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Plant Regeneration from Cell Suspension Culture of Potato (*Solanum tuberosum* L.)

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Abstract: In the present study, for callus production leaf and stem segments of potato cultivar White Desiree were cultured on MS medium supplemented with 2,4-D, NAA and Kinetin (callus production medium). Calli then were transferred in the same liquid medium for cell suspension production. In the next step cell suspensions were transferred back to the callus production medium. Finally, calli derived from cell suspension were cultured on 6 different shoot initiation media (S1-S6). However, on S6 medium with combination of GA3 and BAP more than 80% of the calli produced shoot buds and shoots. Fully grown shoots then were rooted and produced whole plants. Chromosome and morphological analysis showed no somaclonal variation among regenerated plants.

Key words: Potato, plant regeneration, cell suspension

INTRODUCTION

Potato is an important commercial crop world wide. The crop is damaged by many pests and diseases making it an important candidate for genetic manipulation. Creation of novel germplasm through techniques of tissue culture and gene transfer holds great potential for improving the quality, resistance to diseases and agronomic characters of potato (Jayaree *et al*., 2001). Moreover, a narrow genetic background resulted from a long time hybridization within the species makes barriers for potato breeders to rapid breeding program to meet the market demands for new varieties.

In a long history of conventional breeding, it has been proved to be difficult to increase the frequency of desired genes and to improve the selection accuracy and efficiency for target traits since the tetrasomic inheritance of the cultivated potato (*Solanum tuberosum* L.). In recent 20 year, including plant tissue culture and molecular cloning, plant biotechnology has approached an efficient and rapid way for creating new varieties and their reproduction (Dai *et al*., 2000).

Plant cell culture is a desired system for selection of mutants with a simpler protocol than protoplast culture and a higher mutation rate than involving intact plant, so, it is important in the era of biotechnological breeding, particularly in mutants resistant to environmental stress. Until middle of 1990s, there were about 1700 varieties of 154 plant species reported to be bred through mutation strategy (Xu, 1998) and most of which were come from the induction combining together of cell and tissue culture. Using cell culture and artificial induction, potato mutants resistant to *Phytophthora infestance* and *Fusarium oxysparium* were produced (Li and Zeng, 1990). However, it is rather difficult in potatoes to gain mutants than in other crops, which due partly to its less-optimized cell suspension culture techniques and its high ploidy level that usually accompanied with a low mutation rate. Therefore, it is necessary in addition to further improve the techniques for cell suspension culture and selection of single mutant cells (Qi *et el*., 1996).

Qi *et al*. (1996) established the cell suspension culture line from the plantlet leaves of cv. Chunshu 1 and obtained vigor cells. The plants were regenerated from a further research (Qi *et al*., 2000). Feng (1990) constructed cell suspension lines of cvs. Gannongshu 1 and Russet Burbank using leaves and nodes as explants. With the same varieties, Zhang and Dai (2000) researched on the effects of status of callus, periods of subculture of the callus and different kinds of medium on the quality of suspended cells which were originally from leaves and tubers. More recently, Wang and Zhang (2002) looked at the suitable explants for cell suspension and the results showed that cotyledon, hypocotyls and stem tips seemed to be desired explants with a loose structure and rapid growth of the callus and vigor cells isolated. For this, *in vitro* regeneration system of plants via organogenesis or embryogenesis is a prerequisite. Efficient plant regeneration from a range of explants tissues, including leaf, stem and tuber, for several potato genotypes has been reported by Hulme *et al*. (1992).

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The ability of shoot regeneration and organogenesis under *in vitro* condition may vary among species, cultivars (clones) and especially the donor tissue. For example, plants have been regenerated from isolated, protoplasts of potato cultivar Delaware (Ehsanpoue and Jones, 2001). The results indicate that the organogenesis and plant regeneration in potato is highly dependent on the genotype, origin of the explants, growth regulators added to the culture medium and culture conditions. Potato cultivar White Desiree is widely grown locally in Isfahan and so far, no report has been published on plant regeneration using cell suspension culture for this cultivar. The present studies describe optimum plant regeneration system from cell suspension culture of potato cultivar White Desiree. This system can be used fro selection of desirable cell line and subsequent plant regeneration.

MATERIALS AND METHODS

In this study a commercial potato tubers cultivar White Desiree were supplied kindly by Potato Biotechnology Research Center in University of Isfahan. Healthy young plants were grown in the glasshouse under normal day light for 2-3 months. As explant, young internodes with at least one auxiliary bud were harvested from fully grown plants. Explants were then washed with tap water and transferred to 70% ethanol for 30 sec and then were surface sterilized with sodium hypochlorite (5%v/v) containing two drops of Tween 80 for 20 min followed by 3-4 washes with sterile distilled water. Explants were then cultured on MS medium (Murashige and Skoog, 1962) supplemented with sucrose (30 g L^{-1}), agar (8 g L^{-1}). For callus induction, leaf and stem segments of *in vitro* grown plants were transferred to callus proliferation, medium described by Ehsanpour and Amini (2003). Cultures were maintained in the culture room with 16/8 light-dark photoperiod at 25±2°C. The experiments were carried out with 10 replications and 4 explants in each replication. For cell suspension production, calli then were transferred to the liquid callus production medium. The pH of media was adjusted to 5.8 and then were autoclaved for 20 min at 121°C. In the next step, after two subcultures, cell suspensions were transferred to the plant regeneration medium supplemented with different growth regulators according to Table 1.

