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EXPERIMENTAL RESULTS ABOUT POTATO CALLUS INDUCTION

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Abstract

The callus is an unorganized mass of parenchymal proliferate cells that through cultivation, forming groups of meristematic cells, elements of leading system, pigmented cells, etc.. Using other explants than meristem, for regeneration neoplantlets require mandatory completion of a stage of callus culture. To obtain callus is need an agarose to support the cellular mass in growth. In 2012, at Brasov was fitted trifactorial experience, in which two clones of Christian variety were studied, 6 media for callus induction and 2 explants sources consisting of leaf disc and petiole segment. The following results were obtained: medium, explant source (foliar disc, petiole segment) and variety have different influences on callus proliferation. The callus explants responded better to the foliar disk (72.5%) than petiole segments (40%). Media containing 3 mg / l 2,4-D and 3 mg / l BAP x 3 mg / l 2,4-D favored callus induction rate of 90%. Differences obtained by using BAP citokinine are statistically assured, very significant, negative compared to 2,4-D auxine (2mg/l- concentration regarded as control), by -3.5 explants / that induced callus. The callus which was obtained will be used for plantlets regeneration and to identify any somaclonal variation.

Keywords: *leaf disc, petiole segment, callus.*

INTRODUCTION

Callus is an unorganized mass by proliferate parenchymal cells that through cultivation, form centre of meristematic cells, elements of system leading, pigmented cells (Rodica Pop, 2008).

Callus is a particular formation consisting by an undetermined mass of cell holding a uniform histological structure. Usually, when it is young, undifferentiated cells possess, actively dividing.

Friable as possible callus induction its principal purpose when aiming is initiation of cell culture and from this a culture of protoplast.

Callus growth is considered to be indefinite, because it can be multiplied and grown indefinitely when periodically is done subculturing, respectively fragmentation of it.

Callus is often associated with somaclonal variability, variations derived from callus being called caliclones (Skirvin et al., 1976, quoted by Skirvin, 1994).

At the beginning cultivars obtained „in vitro", regardless of species, were called caliclones. Variation is associated, in caliclones case with direct regeneration from callus or cell suspension and not with micropropagation or meristem culture (Karp, 1989).

Somaclonal variation, a common phenomenon in tissue culture includes all variations and derived from all tissue culture (Skirvin et al., 1993). Somaclonal variation is also called tissue variation or culture-induced (Kaeppler, et al., 2000).

Somaclonal variation control represents a challenge (Amirato, 1991). Variations had as result: changes in structure and / or number of chromosomes, a gene changes as a result of structural change in chromosome (Rao *et al*., 1992; Kaeppler, *et al*., 2000). Evans & Sharp (1988) reported four critical variables for somaclonal variation: genotype, explant origin, period of cultivation and culture conditions.

Plant genotype can have significant effects on somaclones regeneration. These effects are very obvious for potatoes: differences are observed in the number of plants regenerated from cultivars grown under identical conditions (Gunn & Shepard, 1981). Explant source is considered most essential variable in somaclonal variation.

Because explants may present different stages of regeneration, selection procedures may differs between different types of explants (De Jong & Custers, 1986).

MATERIALS AND METHODS

In laboratory Plant Tissue Cultures of NIRDPSB Brasov was assembled an experience that followed the influence of growth regulators and explants of Christian variety (two clones).

The experience was by type 2x2x6, made by combining three experimental factors, placed by Latin rectangle method, the number of studied variants was 24, set in three repetitions, and on each experimental variant were inoculated 5 explants.

- Experimental factor A-clone with two graduations:
- a_1- Christian Cl2
- a_2- Christian CHR 01 ROU
- Experimental factor B - explants, with two graduations:
- b_1- leaf discs;
- b_2- petiole segments.
- Experimental factor C - nutrition environment, with six graduations:
- c_1 - MS medium and citokinine BAP (2 mg/l);
- c_2 - MS medium and citokinine BAP (3 mg/l);
- c_3 - MS medium and auxine 2,4-D (2 mg/l);
- c_4 - MS medium and auxine 2,4-D (3 mg/l);
- c_5 - MS medium and BAP x 2,4-D (2 mg/l x 2 mg/l);
- c_6 - MS medium and BAP x 2,4-D (3 mg/l x 3 mg/l).

The proposed objective of this research is to determine the influence of hormonal composition of the culture medium on callus induction of different types of potato explants grown *in vitro*.

Experience in which six medium were used to induce callus was organized in the laboratory on culture vessels, aiming on callus process from leaf discs and petiole segments.

Experimental conditions:

The experience was mounted in the laboratory using conditions required by „in vitro" technology; experimental conditions were those specific to growth chamber of plantlets, provided in the working protocol, sterilization of culture vessels was performed in a drying chamber at 180^0C and culture media was sterilized by autoclaving at 121^0C for 20 minutes at pressure of 1.25 atmospheres.

Cultures were transferred to growth chamber under conditions of darkness; after crossing this period light regime is 4000 lux, with a period of 16 hours light and 8 hours dark at a temperature of 20^0C.

