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Jerusalem artichoke (*Helianthus tuberosus* L.): A review of *in vivo* and *in vitro* propagation

Alla, N. A.,[1,2] * Domokos-Szabolcsy, É.,[2] El-Ramady, H.,[2,3] Hodossi, S.,[4] Fári, M.,[2] Ragab, M.[5] & Taha H.[1]

[1]Plant Biotechnology Dept., Genetic Engineering Div., National Research center, 33-El-Behouth St., 12622 Dokki, Cairo, Egypt (E-mail: neama_ncr@yahoo.com and hussein.taha2@yahoo.com)
[2]Agricultural Botanics, Plant Physiology and Biotechnology Dept., Debrecen Uni., AGTC Böszörményi utca. 138, 4032 Debrecen, Hungary (E-mail: domokosszabolcsy@gmail.com and fari@agr.unideb.hu)
[3]Soil and Water Sciences Dept., Faculty of Agriculture, Kafrelsheikh Uni., 33516 Kafr El-Sheikh, Egypt
[4]Institute of Research and Development, Center of Agricultural Sciences, Debrecen Uni., Böszörményi utca. 138, 4032 Debrecen, Hungary (E-mail: mtkdakania@agr.unideb.hu)
[5]Horticultural Sciences Dept., Faculty of Agriculture, Ain Shams University, Egypt

Summary: Jerusalem artichoke (*Helianthus tuberosus* L.) is an old tuber crop with a recently renewed interest in multipurpose improvement. It is a perennial tuberous plant rich in inulin and is a potential energy crop. During food shortages in times of war Jerusalem artichoke received more attention by scientists and farmers because of its multiple uses as a vegetable, medicinal plant, forage plant and source for biofuel. The energy crisis of the 1970s motivated research on Jerusalem artichoke for biofuel as the aboveground plant biomass and the tubers can be used for this purpose. There are different methods to propagate Jerusalem artichoke using tubers, rhizomes, slips (transplants derived from sprouted tubers), stem cuttings, seeds and tissue culture. So, this review was presented to highlight on propagation of Jerusalem artichoke *via in vivo* and *in vitro* techniques.

Keywords: Jerusalem artichoke, *in vitro* propagation, tuber, somatic embryogenesis

Introduction

Jerusalem artichoke) *Helianthus tuberosus*), which is also called sunchoke, sunroot, topinambur or earth apple, is a perennial species belongs to Asterceae family, native to eastern North America, closely related to the sunflower.. Known for its tubers rich in inulin (Monti *et al.* 2005; Tassoni *et al.* 2010). Inulin is a polymer of monosaccharide fructose ,.Jerusalem artichoke is a healthy choice for diabetics (typ. 2) , therefore mainly cultivated as a vegetable, but also utilized as fodder crop, and a source of inulin for food and industrial purposes (Kays and Nottingham, 2008b). This plant is among the best crops for inulin production. The tubers are seldom bothered by insects and diseases (Splittstoesser, 1990). The inulin used as diabetics, has a positive effects on blood glucose attenuation, lipid homeostasis, mineral bioavailability and immunomodulation effects. Inulin and other polyfructosides are mainly used for the production of high-fructose syrups and artificial sweeteners in food industries. Along with the ability to add texture and improve rheological characteristics and nutritional properties of food allows inulin to be termed a functional food. It can also preferentially stimulate the growth and activity of one or a limited number of desired bacteria in the colon, and thus improves host health. These beneficial microorganisms, such as *Lactobacilli* and *bifidobacteria,* in food are known as probiotics (Gibson *et al.* 1995; Gallaher and Schneeman, 1996). Adding inulin to fermented milk could promote growth and provide protection of *Lactobacillus salivarius* during cold storage. As well inulin improved the viability of *L. salivarius* Alani *et al.* (2009). Furthermore, inulin of Jerusalem artichoke is used as non-food application in the manufacture of biodegradable plastics, cosmetics, and detergents. The tubers of plants can be also considered for succinic acid and butyric acid production (Kays and Nottingham, 2008b). Jerusalem artichoke as plant produces huge green biomass with few inputs, it is considered in silage production (Seiler, 1993).

Moreover, Jerusalem artichoke can be used for bioethanol production and it could potentially also applying for soil remediation *via* removal of heavy metal pollutants (Chen *et al.* 2011). Jerusalem artichoke is propagated using tubers, rhizomes, slips (transplants derived from sprouted tubers), stem cuttings, seeds and tissue culture. Tubers represent the primary choice for commercial production of the crop. While rhizomes are important in the regeneration

of wild populations, rhizomes, slips, and stem cuttings represent secondary methods of asexual propagation that are occasionally used to expand the numbers of a particular clone when tubers are not available. Tissue culture is used for long-term storage of clones in germplasm repositories and for the production of transgenic plants. Unlike sunflower (*Helianthus annuus* L.), where seed represents the primary means of propagation, seed is not a viable method for the production of Jerusalem artichokes and is used only in breeding programs to obtain the progeny from crosses (Kays and Nottingham, 2008a).