Chromosome counting of regenerated plants derived from cell suspension cultures were propagated on hormone free MS medium. Root-tip squash was carried out as described by Karp (1991) from regenerated plants.

Table 1: Combination of growth regulators in MS medium for plant regeneration

Media name	Hormone combination
S1	5 mg L^{-1} BAP
S2	5 mg L^{-1} BAP+0.1 mg L^{-1} IBA
S3	2.5 mg L^{-1} BAP+0.1 mg L^{-1} NAA
S4	2 mg L^{-1} BAP+2.5 mg L^{-1} NAA
S5	2.5 mg L^{-1} BAP+0.18 mg L^{-1} NAA
S6	2.5 mg L^{-1} BAP+5 mg L^{-1} GA3
S7	2 g L^{-1} NAA+2 mg L^{-1} Kinetin+2 mg L^{-1} 2,4-D

RESULTS AND DISCUSSION

Leaf and stem segments of potato cultivar White Desiree were produced friable callus on callus production medium (S7). However, leaf segments produced more callus than stem segments. Cell suspension was also produced from callus on the same liquid S7 medium. Growth curve of cell suspension showed that a lag phase with approx 2-3 days, then cells moved to exponential phase. The interval time between subcultures of cell suspension was 10-11 days. Well grown cell suspension after 2 subcultures were then transferred to regenerated media (S1-S6). On S1 and S2 medium after 3 weeks no shoot bud regeneration was observed and calli became brown. When calli were cultured on S3 medium, after 2 weeks they turned green but after 6-7 weeks no shoot bud differentiation was observed. On S4 and S5 medium we observed similar results as S3 but the calli showed higher growth rate (data are not shown). However, in these media no regeneration was observed even after 8 weeks. When calli were transferred on S6 medium they grow better than other media and 80% of them gradually turned dark green and produced granular callus after 8 weeks post culture in this medium on the surface of the calli shoot buds were initiated. Figure 1 shows different stage of plant regeneration from cell suspension. When regenerated plants were analyzed for chromosome counting, none of them showed chromosome variation. They have also showed normal morphology as parent plants. After acclimatization some of the regenerated plants were transferred to the pot and they grow very well.

Cell suspension cultures are rapidly dividing homogenous suspensions of cells grown in liquid nutrient media. Cell suspensions are used for generating large amounts of cells for quantitative or qualitative analysis of growth responses and metabolism of novel chemicals, as well as for studies of cell cycle and plant regeneration system under standard conditions. In addition, cell suspensions serve as an ideal material for the isolation of protoplasts used intransient gene expression assays and *Agrobacterium*-mediated transformation (Raffata *et al*., 1988). The establishment of suspension cultures of *Arabidopsis thaliana* cells derived from leaf and hypocotyls calli has been reported earlier (Karp, 1991).

