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RESEARCH ON THE PREPARATION A PROTOCOL OF THE DIRECT ORGANOGENESIS THROUGH *"IN VITRO"* CULTURE TECHNIQUES TO *HELIANTHUS TUBEROSUS* L. (JERUSALEM ARTICHOKE)

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Abstract

In the context of the current social and economic effects of the global crisis, it manifests itself with greater intensity and in one of the most important sectors of the Romanian economy - agriculture. Modern technique has transformed farming from a craft mastered in practice daily in a complex science that allows routing of the factors of vegetation and the transition to industrial agriculture. In this paper there will be presented arguments that underline the use of Jerusalem Artichoke (Sun root). Based on general principles of the techniques of "in vitro" culture, whose applicability was verified in the case of most species, our research aims to establish the optimal conditions for developing crops with pronounced morphogenetic capacity. The planting material at tuberous roots of artichoke was studied with greater efficiency by biotechnological methods. The working protocol was developed as a result of research undertaken on apexes "in vitro" cultivation taken from sprouts obtained by forcing the tubers in cold weather to grow shoots in the presence of additional fitohormones (BAP, K, NAA). These tubers were used to fulfill micropropagation requirements through an efficient growth "in vitro" that can succesfully be adapted to improved micropropagation for the varieties of Jerusalem artichoke.

Key words: *organogenesis, „in vitro" culture protocol, Jerusalem artichoke.*

INTRODUCTION

In Romania, Jerusalem artichokes is known under different names: kale, Jerusalem artichoke, carrots earth in Oltenia (SV part of Romania part), apple earth, in areas Brasov and Fagaras, potato crow in northern Transylvania. In Romania it was introduced over 200 years ago, but it was never expanded in culture. It is grown on small areas close to farms or livestock farms being used in pig feed as a steal. Jerusalem artichokes used in feeding these animals contribute to weight gain and changing the meat-fat percentage in favor of meat. Artichoke Romanian market is not developed to the extent properties of this plant. Due to its large strains, which in some varieties exceed 3 meters in height, it can be successfully used to create protection curtains for sensitive crops against cold winds or to prevent moisture evaporation from the soil. Strains can also be used to produce pellets for heating (***MADR, Romania, 2014). In home country (Mexico), Jerusalem artichokes used to be the staple food of the ancient Indians, and in Europe it was introduced at the beginning of the sixteenth century. It was first used in France during the Second World War, as the fermentation substrate for the production of alcohol as fuel.

Helianthus tuberosus perennial herbaceous plant belonging to the family *Asteraceae* and is cultivated as a vegetable for its tubers (Baillarge, 1942, Seiler 1993, Ciofu, 2004). *Astaraceae* family *(Compositae*) is one of the largest family dicotyledonous (approximately 20,000 species, of which 320 with high economic importance including power plants, industrial utilization of medicinal, decorative and honey plants. Type 5 clustering in widened or raised inflorescence (calatidiu) is a main pattern. Dried fruits are achenes (Axinte et al., 2006).

Jerusalem artichoke tubers contain an amount of up to 20% dry matter, which is found in abundance in a polymer of fructose called

inulin. May contain iron, calcium, magnesium, manganese, potassium, sodium, silicon, zinc, proteins, pectins, amino acids, vitamins B1, B2 and C. The stem and the leaves in the composition tryptophan, leucine, and beta-carotene (provitamin A).

Coming into being Jerusalem artichoke as a source for inulin (Gibson et al., 1995, Tassoni et al., 2010), a fructose polymer that may provide dietary health benefits for obesity, diabetes, and several other health issues (Gallaher and Schneeman, 1996). Inulin is a polysaccharide unique natural, with 95% fructose. Apart from artichoke inulin in the chicory roots is, dandelion, and other plants, but in smaller quantities (Monti et al., 2005, Serafini, 2010).

