Using of unconventional methods for obtaining somaclonal variations, having as goal making of new potato varieties with resistance at diseases and pests

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Abstract

The most common factors which affect somaclonal variations are genotype explant source, in vitro period and cultivation conditions in which the culture is established. In this research, calli were induced using leaf disk and leafstalks explants of potato cultivars resistant at potato blight and viroses. Calli were induced with different growing regulators (with different concentrations). Having as objective obtaining of lines with resistance at diseases and pest are necessary: callus induction, callus growing, callus multiplication by subculture, controlling organogenesis processes. Plantlets regeneration from callus is influenced by genotype ad medium variant.

Keywords: callus induction, callus growing, callus multiplication

Introduction

Potato (*Solanum tuberosum* L.) is one of the most cultivated vegetable in Romania and the world‘s fourth most important crop. Potato is a tetraploide species and using of botanical seeds in commercial cultivation is impossible, owing to low germinability and large variability in segregant generations. Synthetic seed is an alternative in solving of this problem. Original definition of synthetic seed is “a single encapsulated somatic embryo”. Synthetic seed is a clonal product, which can be handle and used as a real seed in transport, storage, “ex vitro” and also for “in vitro” planting. The most accepted definition of synthetic seed is: “artificially encapsulated somatic embryos, sprouts and other tissues that can be used *in vitro* or *ex vitro* plantings.

Isolated cell tissue cultures (even from one cell) may present variation after repeated subculturing. Distinct lines may be selected with their particular morphology. This variation (somaclonal variation) may be send to regenerated plants from culture tissue. This is an additional source of a new variation for breeders. Somatic embriogenesis produce somatic embryos and can be used for the production of synthetic seed. Somaclonal variation may be associated with somatic embryogenesis [1].

Somaclonal variation is a common phenomenon on cell plants cultures, including all variation types among plants and cell and derives from all kinds of tissue cultures.

Somaclonal variation is also called induced variation of tissue or cultures. Because the production goal of synthetic seeds is to obtain clonal identity, to control somaclonal variation became a challenge.

Many causes have been identified or proposed for each type of variation; these however, may very from species to species and determining the genetic nature of the observed variation is difficult [1]

These variation causes include: changes in the structure and/or chromosome number, noticeable point mutations, changes in the expression of a gene as a result of structural changes in the chromosome or activation of transposable elements, chromatin loss, DNA amplification, somatic crossing over.

Plant genotype may have important effects on somaclone regeneration and frequency. These effects are very evident on potatoes: differences are observed in the number of regenerated plants of distinct cultivars grown under identical conditions.

Explant source is considered the most frequent critical variable for somaclonal variation. Procedure selection of regeneration depends by different type of explants. Somaclonal variation is influence by genotype, tissue source, and procedure and culture time realization. In present, for obtain somaclonal variation is used a certain number of techniques and tissue cultures.

Among tissue culture techniques, callogenesis becomes an objective aimed at initiating callus cultures or inducing somaclonal variability. At this level the genome altering may involve numerical aberrations or chromosomes rearrangements as well as genes mutations. The genetic variability is generated through cell and tissue culture and the regenerated plants and their descendants inherit more, this variability. After being induced, the callus must have the capacity to generate a big number of plantules [3].

Somaclonal variability is also important for obtain parental forms with special resistance features which may be introduced in a breeding scheme through classical or somatic hybridization [2].

Inducing *in vitro* culture cycle of a callus phase increases significant somaclones frequency. Relating to new solutions it can be specified that medium with a composition on which is predominant auxine (in special NAA), citochinine (zeatine) with different concentration may be a cause of abnormal appearance to potato plants. At potato, explants origin represents other source of somaclonal variation. Existing of "medium micro conditions" (osmotic pressure, light, different substance-natural mutagen) may induces hereditary changes in cells.

Starting with necessity of having varieties resistant to diseases and pests, our researches will be directed in trying of obtain resistant lines using as method plant neogenesis, and having as staring point callus.