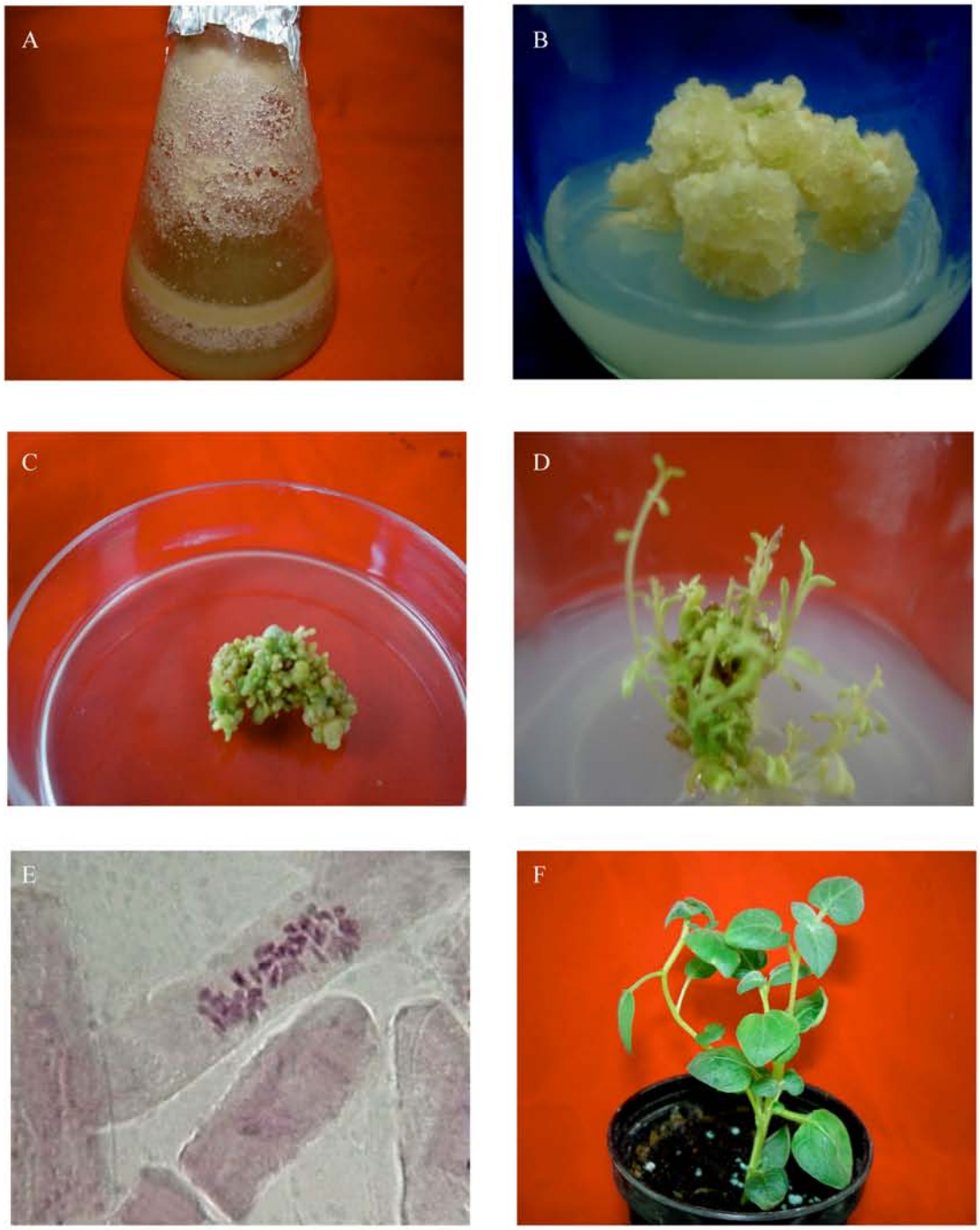

Fig. 1: Different steps of plant regeneration from cell suspension of potato cultivar White Desiree in S6 medium. A: Cell suspension, (B) Callus proliferation derived from cell suspension, (C) Shoot bud initiation, (D) Plant regeneration, (E) Chromosome analysis from regenerated plants and (F) Fully grown regenerated plant in the pot

When carrying out a plant cell culture, there are three important things that must be considered. First the plant part of interest must be isolated from the intact plant. Next the appropriate environment to promote optimal growth must be discovered and applied. This may vary depending on the cells of interest. Finally, these procedures must be carried out in a sterile environment to prevent growth of microorganisms. These conditions have already been applied for callus and cell suspension culture of several different plants. For example callus and cell suspension has successfully been produced in *Medicago sativa* L. using combination of three hormones (Kinetin, NAA, 2,4-D) (Amini and Ehsanpour, 2004). In the same medium (S7) combination of plant growth regulators associated with 1 g L^{-1} yeast extract promoted callus formation from cell suspension of potato cultivar White Desiree. Lindeque *et al.* (1991) have also used similar combination of plant growth regulators for callus formation of potato cultivar BP1. It seems S7 medium can be recommended for callus and cell suspension culture of potato cultivar White Desiree. Anjum and Hakoom (2004) and Anjum (2001) reported that production of cell suspension from explants is highly genotype dependent. They have also reported that combination of plant hormones in the medium is very important too. In the present investigation, when calli derived from cell suspension of potato White Desiree was transferred to S1 to S5 medium despite of presence of auxin and cytokinin in the culture medium no differentiation of shoot bud was observed. In S2, S3, S4 and S5 medium only calli became green. This observation indicating that chloroplast differentiation occurred on the surface of the cells in the presence of cytokinin in the medium. It might be as a result of the interaction of these hormones or interaction of endogenous with exogenous hormones of the cells. In these media no shoot buds were initiated. However, when callus-derived cell suspension was cultured on S6 medium containing BAP and GA3 at the beginning, it turned green and gradually produced green granular callus and finally shoot buds were initiated on the surface of the calli. In this medium it seems the combination and possibly synergism between GA3 and BAP promoted shoot bud differentiation. Regenerated plants did not show any chromosome variation or morphological changes. Patricia *et al.* (2004) detected some somaclonal variation in callus culture of potato cultivars. However, the culture condition for plant regeneration from cell suspension of potato cultivar White Desiree used in this study was optimized. This procedure can be used for regeneration of plant from selected of desirable cell lines in the future.

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