

## GIBBERELIC ACID EFFECT ON TUBER DORMANCY OF JERUSALEM ARTICHOKE

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### Abstract

Tuber dormancy of Jerusalem artichoke (*Helianthus tuberosus* L.) is an important factor hindering breeding progress and production as Jerusalem artichoke tubers do not germinate uniformly and environmental conditions greatly affect germination. Freshly-harvested tubers are normally used for planting of a subsequent crop, and low germination is a problem. The experiments were conducted in greenhouses at Khon Kaen University, Thailand and at Iowa State University, USA to explore other innovative methods to improve germination of Jerusalem artichoke seed tubers. The objective of this study was to determine the effects of Gibberellic acid (GA) on the dormancy of Jerusalem artichoke seed tubers. GA at all concentrations increased germination percentage, shoot length and shoot dry weight of seed tubers. All the varieties of Jerusalem artichoke germinated uniformly under various GA treatments between 4.5 and 8.0 days after planting. The highest rate of germination was noted for GA at a concentration of 1%.

**Key words:** Biological systems, Sunchoke, Environmentally controlled greenhouses, Dormancy, Germination, Seedling.

### Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a carbohydrate containing crop which accumulates inulin in its tubers (Kays & Nottingham, 2008). Inulin is considered to be a functional food ingredient that affects physiological and biochemical processes in human beings, resulting in better health and reduction in the risk of many diseases (Kaur & Gupta, 2002). Moreover, the sugar from 1 acre of Jerusalem artichoke production can produce 500 gallons of alcohol, making the plant an attractive species for use as a raw material for biofuel production (Maet *al.*, 2011).

For breeding of Jerusalem artichoke, it is essential to have access to genetically diverse germplasm. A key barrier in Jerusalem artichoke breeding program is overcoming tuber dormancy, which results in variable germination rate. Dormancy of seed tubers hinders the progress of breeding programs, since diverse Jerusalem artichoke varieties possess different levels of tuber dormancy.

Poor tuber germination is the major limiting factor for large-scale propagation of Jerusalem artichoke. In the rain-fed upland areas of Southeast Asia, farmers usually grow Jerusalem artichoke in double cropping systems the first crop in the early rainy season (March to April) and second crop in the late rainy season (August to September). These farmers also use newly-harvested seed tubers of Jerusalem artichoke from the first crop as planting material for the succeeding crop, and seed tuber dormancy prevents timely and uniform germination.

Due to their dormancy, Jerusalem artichoke tubers cannot be planted immediately after harvest. The type of dormancy of Jerusalem artichoke is termed endo-

dormancy, and is related to physiological factors within plant organs (Kays & Nottingham, 2008). Seed tuber dormancy can be overcome by storing the seed tubers at 2°C for 8 weeks, but this practice is time-consuming and impractical for breeders and farmers (Kantar & Betts, 2012). Application of gibberellic acid (GA) is another way to break dormancy in Jerusalem artichoke. Although this process takes less time than cold treatment, Jerusalem artichoke genotypes respond differently to GA (Kantar & Betts, 2012). It is possible that different concentrations of GA may be optimal for different Jerusalem artichoke genotypes.

Published information on the effects of concentration of GA on emergence of Jerusalem artichoke seed tubers is limited. The goal of this study was to determine responses (germination percentage, days to sprouting, shoot length and shoot dry weight) of Jerusalem artichoke genotypes to different concentrations of GA. Concentrations of GA that induce maximum germination of seed tubers can be used in Jerusalem artichoke breeding programs and commercial production of Jerusalem artichoke.

### Materials and Methods

**Experimental design:** Two experiments were carried out using different seed tuber lots and Jerusalem artichoke varieties. Experiment 1 was conducted in greenhouse at the Field Crop Research Station of Khon Kaen University, Thailand. Four levels of gibberellic acid (GA) (0%, 1%, 2% and 3%) and three Jerusalem artichoke varieties (JA 89, HEL 65 and JA 102×JA 89(8)) were assessed in a 4×3 factorial experiment with a randomized complete block design having four replications.

Experiment 2 was conducted in the greenhouse at the Department of Plant Pathology and Microbiology at Iowa State University, USA. Four concentrations of GA (0%, 1%, 2% and 3%), and three varieties of Jerusalem artichoke (PI 664618, PI 503269 and PI 664596) were tested in a 4×3 factorial experiment with a randomized complete block design having four replications.