This suppose: callus induction, callus growing, callus multiplication by subculture, controlling organogenesis processes [3].

Material and methods

In National Institute of Research and Development for Potato and Sugar Beet (INCDCDZ) Brasov, our research has been directed to plants' regeneration from *in vitro* tissue culture and analysis of factors which compete to their realization:

- genotype
- explant source
- phytohormonale composition
- cultures conditions

- medium
- sterilization of biological material

Sterilization of biological material, harvested from the green house, was accomplished as follows:

- washing on water jet: 5-7 minutes;
- sterilization in mercuric chloride (1%) for 10 minutes;
- washing of biological material in distilled water, for three times, in three waters;
- drying of vegetal material on sterile filter paper [3]
- Explants was represented by a leaf disk (1 cm^2) and leafstalks segments,

(1,5-2 cm length), which was drawn from 4 potato genotypes (table 1). After accomplishing the disinfecting, biological material was transferred in Erlenmeyer flasks, in position with normal polarity, containing a base medium: Murashige-Skoog (1962) with vitamins, 30 g/l sugar, 8 g/l agar and different growth regulators, of different concentrations (table 2).

Hormonal composition of culture medium is presented in table 2, function of different growing regulators and their concentration. pH was 5,8, and medium was sterilized by autoclaving, at 121^0C, for 20 minutes, at 1,25 atmospheres. Explants drawing were accomplished with laminar flow.

20 explants/genotype were inoculated for each medium variant, and after inoculation, cultures were transferred in growing room, in dark conditions for first 2 weeks, with temperature of 23^0C. After this period of time, cultures were maintained at 24^0C, with a photoperiod of 16 hours of light and 8 hours of darkness.

Table 1. Potato genotype

Genotype	Type of explant
R0 99 SASA (Belgium)	Leaf disk, Leafstalks segments
R1 99 SASA (Belgium)	Leaf disk, Leafstalks segments
CHRISTIAN Cl.1 (Lăzarea)	Leaf disk, Leafstalks segments
ROCLAS Cl.2 (Lăzarea)	Leaf disk, Leafstalks segments

It was used varieties resistant to viruses (Christian and Roclas) and lines resistant to potato blight R0 99 SASA and R1 99 SASA. Our objective was to obtain somaclonal variation with resistance to potato blight and viruses.

Table 2. Composition of culture medium from point of view hormonal for calus induction from different potato explants

Medium variant	Used explant type	Growth regulators (mg/l)						
		BA	NAA	AG_3	Kin	Zeatine	IAA	2,4-D
C_1	Disk of foliar limb	0,5	2,0	0,1	-	-	-	-
C_2		-	1,0	-	0,5	-	-	-
C_3		-	-	-	-	0,2	2,0	-
C_4		0,5	-	-	-	-	0,5	3,0
C_5		6,0	2,0	-	-	-	-	2,0
C_6		4,0	2,2	-	-	-	-	4,0
C_7		6,0	2,2	-	-	-	-	4,0
C_8		4,0	2,0	-	-	-	-	2
C_9	Leaf stalk segments	4,0	2,2	-	-	-	-	4,0
C_0		-	-	-	-	-	-	-

Results and discussion

From existing material in greenhouse, in plant vegetation phase, it was manual harvested biological material from aerial part of plants (from leaves and steams). This was tested, as we written in chapter materials and methods. For the first phase, our research was directed to induce callus from inoculum and in the second phase (fig. 1 and 2), it was tried a regeneration of plants from obtained callus. We had in view genotype influence on callus induction and influence of different medium variants used.

