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REGENERATION POTENTIAL OF 6-BENZYL AMINO PURINE (BAP) INDUCED CALLI OF *SOLANUM TUBEROSUM*

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ABSTRACT

Callogenesis is a potential source to create genetic variability in micropropagated plants. The present study was carried out to evaluate the effect of BAP and 2,4-D on callus induction of potato cv. Desiree and its regeneration potential. The shoot tips of Desiree were processed under sterile conditions and cultured on full strength MS basal medium supplemented with different concentrations of BAP (0, 1, 3, 4 and 5 mg L^{-1}) and 2,4-D (0.5, 1.0 and 2.0 mg L^{-1}). The regeneration potential of calli was analyzed on the basis of days taken to induce shoots. In addition callus morphology in both growth regulators (GRs) was recorded and compared. The MS medium containing 5 mg L^{-1} BAP was found optimal for the induction of callus (4.9 days) and shoots in the shortest time (25 days).

Keywords: *Solanum tuberosum,* benzyl amino purine, desiree, callogenesis

INTRODUCTION

The main objective of the most breeding programs is to add genetic variation in the existing gene pool of the crops for sustainable increase in the yield. This is also true to potato (*Solanum tuberosum*), a tetraploid, non-inbred, staple food crop. Although sexual propagation is the ideal source to create and maintain variation, it is not an easy task for tetraploid *Solanum tuberosum.* Plant tissue culture techniques are a potential source of introducing variation (Jain, 2001; Uranbey, 2005; El-sawy *et al*., 2007) that can be utilized in potato improvement programs to overcome the barrier of sexual reproduction of tetraploid *Solanum tuberosum*. Cell and callus culture are the most promising plant tissue culture techniques that can introduce genetic variation in short time. After thorough screening for desirable traits such somaclonal variation derived variants can be maintained by asexual reproductive methods.

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For cell and callus culture plant growth regulators (PGRs) play an important role. According to Dudits *et al*. (1995) PGRs initiate signal transduction pathways that reprograme the expression of explants through the process of de-differentiation. 2,4-D is a synthetic auxin that is well known for its role in callogenesis; when calli are produced using 2,4-D, and kept on this hormone for longer periods they lose their regeneration potential and cannot show organogenesis (El-sawy *et al.,* 2007). Alternatively, BAP (cytokinin) can also be explored for its effect on callogenesis followed by organogenesis of potato. In case BAP do not affect the regenerative potential of calli produced on it; it can be used as potential agent to introduce genetic variation in potato breeding programs.

During this study potato cultivar Desiree was planted on MS solid media containing 4 different levels of benzyl amino purine (BAP: 0, 1, 3, 4 and 5 mg L^{-1}) and 3 levels of dicloro phenoxyacetic acid (2, 4-D: 0.5, 1.0 and 2.0 mg L^{-1}). The main objective of this investigation was to evaluate the regenerative potential of potato on BAP after callogenesis in comparison to 2, 4-D.

MATERIALS AND METHODS

Potato tubers chilled at 10-15^{o}C and formed the sprouts with distinct nodes and internodes in 4-weeks. Dissected segments of sprouts (nodes and internodes) were used as the experimental plant material. Sprouts were surface sterilized with 10% v/v commercial bleach (sodium hypochlorite) for 10 minutes. The surface sterilized sprouts were cut as 1.0-1.5 cm long segments having atleast one bud and planted on full strength MS media (Murashige and Skoog, 1962) containing 3% table sugar and 8g L^{-1} of agar. The pH of the media was adjusted to 5.7 after adding growth hormones. Seven different treatments were studied; four of benzyl amino purine (BAP: 0, 1, 3, 4 and 5 mg L^{-1}) and three of dicloro phenoxyacetic acid (2, 4-D: 0.5, 1.0 and 2.0 mg L^{-1}). The sterile plant material was cultured on the seven different media and cultures were kept in growth rooms at 22$\pm$2^{o}C and 16 hours photoperiod. Experiment was performed in triplate; effect of each treatment was studied using 10 explants. Data was recorded for the days taken to induce callus, its morphology and days taken to start shooting. The texture, color and growth rate of callus was observed as its morphological traits. The mean and standard deviation of the recorded parameters were calculated in the R-software (R Development Core Team, 2010). In addition callus and shoot induction frequency was calculated as described by Rafique *et al.* (2010).

RESULTS AND DISCUSSION

Here the paper reports the use of a cytokinin, BAP (5mg L^{-1}) as an inducer of callogenesis in potato cv. Desiree. Meanwhile 2, 4-D an auxin, was also used for callus production in the same cultivar of potato. The effect of two growth hormones on potato callogenesis and its regeneration potential was evaluated and compared. When BAP was added to MS media at different levels the callus induction was only observed at the concentration of 4 and 5 mg L^{-1}. The induced callus was lush green in color, hard and compact in texture and was non-

embryogenic. It took 25-35 days to start organogenesis in the form of shoots. In the remaining treatments (BAP: 1 and 3 mg L^{-1}) callus was not formed. During this study 5 mg L^{-1} of BAP took minimum time to induce calli that was less than a week (4.9 days) and it grew for 25 days followed by shoot regeneration (Figure 1; Table 1 and 2).

Table 1. Effect of benzyl amino purine on the morphology of callus and organogenesis.