**Seed tuber and GA solution preparation:** Freshly harvested and mature tubers of uniform size were selected for each variety. Tubers were washed and cut into small pieces with two buds per piece. In experiment 1, 11.1 g, 22.2 g, or 33.3 g of the GA powder (gibberellic acid, 90%, Panpan Industry Co., Limited, China) was added to 1000 ml of distilled water in a beaker along with 3.2 ml of potassium hydroxide (KOH) solution (6 mol/liter) to dissolve the GA powder. Distilled water without GA and KOH served as a control. Each experimental unit was consisted of 100 JA seed tubers. The seed tubers were soaked in each GA solution treatments for 2 minutes.

In experiment 2, seed tubers were immersed in GA (4%; ProGibb®; Valent, Memphis, TN, USA) solution with the concentrations (without KOH) mentioned previously for 2 minutes.

**Growing method, data collections and statistical analysis:** In the experiment 1, the pot medium was burned rice hulls. In experiment 2, the pot medium was a mixture of four parts of peat moss and three parts of perlite. After immersion in GA solution, seed tubers were planted in pot trays at 2.50 cm under the soil surface, and all pot trays were placed in a greenhouse with 23.6–30.7°C at KKU and 23.1–25.7°C at ISU. The number of days to observation of >90% germination of seed tubers was recorded until 14 days after planting. Data were also recorded for germination percentage, shoot length (cm) and shoot dry weight (mg/tuber) at 7 days after planting.

Data were analyzed statistically by STATISTIX-8 software program (Statistix 8, 2003). Means were compared by least significant difference (LSD) at 5% significance level.

## Results

**Analysis of variance:** Differences in days to 90% sprouting among GA concentrations were significant ( $p \leq 0.01$ ) in experiment 1, but the differences were not significant in experiment 2 (Table 1). Differences among Jerusalem artichoke varieties were significant ( $p \leq 0.01$ ) for days to sprouting (>90% germination of seed tubers)

in experiment 1 and experiment 2, whereas the interaction between GA and Jerusalem artichoke variety was significant only in experiment 2.

Differences among GA concentrations were significant ( $p \leq 0.01$ ) for germination percentage, shoot dry weight and shoot length in each experiment (Tables 2, 3). However, differences among Jerusalem artichoke varieties were significant ( $p \leq 0.01$ , and 0.05) for these parameters in experiment 1, but in experiment 2 significant difference ( $p \leq 0.05$ ) was observed only for germination percentage. Interactions between genotype and GA concentration were significant ( $p \leq 0.01$ , 0.05) for germination percentage, shoot dry weight and shoot length in experiment 1, but, in experiment 2, significant interaction ( $p \leq 0.01$ ) was observed only for germination percentage.

**Table 1. Mean square values from analysis of variance for days to sprouting of three Jerusalem artichoke varieties grown under four gibberellic acid doses in experiment 1 at KhonKaen University (KKU), Thailand and experiment 2 at Iowa State University (ISU), USA.**

Source of variance	Time to sprouting (days)	
	Experiment 1 (KKU)	Experiment 2 (ISU)
Replication	0.47	6.85
Gibberellic acid (GA)	81.02**	0.30 <sup>ns</sup>
Variety (V)	1.40**	44.44**
GA × V	0.23 <sup>ns</sup>	40.55**
Error	0.24	1.76
CV%	12.52	17.85

<sup>ns</sup> Non-significant at  $p > 0.05$ ; \*\* Significant at  $p \leq 0.01$

**Effect of gibberellic acid on days to sprouting:** Days to sprouting were evaluated when seed tubers for each treatment had reached 90% of sprouted seedlings (14 days after planting). In experiment 1, JA 89, HEL 65 and JA 102 × JA 89 (8), which received 0% of GA, did not reach 90% of sprouting within 14 days after planting, whereas these Jerusalem artichoke varieties receiving 1%, 2% and 3% of GA could reach 90% of sprouting between 4 and 6 days (Fig. 1a).

In experiment 2, PI 664618 of untreated control did not reach 90% of sprouting within 14 days after planting, and PI 503269 and PI 664596 reached 90% of sprouting only after 9 and 11 days, respectively (Fig. 1b). All Jerusalem artichoke varieties treated with GA concentrations of 1%, 2% or 3% sprouted between 6.5 and 8 days, which were significantly faster than the untreated control.

