

## Hormonal and environmental regulation of seed germination in flixweed (*Descurainia sophia*)

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### Abstract

Flixweed is one of the most abundant weeds in North America and China, and causes a reduction in crop yields. Dormancy of flixweed seeds is deep at maturity and is maintained in soil for several months. To identify regulators of seed dormancy and germination of flixweed, the effect of environmental and hormonal signals were examined using dormant and non-dormant seeds. The level of dormancy was decreased during after-ripening and stratification, but long imbibition (over 5 days) at 4 °C in the dark resulted in the introduction of secondary dormancy. The strict requirement of duration of cold treatment for the break of dormancy may play a role in the seasonal regulation of germination. The germination of non-dormant flixweed seeds was critically regulated by red (R) and far-red (FR) light in a photoreversible manner. Sodium nitroprusside, a donor of nitric oxide (NO), promoted germination of half-dormant seeds, suggesting that NO reduced the level of seed dormancy. As has been shown in other related species, light elevated sensitivity to GA<sub>4</sub> in dark-imbibed flixweed seeds, but cold treatment did not affect GA<sub>4</sub>-sensitivity unlike in *Arabidopsis*. Taken together, our results indicate that seed germination in flixweed and its close relative *Arabidopsis* is controlled by similar as well as distinct mechanisms in response to various endogenous and environmental signals.

### Introduction

Flixweed (*Descurainia sophia*), an annual herb, is one of the most abundant weeds in North America (Best 1977; Unger et al. 1999) and China (Zhang 2003), and causes a great loss of crop yield (Blackshaw 1990). Flixweed has also been thought as a potential oil crop, because the oil content of seeds is high (35–40%) and the oil is rich in linolenic acid. Also, the high productivity of seeds (e.g. 2600–3000 kg ha<sup>-1</sup>) (Tkachuk and Melish 1977;

Luo et al. 1997, 1999) supports its potential use as a crop. Seeds of flixweed germinate most readily in late autumn and early spring, but very rarely in summer (Best 1977). As seeds of other grass species, flixweed seeds have long longevity in soil and build up a large seed bank (Conn 1990; Conn and Deck 1995). The ability of flixweed seeds to constitute a bank is largely dependent on their deep primary dormancy imposed in the mother plant and their response to environmental signals that lead to secondary dormancy. It is therefore

important to study how dormancy and germination are regulated in this species in order to reduce the weed population in agriculture, and also to control its growth as an oil crop.

Seed dormancy and germination are complex developmental processes that are regulated by a variety of endogenous and environmental signals. Primary dormancy is imposed genetically during seed maturation on the mother plant. In many grass species, the dormancy level is reduced through after-ripening (dry and warm storage) and stratification (cold treatment) (Bungard et al. 1997; Schutz et al. 2002). Non-dormant seeds germinate upon water uptake if they are exposed to favorable light, temperature and nutrient conditions (Hilhorst et al. 1986; Derkx and Karssen 1993a, b; Hilhorst 1998). Studies in several plant species have shown that these developmental and external signals are in part mediated by phytohormones. Hormonal and environmental regulation of seed dormancy and germination has been best understood at the molecular level in the model species *Arabidopsis* (Bewley 1997; Koornneef et al. 2002), which is also a relative of flixweed. Gibberellin (GA), brassinosteroid and ethylene are positive regulators of seed germination, while abscisic acid (ABA) plays an essential role in establishing seed dormancy (Ketring 1977; Steber and McCourt 2001; Jacobsen et al. 2002; Koornneef et al. 2002). Recent studies have shown that nitric oxide (NO) is also an important regulator for both seed dormancy and germination (Batak et al. 2002; Beligni and Lamattina 2000; Bethke et al. 2004).

Despite its wide distribution as a weed and its potential usefulness as an oil crop, little is known about how seed dormancy and germination are regulated in flixweed. To address these questions, we investigated environmental and hormonal control of seed dormancy and germination in flixweed. Our results indicate that responses of flixweed seeds to various signals are largely similar to those of its close relative *Arabidopsis*, and support the idea that the knowledge from the model species will be useful for the control of seed germination of flixweed in the future. We also provide evidence that duration of cold treatment is an important signal to control the dormancy level in flixweed.