Observations concerning callus growth were made weekly. Also, all sampling operations, inoculation subculturing were performed under sterile conditions in a laminar flow hood.

The biological material used in the production experiences of clonal variability in laboratory conditions, consisted of two clones of the same variety (Christian) from NIRDPSB Brasov. All analyzes were performed in the laboratory plant tissue cultures from NIRDPSB Brasov.

Other materials used in the experiments:

-to initiate callus culture, the biological material used was represented by two types of explants (leaf discs of 1 cm^2 and petiole segments of 1.5-2 cm).

Explant source:

When intended to make somaclonal variability for a new species or cultivar is better to use several types of explants and compare descendants from each. Not to all types of explants are supposed the same capacity for manifestation of variation. In general the variation is more difficult to observe in preformed shoots (derived from axillary buds, shoot tips and meristems), unless meristem explants were not preformed, such as leaves, roots or protoplasts (Rodica Pop, 2008).

Regenerates obtained from organized explants with preformed meristems are genetically stable. For many plant species, cell differentiation is accompanied by qualitative and quantitative changes of genomic DNA. Therefore, when used as explants for callus obtained segments from leaf or root, cells begin to divide (Rodica Pop, 2008).

The culture media used:

To obtain callus is need by a medium with agarose to support cell mass in growth. Periodically, callus must be fragmented and subcultivated in order not enter into senescence. Callus formation can be induced and it proliferates, especially on culture media with 2,4-D. Also, other growth regulators: auxine or citokinine who are in high concentrations in the culture medium can generate callus.

Vegetal explants can be kept alive by detaching their mother's body by growing and raising on aseptic media with a complex chemical composition. The success *in vitro* cultivation of

vegetal explants depends very much on achievement of nutritional composition that best suits with vital requirements of cultured tissues. One of the most common culture media used Murashige-Skoog is (MS) (Rodica Pop, 2008).
In our experience, basic medium used was Murashige-Skoog (1962) supplemented with different growth regulators. As a source of carbohydrates sucrose was used at a concentration of 2% and that phytoagar gelling agent was used in concentration of 0.9%.
Growth regulators used:
Growth regulators are organic compounds other than nutritive substances that in small amounts stimulate, inhibit or modify physiological processes in plants (Rodica Pop, 2008). Growth regulators, particularly 2,4-D (dichlorophenoxyacetic acid 2.4) and benzylaminopurine (BAP), are involved in inducing variability but their direct relation with this phenomenon is still discussed.
Auxins
Auxins are natural compounds that, in small doses, directly or indirectly, can stimulate the growth and development of plants, or forming of vegetative organs and regenerative.
Between auxines, 2,4-dichlorophenoxyacetic acid (2,4-D), in concentrations ranging from 0.5 to 2.0 mg / l, has proven very efficient in callus induction and maintenance of various types of somatic tissues (Tang şi Mullins, 1990 ; Castillo şi colab., 1998, quotation by Rodica Pop, 2008).
Citokinins
Citochinins are substituted with a purine nucleus. They stimulate cell division and have important role in stimulating vegetal cells mature unmeristematic (Cachiţă, 2000).
Caulogenesis is stimulated by the presence in culture substrate of a particular ratio between auxine and citokinine.
Specific technology applied in experiments
From existing material from greenhouse during plant vegetation, aerial plant samples (leaves and stems) were harvested manually which constituted the biological material under experimentation. It was followed the influence of genotype on callus induction and influence of different medium variables used
It was respected the following stages: vegetative material sterilization it requires a few minutes washing, sterilization with sodium hiploclorit - 10 minutes, after which explants are rinsed with doubly distilled water; culture media preparing; sterilization of vessels and growing medium; preparing of plant material for explants sizing, inoculation, incubation either at dark or light, usually at 24^0C, regular observation and passing callus fragments (about 4-6 weeks) in fresh medium.
Explants were represented by foliar disc (1 cm^2) and petiole segments of 1.5 - 2 cm in length (Fig. 1), taken from 4 potato genotypes. After performing disinfection (Figure2), biological material was transferred to Erlenmeyer flask (Fig. 3) on a basic medium: Murashige-Skoog (1962) enriched with vitamins, 20 g / l sucrose, 9 g / l agar and different growth regulators, different concentrations.

Figure 1. Fragment of leaf and petiole segment

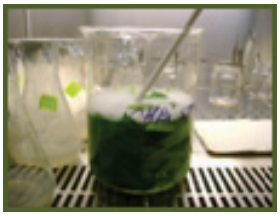

Figure 2. Sterilization of explants

Figure 3. Inoculated explants for callus induction

After incubation of leaf and petiole segment in the dark for two weeks, were registered some features. These include:- Type of callus and callus color:
After callus was initiated (Fig. 4), at about 4-6 weeks, from each recipient was fragmented into three segments and subcultivat on the same type of medium (Fig. 5). Callus was then exposed to UV radiation (Fig. 6), operation repeated three times at an interval of one week.