**Table 2. Mean square values from analysis of variance of percentage of tuber germination shoot dry weight and shoot length of three Jerusalem artichoke varieties (JA 89, HEL 65, JA 89 × JA 102 (8)) treated with four gibberellic acid doses at 7 days after transplanting in experiment 1 at KhonKaen University, Thailand (experiment 1).**

Source of variance	Percentage of tuber germination	Shoot dry weight (mg/tuber)	Shoot length (cm)
Replication	100	49.45	0.31
Gibberellic acid (GA)	10432**	94.71**	6.33**
Varieties (V)	220*	135.18**	1.82**
GA × V	209**	43.62**	0.79*
Error	38	5.30	0.17
CV%	7.88	14.9	14.39

\*, \*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively

**Table 3. Mean square values from analysis of variance of percentage of tuber germination, shoot dry weight and shoot length of three Jerusalem artichoke varieties (PI 66418, PI 503269 and PI 664596) treated with four gibberellic acid doses at 7 days after transplanting in experiment 2 at Iowa State University, USA (experiment 2).**

Source of variance	Percentage of germination	Shoot dry weight (mg/tuber)	Shoot length (cm)
Replication	11	20.41	0.57
Gibberellic acid (GA)	234**	94.92**	8.10**
Varieties (V)	106*	29.35ns	1.34ns
GA*V	148**	24.57ns	2.55ns
Error	26	10.57	1.00
CV%	5.49	16.02	17.22

ns = Non-significant at  $p > 0.05$ ; \*,\*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively

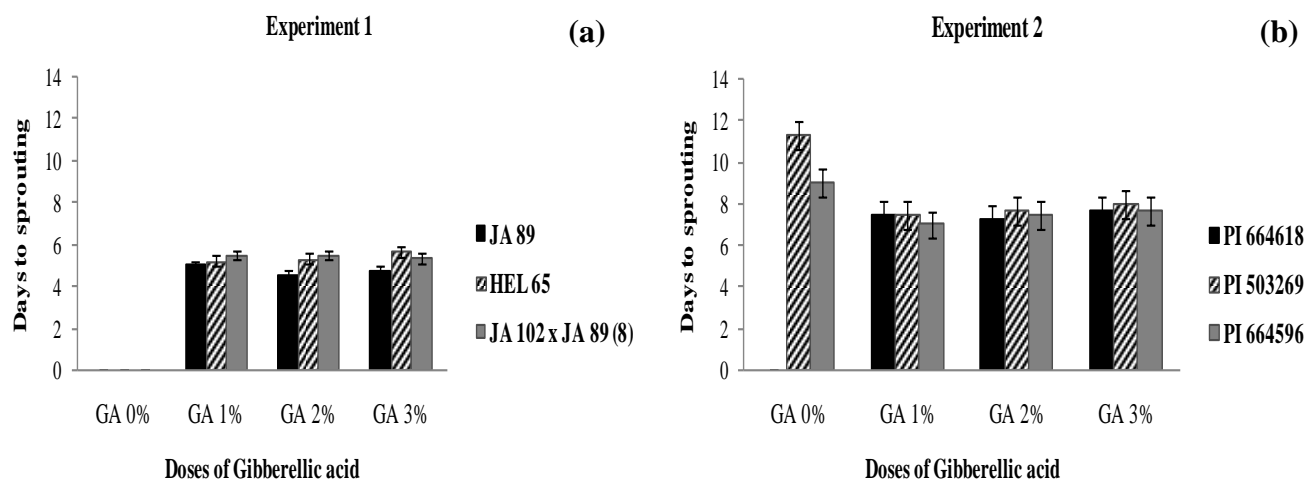


Fig. 1. Effect of difference of gibberellic acid (GA) doses (0%, 1%, 2% and 3%) on days to sprouting in Jerusalem artichoke varieties JA 89, HEL 65 and JA 102 x JA 89(8) in experiment 1 (a), and PI 664618, PI 503269 and PI 664596 in experiment 2 (b)).

**Effect of gibberellic acid on germination percentage:** For the untreated control treatment, variety HEL 65 did not germinate at all by 14 days after planting, whereas JA 89 and JA 102 x JA 89 (8) germinated at 8.5% and 39%, respectively (Fig. 2a). Application of GA increased 14-day germination percentages as indicated by higher germination percentages at concentrations of 1% (98–100%), 2% (99–100%) and 3% (99–100%) (Fig. 2a, b, c, d).

