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In Vitro Studies on Jerusalem Artichoke (*Helianthus Tuberosus*) and Enhancement of Inulin Production

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Abstract: This study aim to establishment promising protocol of calli production and regenerated shootlets from leaf and nodal stem explants of *Jerusalem artichoke*. Also, determination of inulinase activity (u/ml) in the obtained cultures. Supplementation of MS-medium with 1mg/l each of NAA and BAP gave the best results of calli production from nodal stem and leaf explants, respectively. However, MS-medium supplemented with 0.5 mg/l NAA and 3 mg/l BAP gave the highest value of regenerate shootlets from nodal stem and leaf explants, respectively. As will as, the maximum value of inulinase activity was recorded with regenerated shootlets from nodal stem calli cultures as compared with leaf calli cultures.

Key words: *Jerusalem artichoke*, calli, regenerated shootlets, inulinase activity

INTRODUCTION

Plants constitute a major source of natural products for the pharmaceutical, agrochemical, cosmetics and food industry interest. In the past few decades, efforts have been mad to produce valuable plant metabolites using different biotechnological tools, such as plant "*in vitro*" culture techniques which enable the product synthesis and accumulation not only in the whole plant, but mainly in plant tissues, organs or undifferentiated cells[13].

Jerusalem artichoke Family (Asterceae) it is a perennial species known for its tubers rich in inulin, a valuable source of fructose for diabetics[14]. Vegetatively propagated *via* tubers, agricultural plant with nutritional, medicinal and energetic potential. Its desirable to use micropropagation plantlets then microtuberization of *Helianthus tuberosus* for preservation and exchange of varieties devoid of pathogens[4].

Inulin, a non-digestible oligosaccharide, can preferentially stimulate the growth and activity of one or a limited number of desired bacteria in the colon, and thus improves host health. And more, positive effects on blood glucose attenuation, lipid homeostasis, mineral bioavailability and immuno modulation effects, along with the ability to add texture and improve rheological characteristics and nutritional properties of food allows inulin to be termed a functional food[10]. Inulin has been increasingly used in various foods due to its beneficial nutritional attributes and it will be suitable for use in a wide range of food applications[8].

The main aims of this work are establishment protocols of callus, regeneration and inulin production from *Jerusalem artichoke*.

MATERIALS AND METHODS

Plant Materials: Tubers of *Jerusalem artichoke (Helianthus tuberosus L.)* were secured from the Centre of Agriculture Research, Giza, Egypt, and used as a plant materials. Then they were carefully cleaned with sop and tap water and kept under dark conditions. Sprouting tubers had been done within 10 days. The obtained sprouts were surface sterilized by immersion in 70 % ethanol for 10 sec, followed by three washes using sterile distilled water, then immersed in 50 % of commercial Clorox solution containing a drop of Twin 20 for 15 min. The sprouts were subsequently rinsed several times with sterile distilled water. These sprouts were then cultured aseptically on basal solid MS-medium[9]. Cultures were solidified using 0.7 % agar which were added prior autoclaving at 1.2 Kg/cm^2 for 15 min. The pH of the medium was adjusted to 5.8 by addition of 0.1 N HCL or 0.1 N KOH. The cultivation was done in 300 ml glass jars containing 50 ml of basal MS-medium, i.e., hormones free. After one month from culturing the different segments leaf and nodal stem were excised from the obtained shootlets which were used as a source material for callus production.

Callus Production: Two experiments had been done for enhancement of callus induction from leaf or nodal stem explants of *Helianthus tuberosus*.

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First Experiment: The following concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) and N-(2-furanylmethyl)-purine-6-amino (Kinetin) were added to MS-basal medium as follow:-

- MS-medium free hormones
- 0.0 mg/l 2,4-D + 1.0 mg/l Kin.
- 1.0 mg/l 2,4-D + 0.0 mg/l Kin
- 1.0 mg/l 2,4-D + 1.0 mg/l Kin
- 1.0 mg/l 2,4-D + 2.0 mg/l Kin
- 2.0 mg/l 2,4-D + 1.0 mg/l Kin

Second Experiment: The effect of the following concentrations of Naphthalene acetic acid (NAA) and 6-benzylamino purine (BAP) was studied at the following concentrations:

- MS + 0.0 mg/l NAA + 1.0 mg/l BA
- MS + 1.0 mg/l NAA + 0.0 mg/l BA
- MS + 1.0 mg/l NAA + 1.0 mg/l BA
- MS + 1.0 mg/l NAA + 2.0 mg/l BA
- MS + 2.0 mg/l NAA + 1.0 mg/l BA

Culture of all treatments were maintained under light condition 16 h/day photoperiod at intensity of 3000 Lux from cool light fluorescent lamps for 30 days. All cultures were incubated at 26 ±1 °C. Five replicates from each treatment were used.