Concentration BAP(mg L^{-1})	Days taken to induce callus Mean±SD	Callus morphology			Shoot induction from callus
		Growth rate	Colour	Texture	
0.0	No callus	NA	NA	NA	NA
1.0	No callus	NA	NA	NA	NA
3.0	No callus	NA	NA	NA	NA
4.0	8.1±1.1	fair	green	hard/compact	33.9±1.6
5.0	4.9±1.5	excellent	lush green	hard/compact	25.3±0.7

Table 2. Effect of 2, 4-D on the morphology of callus and organogenesis.

Concentration 2,4-D (mg L^{-1})	Days taken to induce callus Mean±SD	Callus morphology			Shoot induction from callus*
		Growth rate	Colour	Texture	
0.0	No	No	NA	NA	No
0.5	19.2±0.6	low	brown	friable	No
1.0	11.0±0.9	fair	brown	friable	No
2.0	7.9±0.4	excellent	brown	friable	No

*: data was collected for 6-weeks and during that time there was no organogenesis on calli induced by 2, 4-D.

Table 3. Effect of BAP on callus and shoot induction frequency.

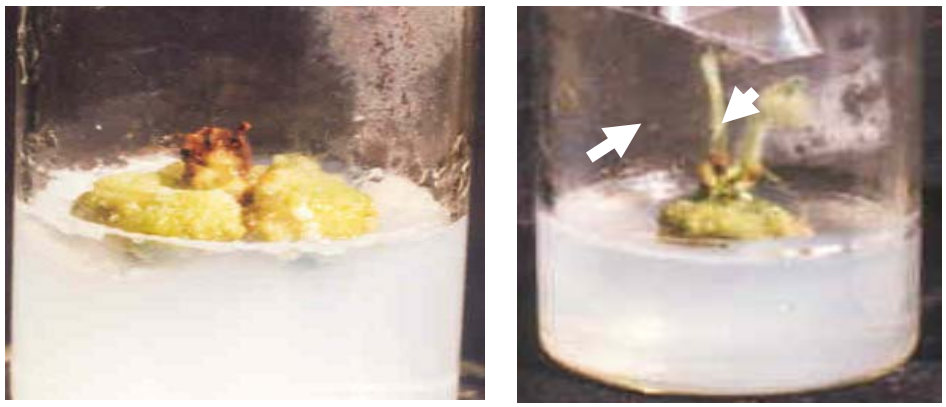
Concentration BAP(mg L^{-1})	Total number of explants inoculated	Callus induction frequency (%)	Shoot induction frequency (%)
0.0	30.0	0.0	0.0
1.0	30.0	0.0	0.0
3.0	30.0	0.0	0.0
4.0	30.0	60.7	80.1
5.0	30.0	100.0	92.7

The calli induced on 2, 4-D were brown in color with friable texture; such calli usually do not regenerate into a plantlet without hormone induction. During the six weeks of this study these calli did not show any sign of shoot induction. 2,4-D

at 2 mg L^{-1} took minimum time (8 days) to induce callus; this time is almost double as compared to BAP which took 4.9 days. In addition, frequency of callus and shoot induction was calculated for both growth hormones. The highest callus (100%) and shoot (92.7%) induction frequency was found in MS media supplemented with 5 mg L^{-1} of BAP as compared to all other treatments (Table 3 and 4).

Table 4. Effect of 2,4-D on callus and shoot induction frequency.

Concentration 2,4-D (mg L^{-1})	Total number of explants inoculated	Callus induction frequency (%)	Shoot induction frequency (%)
0.0	30.0	0.0	0.0
0.5	30.0	34.7	0.0
1.0	30.0	61.5	0.0
2.0	30.0	89.8	0.0



(A) (B)

Plate 1. Effect of benzyl amino purine (5 mg L^{-1}) on potato callusing (a) plate shows the green regenerative callus induced using BAP (5 mg L^{-1}) (b) plate shows white the shoots originated from callus.

In vitro culturing is actually a stressed environment for plantlets (Jain, 2001) that promotes somaclonal variation in long run. The type and nature of this variation can not be predicted. It could be heritable (genetic) and/ or non-heritable (epigenetic) (Jain, 2001; Aryakia and Hamidoghli, 2010). Callogenesis is a potential source to induce such kind of variation that can be used in developing new and improving existing cultivars (El-sawy *et al.*, 2007). Steward and Caplin (1951) reported first successful establishment of a potato callus culture from tuber parenchyma cells in a basal medium containing 2, 4-D and coconut water within 5 weeks. Hassan *et al.* (1990) reported the callus induction at the base of planted shoots in the presence of BAP (0.1-0.5 mg L^{-1}) and NAA (0.5-1.0 mg L^{-1}). In contrast, we observed the callus induction in MS basal media with BAP (5 mg L^{-1}) as shown in Plate (A and B).

CONCLUSION

It can be concluded from the study that the MS media with 5 mg L^{-1} BAP is a good option for studying callogenesis in potato local cultivars as callus induced in BAP have very high regeneration potential when compared to calli produced on media containing 2,4-D. Because of the polyploidy, sexual reproduction in potato is difficult demanding an alternative to introduce variation in existing potato cultivars. Callus culture is one such option. Further studies are required to generate a range of mutants through the process of callogenesis and testing those for phenotypic and genotypic variation *in vitro* and *in vivo*.

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