In experiment 2, initial germination percentages of untreated control plants ranged from 79 to 93% (Fig. 2e). The results suggested that seed tubers of Jerusalem artichoke in this experiment were not truly dormant, as germination rates were relatively high in the absence of GA. Application of GA at 1 or 2% slightly increased germination percentages (98 to 100% for 1% GA and 93 to 99% for 2% GA). However, application of GA at high concentration of 3% reduced germination percentages in PI 503269 (70%) and PI 664596 (92%), whereas it still increased germination percentage in PI 664618 (97%) compare to untreated control.

**Effects of gibberellic acid on shoot length and shoot dry weight:** In experiment 1, shoot length of HEL 65 was recorded as zero (non-germinated), in contrast to 1.94 and 2.11 cm for JA 89 and JA 102 x JA 89 (8), respectively (Fig. 3a). Jerusalem artichoke varieties treated with GA at concentrations of 1%, 2% and 3% had shoot lengths from 2.93 to 4.02 cm, 3.13 to 3.99 cm and 2.82 to 3.63 cm, respectively, which were significantly higher than 0.0 to

2.11 cm for the untreated control (Fig. 3a, b, c, d). HEL 65 shoot dry weight was zero, whereas JA 89 and JA 102 x JA 89 (8) had shoot dry weights of 14.93 and 14.00 mg/tuber, respectively (Fig. 4a). Application of GA at concentrations of 1%, 2% and 3% significantly increased shoot dry weight in JA 89 and HEL 65, but it not in JA 102 x JA 89 (8) (Fig. 4a, b, c, d). Differences among Jerusalem artichoke genotypes were significant for shoot dry weight at GA concentrations of 1%, 2% and 3%; JA 89 had the highest shoot dry weights, followed by HEL 65 and JA 102 x JA 89 (8), respectively.

In experiment 2, shoot lengths were 3.69, 6.55 and 4.55 cm for PI 664618, PI 503269 and PI 664596, respectively (Fig. 3e). In PI 664618, application of GA at concentrations of 1%, 2% and 3% significantly increased shoot lengths to 8.66, 6.14 and 5.51 cm, respectively (Fig. 3e, f, g, h). Application of GA at the same rates for PI 503269 reduced shoot length at the rate of 3%. Application of GA at the rates of 1% and 2% increased shoot lengths to 7.21 and 4.85 cm, respectively, in PI 664596. Shoot dry weights were recorded as 12.45, 20.49 and 21.16 mg/tuber for PI 664618, PI 503269 and PI 664596, respectively (Fig. 4e). Application of 1% GA resulted in a significant increase in shoot dry weight for PI 664618, PI 503269 and PI 664596, but application of gibberellic acid at the concentrations of 2% and 3% resulted lower shoot dry weights (Fig. 4e, f, g, h). Percent germinations of three Jerusalem artichoke varieties treated with four concentrations of gibberellic acid are presented in (Fig. 5.)