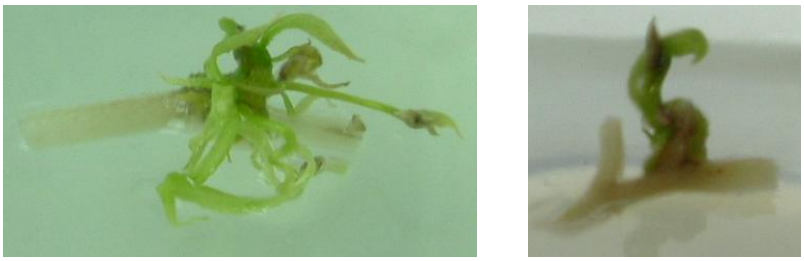

Figure 1. Callus induction from leafstalks segments

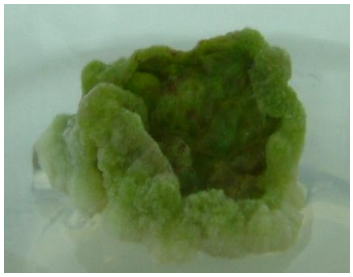

Figure 2. Callus induction from leaf disk

It was observed positive answer of both factors for callus proliferation.

Callogenesis was developed after 8 weeks from inoculation, and regeneration of plantlets from callus, took place at 12-14 weeks (since explants inoculation).

Figure 3. Plantlets regeneration from callus

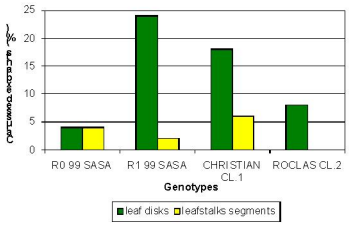

Figure 4. Genotype influence in callogenesis process

Genotype influence on callogenesis process shows R1 99 SASA line (Belgium) (fig.4), as important genetic source. The productivity of this line is big in callus proliferation (24%) from explants of leaf disk and is lower (2%) for explants from leafstalks. Line R1 99 SASA has resistance to potato blight and we can note that proliferate in a big percent (24%). Also line R1 99 SASA has resistance to potato blight, but it proliferates in a smaller percent.

Christian variety was distinguished in callus production for leaf disk (18%) and for leafstalks segments (6%). We can observe importance of this early variety for establishing genetic source in callogenesis process. We want to mention that Christian is resistant to viruses, and so it will be posile to create somaclonal variation with resistance to viruses.

Even if the line R0 99 SASA (Belgia) presented in our research a small rate for callus proliferation (4%), this can be an important material in callogenesis process, because the callus came from leaf disks explants and leafstalks explants.

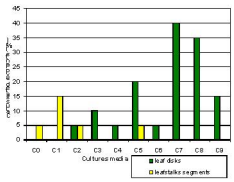

Figure 5. Growth regulators influence over-callusing process

Early variety Roclas, it is the only variety, which in callogenesis process proliferated callus from leaf disc, with an intensity of 8%.Our goal is to obtain new varieties with resistance to diseases, pests.

We can observe (fig.5) about the influence of growing regulators in callusing process, the presence of callusing process on 8 cultures medium variants (for leaf disk) and for 4

culture medium variants (for leafstalks segments). The biggest callusing percent (40%) was observed on medium culture variant C7, for explants with leaf disc (containing 6 mg/l BA, 2,2 mg/, NAA, 4 mg/l 2,4 –D). This was followed by culture medium variant C8 (35%), containing 4 mg/l BA, 2,20mg/, NAA şi 2 mg/l 2,4 –D. It could remark rising of callusing percent once with rising of BA content at 6 mg/l and NAA rising until 2,2 mg/l. After this, we can see a reduction of callus percent at reduction of content in BA and NAA. The values achieved was comprise between 5% and 40%, for leaf disks explants.

About the maximum intensity of callusing proces on explants from leafstalks segments, we note that this took place on culture medium variant C1 (15%-containing 0,5 mg /l BA, 2,0 mg/l NAA and 0,1 mg/l AG_3), followed by medium variant C0 (testefier medium), C2 (5%-containing 1 mg/l NAA + 0,5 mg/l kinetine), C5 (5%, with a content of 6 mg/l BA, 2,0 mg /l NAA, 2,0 mg/l 2,4 – D).

