EFFECT OF AUXINE AND CYTOKININE ON CALLUS INDUCTION IN POTATO (SOLANUM TUBEROSUM L.) EXPLANTS

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Abstract: *Effect of growth regulators for callus induction of potato (Solanum tuberosum L.) genotypes (Christian and Roclas) was studied. The best callus induction was for MS medium supplemented with 1mg/L 2,4 D and 0,5 mg/l BAP for both the genotypes.*

Keywords: somaclonal variations, callus, induction, medium, 2,4-D, BAP

INTRODUCTION

Potato *(Solanum tuberosum)* is one of the most economically important annual vegetable crop of *Solanaceae* family. Cultivated potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world (Solmon-Blackburn and Baker, 2001).

Plant regeneration became a useful technique and is applied to solve the problems of many agriculturas crops. 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). Plant biotechnology has approached an efficient and rapid way for creating new varieties and their reproduction (Dai *et al.*, 2000).

For many cultures, biotechnology is a major tool in breeding. The term „somaclonal variation" is used with reference to the observed variation at regenerated plants from the tissue and culture cells. It is known that the somaclonal variations can take place at isolated protoplasts, calli. Many authors (Choi *et al.*, 1997; Patnaik *et al.*, 1997; Redway *et al.*, 1990) reported on regeneration of plants from embryogenic suspension culture.

The cause of somaclonal variation is assigned to the number modifications and the chromosomes structure. The cytological heterogeneity of the cultures is on principle the result of the next factors:

1. The mosaics chromosomal expression or genetic disorder of the initial explants cells;
2. Irregularity due to the medium conditions.

On tissue culture, this type of modifications, were in general removed, because the main objective was the genetic stability of cultures increase. The researchers have shown that

in cells or tissue cultures can occur genetic modifications (polyploidy, aneuploidy, deletions, translocations, shifting).

Genetic variability is present in cells culture and the obtained plants from these cells are presenting somaclonal variation. In general, the term "somaclonal variation" is used for genetic variability present in cells/plants which are obtained from cells cultivated *"in vitro"*. Somaclonal variation may be observed at potato, sugar beet, tomatoes. In addition of growth regulators to culture medium is known to have influence on the frequency of the karyotype alterations in cell cultures. Frequently, the auxin 2.4-D is considered to be responsible for the chromosome variation (Singh *et al.*, 1975). Callus culture is a good tool on research.

MATERIAL AND METHOD

Christian and Roclas genotypes are from National Institute of Research and Development for Potato and Sugar Beet, Brasov. The explants were cultivated on MS medium supplemented with different growth regulators by different concentrations. The cultures were incubated in growing rooms at 24^0C with a photoperiode of 16 h dark and 8 h light There have been used two Romanian genotypes, with resistance at virosis.

Callus was developed on Murashige-Skoog (1962) medium enriched with vitamines and 1 mg/l BAP and 0,5mg/l 2.4 D for medium 1 (M1); 2mg/l 2,4 D and 0,25 mg/l BAP medium 2 (M2); 2mg/l 2,4D and 0,5 mg/l BAP medium 3 (M3). 2,4 – D in different concentrations and in combination with cytokines inhibits elongation and lateral ramification of explants roads from all tested lines. pH of the culture medium was adjusted to 5,6-5,8 with 1N NaOH before autoclaving at 1.1 atm for 15 minutes.

Table 1.

Medium for callus induction

Mediu 1	Mediu 2	Mediu 3
MS medium enriched with vitamins Sucrose (20 gl^{-1}) 2,4-D (1 mgl^{-1}) BAP (0.5 mgl^{-1}) Phyto - Agar (8 gl^{-1}) pH 5.8	MS medium enriched with vitamins Sucrose (20 gl^{-1}) 2,4-D (2 mgl^{-1}) BAP (0.25 mgl^{-1}) Phyto - Agar (8 gl^{-1}) pH 5.8	MS medium enriched with vitamins Sucrose (20 gl^{-1}) 2,4-D (2 mgl^{-1}) BAP (0.5 mgl^{-1}) Phyto - Agar (8 gl^{-1}) pH 5.8

RESULTS AND DISCUSSION

For callus induction, the most raised value was observed for Christian genotype and for medium 1. Thus for Christian genotype on medium 1 was induced the callus with a percent of 40 %, and for Roclas genotype callus was induced for Roclas genotype on medium 1, with a percent of 40 %. The most raised induction percent has decreased once with the concentration increasing of 2, 4 – D.

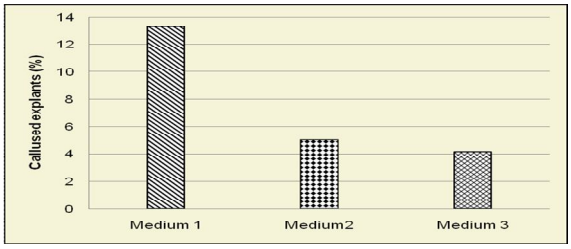

Figure 1. The influence of culture medium for callus induction

Figure 1 presents the influence of medium 1 for development of callused explents in a higher percent (13.33%), by comparison with medium 2 (5%) and medium 3(4.16%). 2,4 – D used in a lowest quantity has formed a highest percent for callused explants.

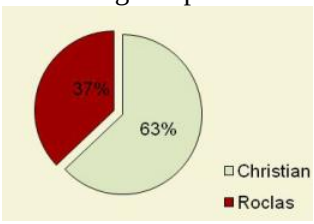

Figure 2. The genotype influence for callus induction

Christian and Roclas genotypes, with virosis resistance, had a different percent for callus induction. For Christian variety was obtained a higher percent of 63%, and for Roclas a percent of 37 %.

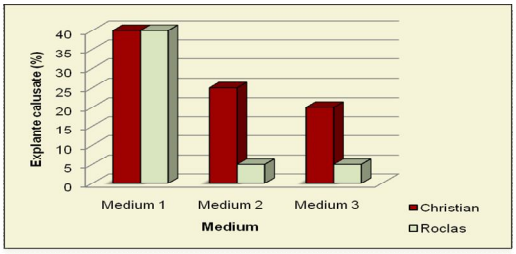

Figure 3. The growing regulators influence on callusing process.

From figure 3, we may observe that for both Christian and Roclas varieties, the medium 1 leads to the callus induction in equal percent of 40%, the medium 2 leads to a higher induction for the Christian genotype (25%), while for Roclas genotype the percent is about 5%; medium 3 induced callus in a percent of 20% for Christian genotype, and for Roclas genotype the percent is 5%.

Figure 4. Christian variety planted on greenhouse

Figure 5. Minutubers of Christian variety

CONCLUSIONS

- the medium which induced callus in the highest percent was medium 1, containing 2,4-D (1 mgl^{-1}), BAP (0.5 mgl^{-1});
- the genotype which had the highest influence over callus induction was Christian.
- the factors that influenced somaclonal variations are: genotype, explant sourse, *in vitro* period and cultivation conditions.

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