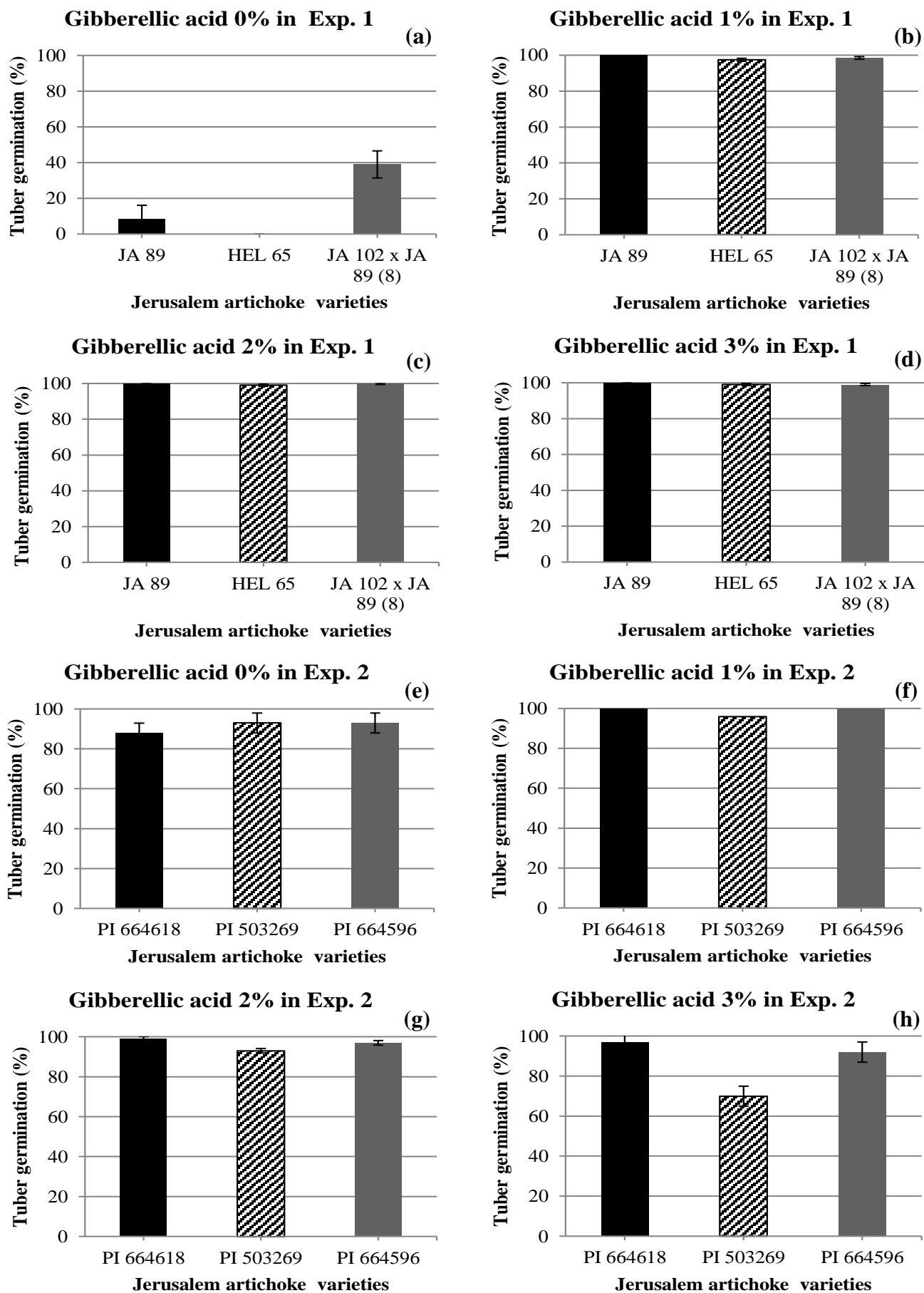


Fig. 2. Effect of four gibberellic acid (GA) doses (0%, 1%, 2% and 3%) on percentage of tuber germination in experiment 1 (Exp. 1) (a – d) and in experiment 2 (Exp. 2) (e – h) at 7 days after planting. Error bars indicate the SE for each individual gibberellic acid doses.