From complex analyse of growing regulators influence over explants of leaf disk and leafstalks segments, it can be establish:

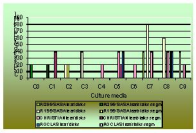

Figure 6. Growth regulators influence

From fig. 5 we may conclude:

1. All culture media induced callus, to all genotypes, with an intensity between 20-80%.
2. On 7 medium variants used, callusing process was at 20%, on 6 medium variants, callusing process was 40%, for one medium variant, the intensity was 60%, and for other one 80%.
3. On 2 medium variants, callusing process was find for leaf disk and leafstalks segments for 2 genotipes.
4. On 4 medium variants callus proliferation took place from leafstalks segments
5. On 8 medium variants, callus proliferation took place from leaf disk
6. On 3 medium variants, callusing process was present at 2 genotypes
7. On 2 medium variants, callusing proces was present at 3 tested genotypes
8. Line R0 99 SASA proliferated callus on 3 medium variants; variety Christian (resistent at viroses) proliferated callus on 7 medium variants, line R1 99 SASA (resistent al potato blight) proliferated callus on 5 medium variants; variety Roclas proliferated callus on 2 medium variants
9. Line R1 99 SASA had the biggest callusing percent on 2 medium variants (80% on C7 medium variant and 60% on C8 culture medium variant.
10. Genotypes R1 99 SASA and Christian induced callus on 2 medium variants for leaf disk and also for leafstalks segments.

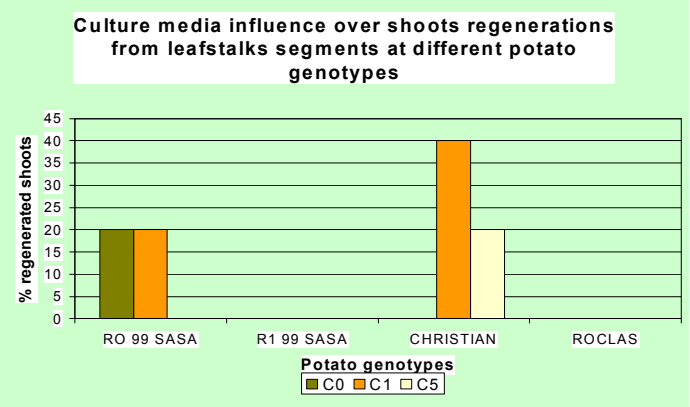

Figure 7. Culture media influence over shoots regenerations from leafstalks segments at different potato genotypes

Christian (resistant to viruses) (fig.6) and R0 99 SASA (resistant to potato blight) regenerated plantlets on three medium variants: C0, C1 and C5.

Christian variety presented the best behavior for bud regeneration, in a percent of 40%, from leafstalks segments produced callus. Regenerated plantlets developed in test tubes with MS culture medium, enriched with vitamins and 0,5 mg/l NAA, 20 g/l sugar, 8 g/l agar, with a 5,8 pH for medium variants (BA 0,5 mg/l, NAA 2,0 mg/l, AG_3 0,1 mg/l –C1; BA 6,0 mg/l, ANA 2,0 mg/l , 2,4 – D 2,0 mg/l –C5).

Line R0 99 SASA (resistant at potato blight) generated buds, even if in a small percentage than Christian variety (20%) on testefier medium variants C_0 (wit MS medium enriched with vitamins, 0,5 mg/l NAA, 20 g/l sugar, 8 g/l agar, with a pH 5,8) and C1 containing BA 0,5 mg/l, NAA 2,0 mg/l, AG_3 0,1 mg/l.

Conclusions

For potato callus induction, is necessary the presence in culture medium of growing regulators – auxine and citochinine in a equilibrate proportion from concentration point of view.

Callus induction takes place at inoculum coming from leafstalk segments or leaf disc, with a biggest percent proliferation for inoculum coming from leaf disk.

Plantlets regeneration from callus is influenced by genotype ad medium variant.

It is possible to create new varieties with resistance at potato blight and viruses. It is necessary to continue the research about callus induction and plants' regeneration, by testing much more varieties and using other kind of different medium by their composition and their concentration.

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