Published January 15, 2016

RESEARCH

Pericarp-Imposed Dormancy in Sunflower: Physiological Basis, Impact on Crop Emergence, and Removal at an Industrial Scale

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ABSTRACT

Sunflower (*Helianthus annuus* L.) achenes often display pericarp-imposed dormancy, which is long-lasting and causes serious problems to crop production and the seed industry. For this study we assessed an extensively used sunflower inbred line that has this type of dormancy. Our goals were (i) to determine the effect of pericarp on germination and to evaluate its impact on crop field emergence, (ii) to provide insight into the physiological basis of pericarp-imposed dormancy by determining the effects of abscisic acid (ABA) accumulation in the embryo and the embryo sensitivity to ABA during incubation at different temperatures, (iii) to assess the effect of oxidant agents and other compounds on dormancy termination, and (iv) to evaluate the feasibility of using oxidants to remove dormancy at an industrial scale. Incubation at high temperatures (i.e., 25 to 30°C) allowed the expression of dormancy, which was imposed by the pericarp and was accompanied by an increase in embryo sensitivity to ABA, but not in ABA concentration. Treated achenes with sodium hypochlorite, or their incubation in presence of an ethylene precursor or gibberellins overcame dormancy. ABA concentration decreased during incubation when treated with sodium hypochlorite. Application of sodium hypochlorite on a commercial seed lot (i.e., washing with 3 and 7%, after additional chemicals used by the industry were applied) resulted in higher germination compared with dormant non-treated controls. Field trials showed that pericarp-imposed dormancy reduced crop emergence in the inbred line tested herein. However, treating achenes with sodium hypochlorite using described industrial procedures improved field emergence.

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Dormancy is an internal condition of the seed that impedes germination under hydric, thermal, and gaseous conditions that, otherwise, would have been appropriate for germination (Benech-Arnold et al., 2000). Dormancy is hereditable and optimizes the distribution of germination over time in nature, preventing its occurrence under unfavorable conditions (Kermode, 2005). In spite of its obvious adaptive importance, dormancy is an undesirable trait for agriculture (Bewley, 1997). For instance, persistent dormancy can affect crop emergence and the number of established plants (Benech-Arnold et al., 2000), impacting crop yield. Because of this, the domestication and breeding of cultivated crops has tended to remove this trait from their wild ancestors (Bewley, 1997). Sunflower is an important oilseed crop (Hussain et al., 2012) with a relatively short domestication history that has not yet resulted in seed dormancy reductions. Sunflower achenes, which are commonly referred to as seeds, of some genotypes often display physiological dormancy at harvest (Rodríguez et al., 2011) that causes difficulties in commercial production impairing crop emergence (Snow et al., 1998; Nasreen et al., 2015).

Physiological dormancy is imposed by the presence of covering tissues surrounding the embryo, referred to as coat-imposed dormancy, by the embryo, referred to as embryo dormancy, or a combination of both factors (Finch-Savage and Leubner-Metzger, 2006). Sunflower achenes usually display both types of dormancy

Published in Crop Sci. 56:1–11 (2016).
doi: 10.2135/cropsci2015.06.0335

at harvest (Benech-Arnold, 2004). If achenes are dried and stored at room temperature (25 ± 1°C, relative humidity 50%) embryo dormancy is removed (Corbineau et al., 1990; Andrade et al., 2015); however, coat-imposed dormancy may persist for several weeks and even months (Benech-Arnold, 2004). "Coat" refers to any embryo-covering tissue, for example the pericarp, seed coat, or endosperm (Finch-Savage and Leubner-Metzger, 2006; Szemruch et al., 2014). The sunflower achene consists of a seed and adhering pericarp, and the seed consists of a seed coat, endosperm and embryo (Seiler, 1997). The seed coat is thin and has three layers with the inner and outer layers parenchymal, and a middle layer of spongy parenchyma. The endosperm consists mostly of a single layer of aleurone cells coalesced with the seed coat. The embryo is made up of mostly cotyledons. It consists largely of palisade parenchyma, with cells containing oil-rich, large aleurone particles and protein crystals (Seiler, 1997). Sunflower achenes display relative dormancy, which means that it is expressed at certain temperatures, but not at others. It has been proposed that coat-imposed dormancy is expressed at high incubation temperatures (i.e., 25 to 30°C), while embryo dormancy is expressed in achenes incubated at low temperatures (i.e., 10 to 15°C) (Corbineau et al., 1990). Most studies on sunflower dormancy have been conducted with genotypes displaying embryo dormancy; however, this type of dormancy is less persistent and easier to remove than pericarp-imposed dormancy.

