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Exogenous Supplementation of Growth Regulators and Temperature Improves Germination of Dormant Jerusalem Artichoke (*Helianthus tuberosus* L.) Seeds under *in vitro* and *in vivo* Conditions

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Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) is an important tuber crop but the seed germination is one of the major limiting factors in propagation due to the dormancy of the seeds. To find a solution, we investigated whether exogenous application of the hormones 6-benzylaminopurine (BAP) and gibberellic acid (GA_3) as well as chilling (4 ºC) alone or in combination can be used to alleviate the problem of dormancy under *in vitro* and *in vivo* conditions. In the present study, JA102xJA89 control seeds showed only 11.7 and 13.3% of germination rate under aseptic and greenhouse conditions, respectively. Exogenous supplementation of 8 ppm BAP for seven and two days scored the highest germination rate of 91.7 and 85.0% under aseptic and greenhouse conditions, respectively. In the presence of BAP it was not necessary to chill the seeds or add GA_3 to increase germination, and, in the presence of chilling, it was not necessary to add GA_3. In addition, no variation was observed in germination percentages of different Jerusalem artichoke genotypes when supplemented with 8 ppm of BAP. Two weeks after germination under aseptic conditions the seedlings had the highest survival rate when transplanted under greenhouse conditions which could be applicable for further genetic characterization.

Keywords: Benzylaminopurine; cytokinin; gibberellic acid; chilling; dormancy; germination; Jerusalem artichoke

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial herbaceous plant that is grown as an annual crop. It originates from the temperate regions of the northern central part of the USA. *Helianthus* belongs to the Asteraceae family and comprises 50 species [1-2]. The upper plant parts are used for biomass and animal feed, whereas the lower parts such as the tubers are used as feedstock for food and for non-food chemical production. The tubers store nearly 20% of inulin, an indigestible carbohydrate that is beneficial to human health and unique on equation vitamin-mineral complex, valuable foodstuff with high fructose syrup, bio-ethanol and prophylactic potential [1, 3-4]. Chicory (*Cichorium intybus* L.) and Jerusalem artichoke are the inulin storage species [1]. Tubers are consumed by people as vegetable because of its food value, flavour, and inulin content [5-6]. Dried Jerusalem artichoke powder is an important source of soluble dietary fibre, which significantly differs from defatted flax seeds, rye and wheat bran [3]. The Jerusalem artichoke was recently introduced into Thailand for commercial production as a food crop.

Seed dormancy is a nuisance in propagation programs; removing or breaking dormancy has always been a challenge for plant physiologists and dormancy is a serious threat to the improvement of the Jerusalem artichoke crop population [1, 4, 7]. Seed dormancy is defined as the lack of germination in a specified period of time under favorable environmental conditions [8-9]. Two major forms of physiological seed dormancy have been described, namely embryo and seed coat dormancy [10-11]. Physiological dormancy can be divided into three levels: deep, intermediate and slight dormancy [8]. Genotypic constraints, physiological inhibitors and morphologically immature embryos appear to cause a combined dormancy in Jerusalem artichoke seeds. In the physiologically dormant seeds, temperature can break dormancy and promote germination, and there is a list of chemicals such as oxidants, nitrites, nitrates, respiratory inhibitors and growth regulators that have been proven to eliminate seed dormancy [12-13]. Levels of endogenous plant growth regulators are believed to play a major role in overcoming seed dormancy of different species with variable success. The gibberellins promote the endosperm weakening and subsequent endosperm rupture (micropylar endosperm in particular) by induction of cell wall hydrolases, whereas abscisic acid inhibits the induction of cell wall hydrolases and thereby inhibits endosperm weakening and endosperm rupture [14-15].