## Materials and methods

### *Plant materials*

Seeds of flixweed were collected in the middle of May in 2000 and 2002 from naturally occurring plants at Nanpi Eco-agriculture Experimental Station of Chinese Academy Science (Cangzhou, China). They were dried for a few days and stored in paper bags at room temperature in the laboratory until germination experiments.

### *Germination tests*

In all experiment for germination, the seeds were washed with 0.02% Triton X solution, rinsed with water and then placed on a layer of wet filter paper (3MM; Whatman, Maidstone, UK) in a sealed plastic Petri dish. Germination tests were performed using triplicate samples (each containing 50–60 seeds). Unless otherwise mentioned, seeds were incubated at 25 °C under continuous white light ( $220\text{--}250\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  from fluorescent lamp). Seeds were scored as germinated when radicle protrusion was visible.

### *Light treatments*

For dark incubation, seeds were handled under a dim green safety light. The far-red (FR) light pulse treatment consisted of 3 min of FR light irradiation ( $91\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ ) supplied from light-emitting diodes (MIL-IF18; Sanyo Biomedical, Osaka, Japan) passed through an FR acrylic filter (Dera-glass A900, 2 mm thick; Asahikasei, Tokyo, Japan). The red-light-pulse treatment consisted of 3 min of red (R) light irradiation ( $120\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ ) supplied from light-emitting diodes (MIL-R18; Sanyo Biomedical).

### *Chemicals*

Uniconazole and sodium nitroprusside (SNP) were purchased from Wako Pure Chemicals (Tokyo, Japan). The NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3 oxide (cPTIO) was purchased from Nacalai Tesque Inc.

(Tokyo, Japan). GAs were purchased from Prof. Lewis Mander (Australian National University, Australia).

## Results

### *Release from seed dormancy by after-ripening and stratification*

Fresh seeds of flixweed are highly dormant (Best 1977). It was previously reported that, when flixweed was seeded in soil in fall, there was 43% germination over a 3-year period, with yearly rates of 24, 8 and 11% (Best 1977). We tested the effect of after-ripening at room temperature on the dormancy level of flixweed seeds that were harvested in 2000 and 2002. As shown in Figure 1, both batches of seeds exhibited similar germination rates at each time point when imbibed under continuous white light at 25 °C. The seeds were released nearly fully from dormancy in 40 months after the harvest (only for the seeds that were

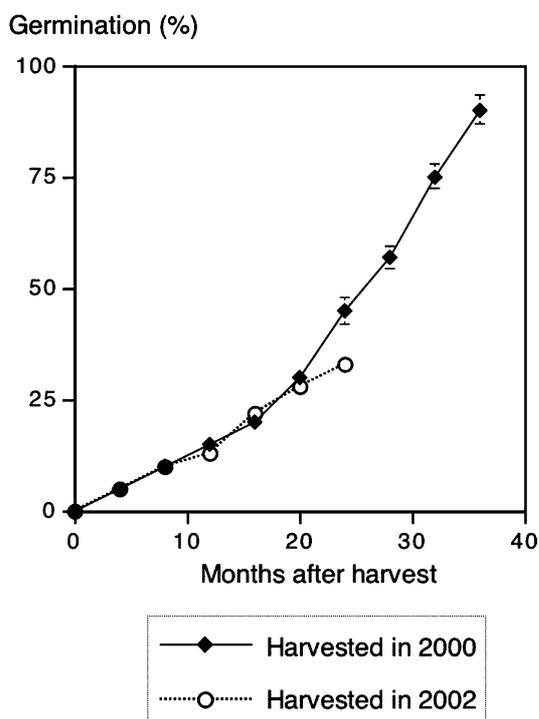


Figure 1. Effect of after-ripening on breaking seed dormancy. Dry seeds were stored at room temperature after the harvest, and then imbibed for 7 days under continuous white light at 25 °C to score germination.

harvested in 2000). In the following experiments, we used seed batches with different levels of primary dormancy as follows: non-dormant seeds (after-ripened for approximately 3 years), half-dormant seeds (after-ripened for 1 year) and deeply dormant seeds (freshly harvested).

