic characters of potato (Haberlach *et al., 1985*). Tetrasomic segregation patterns and low fertility make potato a difficult crop to breed (Ross, 1986).

Potato tissue culture is less straight forward, although plant regeneration from a range of cultured explants and protoplasts is possible. Plant regeneration with intermediate callus formation may produce somaclonal variations (Larkin and Scowcroft, 1981) and proctoclonal variations (Thomos et al, 1982; Sadanandam, 1991) which are normally associated with callus derived regeneration systems. It has therefore become desirable to develop more efficient direct in *vitro* regeneration systems for this crop. One step, two step, three step procedures were used for plant regeneration from various explants. The establishment of efficient plant regeneration system from a range of explant tissues including leaf, stem and tuberdisc for several potato genotypes is reported (Kumar,1994; Sarkar *et al*, 1997). Tissue culture of potato is also known to be strongly genotype dependent (Wenzler *et al*., 1989).

Material & Methods:

The experiments were carried out with virus free tubers of *Solanum tuberosum* Cv Chandramukhi and Jyothi, which were obtained from C.P.R.I, Shimla. Sprouting tubers were placed in pots plants are raised from them. Leaf and stem pieces from 5-6 week old plants were collected and sterilized with commercial bleach (Teepol) and thoroughly washed with distilled water. The explants were sterilized with 5% sodium hypochlorite for 10 min followed by three rinses with sterile distilled water.

Tubers were peeled off and surface sterilized in 5% sodium hypochlorite for 10 min and rinsed 5 times with sterile distilled water. Subsequently using a corkbore cylinder (1 cm) tubers were punched out and cut into discs of approximately.3mm thickness.

There are different media formulations proposed for regeneration from various explants of potato-one step and two steps were adopted. In the two step experiment, step-1 medium was designed with MS medium containing NAA and 2,4-D in combination with BA or kinetin and step II medium was NAA and 2,4-D free, containing GA3 and growth regulators similar to those of step I medium . The two step medium was not suitable for generation from tuber discs. And so, the one step medium encompassing IAA and Zeatin was also employed for the purpose.

Shoots were exciced and placed on basal medium for rooting and extensive rooting was observed in almost all shoots(Fig:2). After four weeks and at length of 6-10cm height, the plantlets were transferred on to vermiculite and placed in green house for 4-weeks(Fig:3) and lastly transferred to garden area.

Results

Two steps method was employed in shoot regeneration from various explants i.e stem, Leaf and tuber disc. Where step I or step II media alone did not yield any substantial shoot formation. One step method was adopted for efficient regeneration of tuber discs.

Stem

A two step method was employed in shoot regeneration from cultured stem explants of both the cultivars (Chandramukhi and Jyothi) (Table-I). The data on the response of stem explants on various media combinations are presented. Initially explants were cultured on step I medium (Murashige& Skoog 1962), where they responded with white callus. BA in combination with NAA,2,4-D(M_1, M_3) in step I medium produced maximum response (95%,90%) in comparision with Kn in combination with NAA,2,4-(M_2,M_4). In cultivar Chandramukhi BA + NAA (M_1), and in cultivar Jyothi 2,4-D + BA(M_3) resulted In maximum percentage of cultures producing callus. Nodular structures in callus cultureswere observed on explants with in two weeks of culture.However shoots were not observed. These explants were subsequently moved onto step II medium. The nodular structures on stem explants developed into shoots with in 2-3 weeks of culture on step II medium. Nodular structures from NAA + BA (M_1), 2,4-D+ BA(M_3) was found to elicit maximum number of shoots per explant (6.4+0.7,8,8+0.5,8-0.5) in cultivars, Chandramukhi and Jyothi respectively in step II media (Plate – IIa). Where as cultures from M_2 and M_4 were failed to develop further kinetin in both step I and step II medium failed to produce shoots.