To increase the rate of germination and to break dormancy, moist chilling and cold stratification have been widely used as pre-sowing treatments. They are cost-effective and simple approaches for overcoming seed dormancy [16]. The mechanism is still not clearly understood, but according to the researchers Nikolaeva, Mozafar and Masoomeh [17-18], moist chilling plays an important role in establishing hormone levels by initiation of appropriate enzyme activity. Gibberellic acid has been shown to promote seed germination, and both GA_3 and pre-chilling also improved the germination rate in the seeds of

Nothofagus obliqua [19-20]. Cold stratification has been found to be an effective method and is also recognized in overcoming physiological dormancy of germinating seeds of many species [21-22]. A previous study reported that 4 ºC treatment was superior to addition of kinetin, BAP and GA_3 in improving seed germination of *Ferula assa-foetida* L. [23]. Removing the hull and seed coat of difficult-to-germinate *Helianthus* species including the Jerusalem artichoke was previously found to be the most effective method and gave rise to 90% germination [24]. BAP is an important growth regulator and plays a key role in breaking of seed dormancy [25]. There are some reports on improvement of sunflower seed germination after application of BAP [26-27]. The aim of this study was to examine the effect of plant growth regulators and the chilling factor both alone and in combination in overcoming seed dormancy of the Jerusalem artichoke under *in vitro* and *in vivo* conditions. For the first time we have established *in vitro* culture protocols for dormancy-breaking of Jerusalem artichoke seeds.

MATERIALS AND METHODS

Effects of the cytokinin 6-benzylaminopurine (BAP), gibberellic acid (GA_3) and chilling at 4 ºC on seed germination under aseptic and greenhouse conditions

Mature achenes of the Jerusalem artichoke (Figure 1a) were harvested from field-grown F_1 hybrids derived from the cross between JA 102 and JA 89 plants. The parental genotypes were kindly donated from the plant genetic resources of Saskatoon Research Centre, Canada (KSRC). All achenes were dry in shade and preserved until used.

In the first experiment, aseptic culture was performed. One thousand two hundred and sixty seeds of JA 102xJA 89 were sterilized with 1.2 and 0.6% sodium hypochlorite for 5 min, and subsequently sodium hypochlorite was removed by washing with sterile distilled water twice for 5 min each. Twenty surface-sterilized seeds were (1) cultured on MS basal medium (pH adjusted to 5.6-5.8) supplemented with various concentrations of BA (Sigma) and GA_3 (Wago, Japan) for seven days (Fig. 1b) and then transferred to plain MS basal medium (MS salt, JRH bio science); (2) subjected to a chilling treatment at 4 ºC for one, two, three or four weeks and then transferred to plain MS basal medium; (3) subjected to combination treatments of BAP (8 ppm) or GA_3 (4 ppm) with/without chilling for four weeks at 4 ºC in a refrigerator and then transferred to plain MS basal medium under the growing conditions 12 hrs photoperiod with approximately 28 $\mu mol.m^{-2}.s^{-1}$ and 25±2 ºC. Non-treated seed culture was used as a control. The detailed treatments are described in Table 1.

In addition, experiments were conducted to determine whether the seeds can germinate sufficiently under greenhouse conditions. Greenhouse experiments were conducted with minor modifications based on the results obtained from the aseptic culture experiments. One thousand one hundred and forty seeds of JA 102xJA 89 were sterilized with 1.2 and 0.6% sodium hypochlorite for 5 min and followed by washing with sterile water twice for 5 min each. Twenty seeds of the Jerusalem artichoke were placed on Whatman No.1 filter paper in Petri dishes, and were imbibed with sterile distilled water supplemented with various concentrations of BAP and GA_3 for 48 hours as well as combinations of BAP (8 ppm) or GA_3 (6 ppm) with/without chilling (4 ºC) for four weeks (Table 1). Pure water-supple- mented cultures which were unchilled were used as a control treatment. The seeds were also incubated at 4 ºC for one, two, three or four weeks in a refrigerator. Seeds were transferred to a peat moss medium (Pindstrup) under greenhouse conditions and water was added once a day.

