Abscisic acid content of a nondormant sunflower (*Helianthus annuus* L.) mutant

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ABSTRACT

A sunflower (*Helianthus annuus* L.) mutant was observed in the progeny of a cross between the sunflower cultivar HA 89 and an amphiploid of a *H. divaricatus* L. × P21 cross that exhibited loss of dormancy induction in the developing embryo. Seeds of this mutant frequently germinate on the head about 40 d after pollination (DAP). In contrast to other nondormant sunflower mutants reported previously, the cotyledons of this mutant remain green, whereas other nondormant mutants exhibit loss of pigmentation. The objective of this investigation was to compare the level of abscisic acid, a plant hormone that induces dormancy in developing embryos, in the nondormant green mutant (*ndg*) and HA 89 from which *ndg* was derived. Immunoassays showed that abscisic acid was present in *ndg* and the levels decreased from a maximum at 5 to 20 DAP to basal levels at 25 DAP. The levels of abscisic acid were not significantly different from those in the control plant HA 89. We conclude that the nondormancy trait is due to a mutation that renders *ndg* insensitive to abscisic acid.

Key words: abscisic acid – *Helianthus annuus* – mutant – nondormancy – sunflower.

INTRODUCTION

Seed dormancy is a physiological strategy evolved by plants to ensure survival of the species. A dormant state prevents germination into a temporarily favorable, but unstable, environment that could become adverse shortly after germination and lead to plant death. Of the several types of dormancy, physiological dormancy is the most common mechanism that has evolved and is present in both gymnosperms and angiosperms. It can occur at a deep, intermediate, or non-deep level (Baskin and Baskin, 2004).

Cultivated sunflower seeds (*Helianthus annuus* L.) undergo a Type 2 non-deep physiological dormancy period (Baskin and Baskin, 2004). In Type 2 dormancy, seeds initially have greater germination potential at higher temperatures, but gradually improve their ability to germinate at lower temperatures during the progression from dormancy to nondormancy. Abscisic acid (ABA) is a known inducer of dormancy in sunflower as it is in many plants (Le Page-Degivry and Garello, 1992). Dormancy in sunflower seed can be broken by application of gibberellic acid or ethylene, by cold stratification, or by excision and culture of the embryo on appropriate medium (Fick, 1978; Corbineau et al., 1990; Jridi et al., 2004).

An albino sunflower mutant, *nd-1*, that exhibited loss of seed dormancy was previously reported by Fambrini et al. (1993). The mutant was found in the selfed progeny of an *in vitro*-regenerated plant and displayed visibly reduced pigmentation by carotenoids. Analysis showed that a defective ζ-carotene desaturase caused the loss of pigmentation (Conti et al., 2004). Because carotenoids are precursors of ABA, altered ABA biosynthesis was likely responsible for nondormancy in *nd-1*.

Within our sunflower germplasm enhancement program, we recently identified a nondormant sunflower mutant that occurred during an interspecific gene transfer from a wild *Helianthus* species into cultivated sunflower to find resistance to the newly evolved broomrape (*Orobanche cumana* Wallr.) race F in Spain. Resistance genes were found in an interspecific cross with the pedigree of *H. divaricatus* 830/P21 amphiploid//P21/2/HA 89. In 1999, a single plant among the sib-pollinated progeny with 2n=34 chromosomes of this pedigree was observed to have seed germinated on the head. The amphiploid *H. divaricatus* 830/P21 has 2n=68 chromosomes; therefore, it took several backcrosses and sib- or self-pollinations to reduce the 2n chromosome number to 34, the same as cultivated sunflower, while continuing to monitor the broomrape resistance. Continued self-pollination maintained the nondormancy trait until F_{14}, and one homozygous F_{14} nondormant line was selected in 2003 for this study.

In this mutant, dormancy was not induced in the developing embryo. Instead, developing seeds of the mutant sunflower began to germinate in the head about 40 d after pollination (DAP). This nondormant mutant differed from *nd-1* in that pigmentation appeared normal. Hence, we use the term *ndg* to describe this *nondormant green* sunflower mutant. Because ABA is known to induce dormancy in physiological dormancy, we investigated the levels of ABA in the developing seeds of *ndg* at various stages after

pollination. When the ABA levels in *ndg* were compared to those in HA 89, which is in the pedigree of *ndg*, we found that the ABA content of *ndg* was not significantly different from HA 89.

MATERIALS AND METHODS

Plant material

Both HA 89 and the mutant *ndg* sunflower were grown in the greenhouse (16-h light) and self-pollinated. After pollination, developing seeds of HA 89 and *ndg* were removed from the head of a single plant at 5, 10, 15, 20, 25, 30, 40, 50, and 60 DAP. At each harvest date, 20 to 40 achenes of *ndg* and 25 to 50 achenes of HA 89 were removed from the head and stored at -80° C prior to ABA analysis.