Leaf

The data on the response of leaf explants on various media combination are presented(Table-I). Leaf explants were initially cultured on step I medium resulted in producing nodular callus after two weeks. The maximum response of explants to callusing was observed in M_1 for cultivar Chandramukhi (90%) and M_3 in cultivar Jyothi (98%). These green nodular callus explants were subsequently transferred on to step II medium where the shoot buds on leaf explants of cv Chandramukhi were failed to develop further. However, those of cultivar Jyothi developed into shoots with in 2-3 weeks. Further more leaf explants from kinetin supplemented (M_2,M_4) step I medium were failed to develop shoots on step II medium. (M_2,M_4) the maximum number of shoots per explant (4.4±0.7,4.4±0.7) were elicited onM_3R_1 medium.

Tuber Disc

Both two-step and one-step procedures were applied for tuber disc regeneration.

a) **Two-step method :** Initially tuber disc explants were cultured on step I medium resulted in the production of callus. 2,4-D + BA(M_3) resulted in the maximum number of explants with callus (80%,85%) in both the cultivars Chandramukhi and Jyothi respectively. The callus from step I medium was subsequentelysubcultured into step II medium.Explants transferred to M_1R_2 and M_4R_2 media failed to produce shoots. However, explants cultured on M_1R_1 and M_3R_1 media

produced shoots. M_1R_1 media induced regeneration in 6% and 10% explants with 1.3 + 0.6, 1.3-0.6 and 2.6+0.2, 2.6-0.2 shoots/explants. Explants on M_3R_1 media responded with 10% and 12% explants with 2.2+0.4; 2.8+0.2 shoots/explants.

b) One-step method: The two step medium was not suitable for regeneration from tuber discs. The frequency of regeneration and number of shoots were very much reduced. And so, the one-step medium encompassing IAA and Zeatin was employed for the purpose.

Zeatin (1.0 mg/L) was found the best in producing high frequency and maximum number of shoots per explant, of tested levels in combination with 0.1 mg/L(1.5mg/L) resulted in the decrease of frequency and number of shoots per explant. Moreover no response was observed at Zeatin (2.0 mg/L). Shoot regenerated directly from tuber discs without an intervening callus phase with in four weeks of culture.

In this evaluation:

- NAA(0.2 mg/L) + BA(2.25 mg/L) as step I and GA_3(5.0 mg/L) + BA(2.25 mg/L) as step II media were found to be suitable for shoot production from step explants of cv Chandramukhi. Where as leaf explants were failed to develop shoots on step II medium.
- 2,4-D(0.2 mg/L) + BA(2.25 mg/L) as step I and GA_3(5.0mg/L)+BA(2.25 mg/L) as step II media were found to be suitable for shoot production from stem and leaf explants of cultivar Jyothi.
- Stem explants were found to be superior in response, compared to leaf.
- The leaf cultures of cv Jyothi were found to be embryogenic after culturing in step II medium comprising of Zeatin (5.0 mg/L)+BA(2.25 mg/L) from step I medium of 2,4-D(0.2 mg/L) + BA(2.25 mg/L) or IAA (0.2 mg/L) + TDZ(0.5 mg/L).
- The leaf cultures of cv Jyothi were found to be embryogenic after culturing in step II medium comprising of Zeatin (5.0 mg/L) + BA (2.25 mg/L) from step I medium of 2,4-D (0.2 mg/L) + BA (2.25mg/L) or IAA (0.2 mg/L) + TDZ (0.5 mg/L).
- One step medium encompassing IAA + Zeatin was employed and high frequency regeneration was obtained from the tuber discs of both cultivars.

Discussion

A number of different protocols have been published for the regeneration of various explants of potato (Mitten *et al*,1990). However it has frequently been shown that regeneration in a number of species including potato is genotype dependent (Wheeler *et al.*, 1985 Hulme *et al*, 1992) the published protocols for potato regeneration can be classified into one-step method described by Tavazza *et al* , (1988), keill*et al* (1989). Two-step method first introduced by webb*et al*.(1983) and modifications of the procedure were subsequently used by others (Wheeler *et al*, 1985, Yadav and sticklen,1995), three-step method described by Deblock (1988),Hulme et al(1992).