Table 1. Treatments to study the effect of plant growth regulators and chilling on seed germination of the Jerusalem artichoke JA 102xJA 89 under aseptic culture conditions and greenhouse conditions

Treatment	BA (ppm)	GA_3 (ppm)	Chilling (week)
1	0	0	0
2*	1	0	0
3	2	0	0
4	4	0	0
5	6	0	0
6	8	0	0
7	10	0	0
8*	0	1	0
9	0	2	0
10	0	4	0
11	0	6	0
12	0	8	0
13	0	10	0
14	0	0	1
15	0	0	2
16	0	0	3
17	0	0	4
18	8	4* (6)	0
19	8	0* (0)	4
20	0	4* (6)	4
21	8	4* (6)	4

* Treatment used for aseptic culture conditions only
() Treatment used for greenhouse culture conditions only

Effect of BAP on seed germination of 12 Jerusalem artichoke genotypes

The treatment that gave the best germination results (Table 2) was further tested with seeds of 12 Jerusalem artichoke varieties (Table 3), to determine if there are different responses among varieties. In this case, BAP at the concentration of 8 ppm without chilling was selected to treat the 12 Jerusalem artichoke varieties both under aseptic and greenhouse conditions. Seed preparation and growing conditions were similar to those described previously. The treatments were replicated three times. Germination, dormancy and dead seed percentages were recorded.

Transplanting study of *in vitro* grown seedlings

Pericarps of the Jerusalem artichoke hybrid (JA 102xJA 89) were removed and seeds were cultured under the growing conditions described above. For hardening, seedlings of the *in vitro* germinated seeds that were one, two, three and four weeks old were transferred to plastic containers filled with peat moss. The pots were covered with transparent plastic bags provided with ventilation holes for three days. After hardening, the plastic bags were removed and water was supplied daily. The surviving

plants were counted weekly until four weeks and then transferred to pots with high-quality soil.

Data analysis

We define 'germination' as the stage when the root tips emerge from the seeds [27]. The germination was recorded eight times at 3-day intervals. Ungerminated seeds were then tested for their viability using 0.5% tetrazolium (Sigma) for 12 hours. Final germination rate, dormancy and dead seed percentage were recorded.

Germination, dormancy and dead seed data were analysed using the Statistic 8 software program [28] and mean separation was performed by the Least Significant Difference (LSD) if the F-test was significant at the 0.05 probability level. Graphical presentation was done in Microsoft Excel.

RESULTS

Effects of BAP and GA_3

Supplementation of 1-10 ppm of BAP to the seeds of the Jerusalem artichoke hybrid JA102xJA89 significantly increased seed germination under aseptic (45.0 to 91.7%) and greenhouse conditions (38.3 to 85.0%) when compared to untreated controls (11.7% and 13.3%) after twenty-four days (Table 2). The highest seed germination of 91.7% and 85.0% was recorded at 8 ppm of BAP under both aseptic and greenhouse conditions (Table 2; Figures 2a & 2b).

Supplementation of GA_3 also significantly increased the germination rate except at the lowest concentration of 1 ppm under aseptic conditions (Table 2). However, the germination percentages were generally much lower than those of BAP, ranging from 13.3 to 38.3% for aseptic conditions and 25.0 to 58.3% for greenhouse conditions. There was no significant difference among concentrations from 2 to 10 ppm under aseptic conditions; therefore, the best concentrations could not be clearly determined. The best concentration for greenhouse conditions was recorded at 6 ppm (58.3% germination) (Figure 2d).

For all concentrations tested under aseptic conditions, BAP and GA_3 can significantly reduce dormancy of Jerusalem artichoke seeds (Table 2). The percentages of seed dormancy ranged from 0 to 40% for BAP and 38.3 to 50% for GA_3 compared to 71.7% of the untreated control. BAP at the concentration of 8 ppm gave the lowest percentage of dormancy (0%), whereas GA_3 at all concentrations gave similar results. The total percentage of dead seeds in BAP-treated experiments were much lower than that of GA_3 treatments under aseptic conditions.

Effects of Chilling

Chilling at 4 ºC for one to four weeks can also significantly increase germination (Table 2, Figures 2e & 2f). The germination percentages ranged from 31.7 to 76.7% for aseptic conditions and 33.3 to 75.0% for greenhouse conditions, when compared to 11.7 and 13.3% of the untreated controls. The best treatment for both culture conditions was chilling for four weeks that can give germination rates of 76.7 and 75%.

In contrast to germination, chilling at 4 ºC can reduce seed dormancy and the lowest percentages of dormancy (6.7% for aseptic conditions and 5.0% for greenhouse conditions) were found when the seeds were chilled for four weeks, whereas untreated controls had seed dormancy of 71.7 and 36.7%.

Combined effects of BAP, GA_3 and chilling

Supplementation of BAP, GA_3 and chilling alone significantly improved seed germination under both aseptic and greenhouse conditions. The combined treatment of BAP, GA_3 and chilling also significantly increased seed germination rate under aseptic conditions (33.3 to 86.7%) and greenhouse conditions (30.0 to 55.3) when compared to 11.7 and 13.3% in untreated controls (Table 2). The maximum synergistic treatment effect (86.7%) was observed at 8 ppm BA with combination of chilling at 4 ºC for four weeks. However, BAP at a concentration of 8 ppm alone showed a higher seed germination than combination with chilling. The combination of GA_3 and chilling resulted in less improved germination when compared to other combinations (Table 2 and Figure 2). Treatment with co-applied GA_3 showed significantly lower seed germination compared to treatment without GA_3 under greenhouse conditions (Figure 2h).

Variation in seed germination of Jerusalem artichoke varieties

According to our experiments, 8 ppm BAP concentration was the most effective in breaking seed dormancy and improving germination rate. In this experiment, twelve accessions of Jerusalem artichoke seeds were treated with 8 ppm BAP for scoring of germination and dormancy rate under both aseptic and greenhouse conditions. Seed germination (85.0 to 93.3% and 83.3 to 93.3%) (Figures 3a & 3b), dormancy (0.0% to 5.0% and 0%) and dead seeds (1.7% to 15% and 6.7% to 16.0%) were recorded under aseptic and greenhouse conditions (Table 3). The difference of seed germination percentages among the genotypes was not significant. However, a significant difference was found in the percentage of dead seeds under *in vitro* conditions. The application of 8 ppm BAP alone gave the highest germination percentages from 9 to 18 days under aseptic conditions (Figure 3a) and 6 to 12 days under greenhouse conditions (Figure 3b).

Transplanting of seedlings

Transplanting of seedlings germinated under aseptic conditions to ambient environments usually has a low survival rate and depends on the age of the seedling. The highest survival rate of JA102xJA89 Jerusalem artichoke seedlings was recorded for the seedlings transferred two weeks after germination, followed by seedlings transferred one week, three weeks and four weeks after germination (Figure 3c). The final survival rates were 100%, 95%, 80% and 72% for seedlings of two weeks, one week, three weeks and four weeks, respectively. After transplanting the survival was constant from two to four weeks.

Table 2. Effect of treatments of Jerusalem artichoke JA 102xJA 89 seed germination