Determination of Callus Growth: Callus growth patterns of all type cultures were determined after 30 days from incubation as follow:-

- Fresh weight (gm).
- Dry weight (gm).
- Dry matter content(%).

Shootlets Regeneration: In this experiment four concentrations of NAA and BAP as well as MS-medium free hormone were used for enhancement of shootlets regeneration from leaf or nodal stem calli as follow:

- MS-medium free hormones
- 0.5 mg/l NAA + 1.0 mg/l BA
- 0.5 mg/l NAA + 3.0 mg/l BA
- 0.5 mg/l NAA + 5.0 mg/l BA
- 0.5 mg/l NAA + 7.0 mg/l BA

Determination of Shootlets Growth Parameters: Shootlets growth patterns of all type of cultures were estimated after 30 days from incubation as follow:-

- Percentage of shoot induction
- Number of shootlets/jar
- Fresh weight (gm).
- Dry weight (gm).
- Dry matter content (%).

The design of all experiments was completely randomized and the obtained data were statistically analyzed using standers error (SE) according to the method described by Snedecor and Cochran[15].

Determination of Inulinase Activity: Determination of inulinase activity had been done through the following steps:

Determination of Total Carbohydrate: The total carbohydrate was determined according the method described by Grootwassink and Fleming[5].

Assay of Inulinase Activity: The inulinase activity was tested as described by Parekh and Margaritis[12], with the inulin (Sigma, MW 4000). The reduce carbohydrates was analyzed by the 3,5-dinitrosalicylic acid[1]. One unit of inulinase activity was defined as the amount of enzyme that liberate 1 µmol of fructose equivalent from inulin / min.

RESULTS AND DISCUSSIONS

Effect of 2.4-d and Kin on Callus Production: Data in Table (1) shows the effect of different concentration of 2,4-D and Kin on callus production from nodal stem and leaf explants of *Helianthus tuberosus*. MS-medium free hormones gave no effect on callus initiation from different explants. However, the supplementation of MS-medium with low concentration from 2,4-D or Kin alone gave a low effect on callus induction. Furthermore, the optimum value of callus production was obtained from using mixture of 2,4-D and Kin. The highest value of callus production 7.53 and 5.26 (gm/jar) at the same sequence were recorded for nodal stem and leaf explants, respectively. The addition of (1mg/l) each of 2,4-D and Kin to MS-medium was more suitable for callus production than other supplementations. Fresh weight (gm/jar) of nodal stem and leaf calli cultures were recorded after 8 weeks. However, the dry weight (gm/jar) of different cultured explants was 0.59 and 0.35 for nodal stem and leaf calli cultures, respectively. The descending order of dry matter content (%) was 7.80 % and 6.75 % for nodal stem and leaf calli cultures of *Helianthus tuberoses* respectively.

Effect of NAA and BAP on Callus Production: The effect of supplementation of MS-medium with different concentrations of NAA as auxin alone or in a combination with BAP as cytokinin on callus

Table 1: Effect of MS-medium supplemented with different concentrations of 2,4-D and Kin or NAA and BAP (mg/l) on calli fresh, dry weights (gm/jar) and dry matter content (%) of nodal stem and leaf explants of *Helianthus tuberosus* L. cultured under light condition