Stratification treatment (dark imbibition at 4 °C) is often effective to break seed dormancy (Karssen 1980/1981, 1982). As shown in Figure 2, such cold treatment induced germination of half-dormant flixweed seeds partially, but not that of freshly harvested seeds. The duration of cold treatment was critical for the induction of germination. For the 2002 harvest, germination was promoted only when seeds were stratified for exactly 3 days, whereas for the 2000 harvest stratification for 1 to 4 days was effective even through

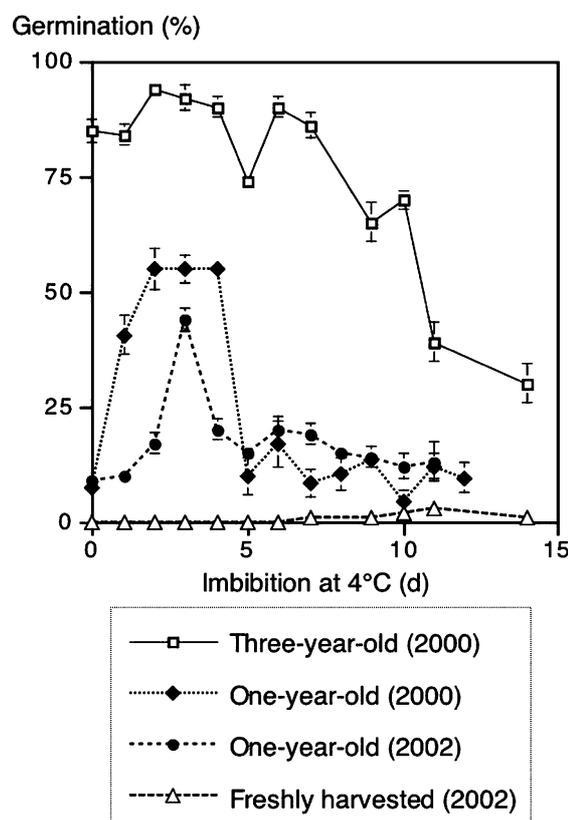


Figure 2. Effect of cold treatment on breaking seed dormancy. Seeds were imbibed in the dark, incubated at 4 °C for the indicated period, and then incubated at 25 °C under continuous light for 7 days before scoring for germination. Results from four different seed batches are shown; non-dormant 3-year-old seeds (harvested in 2000), 1-year-old half-dormant seeds harvested in 2000 or 2002, freshly harvested dormant seeds.

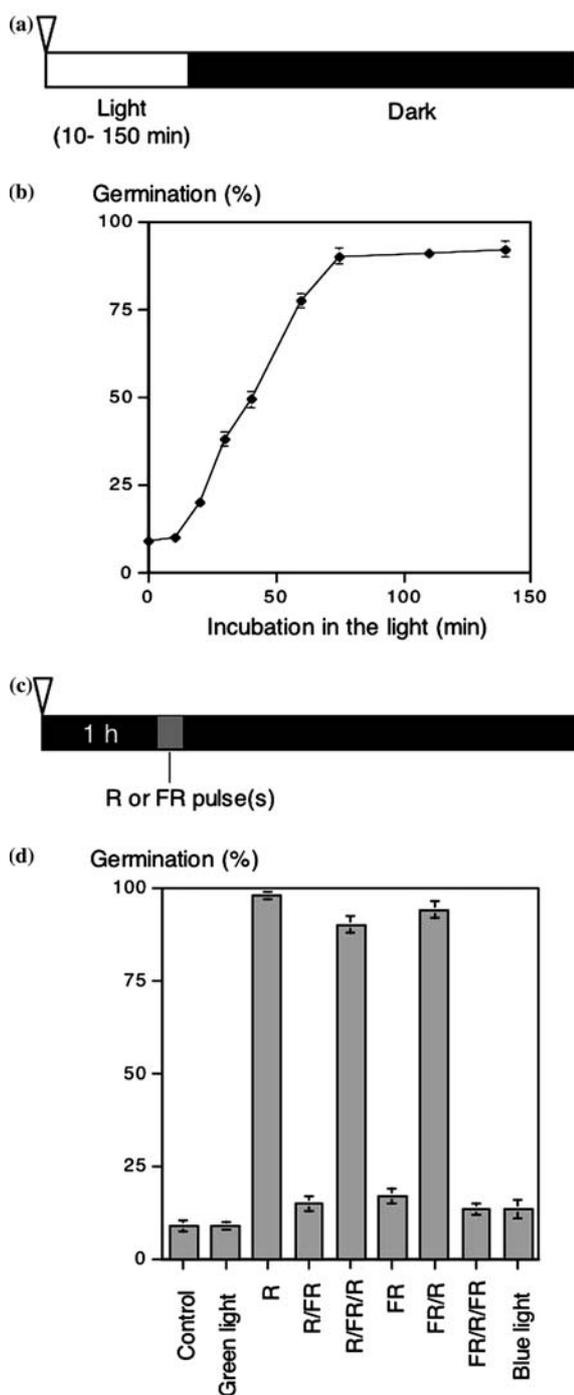
the two batches were stored for same time (1 year). Continuous dark incubation at 4 °C for more than 5 days resulted in a reduction in germination rate. This appears to be a result of the onset of secondary dormancy, but not that of a loss of viability, because the seeds germinated if GA<sub>4</sub> is applied exogenously (data not shown, the activity of GAs is discussed below). Germination of freshly harvested seeds remained less than 5% after stratification treatment of up to 12 days, suggesting that partial release from the primary dormancy is necessary for being responsive to cold treatment.