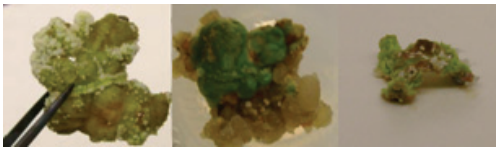

Figure 4. Initiated callus

Figure 5. Recipents with callus fragmented

Figure 6. The exposure callus on UV radiation

RESULTS AND DISCUSSIONS

Analyzing Table 1, we see that when using citokinine BAP, callus induction did not take place. In the case of auxine 2,4-D callus was brittle, with a brownish color, and if the used medium contained both growth regulators, callus was hard, its color being green.

Clones explants of Christian variety were cultured on MS medium containing different concentrations of 2,4-D, BAP or in combination. Explants which produced callus were analyzed at 6 weeks, and the results show that there is a wide variation in the percentage of explants which initiated callus, callus texture, color callus, it depends on the culture.

It was found a positive response of leaf fragments and auxine 2,4-D and its combination with citokinine BAP in callus proliferation. Reaction manifested callus after 8 weeks after inoculation.

Medium did not contain 2,4-D (which contained the BAP 2mg, 3mg respectively - media c1 and c2) did not produce callus induction (0%).

Leaf segments and internode segments explants were cultured on MS medium containing different concentrations of 2,4-D, BAP and combinations thereof.

Table 1. Effects of substances 2,4-D and BAP on callus induction

Growth regulator (mg/l)		Callus texture	Color callus
2,4-D	BAP		
0	2	-	-
0	3	-	-
2	0	friable	brownish
3	0	friable	brownish
2	2	strong	green
3	3	strong	green

Table 2. Influence of variety and medium in the callus culture

Explant source, b	Culture medium, c	Callus induction/variety (clones) (%)		Average % variety Christian
		CHRISTIAN Cl 2	CHRISTIAN CHR 01 ROU	
Fragment of leaf	c_3	100	40	70
	c_4	80	100	90
	c_5	60	20	40
	c_6	100	80	90
Average		85	60	**72,5**
Segment of petiole	c_4	40	40	40

Note:
c3= Medium MS – 2 mg/l 2,4-D
c4= Medium MS – 3 mg/l 2,4-D
c5= Medium MS - 2 mg/l BAPx2 mg/l 2,4-D
c6= Medium MS - 3 mg/l BAPx3 mg/l 2,4-D

From Table 2, it can be noted the superiority of the Christian variety clone 2 of the callus, in case of disc explant foliar (85%).

About the influence of culture medium on callus, it appears that for clone 2, using media containing: 2.0 mg / l 2,4-D, and 3 mg / l BAP x 3 mg / l 2.4 -D is achieved highest percentage of callus (100%), followed by media containing 3 mg / l 2,4-D and 2 mg / l BAP x 2 mg / l 2,4-D (80% and 60%).

For CHR 01 ROU clone, of Christian variety, the best results were registered in callus induction on c4 medium using, leading to callus proliferation at 100%.

On the variety, the best results were registered using c4 and c6 the media, achieving a rate of 90%.

In case of using petiole segment, the callus induction had low intensity of only 40% (average of two clones), only at variants that experienced the quantity of 3 mg 2,4-D/l; the remaining samples were affected by infections.

Statistical analysis of the influence of growth regulators on callus induction shows that all

data derived from experimentation showed statistically differences. Considering auxine 2,4-D (the quantity of 2mg / l) control, the results showed significant increases in the number of explants (1 explant) for citokinine BAP (in the case of both concentrations) decreases of explants numbers are significant of -3.5 explants; the combination of 2,4-D * BAP (2mg / l) are distinct differences significant negative (-1.5 explants) and for 2,4-D * BAP (3 mg / l) increases of explants number are significantly positive (1explant), resulting that induction of callus was directly influenced by growth regulator and quantity of this (Table 3).

Table 3. Influence of growth regulators used in the callus induction

Growth regulator used	Number of explants that induced callus	Procent (%)	Differences (explants number)	Signification
2,4-D (2 mg) (ct)	3,5	100.00	-	-
2,4-D (3 mg)	4,5	128.57	1	*
BAP (2 mg)	0	0	-3.5	ooo
BAP (3 mg)	0	0	-3.5	ooo
2,4-D*BAP (2 mg)	2	57.14	-1.5	oo
2,4-D*BAP (3 mg)	4,5	128.57	1	*

DL 5% =0.80 (explants)
DL 1% =1.13 (explants)
DL 0,1% =1.64 (explants)

CONCLUSIONS

Medium, explant surse (foliar disc, petiol segmente) and variety have different influences in callus proliferation.

The callus induction responded better to explants from foliar disk (72.5%) than petiole segments (40%).

C4 (3 mg / l 2,4-D) and c6 (3 mg / l BAP x 3 mg / l 2,4-D) media favored callus induction in proportion of 90

Differences obtained using BAP citokinine are statistically very significant negative towards auxine, 2,4-D (concentration of 2mg/l-considered control), -3.5 explants / that induced callus.

Medium that contained 3 mg / l 2,4-D * BAP determined obtaining an increase in the number of expants that induced callus (1 explant).

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