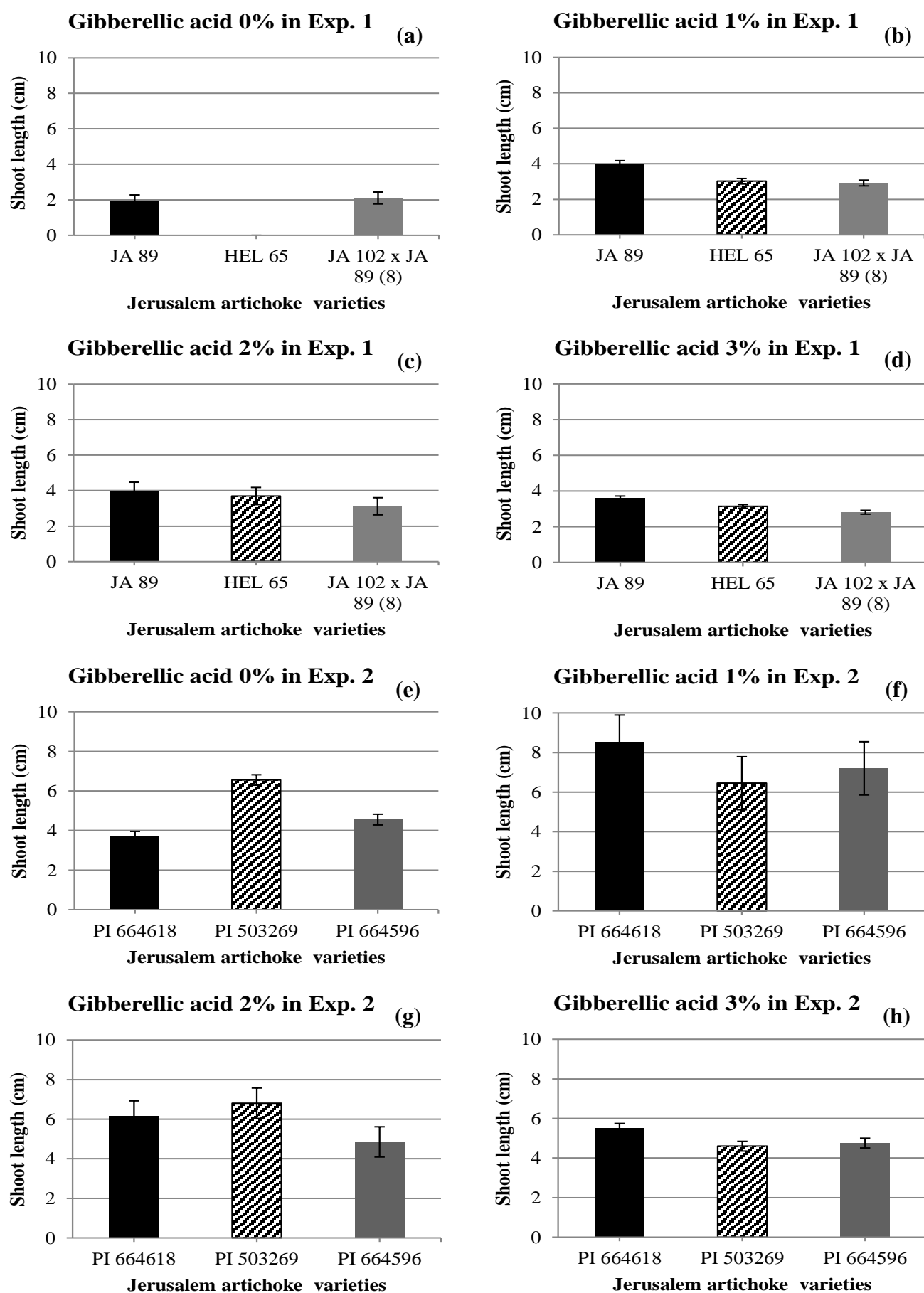


Fig. 3. Effects of four gibberellic acid (GA) doses (GA 0%, GA 1%, GA 2% and GA 3%) on shoot length (cm) in experiment 1 (Exp. 1) (a–d) and in experiment 2 (Exp. 2) (e–h) at 7 days after planting. Error bars indicate the SE for each individual gibberellic acid doses.