Wheeler *et al.,* (1985) reported a two step procedure for plant regeneration but they found significant differences in the response of different cultivars. In the experiments reported here shoot regeneration has been achieved using two-step procedure based on that of wheeler *et al*, (1985), where

step I and step II media alone did not yield any substantial shoot formation. Explants were initially cultured on step I medium (2,4-D/NAA+Kn/BA) where callus induction occurred. The explants then moved to step II medium (GA_3+Kn/BA) resulting in shoot production. However leaf explants of cv Chandramukhi were failed to produce shoots. The addition of Ga_3 to culture medium was required for shoot development and elongation, in its absence only nodule like structures that usually did not develop further were formed on the callus surface. The role of GA_3 appears to be promoting shoot development from previously formed shoot meristems.

One step procedure is more efficient than two step procedure in shoot regeneration from tuber disc explants. The media encompassing Zeatin + IAA promoted shoot regeneration. Shoot formation in potato tuber discs was first reported by Lam (1975). The importance of cytokinin in the promotion of shoot bad formation from in *vitro* cultured explants has been reported (Rao *et al*,1973). Zeatin was found to promote the tissue culture response of potato (cardi*et al.*, 1993). Jarret*et al*, (1981) reported that while GA_3 inhibited the initiation of shoot meristems from cultured tuber discs. However, it was absolutely necessary for shoot growth and development in two step procedure.

Acknowledgement: Author thankful to the Prof A Sadanandam of Kakatiya university, Hanamkonda, Telangana for providing facilities and guidance

TABLE-1: RESPONSE OF VARIOUS EXPLANTS OF POTATO CULTIVARS CHANDRAMUKHI (C) AND JYOTHI(J) IN TWO STEP METHOD

STEP	MEDIUM	Growth regulator(mg/L)	STEM						LEAF						TUBER DISC					
			%Explants with Callus		% Regeneration		No.Of shoots /Explants±SE		%Explants with Callus		% Regeneration		No.Of shoots /Explants±SE		%Explants with Callus		% Regeneration		No.Of shoots /Explants±SE	
			C	J	C	J	C	J	C	J	C	J	C	J	C	J	C	J	C	J
STEP-I	M1	NAA(0.2)+BA(2.25)	95	90	-	-	-	-	90	86	-	-	-	-	70	75	-	-	-	-
	M2	NAA(0.2)+Kn(2.25)	70	65	-	-	-	-	50	45	-	-	-	-	50	60	-	-	-	-
	M3	2,4-D(0.2)+BA(2.25)	80	90	-	-	-	-	80	98	-	-	-	-	80	85	-	-	-	-
	M4	2,4-D(0.2)+Kn(2.25)	75	70	-	-	-	-	60	60	-	-	-	-	65	65	-	-	-	-
STEP-II	M1R1	GA3(5.0)+BA(2.25)	75	83	23	15	64±0.7	5.6±0.6	90	85	-	13	-	2.8±0.5	65	60	6	10	1.3±0.6	2.6±0.2
	M2R2	GA3(5.0)+Kn(2.25)	70	60	-	-	-	-	48	45	-	-	-	-	70	58	-	-	-	-
	M3R1	GA3(5.0)+BA(2.25)	85	70	19	28	2.4±0.2	8.8±0.5	85	73	-	24	-	4.4±0.7	72	70	10	12	2.2±0.4	2.8±0.2
	M3R2	GA3(5.0)+Kn(2.25	75	65	-	-	-	-	60	58	-	-	-	-	68	63	-	-	-	-

Data was scored after Four weeks in Step-II medium

TABLE-2: RESPONSE OF TUBER DISC EXPLANT OF POTATO CULTIVARS CHANDRAMUKHI (C) AND JYOTHI(J) IN ONE STEP METHOD

Growth regulators(mg/L)	%Explants with Callus		% Regeneration		No.Of shoots /Explants±SE	
	C	J	C	J	C	J
IAA(0.1)+Zn(0.5)	-	-	50	70	4.4±0.4	6.1±0.1
IAA(0.1)+Zn(1.0)	-	-	60	79	6.2±0.2	10.8±0.3
IAA(0.1)+Zn(1.5)	-	-	45	56	3.1±0.1	5.3±0.2
IAA(0.1)+Zn(2.0)	-	-	-	-	-	-