Type of growth regulators (mg/l)		Fresh weight (gm/jar)		Dry weight (gm/jar)		Dry matter content %	
	Nodal stem	Leaf	Nodal stem	Leaf	Nodal stem	Leaf	Nodal stem
2,4-D	Kin.						
0	0	---	---	---	---	---	---
0	1	2.65±0.45	2.14±0.54	0.08±0.0015	0.06±0.0025	5.25±0.23	4.84±0.58
1	0	1.00±0.14	0.82±0.02	0.06±0.0002	0. 04±0.0001	4.17±0.58	3.15±0.47
1	1	7.53±0.75	5.26±0.87	0.59±0.0024	0.35±0.0032	7.80±0.69	6.75±0.73
1	2	5.42±0.66	4.76±0.42	0.43±0.0034	0.35±0.0045	6.35±0.33	5.19±0.84
2	1	3.93±0.33	3.25±0.66	0.25±0.0009	0.24±0.0065	6.18±0.46	5.09±0.44
NAA	BAP						
0	1	3.45±0.35	2.25±0.25	0.29±0.005	0.18±0.002	6.2±0.45	5.8±0.47
1	0	2.12±0.15	1.56±0. 12	0.12±0.003	0.08±0.004	5.4±0.32	4.9±0.45
1	1	8.65±1.24	6.35±0.85	0.79±0.0015	0.51±0.005	9.3±0.52	8.2±0.33
1	2	6.85±0.86	5.45±0.56	0.54±0.0017	0.43±0.001	7.9±0.35	8.1±0.25
2	1	4.25±0.25	3.17±0.33	0.35±0.0015	0.29±0.002	7.2±0.18	6.5±0.18

Each treatment is the average of 5 replicates ± SE (Standard error).

Table 2: Effect of MS-medium supplemented with 0.5 (mg/l) of NAA in combinations with 1,3,5 and 7 (mg/l) of BAP on percentage; fresh ; dry weights (gm/jar) and dry matter content (%) of shootlets regeneration, from nodal stem and leaf calli cultures of *Helianthus tuberosus* L. cultured under light condition.

MS-medium supplemented with NAA and BAP(mg/l)		Shootlets regeneration parameters *of Helianthus tuberosus* L							
		Percentage of shootlets regeneration		fresh weight (gm/Jar)		Dry weight (gm/jar)		Dry matter content %	
		Nodal stem	Leaf	Nodal stem	Leaf	Nodal stem	Leaf	Nodal stem	Leaf
0.0	0.0	---	---	---	---	---	---	---	---
0.5	1.0	30±7.5	15±5.16	4.85±0.95	3.17±0.45	0.25±0.012	0.19±0.014	5.15±1.25	5.99±1.85
0.5	3.0	55±5.25	32±8.0	7.25±1.25	5.15±0.85	0.69±0.025	0.46±0.015	9.51±0.75	8.93±0.25
0.5	5.0	42±6.15	25±5.12	5.17±0.82	4.12±0.25	0.43±0.015	0.35±0.012	8.31±0.72	8.49±0.95
0.5	7.0	25±4.6	12±3.17	2.65±0.75	3.00±0.23	0.17±0.009	0.11±0.009	6.41±0.55	3.66±0.66

Each treatment is the average of 5 replicates ± SE (Standard error).

production from leaf and nodel stem explants of *Helianthus tuberosus* L. are tabulated in Table (1). The conditioned of MS-medium with low concentration of NAA or BAP alone gave some little effect on callus production. On other hand, the optimum value of callus production was obtained from the addition of both NAA and BAP to MS-medium.

As mentioned above in the first experiment, the maximum value of callus fresh weight was 8.6 and 6.35(g/ jar) for nodal stem (Fig.1), leaf explants, respectively. MS-medium supplemented with 1 mg/l both of NAA and BAP resulted in highest values of callus production as compared with other supplementations. Furthermore, dry weight as growth dynamic recorded 0.79 and 0.51 (g/jar) for nodal stem and leaf calli cultures, respectively. However, concerning dry matter content (%) it was 9.33 and 8.23 for nodal stem and leaf calli cultures of *Helianthus tuberosus.* L. *respectively*.