1. Propagation of Jerusalem artichoke

Jerusalem artichoke is usually propagated vegetatively, from tubers or tuber pieces. Reproduction by seed, although of no consequence in commercial production, is a means of dispersal for wild populations and is vital when crossing in plant breeding programs. Propagation is also possible from rhizomes, slips, and cuttings. Jerusalem artichoke is amenable to propagation by tissue culture, being a model species used in pioneering micropropagation studies (Kays and Nottingham, 2008c).

In vivo propagation of Jerusalem artichoke

Jerusalem artichokes are propagated from tubers or tuber pieces typically 45 to 60 g in size; large tubers can be cut into smaller pieces that sprout under normal conditions as well as intact tubers (Baillarge, 1942; Milord, 1987). Rhizomes are specialized underground stems that arise from belowground portions of the aerial stem. They facilitate dispersal in that they can form new vegetative shoots as far as 50 cm outward from the parent plant (Swanton, 1986). Furthermore, rhizomes are generally white, vary in length, and have nodes that possess axillary buds from which branches and vegetative shoots may arise. Regeneration of plants *via* rhizomes is substantially less than that for tubers; however, their reproductive potential is significant and varies depending upon a number of factors (e.g., clone, age, depth in the soil, size, and environmental conditions). Moreover, slips (transplants), derived from sprouted tubers, can be used to increase the plant population of a clone over what would be realized *via* direct field planting of the tubers. Whereas, stem cuttings can be readily rooted under appropriate conditions (Phillips, 1985; Norcini and Aldrich, 2000). On the other hand seed reproduction is important in wild populations and is an essential part of Jerusalem artichoke plant breeding programs. Jerusalem artichoke is an obligate out crosser that exhibits a high level of self-incompatibility (van de Sande Bakhuyzen and Wittenrood, 1950; Toxopeus, 1991).

In vitro propagation of Jerusalem artichoke

Plant tissue culture is the culturing of cells, tissues, or organs from plants under aseptic conditions. The material cultured ranges from cells or cell aggregates in suspension to mature or immature embryos, segments, or explants of plant organs, and isolated plant organs, shoot, or root tips. *Helianthus* species have been raised from diverse material, although Jerusalem artichoke has been predominantly micropropagated through the culturing of tuber tissue explants. Plant tissue culture is an important tool in plant breeding programs and in the conservation of genetic resources. Moreover, tissue culture facilitates the screening of *in vitro* germplasm for desirable traits, such as *Sclerotinia* resistance or cold and salt tolerance (Escandon and Hahne, 1991; Cassells and Walsh, 1995). Also, tissue culture enables germplasm of Jerusalem artichoke to be preserved in biodiversity conservation programs.

Jerusalem artichoke can be *in vitro* propagated efficiently on relatively simple nutrient medium through repeated subcultures. Only one bud of two per node produced a shoot suitable for the obtaining segments for the next subculture. The dominance of one bud over the other has been observed in *in vitro* propagated plants of other species with two buds at the node such as *Dianthus* and *Theobroma cacao* (Esan, 1992). *In vitro* propagated Jerusalem artichoke can be used as a model for the study of correlative bud inhibition. Moreover, clonal *in vitro* propagation can be used for prolonged maintenance of Jerusalem artichoke lines and varieties devoid of pathogens. Furthermore, *in vitro* propagated plantlets provide nodal stem segments for microtuber induction (Gamburg *et al.* 1999).

- Clonal micropropagation and microtubers induction of Jerusalem artichoke

Clonal micropropagation of Jerusalem artichoke was initiated from axillary meristems of lateral shoots of field-grown plants on medium with MS salts, 2% sucrose, 1 mg l^{-1} thiamine-HCl, 1 mg l^{-1} IAA and 0.6% agar. Plantlets were cut into nodal sections and used for subsequent subcultures and for Microtubers induction. Microtubers were induced from axillary meristems on medium with half-strength of MS salts, 8% sucrose and 0.5 mg l^{-1} BA in darkness at 18 ºC. They had near to 30% of dry matter. Microtubers resumed growth in light room at 23 ºC after 4–6 months of cold storage (Gamburg *et al.* 1999). Microtubers are the best plant material for germplasm conservation of tuber-producing plants because they are pathogen-free and are suitable for prolonged preservation at low temperature. Potato microtubers are used widely for international exchange of cultivars and germplasm. *In vitro* tuberization was used to study hormonal regulation of tuber formation (Melis and Van Staden, 1984; Vreugdenhil and Struik, 1989) and tuber protein gene expression (Bourque *et al.* 1987) and as a simple and effective means of screening potato genotypes capable to form tubers at high temperature (Nowak and Colborne, 1989). Methods for microtuber induction from micropropagated plantlets are established for potato and yam (Dodds *et al.* 1992; Ng, 1992). The study of microtuber formation in Jerusalem artichoke as in other tuber-producing plants may be useful for elucidation of common characteristics of tuberization in plants. However,