The possible use of the crop for biofuels is drawing tremendous recent interest (Hergert, 1991, Kays and Nottingham, 2008a, Diederichsen, 2010). With its ready cultivation and minimal pest and disease problems (Cassells and Walsh, 1995), Jerusalem artichoke is an underutilized resource that possesses the potential to meet major health and energy challenges (Kays and Nottingham, 2008a, 2008b, 2008c).

In vitro propagated techniques were used for propagating plants *Helianthus tuberosus*, explants of leaves was used in experiments to induce somatic embryogenesis (El Mostafa, 2006, Alla et al., 2014) and also in other research initiated on the formation of tubers of Jerusalem artichoke, using fragments of stems (Wissmann and Tripathi, 1977, Gamburg et al., 1999) in order to increase efficiency in obtaining biological material for planting. Taha et al., (2006) applied *"in vitro"* propagated techniques on Jerusalem artichoke (*Helianthus tuberosus*) for enhancement of inulin production. Preservation methods also are evalueted to facilitate the back-up of field collections of clonally propagated *H.tuberosus* (Volk, 2006, Diederichsen A., 2010, Alla et al., 2014).

The purpose of this study was the development of protocols for obtaining morphogenetic *Helianthus tuberosus* cultures and to assess the efficiency of multiplication obtained by using somatic explants taken from the tubers.

MATERIALS AND METHODS

Plant material

Tuberous root of the Jerusalem artichoke, which are the vegetative parts, spare and organs used for propagation of the plant presents adventitious buds that can be induced in a controlled manner as to produce sprouts. This is a major pattern to obtain the planting material in areas with cold seasons.

The tuberous roots used in this study to produce sprouts in order to initiate "*in vitro*" cultures, were not characterized in terms to belonging to a particular variety, it was chosen one depending on the periderm and pulp color phenotype harvested from local population plants grown in Cernatesti village near Buzau town, (located in the E region of Romania country). The Jerusalem artichoke tubers roots used to obtain shoot in aseptical conditions had purple and white periderm color, but in both cases the pulp was white (Figure 1).

Figure 1. Aspect of tuberous roots artichoke crops (local population from Cernăteşti village) with purple and white periderm color, used as a source of biological material for the initiation of *"in vitro"* cultures.

The roots tuberous had medium size, were placed on rockwool (Grodan type) moistened and kept in laboratory conditions at about 25°C and moderate atmospheric humidity for 3 weeks long. This time was necessary so that the level adventitious buds would be in the state of physiological rest to begin the shoot elongation. Therefore, depending on the size of the tuberous root after thermal pretreatment there were formed between 10-30 adventitious buds/1 piece.

Aseptic protocol for culture initiation

Tuberous roots were first cleaned by immersion for 8 hours in a dilute solution prepared from concentrated dishwashing detergent with tap water rinse followed by stirring in a stream supported by tap water for 20 minutes. Each root was cut crossectionally and then in quarters (Figure 2a). In order to sterilize the area under aseptic conditions fragments of Jerusalem artichoke as inocula were surface sterilized first of all with ethyl alcohol solution of 70% concentration (1 minut imersion) and after one rinsing in sterile distilled water (5 minutes) we continues the sterilization with another product represented by Na hypochlorite solution (under the form of the commercial product, Hey) 10% (w/v) concentration for 20 minutes. The explants were triple-washed in sterile water (3 times for 10 minutes each), dried on filter paper and after that was made the inoculation of the explant. In a first stage was carried out in culture vessels type Petri dishes of 10 cm diameter insulated with Parafilm tape (Figure 2b) and later, with the development of cultures, they were transferred (at the time of passages) in vessels with higher capacity (100 ml Erlenmeyer vessels).