#### *Light regulation of seed germination via phytochrome*

To examine if light is required for germination of flaxweed seeds, after-ripened seeds were exposed to fluorescent white light upon imbibition for given periods, and then incubated in the dark (Figure 3a). As shown in Figure 3b, dark-imbibed seeds germinated only poorly, while light irradiation during the first 70 min after imbibition was sufficient to induce germination nearly completely. Light irradiation as short as 20 min from the start of imbibition increased germination rate in this experiment.

It has been well documented that R- and FR-light control seed germination in some plant species via phytochrome (Phy) (Shinomura 1997; Yamaguchi and Kamiya 2002). The involvement of Phy in the control of flaxweed seed germination was studied using non-dormant seeds that were imbibed in the dark and then exposed to different combinations of R- and/or FR-light pulses

(Figure 3c). Figure 3d illustrates that a pulse of R-light stimulated germination of dark-imbibed seeds, while a following FR pulse inhibited germination in a photo-reversible manner. Results of repeated pulse treatments (R/FR/R and FR/R/FR)



**Figure 3.** Light-regulation of seed germination. (a) Diagram showing light conditions. White bar indicates light-imbibition and black bar depicts incubation in the dark. The start of imbibition is shown by a reverse triangle. (b) As outlined in (a), imbibed non-dormant seeds were irradiated with fluorescent white light for a given period (10–150 min), and then incubated in the dark until germination was scored at 7 days. (c) Diagram showing light treatments for phytochrome-regulation. Non-dormant seeds were imbibed in the dark, and then irradiated with a pulse of FR-light, R-light or a combination of pulses of FR- and R-light. Black bar indicates dark-imbibition and the start of imbibition is shown by a reverse triangle. (d) Germination rates under different light conditions. FR/R; an R-pulse was given following an FR-pulse.

also were consistent with the involvement of Phy in the control of germination in this species (Figure 3d).

#### Effect of nitric oxide on seed germination

Recent work has shown that NO plays a regulatory role in breaking seed dormancy in *Arabidopsis* (Bethke et al. 2004). To examine the effect of NO on seed dormancy in flaxweed, half-dormant seeds were treated with SNP, an NO donor, during imbibition. Figure 4 shows that exposure to SNP results in the induction of germination in a dose-dependent manner. Imbibing flaxweed seeds with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) at 100  $\mu\text{M}$  strengthens dormancy of half-dormant seeds. In the presence of 400  $\mu\text{M}$  SNP, germination of half-dormant seeds was near 100%, but it did not happen with nitrate and nitrite, two other oxides of nitrogen, at a similar concentration range (data not shown). These observations suggest that NO functions as an effective signal to break seed dormancy in flaxweed as in *Arabidopsis*.

#### Effect of phytohormones on seed germination

ABA is important for the maintenance of seed dormancy and inhibits germination of non-dormant seeds during imbibition. Figure 5a shows

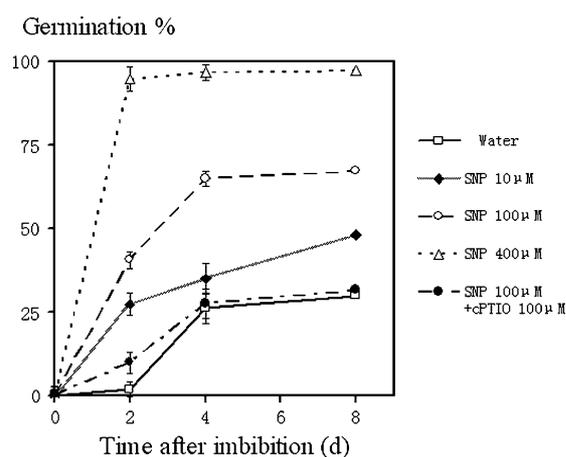


Figure 4. Effect of NO on breaking seed dormancy. Half-dormant seeds were imbibed under continuous white light at 25  $^{\circ}\text{C}$  in the presence of varying concentrations of SNP or with 100  $\mu\text{M}$  cPTIO as indicated.

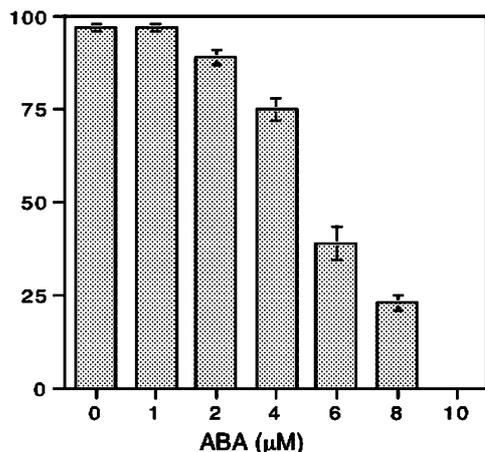
that germination of non-dormant flaxweed seeds was inhibited by exogenous ABA in a dose-response manner. GA is an essential hormone that promotes seed germination in *Arabidopsis* (Kornneef and van der Veen 1980). Inhibition of *de novo* GA synthesis by uniconazole or paclobutrazol (inhibitors of *ent*-kaurene oxidase) results in no germination in *Arabidopsis* (Nambara et al. 1991). Figure 5b illustrates that uniconazole inhibited seed germination completely at 10  $\mu\text{M}$  in flaxweed. Although more than 100 different GA structures have been identified in plants, only a few of them, such as GA<sub>1</sub>, GA<sub>3</sub> and GA<sub>4</sub>, are biologically active forms (Crozier et al. 2000). Exogenous GA<sub>4</sub> at 1–10  $\mu\text{M}$  was able to fully rescue the inhibitory effect of uniconazole on germination. However, GA<sub>1</sub> and GA<sub>3</sub> were not active in inducing germination of uniconazole-treated flaxweed seeds even at the highest dose (100  $\mu\text{M}$ ) (data not shown). NO (provided as SNP) was able to induce germination of half-dormant seeds (Figure 4), but was unable to rescue germination of uniconazole (10  $\mu\text{M}$ )-treated non-dormant seeds (data not shown). On the other hand, exogenous GA<sub>4</sub> promoted germination of half-dormant seeds (Figure 5c).

It has previously been shown in several plant species that the effectiveness of exogenous GA to stimulate germination is altered under different environmental conditions (Hilhorst et al. 1986). Experiments were undertaken to determine the effect of light and temperature on relative tissue sensitivity of non-dormant flaxweed seeds in the presence of 10  $\mu\text{M}$  uniconazole. As shown in Figure 6, light-imbibed seeds were much more sensitive (nearly 100-fold) to exogenous GA<sub>4</sub> than dark-imbibed seeds. In contrast, in both light- and dark-imbibed seeds of flaxweed, chilling treatment did not change GA sensitivity (Figure 6), unlike in *Arabidopsis* seeds (Derckx and Karssen 1993b).

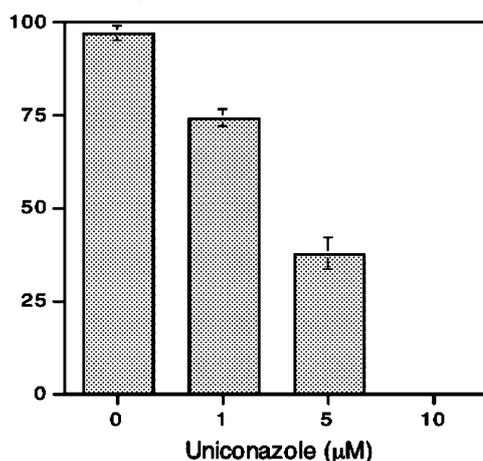
## Discussion

In this study, we addressed environmental and hormonal regulation of seed dormancy and germination in flaxweed. It has been thought that seeds of some grass species control their dormancy levels to build a large seed bank and survive in soil for a long period. In such species, the level of dormancy is modulated seasonally to allow

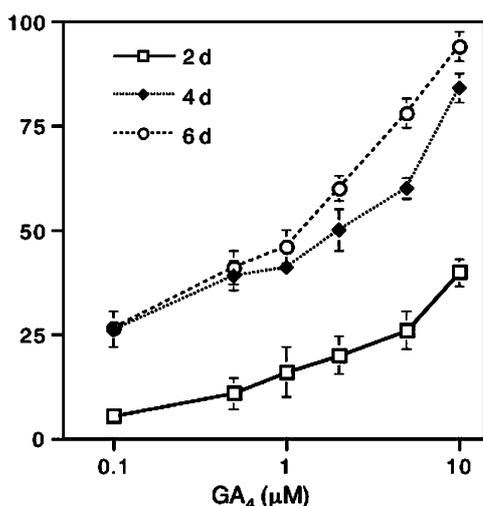
(a) Germination (%)



(b) Germination (%)



(c) Germination (%)



subsequent seedling development under favorable growth conditions (Karszen 1982). Besides seasonal regulation, the seeds are thought to monitor their location in soil by sensing the quality and quantity of light. Our current experimental data on flaxweed seeds are consistent with all of these ideas. As shown in Figure 1, fresh flaxweed seeds were highly dormant, and after-ripening in our experimental condition proceeded very slowly over 3 years. Our results using half-dormant seeds illustrate that prechilling treatment (4 °C in the dark for 2–4 days) was effective to break seed dormancy (Figure 2). After-ripened non-dormant seeds germinated only poorly in the dark, and phytochrome is likely to be involved in light-regulation of germination of flaxweed seeds (Figure 3). The strict light requirement for germination would lead after-ripened flaxweed seeds to the secondary dormancy when they are buried too deep in soil.

Notably, cold treatment for more than 5 days resulted in the onset of secondary dormancy (Figure 2). The role of this duration-dependent response to cold temperature in seasonal control of seed germination in nature remains to be investigated further. Because temperature fluctuates both diurnally and seasonally in nature, it would be beneficial for seeds to monitor the length of cold temperature in order to recognize the season accurately. Continuous exposure of seeds to 4 °C for over 5 days might act as a signal of the middle of winter, which is not an appropriate time for germination. On the other hand, exposure to cold temperature for a shorter time (2–3 days) might be recognized as being in late fall, in which germinated seedlings would then grow into rosette plants before the coldest time of the year and survive.

The role of GA in stimulating seed germination has been well established in many plant species (Bewley 1997; Koornneef et al. 2002). In our experiments, GA<sub>4</sub> promoted germination of half-dormant seeds (Figure 5c) and uniconazole-treated non-dormant seeds (Figure 6). Bioactive

Figure 5. Effect of ABA and GA on seed germination. Seeds were imbibed under continuous white light at 25 °C. (a) Non-dormant seeds were treated with ABA. (b) Non-dormant seeds were treated with uniconazole (a GA biosynthesis inhibitor). (c) Half-dormant seeds were treated with GA<sub>4</sub>.

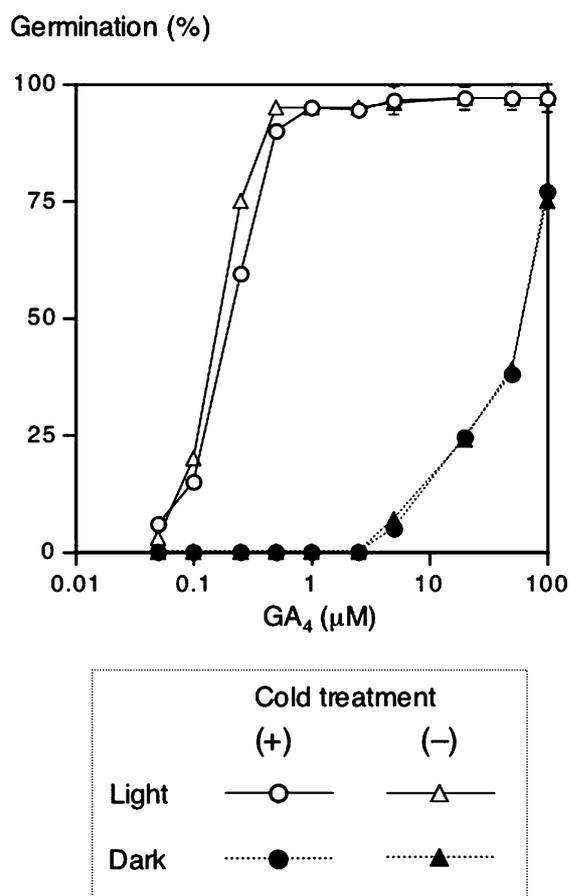


Figure 6. Sensitivity to exogenous GA<sub>4</sub>. Non-dormant seeds were treated with exogenous GA<sub>4</sub> in the presence of 10 μM uniconazole. Seeds were imbibed in the dark or light with or without prechilling treatment (imbibition in the dark at 4 °C for 2 days).

13-hydroxylated GAs (such as GA<sub>1</sub> and GA<sub>3</sub>) are 10-fold less active than GA<sub>4</sub> (a non-13-hydroxylated GA) in *Arabidopsis*, but can induce near 100% germination of uniconazole-treated *Arabidopsis* seeds at 100 μM (Yamaguchi unpublished data). This contrasts with our finding that GA<sub>1</sub> and GA<sub>3</sub> at up to 100 μM did not exhibit any germination promoting activity in flixweed in our experimental conditions (data not shown). Both GA<sub>4</sub> and GA<sub>1</sub> are bioactive GAs that are synthesized through similar, but separate parallel pathways. In many plant species, both pathways exist, with one of them playing a predominant role. The strict distinction of GA<sub>4</sub> from GA<sub>1</sub> and GA<sub>3</sub> observed in flixweed seeds is not common, but is similar to the structure–activity relationships of

GAs for the growth promotion of cucumber hypocotyls (Saito et al. 1998). For pea, rice, barley and maize, it is GA<sub>3</sub> or GA<sub>1</sub> and not GA<sub>4</sub> that is the active form of GA (Crozier et al. 2000).

As has recently been shown in lettuce, *Lupinus luteus*, Empress tree and *Arabidopsis*, (Giba et al. 1998; Beligni and Lamattina 2000; Kopyra and Gwód 2003; Bethke et al. 2004), NO (provided as SNP) promoted germination of half-dormant flixweed seeds (Figure 4). The activity of NO in inducing germination of half-dormant seeds was reduced by uniconazole (data not shown). In addition, only GA<sub>4</sub> (Figure 6), but not NO (data not shown), restored the inhibition of germination of non-dormant seeds by 10 μM uniconazole. These results suggest that normal GA synthesis is required for the action of NO that induces seed germination in flixweed. However, this result should be carefully interpreted, because uniconazole affects other hormone metabolism pathways as well at a high concentration.

There are at least two possible ways by which GA could mediate responses to environmental changes; (1) the environmental signal modulates the level of endogenous GA, and (2) it affects the ability of cells to respond to the hormone. In *Arabidopsis*, both endogenous GA levels and GA sensitivity are altered in response to both light (via Phy) and temperature (cold treatment) (Yamaguchi and Kamiya 2002; Yamauchi et al. 2004). On the other hand, in germinating lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds, R light increases bioactive GA levels without changing the sensitivity to exogenous GA (Toyomasu et al. 1993, 1994, 1998). Our study revealed that tissue sensitivity to exogenous GA in flixweed is substantially increased in light-imbibed seeds relative to dark-imbibed seeds. However, unlike in *Arabidopsis*, cold treatment did not affect GA sensitivity of flixweed seeds in our experimental conditions. Future studies will be necessary to elucidate how GA biosynthesis is regulated in response to light and temperature in flixweed seeds. Such investigations will be helpful to understand how GAs, common germination regulators in plants, are utilized in various environmental responses in different species.

In conclusion, we have studied environmental and hormonal regulation of seed dormancy and germination in flixweed. Our data indicate the

presence of distinct as well as common mechanisms for the control of seed dormancy and germination in *Arabidopsis* and flaxweed.

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