ABA determination

ABA content was determined in both mutant *ndg* and HA 89 achenes. The frozen achenes from each harvest date were thawed and the hulls separated from the kernels when possible. Both the hulls and kernels were weighed and placed in a desiccator (Moisture Gone desiccant, Hiatt Distributors, Ltd., Long Beach, CA, USA)[1] and allowed to dry overnight. For each sampling date the hulls and kernels were weighed separately and then the hulls or kernels from each were pulverized in liquid nitrogen using a mortar and pestle and extracted with 4 mL of 80% (v/v) aqueous acetone using a Polytron homogenizer. After evaporation of the acetone with a stream of nitrogen and extraction of oil with hexane, the homogenate was diluted with 5 mL of 1 M formic acid and partially purified by separation on an Oasis SepPak (Waters, Milford, MA). The ABA was eluted with 5 mL of methanol and the eluate was evaporated to dryness using a stream of nitrogen. The samples were reconstituted in 1.0 mL of Millipore-purified water. Dilutions of 10× or 100× in Millipore-purified water were used for immunoassay. Samples containing ABA were subjected to quantitative analysis for ABA content by an enzyme-linked immunosorbant assay (Suttle and Hultstrand, 1994; Walker-Simmons, 1987). The (±)ABA used for the standard curve was purchased from Sigma (St. Louis, MO, USA) and the (±)ABA concentrations were doubled for calculation of the physiologically active (+)ABA racemate. (+)ABA concentrations in the embryos and the hulls were expressed as nmol·g dry wt^{-1}. ABA analyses from at least three different plants were conducted for the time points between 5 and 25 DAP, and two ABA determinations were typically made for 30 to 60 DAP. Between 5 and 15 DAP, the ABA content of *ndg* was determined on the whole achene because the embryos were too small to be successfully separated from the hull. From 20 DAP and later, the ABA contents in the embryos and hulls were determined separately.

RESULTS

ABA levels in ndg mutant and HA 89 sunflower

The quantitation of ABA levels at the early stages of seed development showed high variability among samples, likely due to the small size of hulls and embryos and varying stages in initiation of development. Both HA 89 and the *ndg* mutant showed similar patterns of ABA levels in the achenes during the period following pollination (Fig. 1). ABA concentrations in each declined during achene development until basal levels were reached by 30 DAP. We detected a slightly higher ABA concentration in HA 89 compared to *ndg* at 15 DAP, but the differences were otherwise not statistically significant. For both HA 89 and mutant *ndg*, the concentrations of ABA in the hulls and embryos were the same (Fig. 2). By 40 DAP, the developed seeds of *ndg* typically began to germinate on the sunflower head.

DISCUSSION

Sunflower normally undergoes a dormancy period during and following seed development. The length of dormancy is dependent on cultivar and on the environment. Under normal conditions at room temperature, gradual dormancy release for cultivated sunflower typically begins about 45 to 50 DAP (Fick, 1978). For wild sunflower species, the dormancy period is often much longer and highly variable in length.

[1] Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Several studies on sunflower dormancy have been reported. By inhibiting ABA synthesis with fluridone, Le Page-Degivry et al. (1990) demonstrated that sunflower seed dormancy was dependent on ABA synthesis, but that dormancy induction was not concomitant with ABA accumulation. Their results for developing seeds showed an increase in embryo ABA content that reached a maximum at 13 DAP and then decreased to low levels by 25 DAP. They concluded that ABA induced embryo dormancy during seed maturation.

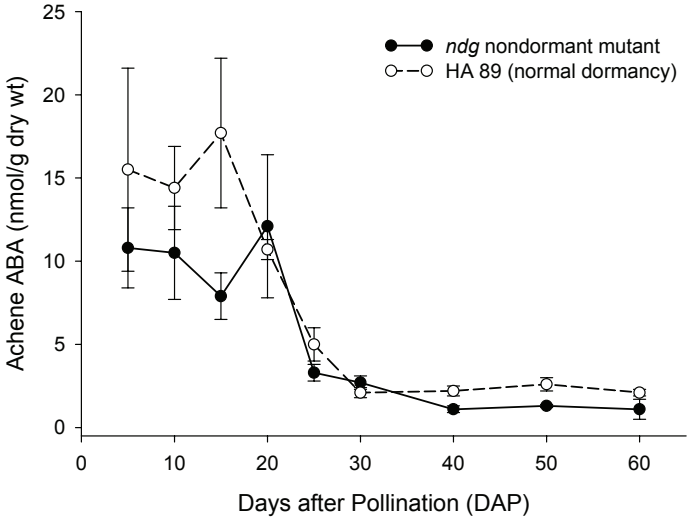

Fig. 1. ABA content (dry weight basis) of achenes of sunflower mutant *ndg* (●) and HA 89 (○) cultivated sunflower at various stages of achene development. Data are means of 3 or more individual plants for 5 to 25 DAP, and of 2 individual plants for 30 to 60 DAP ± SE.