Treatment	%Germination		%Dormancy		%Dead seed	
	In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*
Control	11.67 ^{k}	13.33 ^{k}	71.67 ^{a}	36.67 ^{c}	16.67 ^{efg}	50.00 ^{bc}
BA 7 days (2 days)						
1 ppm*	45.00 ^{fg}	ND	40.00 ^{c}	ND	15.00 ^{fg}	ND
2 ppm	48.33 ^{f}	38.33 ^{k}	36.67 ^{cd}	30.00 ^{d}	15.00 ^{fg}	31.67 ^{def}
4 ppm	65.00 ^{e}	46.67 ^{de}	23.33 ^{ef}	23.33 ^{ef}	11.67 ^{ghi}	30.00 ^{efg}
6 ppm	71.67 ^{de}	58.33 ^{c}	15.00 ^{g}	16.67 ^{g}	13.33 ^{gh}	25.00 ^{fght}
8 ppm	91.67 ^{a}	85.00 ^{a}	0.00 ^{j}	3.33 ^{hi}	8.33 ^{hi}	11.67 ^{j}
10 ppm	78.33 ^{cd}	55.00 ^{c}	11.67 ^{ghi}	18.33 ^{fg}	13.33 ^{gh}	26.67 ^{fgh}
GA_3 7 days (2 days)						
1 ppm*	13.33 ^{k}	ND	50.00 ^{b}	ND	36.67 ^{a}	ND
2 ppm	30.00 ^{ij}	26.67 ^{ij}	38.33 ^{c}	51.67 ^{a}	31.67 ^{abc}	21.67 ^{hi}
4 ppm	38.33 ^{gh}	33.33 ^{ghi}	40.00 ^{c}	36.67 ^{c}	21.67 ^{de}	30.00 ^{efg}
6 ppm	35.00 ^{hi}	58.33 ^{c}	38.33 ^{c}	23.33 ^{ef}	26.67 ^{cd}	18.33 ^{ij}
8 ppm	25.00 ^{j}	45.00 ^{ef}	41.67 ^{c}	23.33 ^{ef}	33.33 ^{ab}	31.67 ^{def}
10 ppm	30.00 ^{ij}	25.00 ^{j}	41.67 ^{c}	26.67 ^{de}	28.33 ^{bc}	48.33 ^{c}
Chilling 4ºC						
1 wk	31.67 ^{hij}	33.33 ^{ghi}	40.00 ^{c}	31.67 ^{cd}	28.33 ^{bc}	35.00 ^{de}
2 wks	50.00 ^{f}	53.33 ^{cd}	30.00 ^{de}	28.33 ^{de}	20.00 ^{ef}	18.33 ^{ij}
3 wks	65.00 ^{e}	60.00 ^{c}	18.33 ^{fg}	20.00 ^{fg}	16.67 ^{efg}	20.00 ^{hi}
4 wks	76.66 ^{cd}	75.00 ^{b}	6.67 ^{hij}	5.00 ^{hi}	16.67 ^{efg}	20.00 ^{hi}
Combination						
8 ppm BA + 4 (6) ppm GA_3 for 7 (2) days	80.00 ^{bc}	36.67 ^{gh}	13.33 ^{gh}	6.67 ^{hi}	6.67 ^{i}	56.67 ^{b}
8 ppm BA + 4 ºC for 4 wks	86.67 ^{ab}	53.33 ^{cd}	5.00 ^{ij}	8.33 ^{h}	8.33 ^{hi}	38.33 ^{d}
4 (6) ppm GA_3 + 4 ºC for 4 wks	33.33 ^{hi}	33.33 ^{ghi}	51.67 ^{b}	43.33 ^{b}	15.00 ^{fg}	23.33 ^{ghi}
8 ppm BA +4 (6) ppm GA_3 +4 ºC for 4 wks	81.67 ^{bc}	30.00 ^{hij}	6.67 ^{hij}	1.67 ^{i}	11.67 ^{ghi}	68.33 ^{a}

*Treatments used for aseptic culture condition only
() Treatments used for greenhouse condition only
ND: Not determined
Numbers in the same column with the same letter(s) are not significantly different at 0.05 probability level by the Least Significant Difference (LSD).

Table 3. Percentages of germination and dormancy of seeds of Jerusalem artichoke varieties treated with BAP at concentration of 8 ppm cultured under aseptic and greenhouse conditions

Genotype	% Germination		% Dormancy		% Dead Seed	
	Aseptic	Greenhouse	Aseptic	Greenhouse	Aseptic	Greenhouse
CN 52867	93.3	93.3	0.0	0.0	6.7^{bcd}	6.7
HEL 53	93.3	91.7	5.0	0.0	1.7^{d}	8.3
HEL 54	91.7	93.3	5.0	0.0	3.3^{cd}	6.7
HEL 65	91.7	90.0	1.7	0.0	6.7^{bcd}	10.0
HEL 66	91.7	93.3	3.3	0.0	5.0^{bcd}	6.7
HEL 321	90.0	91.7	1.7	0.0	8.3^{bc}	8.3
HEL 322	93.3	90.0	3.3	0.0	3.3^{cd}	10.0
HEL 335	90.0	83.3	3.3	0.0	6.7^{bcd}	16.0
JA 37	93.0	85.0	0.0	0.0	6.7^{bcd}	15.0
JA 38	91.7	91.7	0.0	0.0	8.3^{bc}	8.3
JA 41	85.0	91.7	0.0	0.0	15.0^{a}	8.3
JA 81	90.0	88.3	0.0	0.0	10.0^{ab}	11.7

Numbers in the same column with the same letter(s) are not significantly different at 0.05 probability level by the Least Significant Difference (LSD).