Various combinations of induction media were used, all based on Murashige and Skoog (MS) medium, supplemented with 30.0 g/l sucrose, 7.5 g/l Difco Bacto Agar (Sigma) and different concentrations of phytohormones (0.01 mg/l) of naphthaleneacetic acid and 2 mg/l for cytokinins: kinetin (K) (6 furfuryl-aminopurine) and BAP (6-benzylaminopurine) in comparison with Control variant without added hormones in composition of MS (1962) basal medium (Table1).

a) b)

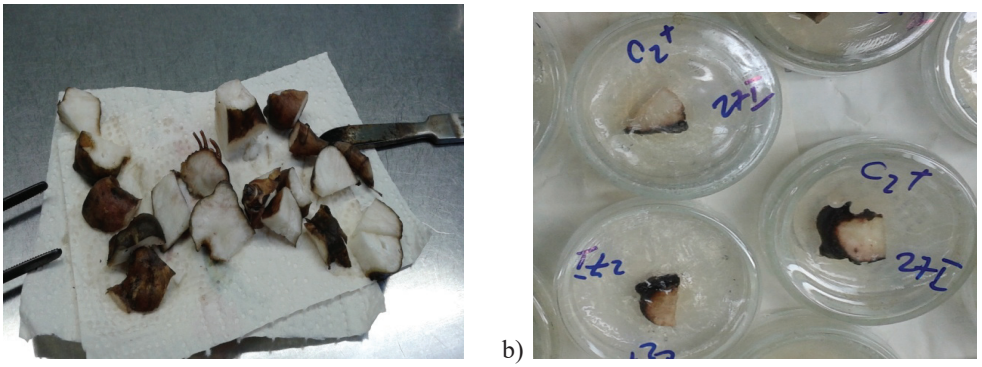

Figure 2**.** Appearance artichoke tuberous roots fragments used as a source of biological material before (a) and after inoculation (b) inoculare

Table 1. Experimental variants used in recipes to initiate artichoke organogenesis

Variants	The basal culture medium and hormonal balance	Other component
Control	MS (1962)	7.5 g./l agar+ 30 gr/l sucrose
C2	MS (1962) +2.0 mg/lBAP+0.01 mg./l NAA	7.5 g./l agar+ 30 gr/l sucrose
C 3	MS (1962) +2.0 mg/l K+0.01 mg./l NAA	7.5 g./l agar+ 30 g./l sucrose

Legend: MS – *Murashige&Schoog(1962);* BAP - 6- benzylamino purine*;*
NAA – *1-Naphthaleneacetic acid;* K- chinetine

All the explants were cultivated under illumination (16 h light/8 h dark) with light intensity of 110 µmol/m^{-2}.s^{-1} (ROMLUX fluorescent lamps,LFA 40W daylight-230V).
Explants transfers on fresh media with the same hormonal balance or medium other variants was performed observing aseptic conditions under the same conditions with sub-culturing period of 21 days.

RESULTS AND DISCUSSIONS

Observations made in the multiplication phase.
Inoculation of explants consisted of inoculating the culture medium variants selected for experimentation (Table 1), distributed into the culture dish, operations being performed in a sterile laminar flow hood (Activa HF Miniflo 120 model) by biotechnological methods developed on following standard operating plant requirements for explant cultures "*in vitro*" (Cachiţă-Cosma, 2007), with some adaptations to the specific conditions of the Laboratory of Plant biotechnology at the Faculty of Biotechnology (UASVM from Bucharest) described in the previous chapter Material and methods.
Subcultivation cultures initiated by us were made at intervals of 21 days on the variant culture medium used to originate (Variant C2), as it has proven effective in both stimulating the development of shoots multiples and in terms of their elongation. On the occasion of each subcultivations shoots elongated at longer dimensions than 3-4 cm were detached and used to manufacture new meristematic explants apexes and uninodal fragments (Figure 3). Also there were inoculated 3 series of explants on each variant / recipe used for fragments of the Jerusalem artichoke tubers. These three series are considered repetitions of used variants, the following results presented in the Table 2 represent the average of this assembly line. Inoculation of explants from all three series / variant culture medium was carried out simultaneously with Control date (in 3 repetition).
Inoculated plants reaction to the different hormonal combinations of cytokinins and auxins added to the culture medium Murashige Skoog (1962) was shown in the first three weeks of the initiation of culture through a hypertrophy of the explants for the Jerusalem artichoke tubers fragments. and poor axillary bud elongation in both variants C2 and C3 culture used. Slight differences were highlighted as compared to their control samples (Table 2).