The above results clearly show that, the supplementation of MS-medium with NAA as auxin and BAP as cytokinin was more suitable for callus

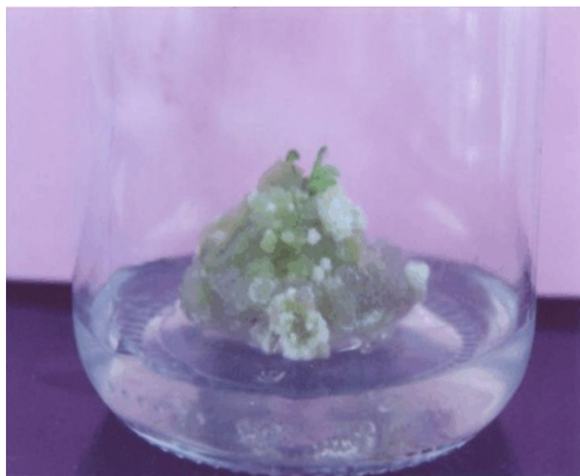

Fig. 1: Callus induction form nodal explant of Jerusalem artichoke cultured on MS-medium supplemented with 1 mg/l each of NAA and BAP.

Fig. 2: Shootlets regeneration form nodal stem explant of Jerusalem artichoke cultured on MS-medium supplemented with0.5 mg/l NAA + 3 mg/l BAP

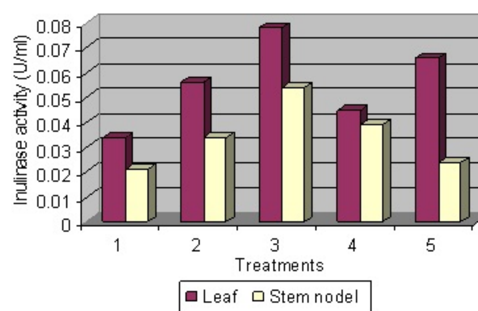

Fig. 3: Inulinase activity (u/ml) of leaf or nodel calli cultures of Jerusalem artichoke induced from MS-medium supplemented with different concentrations of 2,4-D and Kin as follow: (1) 1 mg/l Kin, (2) 1 mg/l 2,4-D, (3) 1 mg/l 2,4-D + 1 mg/l Kin, (4) 1 mg/l 2,4-D + 2mg/l Kin and (5) 2 mg/l 2,4-D + 1 mg/l Kin.

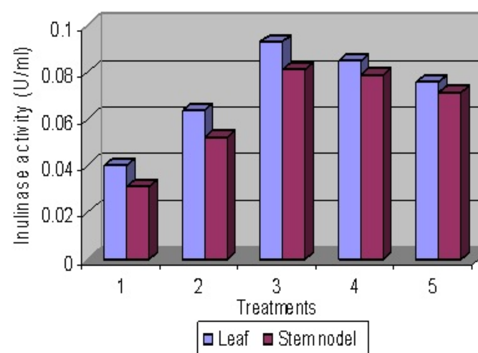

Fig. 4: Inulinase activity (u/ml) of leaf or nodel calli cultures of Jerusalem artichoke induced from MS-medium supplemented with different concentrations of NAA and BAP as follow: (1) 0 mg/l NAA +1 mg/ l BAP, (2)1 mg/l NAA + 0mg/l BAP, (3) 1 mg/l NAA and 1mg/l BAP. (4) 1 mg/l NAA + 2mg/l BAP and (5) 2 mg/l NAA + 1mg/l BAP.

production from leaf and/or nodal stem explants of *Helianthus tuberosus* L. than using 2,4-D and Kin. Also, it was found that, addition of 1 mg/l of NAA and BAP to MS-medium gave the highest values of either fresh, dry weights (gm/jar) or dry mater content (%) as compared with other concentrations.
In this respect, obtained results were in close with Gamborg and Shyluk[3], concluded that, callus initiation and production was depending on the presence of auxin and cytokinin, which stimulates cell division and cell elongation, respectively. Moreover, the obtained results by Nussbaumer *et al.*[11] they reported that supplementation of B_5 medium with 1 mg/l of each of NAA and BAP gave the best results of growth value for *Datura,* and this result are in agreement with the obtained results

Shootlets Regeneration: As shown in Table (2) one concentration of NAA (0.5 mg/l) in combinations with 1 or 3 or 5 or 7 (mg/l) of BAP were used for enhancement of shootlets regeneration from leaf or nodal stem calli cultures of *Helianthus tuberosus.* L The highest percentage of shootlets regeneration was recorded with nodal stem calli cultures (Fig. 2) as

compared with leaf. MS-medium supplemented with 0.5 mg/l NAA + 3.0 mg/l BAP was the best medium for enhancement of shootlets regeneration which gave 55% and 32 % from stem nodal and leaf calli cultures, respectively.