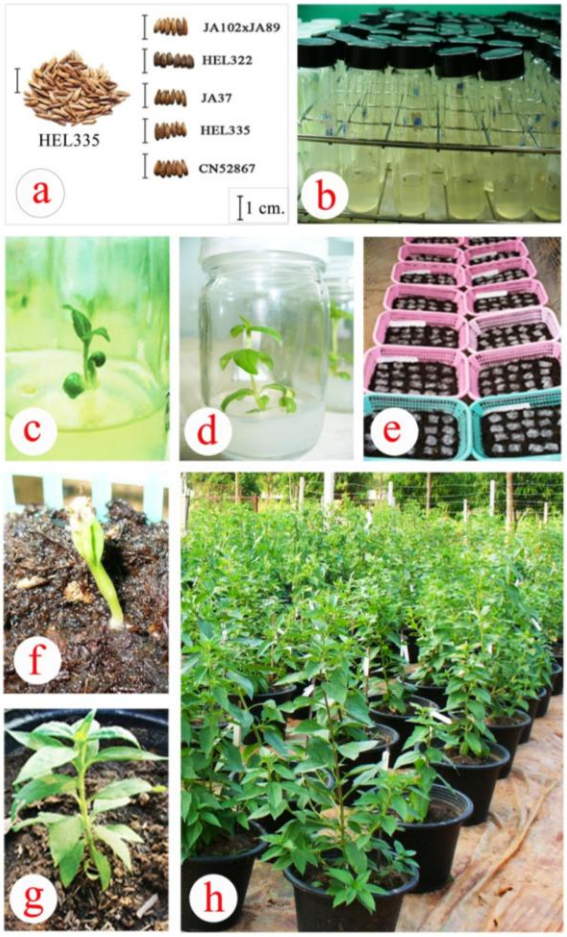

Figure 1. Seed germination in the Jerusalem artichoke: achenes (a); seeds treated under aseptic conditions (b); *in vitro* seedlings on MS basal medium free hormone (c, d); treated seeds transferred to peat moss medium in the greenhouse (e); seedling growth in peat moss medium (f); plants of Jerusalem artichoke in pots (g, h).

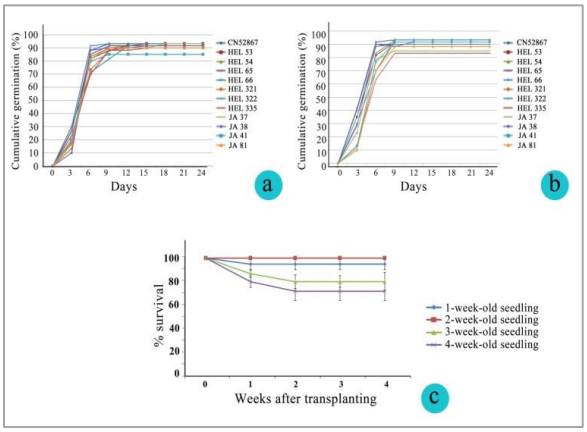

Figure 3. Percentages of cumulative germination of Jerusalem artichoke varieties treated with BAP at a concentration of 8 ppm under aseptic conditions (a) and under greenhouse conditions (b); percentage of survival of JA102xJA89 Jerusalem artichoke seedlings grown *in vitro* and transplanted at different ages under greenhouse conditions (c).