clonal micropropagation of Jerusalem artichoke has not been described and *in vitro* tuberization was performed only with stem segments excised from intact plants (Wyssmann and Tripathi, 1977; Koda and Kikuta, 1991). The requirements for microtuber induction were similar in Jerusalem artichoke and potato (Dodds *et al.* 1992). Further work must be done to study the dependence of microtuber formation on light/dark regime, type of explant, sucrose concentration, type and concentration of cytokinin and incubation temperature. Microtubers can be obtained in Jerusalem artichoke in darkness below 20 ºC on medium with high sucrose (8% may be optimal). High concentration of sucrose was shown to be necessary for microtuber formation in potato with 8% being optimal level (Garner and Blake, 1989; Dodds *et al.* 1992) and in white yam (Ng, 19**92**). Cytokinin (BA) (0.5 mg l^{-1} being optimal concentration) increased the number of microtubers possibly due to the weakening of correlative growth inhibition of one lateral bud by another. However, microtuber induction was high enough also in the absence of BA, whereas BA did not induce microtubers at 2–4% sucrose. Thus, BA must be considered as an improving but not an inducing factor in microtuber formation in Jerusalem artichoke (Garner and Blake, 1989).

- Somatic embryogenesis of Jerusalem artichoke

Somatic embryogenesis resulting in regeneration of whole plant is an important step in a plants transformation method. Successful and stable transformation requires mechanism by which a single cell is able to give rise to a plant. Ideal transformation scheme is issued from somatic embryogenesis, as in a callus each transformed cell is able to produce a plant. *In vitro* morphogenesis and somatic embryogenesis was studied in relation to genetic basis and showed the genetic control of organogenesis. Somatic embryogenesis and subsequent plant regeneration have been reported in most of main major crop species (Evans and Sharp, 1981)

El Mostafa *et al.* (2008) studied the effect of plant growth regulators on somatic embryogenesis from leaf segment of *in vitro* cultures of *Helianthus tuberosus* L. They found that the highest frequency of somatic embryos (41.6) was observed on MS medium supplemented with 0.1 mg l^{-1} NAA. In the genus *Helianthus* plant regeneration has been achieved through organogenesis and somatic embryogenesis, whereas, some studies reported a strong correlation between the genetic background of the donor plant and its *in vitro* regeneration response (Paterson *et al.* 1985; Pelissier *et al.* 1990; Knittel *et al.* 1991; Sarrafi *et al.* 1996).

- Callus cultures production and plantlet regeneration of Jerusalem artichoke Taha *et al.* (2007) established a promising protocol of calli production and regenerated shootlets from leaf and nodal stem explants of Jerusalem artichoke. They stated that MS-medium supplemented with 1mg L^{-1} each of NAA and BAP gave the best results. However, MS-medium fortified with 0.5 mg L^{-1} NAA + 3 mg L^{-1} BAP was the best medium for enhancement of shootlets regeneration which gave 55% and 32 % from stem nodal and leaf calli cultures, respectively.

Fig. 1: *In vivo* propagation of Jerusalem artichoke using tubers: Storing of tubers in the soil during winter season (photos 1 and 2); preparing soil for cultivation (photo 3); sprouted tubers, which cultivated in the field in April (photos 4 and 5) (Photos by N. Abd Alla, Demonstration Garden, Agricultural Botanics, Plant Physiology and Biotechnology Dept., Debrecen Uni., Hungary, 2013-2014)

Fig. 2: *In vivo* propagation of Jerusalem artichoke using transplants: cultivated tubers in pots in green house (photo 1); produced transplants ready for cultivation (photo 2); cultivated transplants in the field (photos 3 and 4) (Photos by N. Abd Alla, Demonstration Garden, Agricultural Botanics, Plant Physiology and Biotechnology Dept., Debrecen Uni., Hungary, 2013-2014)

Fig. 3: *In vitro* propagation of Jerusalem artichoke using stem nods: clean and surface sterilized harvested tubers (photo 1); transplants produced from tubers as a source of stem nod explants (photo 2); disinfected stem nod explants in laminar air flow (photos 3); acclimatization of *in vitro* plants (on MS medium containing starch, Photo 4) and using micro-farm (Photo 5) (Photos by N. Abd Alla, Demonstration Garden, Agricultural Botanics, Plant Physiology and Biotechnology Dept., Debrecen Uni., Hungary, 2013-2014).

Conclusion

It could be summarized that, Jerusalem artichoke can be propagated using *in vivo* techniques (tubers, rhizomes, slips, stem cuttings, seeds) and *in vitro* by tissue culture techniques. There are limit literature reviews about *in vitro* propagation of this important plant and this may be due to some restrictions such as a long period of dormancy (from 4 to 6 months), *in vitro* vitrification and contamination. Therefore, further studies should be achieved to overcome these previous problems. Moreover, we aim in the future to produce biofortified and tolerant Jerusalem artichoke for human nutrition and soil remediation purposes, respectively.

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