Furthermore, the best results of fresh, dry weights and the percentage of dry matter content were recorded with derived shootlets regeneration form nodal stem and leaf explants respectively. MS-medium supplemented with 0.5 mg/l NAA and 3.0 mg/l BAP gave the best results capacity of shootlets regeneration as compared with other BAP supplementations. Concerning, the growth parameters, fresh and dry weights recorded 7.25, 4.12 and 0.69, 0.46 (g/jar) for nodal stem and leaf calli cultures, respectively. Similarly, the percentage of dry matter content 9.51 and 8.93 (%) was recorded with nodal stem and leaf calli cultures, respectively.

Jerusalem artichoke can be micropropagated efficiently on relatively simple nutrient medium through repeated subcultures[4]. The effect of cytokinin BAP on shootlets regeneration was studied by Dodds *et al.*[2] they reported that cytokinin BAP is more suitable for multiplication and micropropagation of potato and this results are in agreement with our obtained results. And with close with our obtained results by Wagner and Schittenhelm[17] they reported that explants taken from shoots and tubers of 20 genotypes of *Jerusalem artichoke* were successful regenerated during *in vitro* cultured period.

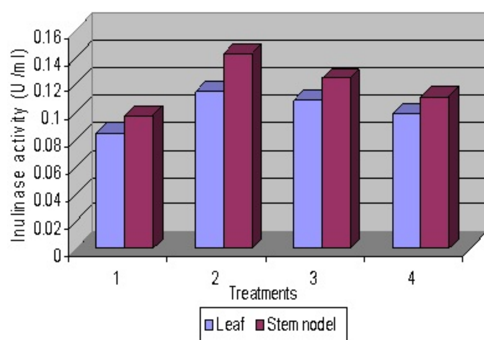

Fig. 5: Inulinase activity (u/ml) of derived shootlets of leaf or nodel calli cultures of Jerusalem artichoke induced from MS-medium supplemented with different concentrations of NAA and BAP as follow:
(1) 5 mg/l NAA +1 mg/l BAP,
(2) 0.5 mg/l NAA + 3.0 mg/l BAP,
(3) 0.5 mg/l NAA +5.0 mg/l BAP and
(4) 0.5 mg/l NAA + 7mg/l BAP.

Inulinase Activity: The calli derived from nodel stem and leaf explants as well as the obtained regenerated shootlets from previously mentioned treatments were subjected to determination of inulinase activity as indicator for inulin accumulation. The powder of *Jerusalem artichoke* tubers was used as comparative study for inulinase activity determination, whereas the inulinase activity was 15.7 (u/ml). Data illustrated in Fig. (3) clearly shows the highest value of inulinase activity i.e., 0.078 then 0.054 (u/ml) were produced from leaf nodal stem calli cultures, respectively. Moreover MS-medium supplemented with 1 mg/l each of 2,4-D and Kin. gave the best result of inulinase activity as compared with other supplementations. On other hand, the effect of supplementation of MS-medium with different concentrations of NAA and BAP on inulinase activity determination for leaf and nodel stem callus cultures was illustrated in Fig. (4). Moreover, the highest value of inulinase activities 0.093 then 0.081 (u/ml) were recorded with leaf and nodal stem calli culture, respectively. Furthermore, MS-medium supplemented with 1 mg/l each of NAA and BAP gave the best result of inulinase activity as compared with other supplementations. From above mentioned results, it can be concluded that, the supplementation of MS-medium with 1 mg/l each of NAA and BAP more effective on enhancement of inulinase activity as compared with supplementation of MS-medium with 1 mg/l each of 2,4-D and Kin.