Data was scored after Four weeks in medium

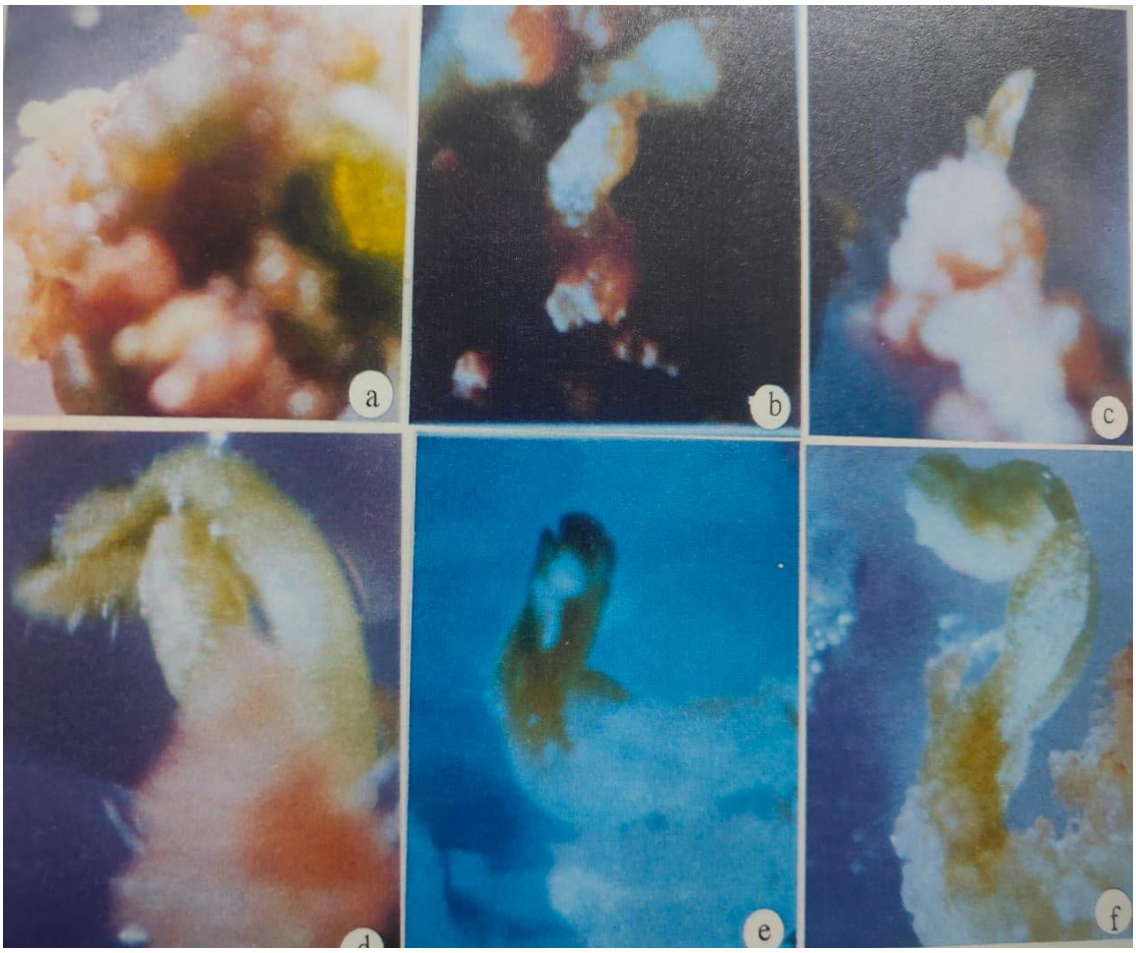

Figure-1: a - f different stages of *in vitro* development of different explants

Figure:2-In vitro rooting on basal medium

Figure 3: Acclimatized and Hardened plants

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