Physiological dormancy is under hormonal control. There is evidence that ABA is involved in the establishment and maintenance of dormancy (Bewley, 1997). Endogenous ABA levels are regulated through the balance of its biosynthesis and catabolism (Millar et al., 2006), as ABA accumulation in seeds is low during early developmental stages, increases during mid-development, and begins to descend at maturation drying (Bewley, 1997). Prevention of germination during development may be due to the endogenous ABA content of the seed (Berry and Bewley, 1992). In mature seeds, dormancy release is associated with a reduction in ABA content (Millar et al., 2006) combined with reduced embryo sensitivity to ABA (Finch-Savage and Leubner-Metzger, 2006). However, in some cases, dormancy of mature seeds is maintained due to an increase in the concentration of ABA via de novo synthesis during imbibition of dormant seeds, which does not occur in non-dormant seeds (Le Page-Degivry and Garello, 1992). Pericarp-imposed dormancy is presumably more troublesome than embryo dormancy to crop production, though little is known about the underlying mechanisms for dormancy maintenance. This lack of knowledge hinders designing technologies for removing pericarp-imposed dormancy at an industrial scale. Embryo-imposed dormancy in sunflower is usually removed with ethylene (C_2H_4), which antagonizes the effect of ABA (Corbineau et al., 1990), but methodologies to remove pericarp-imposed dormancy do not exist.

In sunflower, dormancy imposed by the covering tissues surrounding the embryo may involve the establishment of embryo hypoxia, possibly interfering with ABA metabolism and enhancing ABA signaling. This hypothesis is based on a mechanism described for barley, whose grains have different structures surrounding the embryo (i.e., endosperm, pericarp, lemma, and palea) (Benech-Arnold et al., 2006). As in the case of the sunflower pericarp, barley structures are rich in polyphenols and act as "traps" for oxygen, thus imposing hypoxia to the embryo and interfering with ABA metabolism, increasing ABA synthesis and/or reducing its catabolism, and also interfering with ABA signaling, increasing the sensitivity of embryos to ABA (Benech-Arnold et al., 2006; Mendiondo et al., 2010). Therefore, similar mechanisms may be operating in sunflower in which pericarp imposes achene dormancy. If this assumption is correct, the use of oxidants to overcome the hypoxia imposed by the pericarp appears promising at an industrial scale to improve germination. The use of hydrogen peroxide (H_2O_2) as an oxidant (Wang et al., 1998) reduced the level of endogenous ABA, via an alternative mode of action (Bahin et al., 2011), thus terminating dormancy in barley. Moreover, Liu et al. (2010) found in *Arabidopsis* that both ABA and gibberellins (GA), a hormone that acts on seed dormancy antagonistically to ABA, are under regulation of H_2O_2 in dormant seeds such that exogenous H_2O_2 increased ABA catabolism and GA biosynthesis.

This work was performed on a sunflower inbred line with pericarp-imposed dormancy to (i) determine the effect of pericarp on germination and to evaluate its impact on crop field emergence, (ii) provide insight into the physiological basis of pericarp-imposed dormancy by determining the effects of ABA accumulation in the embryo and the embryo sensitivity to ABA during incubation at different temperatures, (iii) assess the effect of oxidant agents and other compounds on dormancy termination, and (iv) evaluate the feasibility of using oxidants to remove dormancy at an industrial scale.

MATERIALS AND METHODS

Plant Material

Sunflower achenes of an oil-producing parental line were received from Dow AgroSciences soon after harvest, dried at 6.6% moisture content, and stored at −30°C to preserve the initial dormancy level, as at this temperature sunflower achenes dormancy level does not change (Oracz et al., 2008; Bazin et al., 2011). This inbred line is used as male parent for pollen donor of many commercial hybrids and develops long lasting dormancy (M. Gerbaldo, Dow AgroSciences, personal communication, 2010). Achenes were produced in two regions in Argentina in multiple years: Luján de Cuyo (33°1′ S, 68°52′ W) in the province of Mendoza in 2010 and in Ascensión (34°13′ S, 61°7′ W)

in the province of Buenos Aires in 2010 and 2012. For field trials, a non-dormant batch of this same genotype was included as a control.

Incubation and Germination of Sunflower under Laboratory Conditions

In the present manuscript achene refers to a seed and adhering pericarp, seed refers to a seed coat, endosperm and embryo, and embryo refers to an isolated embryo, following Seiler (1997).

Each experiment was performed in darkness in 9-cm petri dishes on two discs of filter paper moistened with 5 mL of distilled water or hormone solution at different concentrations. Each replicate consisted of 25 achenes, seeds, or embryos per petri dish. A completely randomized design was used in each laboratory experiment.

Incubation temperatures of 12°C (low temperature), 25°C, and 30°C (high temperatures) were imposed to allow pericarp-imposed dormancy to be differentially expressed (Rodríguez et al., 2011). Germination was defined as when the radicle elongation reached 2 to 3 mm (Oracz et al., 2007; Bazin et al., 2011) and was recorded daily for 20 days. Four replicates of achenes, seeds, and embryos were germinated with distilled water at each of the three incubation temperatures.

Embryo-Covering Tissues Dissection

Embryo-covering tissues were removed or damaged mechanically to assess their role on the expression of dormancy at different incubation temperatures. Seed coat and endosperm were first hydrated for 30 min in petri dishes on a disc of filter paper moistened with 3 mL of distilled water. A scalpel was used to make the removals and damages using a magnifying glass. Four replicates were incubated at 25 and 30°C in distilled water for achenes (A), achenes with a lateral cut affecting half of the pericarp length and towards the embryonic axis end (i.e., pericarp damage, