The effects of gibberellic acid (GA3), Ethrel, seed soaking and pre-treatment storage temperatures on seed germination of *Helianthus annuus* and *H. petiolaris*

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ABSTRACT

A moderate concentration of GA3 (500 ppm) with one wk stratification at 4°C was very effective in increasing the germination rate of recalcitrant native sunflower seeds (80% vs. 30% control). Stratification (1 wk at 4°C) increased germination, regardless of the seed treatment. Ethrel (25 ppm) treatment was effective, but not as much as GA3 (500 ppm). Soaking sunflower seed in water for 12 or 16 hr resulted in no seed germination. Published on-line www.phytologia.org *Phytologia 98(3): 213-218 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Helianthus annuus, H. petiolaris*, seed germination, dormancy, gibberellic acid (GA3), Ethrel, Florel, Ethephon.

Seed dormancy in wild sunflowers can be a problem for studies of hybridization and in crop breeding. In a recent seed shipment from GRIN, USDA, 15 accessions of *Helianthus annuus* had germination rates ranging from 13% (PI673305, Utah) to 91% (PI413066, Obregon, Mexico). Two *H. petiolaris* accessions (PI451978, Ellsworth, KS, PI413175, Spencer, NE) both had 14% germination rates. Seiler (1993) reported germination rates of 84% (*H. agrophyllus*), 76% (*H. debilis* ssp. *silvestris*), 51% (*H. petiolaris*) and 44% (*H. annuus*). He noted that dry storage at 20, 0, or -20°C did not affect the germination rate. In addition, Seiler, also found that the length of storage (0, 120, 360 days) did not significantly affect the germination rate.

Seed stratification (5°C) was found (Bratcher, Dole and Cole, 1993) to increase the germination of *H. maximiliani* from 35% (no stratification) to 87% (2 weeks, 5°C), but longer stratification (up to 10 weeks) made little difference in germination.

Soaking in Ethrel (that produces ethylene), water , 25% acetone soaking, or potassium nitrate (0.2%) all increased germination in *H. annuus*, with soaking in plain water (24-36h, 20°C) giving the largest increase in germination (Maiti et al. 2006a). Interestingly, tests based on 12 genotypes, gave mixed results, with most genotypes improving germination by 10 to 43%. However, the germination of two of the genotypes actually declined with water soaking from 27% to 20% and 33% to 17%. Maiti et al. (2006b) extended their study on water soaking (priming) by the examination of germination after 5, 10, 15, 20, 25, 30, 35 and 40 hrs of soaking (at RT?). They found, with genotype VSF-15046 (*H. annuus*), the maximum germination occurred for seeds soaked 15 and 20 hrs, then declined with increaded soaking time. However, 15 hr water soaking using seed of VSFH-1008 from 13 locations gave greater germination in 10/13 seed lots, no difference for one location and a decline in germination for seeds from two locations.

Ethephon (Ethrel) has been used to break dormancy in sunflower. Kumari and Singh (2000) sprayed seed heads (21 Days After Anthesis, DAA) and obtained an increase in germination from 35.5% to 69.1% at 250 ppm spray. Gibberellic acid (GA3) has been used to enhance germination (Deno, 1993). Pallavi et al. (2010) tested gibberellic acid (GA3), Ethrel (Ethephon),potassium nitrate, water soaking, dry heating, microwave and smoking treatments on the germination of sunflower seed (hybrid KBSH-44).

They reported that GA3 (100 ppm); Ethrel (25 ppm); water soaking (24h); dry heating (80°C, 10 min); and smoking (3h) were all very effective (230 to 240% increase in germination) for KBSH-44 seed.

This is a brief, but sufficient review of methods to enhance sunflower seed germination for the reader to grasp that there are many methods, and some work better than others, but seldom does one method work on all genotypes of a species. Thus, the search for a universal method to apply to recalcitrant seed collections appears to be near, but not quite attained.

The purpose of the present paper is to report on sunflower seed germination tests using various concentrations of GA3 and Ethrel as well as water priming (soaking in water) and temperature stratification.

MATERIALS AND METHODS

All seeds were obtained from GRIN (Germplasm Resources Information Network), USDA.
H. annuus: PI413039, Gettysburg, SD; PI413035, Kearney, NE.
H. petiolaris, PI451978-NC7, Ellsworth, KS.
All seeds were surface sterilized by:
1. Washing with soap/tap water;
2. Dipping in 70% ethanol, 30 sec;
3. Sterilizing by soaking in 20% Chlorox (8.25% sodium hypochlorite) for 30 min.;
4. Thoroughly rinsing in sterilized ddwater (Protocol from Singhung Park, Kansas State University).

Experiment 1. Effects of soaking in 500 and 1000 ppm GA3 at RT(21°C), and soaking in DI (deionized) water, 12 h vs. 16 hr. GA3, gibberellic acid, PlantHarmones.net, 90%. dissolved in 1.0 g in 5 ml ethanol, add to 1 995 ml DI water to produce 1000 ppm stock. Dilute 1/2 with DI water for 500 ppm stock.
Experiment 2. Effects of stratification in 500 and 1000 ppm GA3 for 1 week at 4°C vs. 21°C.
Experiment 3. Effects of stratifying in 29, 100, and 200 ppm Ethrel, 1 week at 4°C. Florel is used to prevent nuisance fruit, remove mistletoe, induce flowering, reduce plant height, increase branching and increase seed germination. Florel is sold as a mixture of 3.9% Ethel [(2-chloroethyl) phosphoric acid] and 96.15 'inert' ingredients. Ethrel ex Florel, Monterrey Florel Brand Growth Regular, HydroGalaxy.com. Florel.

RESULTS

GRIN reported that the germination of *H. petiolaris* (PI451978), ex Ellsworth, KS, was low (14%) using their sunflower germination test protocol: seed soaked in hydrogen peroxide (3%), 5 min., rinsed in water, then soaked in 25 ppm Ethrel, 12hr at RT, then chilled 7-14 days (4° C), then planted onto wetted filter paper (Laura Marek, Lisa Pfiffner, GRIN, pers. comm.). No doubt, germination in *H. petiolaris* would have been lower if Ethrel and stratification were not used (Maiti et al. 2006a; Kumari and Singh, 2000).

Table 1 shows that germination of *H. petiolaris,* on DI (deionized water) wetted filter paper at RT, gave a very low germination rate (10% vs. 14% for GRIN treatment). Saturating the filter paper with 1000 ppm GA3 gave a large increase in germination rate (35%) and an even larger rate (70%) using 500 ppm GA3. Soaking in DI, 12 or 16 hr did not produce any germination (Table 1).

Varying gibberellic acid (GA3) concentration (Deno, 1993) and stratification temperatures using *H. annuus*, PI413039, ex Gettysburg, SD, produced considerable differences (Table 2, Fig. 1). The DI control had 30% germination vs. 50% (1000 ppm GA3, 1 wk, 21°C), 70% (1000 ppm GA3, 1 wk, 4°C), 65% (500 ppm GA3, 1 wk, 21°C) and 80% (500 ppm GA3, 1 wk, 21°C). Notice (Fig. 1) that germination

for both 1000 and 500 ppm GA3 increased when stratification was cold (4°C). Likewise, both 21°C and 4°C tests increased with lower GA3 (500 ppm, Fig. 2). At least in these preliminary tests, the optimum conditions are 500 ppm GA3 with 1 wk at 4°C. Additional, replicated tests are needed (in progress) to determine if a lower concentration of GA3 might be even more effective.

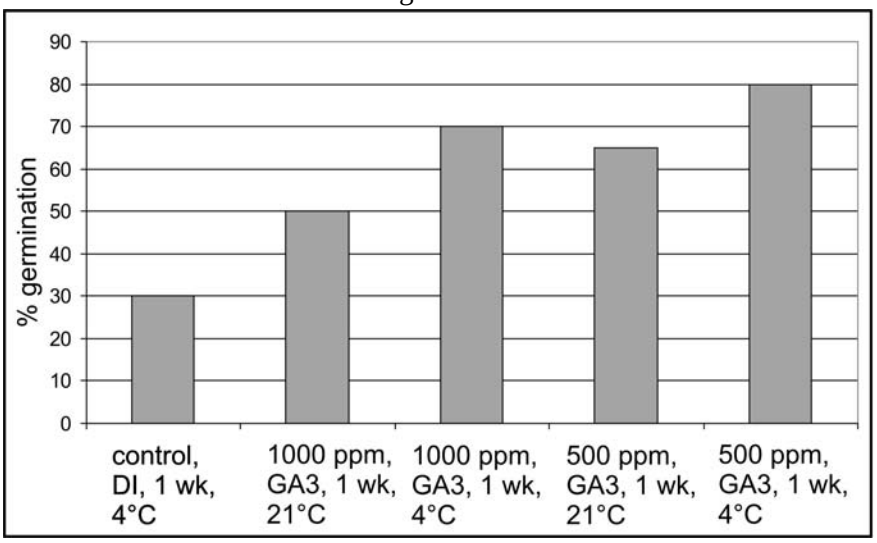

Figure 1. The effects of GA3 concentration and stratification temperature on germination, *H. annuus*, PI413039, ex Gettysburg, SD.

Testing the effects of Ethrel concentrations found the highest germination of *H. annuus*, PI413035, ex Kearney, NE, to be 25 ppm Ethrel (Table 3, Figure 2). This is the concentration used by GRIN and their germination of this lot of PI413035 was 51% (vs. 55% in our test).

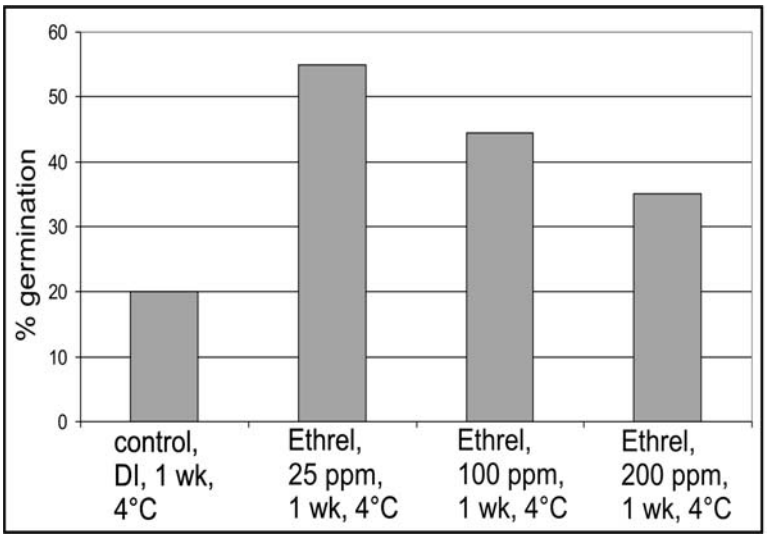

Figure 2. The effects of Ethrel concentration and stratification temperature on germination, *H. annuus*, PI413035, ex Kearney, NE.

In summary, this preliminary report found that a moderate concentration of GA3 (500 ppm), with 1 wk at 4°C, was very effective in increasing the germination (80% vs. 30% control) of recalcitrant native sunflower seeds. Stratification (1 wk at 4°C) increased germination, regardless of the seed treatment. Ethrel (25 ppm) treatment was effective, but not as much as GA3 (500 ppm). Soaking sunflower seeds in water for 12 or 16 hr resulted in no germination.

ACKNOWLEDGEMENTS

This research funded by Baylor University. Thanks to Laura Marek and Lisa Pfiffner, GRIN, USDA, for helpful discussions.

LITERATURE CITED

Bratcher, C. B., J. M. Dole and J. C. Cole. 1993. Stratification improves seed germination of fire native wildflower species. HortScience 28: 899-901.

Deno, N. C. 1993 Seed germination theory and practice. 2nd ed. Privately published. free pdf available from USDA by Google "Deno Seed germination 1993".

Kumari, C. A. and B. G. Singh. 2000. Ethephon adequacy in release of innate dormancy in sunflower. Indian J. Plant Physiol. 5: 277-280.

Maiti, R. K., P. Vidyasagar, S. C. Shahapur, S. K, Ghosh and G. J. Seiler. 2006a. Development and standardization of a simple technique for breaking seed dormancy in sunflower (*Helianthus annuus*). Helia 29: 117-126.

Maiti, R. K., P. Vidyasagar, S. C. Shahapur and G. J. Seiler. 2006b. Studies on genotype variability and seed dormancy in sunflower genotypes (*Helianthus annuus* L.). Indian J. Crop Sci. 1: 84-87.

Pallavi, H. M., R. Gowda, Y. G. Shadakshari and V. Vishwanath. 2010. Study on the occurrence and safe removal of dormancy in sunflower (*Helianthus annuus* L.). Res. J. Ag. Sci. 1: 341-344.

Seiler, G. J. 1993. Wild sunflower species germination. Helia 16: 15-20.

Table 1. Tests of the effects of gibberellic acid (GA3) concentration and soaking on germination of *H. petiolaris*, PI451978-NC7, Ellsworth, KS. Tests: 21 days, RT, ambient room light, 20-21° C.