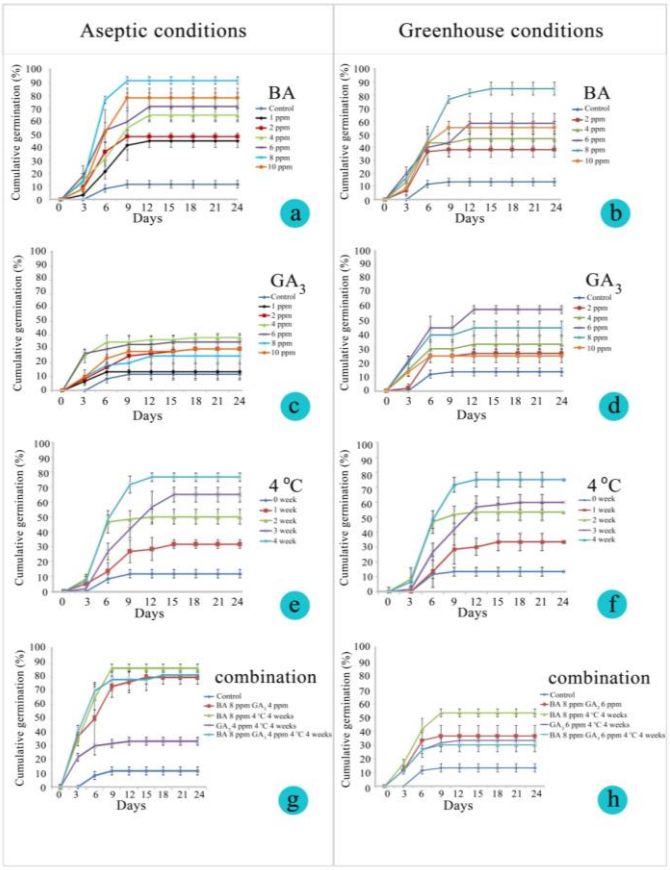

Figure 2. Percentages of cumulative germination of the Jerusalem artichoke hybrid 102xJA89 treated with BAP (a and b), GA_3 (c and d), chilling (e and f), and a combination of growth regulators and chilling at 4 ºC (g and h) under aseptic conditions (a, c, e and g) and greenhouse conditions (b, d, f and h).

DISCUSSION

Plant growth regulators play an important role in the regulation of seed germination and plant growth by breaking seed dormancy. The method to supplement plant growth regulators is one of the key successes in seed germination under laboratory conditions or when cultivation is attempted [29]. Present results demonstrate that supplementation of BAP, GA_3 and chilling is involved in breaking the dormancy of Jerusalem artichoke seeds under both *in vitro* and *in vivo* conditions. Our findings were confirmed by a number of earlier reports in other plants [30-31]. In one report, a treatment of lotus nuts (*Nelumbo nucifera*) supplemented with 0.8-3.5 µM BAP followed by chilling at 4 ºC for four weeks and addition of GA_3 induced the highest germination [31].

Cytokinins regulate diverse processes from embryonic development to adult plant growth [32-33]. Increase in cytokinins may result in the breaking of dormancy, perhaps by overcoming the effect of endogenous inhibitors [34]. Our results show that 8 ppm concentration of BAP (cytokinin) significantly reduces dormancy in the Jerusalem artichoke both *in vivo* and *in vitro* (Table 2 and Figures 2a

& 2b). These results are consistent with those of Nikolic et al [31], who reported that most of the cytokinins including BAP and TDZ influenced seed germination in *Lotus corniculatus* and *Carica papaya*. The cytokinins probably penetrate through the testa and neutralise the inhibitors present in the embryo, thus enabling it to rupture the seed coat [35]. Cytokinins are not necessary for germination, but they can affect the germination inhibitor ABA [36].

Several growth regulators including gibberellins promote germination in dormant or partially dormant seeds. GA is effective in stimulating germination in seeds with a shallow dormancy. Our study showed that under greenhouse conditions GA_3 at 2 and 4 ppm did not markedly affect Jerusalem artichoke seed dormancy, but a 6 ppm concentration of GA_3 significantly reduced dormancy. Application of GA_3 to seeds in a greenhouse at 6 and 8 ppm for two days showed much higher germination and a decrease of dormant and dead seeds compared to the same concentration treated for seven days under aseptic conditions. In contrast to our own findings, Yang et al [37] reported a slightly higher germination percentage of 95% in seeds of the trianda palm (*Areca trianda*) treated with GA_3 (250 mg/l) compared to a germination percentage of 85% when treated with BAP (5-25 mg/l). The supplementation of gibberellins improves seed germination rate by increasing the amino acid availability in embryo and they cause the releasing of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination [38].