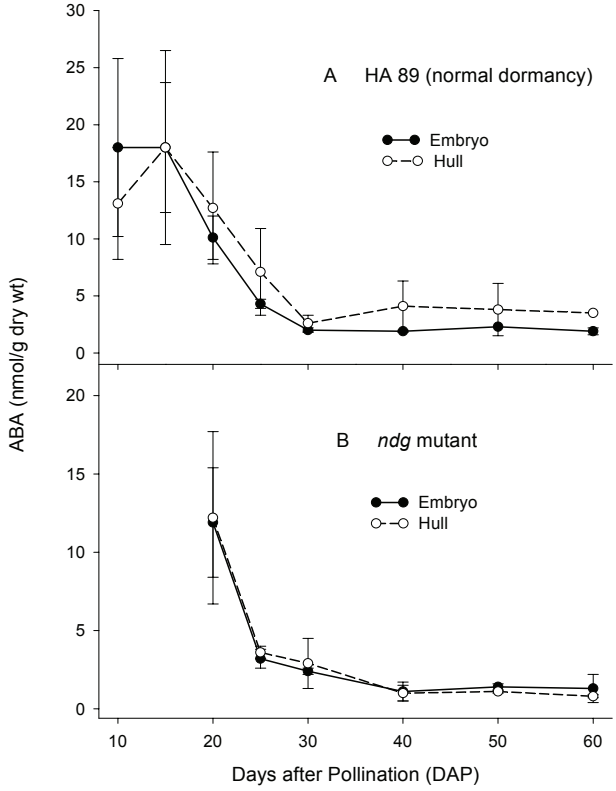

Fig. 2. ABA content (dry weight basis) of embryos (●) and hulls (○) in A) cultivated sunflower HA 89 and B) mutant *ndg* sunflower. Data are means of 3 or more individual plants for 5 to 25 DAP, and of 2 individual plants for 30 to 60 DAP ± SE.

Because expression of total ABA content in an embryo is dependent on the size of the embryo, we avoided using total ABA per embryo. Instead, we expressed ABA content on a dry weight basis of the whole achene so that equivalent comparisons could be made between ABA concentrations in the *ndg* mutant and HA 89. Our results clearly showed the presence of ABA during early achene development in both HA 89 and the *ndg* mutant (Fig. 1). The differences in ABA content between the *ndg* mutant and HA 89 were not statistically significant at most stages of achene development.

We believe that the nondormancy observed in the *ndg* mutant is due to loss of sensitivity to ABA. In our terminology, loss of sensitivity is used in the broad sense to include a defect in any component of the dormancy induction mechanism. A mutation in the ABA receptors that results in reduced affinity to ABA, or a mutation in any of the proteins involved in ABA signal transduction could lead to impaired transcriptional activation of ABA-inducible gene expression. Koornneef et al. (1984) have reported a similar ABA-insensitive mutant (*abi-3*) of *Arabidopsis thaliana* that is green and exhibits nondormancy that also is not reversed by exposure to ABA.

While the synthesis of ABA in the mutant *ndg* embryo appears to be normal, or at least near normal, we cannot rule out the effect of a slightly reduced capacity for ABA synthesis. White et al. (2000) proposed that an adequate ABA:GA ratio is critical for suppression of germination and induction of dormancy, rather than the absolute amounts of the two hormones. In the case of *ndg*, it may be that a slightly reduced content of ABA leads to a ratio shift in favor of GA, and the result is a failure to induce dormancy at a critical time during embryo development. Indeed, Fong et al. (1983), in a study on maize vivipary, proposed that there is a narrow window of embryo development in which ABA is able to induce dormancy. Accelerated catabolism of ABA during after-ripening or a reduced rate of seed desiccation might also result in loss of dormancy.

We did not investigate these alternative possibilities for the observed nondormancy of *ndg*. The aim of this study was to examine whether ABA synthesis was altered in seeds of the nondormant mutant. Our results showed that ABA levels in the mutant *ndg* were the same as in the control line HA 89. Thus, we believe that the *ndg* mutant is defective in the signaling pathway of ABA recognition and subsequent induction of gene expression leading to dormancy.

Introduction of the nondormancy trait into a breeding program could be a useful tool for sunflower breeders. We have determined that nondormancy in the *ndg* mutant is under the control of a dominant gene(s) (unpublished), and if introduced during development of a germplasm line could be a useful tool to advance generations quickly. Use of the nondormancy trait could circumvent the utilization of embryo rescue techniques to avoid the normal dormancy period between germplasm generation advances. Seeds could simply be transferred from the sunflower head directly to large pots without the need for chemical treatment to break dormancy. Finally, the nondormancy trait could be eliminated in the last phase of germplasm line development by selection for segregating lines having normal seed dormancy.

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