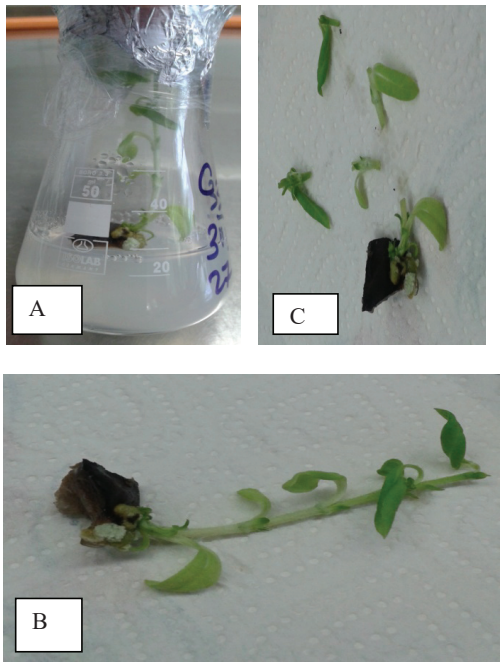

Figure 3. Induction development of shoots (A) to *H.tuberosus* by cultivating the tuberous roots sections on the recipe variant C3 (MS (1962) 2.0 mg/l K + 0.01 mg./l NAA) and multiplying elongated sprouts 3-4 cm long (B) by uninodal fragments explant (C)

Observations on the influence of the culture medium and hormonal balance composition.
Positive results in inducing caulogenesis (formation of adventitious shoots) to explants fragments tuber artichoke used as a source of biological material from plants grown in the area Cernatesti, Buzau county, did we obtain only on hormonal alternative C2 2.0 mg / lBAP + 0.01 mg / l NAA on the same basal medium Murashige & Skoog (1962) and added 7.5 gr./l agar Difco Bacto Agar (Sigma) + 30 gr/l sucrose after 55 days to initiation of the "*in vitro"* culture (Figure 4).

Table 2. The comparative effects of variants (C2 and C3) on *in vitro* morphogenesis leadership at Jerusalem artichoke explants (explants inoculated in 3 series of experimental compared to control)

The series of inoculation (Repeatition)	Recipe culture medium	Number of plants inoculated	Total number of cultures derived morphogenetic	%
Series I	**C2**	12	10	83.33
	C3	7	4	57.14
Series II	**C2**	10	10	100.00
	C3	10	8	80.00
Series III	**C2**	12	12	100.00
	C3	9	9	100.00
Control	MS (1962) for series I	5	0	0
	MS (1962) for series II	5	2	40.00
	MS (1962) for series II	5	1	20.00

Legend: MS – *Murashige&Schoog(1962);* C2=MS(1962) +30g/l sucrose +7.5 g/l agar +2.0 mg/lBAP+0.01 mg./l NAA; C3=MS(1962) +30 g/l sucrose +7.5 g/l agar+2.0 mg/l K+0.01 mg./l NAA; Control= MS (1962) without added hormones

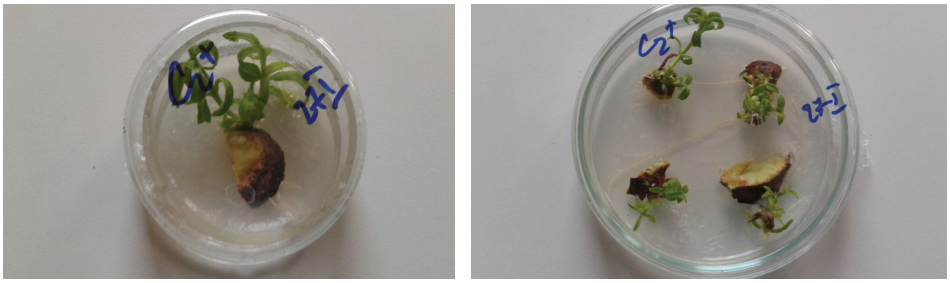

Figure 4. Morphogenetic cultures developed on the C2 recipe variant of culture medium (MS (1962) + 30g / l sucrose 7.5 g / l agar +2.0 mg/l BAP + 0.01/ mg./l NAA) after 55 days to initiation of the *in vitro* artichoke experiment