As shown in Fig.(5) regenerated shootlets of leaf and nodel stem calli cultures showed promising quantitative determination of inulinase activity than leaf or nodel stem calli cultures. The highest value of inulinase activities 0.143 and 0.115 (u/ml) were recorded with regeneration shootlets from nodal stem and leaf calli cultures, respectively. On other hand MS-medium supplemented with 0.5 mg/l NAA and 3 mg/l BAP showed the best results as compared with other supplementations. There is a few literature discussed the inulinase activity in either calli or regenerated shootlets of *Jerusalem artichoke* plant. Hase,[6] reported that ATPase activity and the level of a polypeptide with a molecular weight of 97 KDa had increased more than 3.5 fold in calli derived from tuber tissue discs of *Jerusalem artichoke* when cultured onto MS-medium containing auxin. Moreover, Ueoke and Hase,[16] reported that H^+ translocation activity of tonoplast vesicles increased about 8 fold in *Jerusalem artichoke* tuber calli cultures after 3 days of cultivation in the presence of 2,4-D in the culture medium. In general, the obtained results may be due to enhancement of inline production from different *Jerusalem artichoke* cell lines through the advanced techniques of scaling up through bioreactors.

REFERNCES

1. Chaplins, F. and F. Kennedy, 1986. Carbohydrate Analysis. IREL Press, Oxford, Washington, 1-228.

2. Dodds, J.H., D.R. Silva and P. Tovar, 1992. Micropropagation of potato (*Solanum tuberosum* L.) In: Bajaj YPS (ed.) Biotechnology in Agriculture and Forestry, 19: 91-106. Springer Verlag. Berlin.
3. Gamborg, O.L. and J.P. Shyluk, 1981. Nutrition, media and characteristicsof plant cell and tissue cultures. *In*" Plant Tissue culture: Methods and Application in Agriculture". By. T.A. Thorp (ed.). 21-44, Academic Press, New York.
4. Gamburg, Z., E.F. Vysotskaya and L.V. Gamanets, 1999. Microtuber formation in micropropagated *Jerusalem artichoke* (*Helianthus tuberosus*). Plant Cell, Tissue and Organ Culture, 55: 115-118.
5. Grootwassink, D. and E. Fleming, 1980. Nonspecific B- fructofuranosidase (inulinase) from *Kluyveromyces fragilis*: batch and continuous fermentation, simple recovery method and some industrial. Microb. Technol., 2: 45-53.
6. Hase, A., 1993. Change in protein composition and the H^+ - ATPase activity of the plasma membrane during induction of callus from tuber tissues of *Jerusalem artichoke*. Plant Cell-Physiology. 34(1): 67-74.
7. Kaneko, T., 1967. Comparative studies on various calluses and crown gall of *Jerusalem artichoke* II. Some biochemical properties.Plant cell physiology, 8(3): 375-384.
8. Molina, L.D., M.N. Martinez, R.F. Melgarejo, A.N. Hiner, S. Chazarra and J.R. Lopez, 2005. Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus* L.). Phytochemistry, 66(12): 1476-1484.
9. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth of bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473.
10. Niness, K.R., 1999. Inulin and oligofructose: What are they? Journal of Nutrition., 129: 1402S–1406S
11. Nussbaumer, P., T. Kapetanidis and P. Christen, 1998. Hairy roots of *Datura candida* X *D. aurea* : Effect of culture medium composition on growth and alkaloid biosynthesis. Plant Cell Reports, 17(5): 405-409.
12. Parekh, S. and A. Margaritis, 1986. Production of inulinase (B- fructanhydrolase) by *Kluyveromyces marxianus*. Agric. Bio. Chem., 50: 1085-1087.
13. Phillipson, J.D., 2005. Plants as source of valuable products: In. Secondary Products from Plant Tissue Culture. (eds) Charwood, B.V. and Rhodes, M.J.C. pp: 1-23 Press in Oxford, New York, Toronto.
14. Rani, S., 1997. Genetic improvement of sunflower (*Helianthus annuus* L.) through tissue culture methods. Ann. Bot., 83(4): 347-354.
15. Snedecor, G.M. and W.G. Cohchran, 1967. Statistical Methods 5 th Ed. Iowa State Collage, Press, Amcr Iowa, U.S.A.
16. Ueoka, H. and A. Hase, 1997. Tonoplast H^+ pumps are activated during callus formation of tuber tissues of *Jerusalem artichoke* (*Helianthus tuberosus*). Physiologia plantarum, 100(1): 91-101.
17. Wagner, M.G. and S. Schittenhelm, 1992. *In vitro* propagation methods of *Jerusalem artichoke* (*Helianthus tuberosus* L) in medium term storge. Plant Genetic Resources- Newsletter., 86: 5-7.