Stratification is the most efficient factor and that in some plants imbibed seeds germination with the highest rate only occurs in chilling temperature. It indicates that glycolysis, respiratory system, pentose phosphate pathway, citric acid cycle and protein synthesis do not start in dry seeds at room temperature [39]. Generally for seed germination, seed imbibition, respiration and protein synthesis will provide necessary energy (ATP) [40]. Evidence shows that under moist-chilling conditions many genes in dormant seeds are active and some of these genes are under control of plant hormones [41]. El-Dengawy also found that chilling at 4 ºC for 60 days lead to high germination of 93% although the differences were not significant when compared with those treated with GA_3 and BAP [41]. In the present work, following stratification for four weeks, 76.7% and 75% seed germination was achieved in aseptic and greenhouse conditions. The results shown in Table 2 and Figures 2e and 2f indicate that four weeks of exposure to chilling significantly improved germination compared to shorter exposure. Figures 2e and 2f showed that cold-exposure for four weeks was the best chilling treatment for Jerusalem artichoke seed germination, even if it is still less effective than BAP treatment. The highest germination percentages were observed in both aseptic and greenhouse conditions as early as 12 days after initiation of germination. Durations of chilling for two and three weeks could be confounded at different evaluation times, but in general seeds chilled for two weeks showed a clearly higher germination percentage than the seeds chilled only for one week.

The participation of more than one plant growth regulator in biological systems is not un-common. An interplay of hormones may result in synergistic, antagonistic, additive, and permissive effects. BAP acts synergistically with gibberellins, chilling and probably also with other plant growth regulators; which might be called a system approach or synergism. Cytokinins (CKs) and gibberellins (GAs) are actively involved in dividing of seed tissues and breaking of dormancy after seed imbibitions which results in germination and growth of the dormant embryos [42-43]. In our study, concomitant application of growth regulators (BAP and GA_3) and chilling all successfully increased the seed germination of the Jerusalem artichoke. The ineffectiveness of exogenous GA_3 along with cold-stratification has previously been reported in *Ferula assa-foetida*, *Heracleum sphondylium* and *Chaerophyllum temulum* [23, 44-45]. We found that seeds treated with 6 ppm GA_3 for two days under greenhouse conditions showed lower percentage of dormancy than under aseptic conditions when seeds were treated with 4 ppm GA_3 for seven days (Table 2). This observation is confirmed by the results of the combined effects of concomitant application of BAP, GA_3 and chilling. The positive effect of BAP (8 ppm) on seed germination was decreased when it was co-applied with GA_3 or chilling at 4 ºC. This is in contrast to Sharifi and Pouresmael [18] who found that the positive effect of BAP was increased when it was co-applied with GA_3 in breaking the seed dormancy of *Bunium persicum*. The exact role of cytokinins in seed germination has yet to be solved. However, cytokinins certainly can break dormancy in seeds with a chilling requirement or with light requirement [46]. Our results indicated that when BAP was present, it was not necessary to include GA_3 and chilling. With chilling it was not necessary to include GA_3. Inclusion of these treatments results in lower germination.

Twelve Jerusalem artichoke varieties were treated with 8 ppm BAP and tested for germination percentages under aseptic and greenhouse conditions. They showed very similar germination percentages, indicating that there was no significant variation in response to 8 ppm BAP (Table 3 and Figures 3a & 3b). The plants later developed well in pots (Figures 1g and 1h).

In conclusion, the results of our investigation clearly demonstrated that plant growth regulators such as BAP and GA_3 as well as chilling can successfully enhance the seed germination of the Jerusalem artichoke. Among the tested growth regulators, 8 ppm BAP was the most effective in increasing seed germination and reducing dormancy. Our findings may also apply to other plant species with low germination rates.

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