Our observations on the effect of plant hormones on artichoke explant evolution of grown "*in vitro*" variants C2 and C3 have concluded that both cytokinins used (BAP and K) in 2 mg/l concentrations stimulated elongation of axillary stems and development of morphogenetic culture with multiple adventitious shoots. After three months duration of making subcultivation on the initial inoculation environments the efficiency of developing new shoots doubled (5-7 adventitious shoots/initial inoculated explants) as you can see in Figure 5 and Table 3.

Figure 5. Morphogenetic cultures developed the recipe C3 variant of culture medium (MS (1962) + 30g / l sucrose 7.5 g / l agar 2.0 mg / NAA mg./l lBAP + 0.01) after 90 days of initiation of the artichoke experiment.

Table 3. The comparative effects of variants (C2 and C3) on "*in vitro*" morphogenesis at Jerusalem artichoke explants (explants inoculated in 3 series of experimental compared to control) after 90 days

The series of inoculation (Repeatition)	Recipe culture medium	Number of plants inoculated	Total number of cultures derived morphogenetic	%
Series I	C2	13	4	30.76
	C3	11	10	90.90
Series II	C2	28	16	57.14
	C3	23	16	69.56
Series III	C2	2	2	100.00
	C3	12	6	50.00
Control	MS (1962) for series I	5	5	100.00
	MS (1962) for series II	9	5	55.55
	MS (1962) for series III	15	5	33.33

Legend: MS – *Murashige&Schoog(1962);* C2=MS(1962) +30g/l sucrose +7.5 g/l agar +2.0 mg/lBAP+0.01 mg./l NAA; C3=MS(1962) +30 g/l sucrose +7.5 g/l agar+2.0 mg/l K+0.01 mg./l NAA; Control= MS (1962) without added hormones

CONCLUSIONS

In this work, we have established for the fist time protocol caulogenessis induction of *Helianthus tuberosus* L. (Jerusalem artichoke). The responsiveness of explants was optimal and was not recorded substantial losses due to contamination of biological material. The best morphological characteristics and growth behavior were observed for tuberous routs fragment culture with multiple adventitious shoots cultivated on MS(1962) basal media supplemented with low concentration of NAA (0.01 mg./l) and high concentration of K chinetine (2.0 mg/l) after a previous subcultivation on variant C2 (3-4 times). After this transfer on C3 recepies the yield was 10-12 multiplier adventitious shoots/explant which was inoculated and adventitious roots developed (Figure 6).

Figure 6. Explants with adventitious roots morphogenetic artichoke recipe developed on C3 [MS(1962) +30 g/l sucrose +7.5 g/l agar+2.0 mg/l K+0.01 mg./l NAA] environment.

REFERENCES

Alla N.A., Domokos-Szabolcsy É., El-Ramady, H., Hodossi, S., Fári M., Ragab M., Taha H.., 2014. Jerusalem artichoke (*Helianthus tuberosus* L.) -A review of *in vivo* and *in vitro* propagation, International Journal of Horticultural Science, 20(3–4): 131–136.

Axinte M., Roman G.V., Borcean I., 2006. Fitotehnie, ED. Ion Ionescu de la Brad, Iaşi.

Baillarge E., 1942. Le Topinambur: Ses Usages, Sa Culture, Flammarion, Paris.

Cachița-Cosma D., 2007. Micropropagarea speciilor vegetale- Lucrarile celui de-al XX lea Simpozion National de Culturi de Tesuturi şi Celule Vegetale, Ed. Risoprint, Cluj-Napoca.