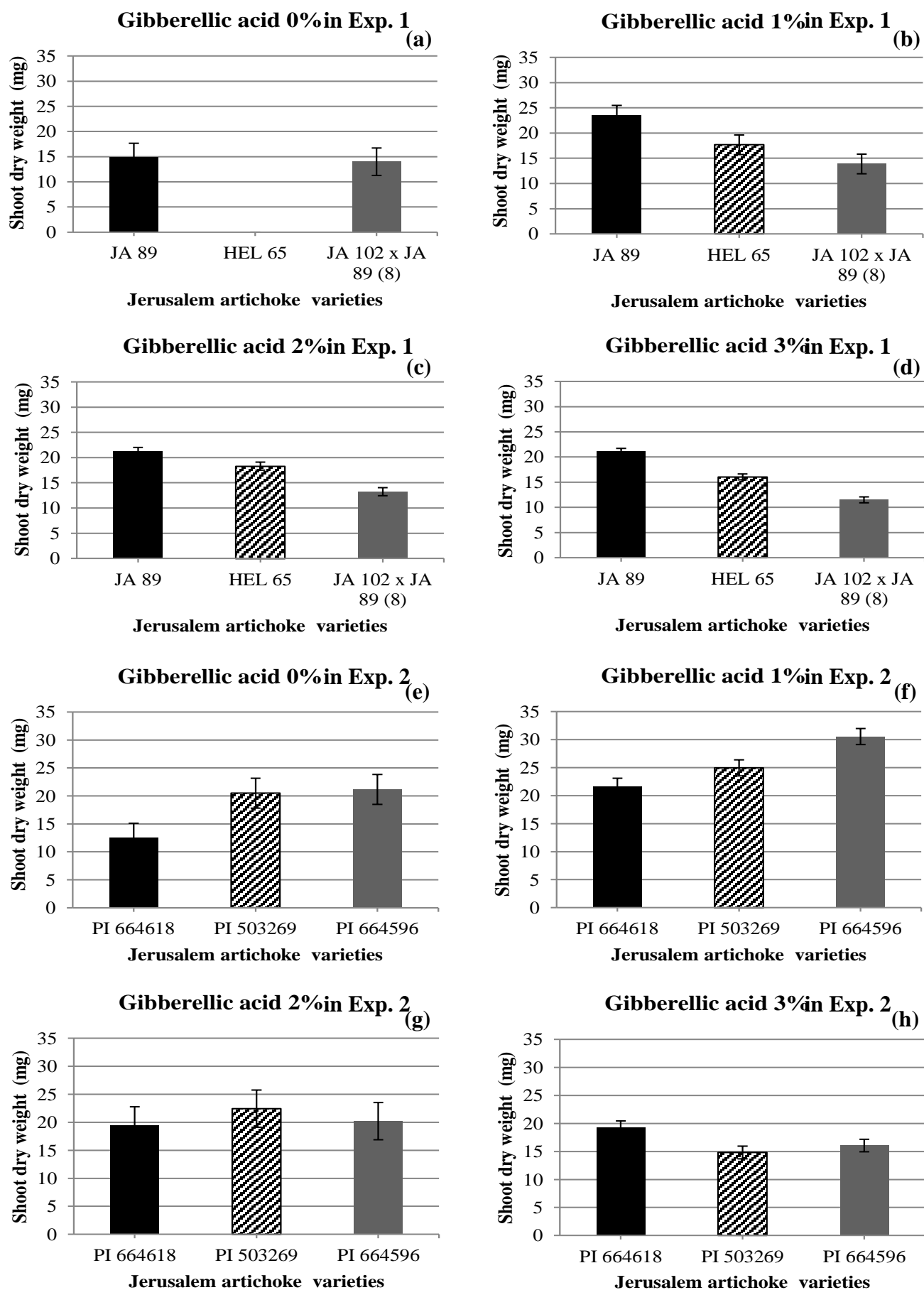


Fig. 4. Effect of four gibberellic acid (GA) doses (GA 0%, GA 1%, GA 2% and GA 3%) on shoot dry weight (mg/tuber) in experiment 1 (Exp. 1) (a – d) and in experiment 2 (Exp. 2) (e – h) at 7 days after planting. Error bars indicate the SE for each individual gibberellic acid doses.



Fig. 5. Effect of four gibberellic acid (GA) doses (GA 0%, GA 1%, GA 2% and GA 3%) on sprouting in three Jerusalem artichoke varieties of experiment 2 at 7 days after planting.

## Discussion

Seed tuber dormancy of Jerusalem artichoke inhibits its varietal evaluation for breeding programs because seed tubers do not germinate uniformly. Ideally, seed tubers should germinate uniformly in a relatively short period of time, so experiments were limited at 14 days after the seed tubers were treated with gibberellic acid. In this study, seed tuber dormancy of six Jerusalem artichoke varieties was overcome by application of gibberellic acid at different concentrations from 0 to 3% under greenhouse conditions in two independent experiments. When germination percentage is considered, only HEL 65 was truly dormant because it did not germinate in the absence of GA, and dormancy was overcome by 1%, 2% and 3% GA. Other varieties were not dormant because they germinated at relatively high rates in the absence of added GA. For these varieties, application of GA at low concentration resulted in small increase in germination percentage, but inhibited germination at higher.

The gibberellic acid was used to break tuber and seed dormancy in many crops such as potato (Alexopoulos *et al.*, 2008) and *Trifolium rubens* (Jeremi, 2018). Kantar & Betts (2012) showed that gibberellic acid at 2% could break dormancy of most Jerusalem artichoke varieties in their study except for JA 318F. The genetic and physiological mechanisms of inheritance of seed tuber dormancy have not been studied in Jerusalem artichoke. Analyses for quantitative trait loci (QTL) in potatoes found between six (Freyre *et al.*, 1994) and nine (Van Den Berg *et al.*, 1996) genes that affected seed tuber dormancy. Based on our results and previous investigations (Kantar & Betts, 2012), seed tuber dormancy in Jerusalem artichoke may be classified into three categories: no dormancy, normal dormancy and recalcitrant dormancy, and the inheritance of seed tuber dormancy is hypothesized to be polygenic. Non-dormant seed tubers are preferable in terms of production and multiplication. However, non-dormant tubers can sprout during storage. If the inheritance of seed tuber dormancy is well understood, selection against dormancy would be a promising mean to resolve this problem in Jerusalem artichoke breeding.