Treatment	germination	reference
GRIN (germination basis): soak in hydrogen peroxide (3%), 5 min., rinse in water, soak in 25 ppm Ethrel, 12hr at RT, chill 7-14 days (**4° C**?), plant on filter paper (GRIN, USDA)	**25 ppm Ethrel, 1 wk, 4° C** GRIN, Control #1: 14% (% viable = 93%)	GRIN, USDA, Laura Marek and Lisa Pfiffner, (pers. comm.)
1. dry seed, control, filter paper saturated with DI water	**DI water, RT** Lab, Control #2: 2/20 (10%)	Seiler (1993)
2. filter paper saturated with 1000 ppm GA3, RT	**1000 ppm GA3, RT** 7/20 (35%)	Deno, 1993
3. filter paper saturated with 500 ppm GA3, RT	**500 ppm GA3, RT** **14/20 (70%)**	Deno, 1993, 1/2 X GA3
4. seed soaked in DI water, 12 hr., then placed on DI saturated filter paper, RT	**soaked DI, 12 hr, RT** 0/18 (0%)	Maiti, et al. (2006a)
5. seed soaked in DI water, 16 hr., then placed on DI saturated filter paper, RT	**soaked DI, 16 hr, RT** 0/17 (0%)	Maiti, et al. (2006a)

Table 2. Tests of the effects of gibberellic acid (GA3) concentration on germination of *H. annuus*, PI413039, Gettysburg, SD. Tests: 21 days, RT, ambient room light, 20-21° C.

Treatment	germination	reference
GRIN (germination basis): soak in hydrogen peroxide (3%), 5 min., rinse in water, soak in 25 ppm Ethrel, 12hr at RT, chill 7-14 days (**4° C**?), plant on filter paper (GRIN, USDA)	**25 ppm Ethrel, 1 wk, 4° C** GRIN, Control #1: 49% (% viable = 97%)	GRIN, USDA, Laura Marek and Lisa Pfiffner, (pers. comm.)
1. seed stored on filter paper saturated with DI water in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**DI water, 1 wk., 4° C** Lab, Control #2: 6/20 (30%)	Seiler (1993)
2. seed stored on filter paper saturated with1000 ppm GA3, in a plastic bag, 1 wk., **21°C**, then planted on filter paper, sat. with DI water, RT	**1000 ppm GA3, 1 wk., 21° C** 10/20 (50%)	Kumari and Singh (2000) Maiti et al. (2006b)
3. seed stored on filter paper saturated with 1000 ppm GA3, in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**1000 ppm GA3, 1 wk., 4° C** **14/20 (70%)**	Deno, 1993
4. seed stored on filter paper saturated with 500 ppm GA3, in a plastic bag, 1 wk., **21°C**, then planted on filter paper, sat. with DI water, RT	**500 ppm GA3, 1 wk., 21° C** 13/20 (65%)	Deno, 1993
5. seed stored on filter paper saturated with 500 ppm GA3, in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**500 ppm GA3, 1 wk., 4° C** **16/20 (80%)**	Deno, 1993

Table 3. Tests of the effects of Ethrel concentration on germination of *H. annuus*, PI413035, Kearney, NE. Tests: 21 days, RT, ambient room light, 20-21° C.

Treatment	germination	reference
GRIN (germination basis): soak in hydrogen peroxide (3%), 5 min., rinse in water, soak in 25 ppm Ethrel, 12hr at RT, chill 7-14 days (**4° C**?), plant on filter paper (GRIN, USDA)	**25 ppm Ethrel, 1 wk, 4° C** GRIN, Control #1: 51% (% viable = 96%)	GRIN, USDA, Laura Marek and Lisa Pfiffner, (pers. comm.)
1. control, seed stored on filter paper saturated with DI water in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**DI water, 1 wk., 4° C** Lab, Control #2: 4/20 (20%)	Seiler (1993)
2. seed stored on filter paper saturated with 29 ppm Ethrel (to prod. ethylene), in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**29 ppm Ethrel, 1 wk., 4° C 11/20 (55%)**	Kumari and Singh (2000)
3. seed stored on filter paper saturated with 100 ppm Ethrel (to prod. ethylene), in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**100 ppm Ethrel, 1 wk., 4° C** 8/18 (44.4%)	Kumari and Singh (2000)
4. seed stored on filter paper saturated with 200 ppm Ethrel (to prod. ethylene), in a plastic bag, 1 wk., **4°C**, then planted on filter paper, sat. with DI water, RT	**200 ppm Ethrel, 1 wk., 4° C** 7/20 (35%)	Kumari and Singh (2000) Maiti et al. (2006b)