Cassells, A.C. and Walsh M., 1995. Screening for *Sclerotinia* resistance in *Helianthus tuberosus* L. (Jerusalem artichoke) varieties, lines and somaclones, in the field and *in vitro*, *Plant Pathol.*, 44: 428–437.

Ciofu R., 2004. Tratat de legumicultură, Ed. Ceres, Bucureşti.

Diederichsen A., 2010. Phenotypic diversity of Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm preserved by the Canadian Genebank, In: Helia, 33(53),1-16.

El Mostafa, N., Fakiri M., Benchekroun M., Amzil J., El Arbaoui A. and Hilali S., 2008. Effect of plant growth regulators on somatic embryogenesis from leaf *in vitro* cultures of *Helianthus tuberosus* L. Journal of Food, Agriculture & Environment, 6 (2): 213-216.

Gallaher, D.D. and Schneeman B. O., 1996. Dietary-fiber. [In: present knowledge in nutrition.] (Ziegler, E. E & Filer, L. J., eds).

Gamburg K.Z., Vysotskaya E.F., and Gamanets L.V. 1999. Microtuber formation in micropropagated Jerusalem artichoke (*Helianthus tuberosus*). Plant Cell, Tissue and Organ Culture, 55: 115–118.

Gibson G.R., Bear E.R., Wang X., and Cummings J.H. , 1995. Selective stimulation of *Bifidobacteria* in the human colon by oligofructose and inulin. Gastroenterology. 108: 975–982.

Hergert G.B., 1991. The Jerusalem artichoke situation in Canada. Alternative Crops Notebook. 5: 16-19. International life science institute, Washington, DC.

Kays S.J. and Nottingham S.F., 2008a. Biology and chemistry of Jerusalem artichoke. CRC Press Taylor & Francis Group.

Kays, S.J. and Nottingham S.F., 2008b. Classification, Identification, and Distribution. In: Biology and chemistry of Jerusalem artichoke *Helianthus tuberosus* L., CRC Press Taylor & Francis Group, pp: 29-34.

Kays S.J. and Nottingham S.F., 2008c. Introduction: Anunder utilized resource. In: Biology and chemistry of Jerusalem artichoke *Helianthus tuberosus* L., CRC Press Taylor & Francis Group, pp: 1-6.

Monti A, Amaducci M.T., Venturi G., 2005. Growth response, leaf gas exchange and fructans accumulation of Jerusalem artichoke (*Helianthus tuberosus* L.) as affected by different water regimes. Eur.J. Agron., 23 (2): 136-145.

Serafini F.D., 2010. *Helianthus tuberosus* and polyamine research: Past and recent applications of a classical growth model. Plant Physiol. Bioch., 48 (7): 496-505.

Seiler G.J., 1993. Forage and tuber yields and digestibility of selected wild and cultivated genotypes of Jerusalem artichoke. Agron. J. 85: 29-33.

Taha H.S., El-sawy A.M. and Bekheet S.A., 2007. *In Vitro* Studies on Jerusalem artichoke (*Helianthus tuberosus*) and Enhancement of Inulin Production. Journal of Applied Sciences Research, 3 (9): 853-858.

Tassoni A., Bagni N., Ferri M., Franceschetti M., Khomutov A., Marques M.P., Fiuza S.M., Simonian A.R., Toxopeus, H., 1991. Improvement of Plant Type and Biomass Productivity of *Helianthus tuberosus* L., Final Report to the EEC, DGXII.

Volk G.M., 2006. Preservation methods for Jerusalem artichoke cultivars, Hortscience, 41(1):80-83.

Wissmann A.M. and Tripathi B.K., 1977. Recherches sur la tuberization de fragments de tiges de topinambour cultives "*in vitro*". C. R. Acad. Sci. (Paris) D285: 1431–1433.

***Energii regenerabile în agricultură, 2014. In: Publicatia tematica nr.10, Anul II, 7-8, MADR, Romania.


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