When days to sprouting were considered, it was apparent that three Jerusalem artichoke varieties in experiment 1 and one in experiment 2 showed seed tuber dormancy as they did not reach 90% of sprouting at 14 days after seed tubers were treated with GA. Application of gibberellic acid at concentrations of 1%, 2% and 3% reduced the number of days to sprouting (within 4.5–8 days after planting) in all Jerusalem artichoke varieties.

Evaluation of sprouting process in this study was limited at 14 days, if seed tubers are used directly as planting materials, they should sprout early and uniformly for good establishment of the crop. Time to sprouting is therefore another criterion for evaluation of seed tuber dormancy in Jerusalem artichoke. The results indicated that all Jerusalem artichoke genotypes required GA to reduce sprouting time.

In previous investigations on Jerusalem artichoke, application of GA required 6.5 to 11.5 days to sprout in the majority of genotypes tested and was shorter time than the treatment at 2°C for eight weeks, showing 63.6 to 67.5 days to sprout (Kantar & Betts, 2012). In potatoes, application of gibberellic acid induced sprouting within 21 days, which was earlier than for the untreated control (Shibairo *et al.*, 2006; Alexopoulos *et al.*, 2008). In this study, days to sprouting of gibberellic-treated tubers were in the range of 4.5–8 days.

Days to sprouting in this study were also in the range of previous finding for Jerusalem artichoke but much earlier than those for potatoes. Days to sprouting at this range were sufficient for uniform establishment of the crop, and application of GA at the rate of 1% was sufficient to reduce germination time satisfactorily.

Application of GA at low concentrations generally increased shoot length. However, application of GA at high concentrations reduced shoot length, especially for PI503269, which was not dormant in the absence of added GA. This pattern was similar for shoot dry weight. Percentage of tuber germination, shoot length and shoot dry weight at 14 days were lower than at 7 days and the sprouted seedlings showed tip injury (data not shown).

Seedlings of all the varieties treated with gibberellic acid were also thinner and longer than untreated control (Fig. 5). Seedlings of potato treated with gibberellic acid were thinner and longer than those that sprouted naturally (Alexopoulos *et al.*, 2008). This difference may result from the fact that breaking dormancy naturally requires greater physiological age, which affects membrane integrity (Knowles & Knowles, 1990) and metabolic activity of cell division and elongation (Taiz & Zeiger, 2002). High doses of GA and longer duration of exposure to gibberellic acid may cause injury to the seedlings. Gibberellic acid plays an important role in cell elongation of stems in pea (Arney & Mancinelli, 1966). In potatoes, application of GA at low concentration (< 20 mg/l) was sufficient to break dormancy and promote sprouting of potato tubers (Van Ittersum, 1992; Shibairo *et al.*, 2006) at GA concentrations of 5 to 100 mg/l (Bryan, 1989). The results from this study and other studies suggested that a concentration of gibberellic acid at 1% may be too high for achieving an optimal balance between disruption of dormancy and stimulation of normal shoot growth; further research is required to determine an optimal concentration for Jerusalem artichoke.

Interestingly, some Jerusalem artichoke varieties used in this study were not dormant. However, selection against seed tuber dormancy may be possible once inheritance for this trait is clearly understood. Selection against dormancy is likely to be the most effective mean to eliminate seed tuber dormancy in Jerusalem artichoke breeding populations. Information to guide selection against dormancy of Jerusalem artichoke seed tubers is not available in the literature. However, some study in annual ryegrass (*Lolium rigidum*) found that the mutual selection of low dormancy and constitutive  $\alpha$ -amylase activity could enhance establishment of the seedling (Goggin & Powles, 2012). Further study of inheritance of tuber dormancy in Jerusalem artichoke may lead to advances that benefit both breeders and farmers.

The interactions between GA and variety were significant for days to sprouting and percentage of tuber germination in experiment 2 only. The results indicated differential responses of Jerusalem artichoke genotypes to varying concentrations of GA for these parameters. Based on this finding, the optimum treatment for one genotype may not be optimum for others; therefore, it may be necessary to customize dormancy treatments to genotypes in order to optimize their effectiveness.

Application of 1% GA had the highest tuber germination percentage, shoot length and shoot dry weight in all Jerusalem artichoke varieties. GA concentrations of 2% and 3%, however, inhibited germination percentage and growth parameters, possibly due to toxicity at high concentrations. Therefore, application of GA at 1% was most effective for breaking seed tuber dormancy in Jerusalem artichoke. Application at lower concentrations may yield better results, but application at >1% is not recommended.

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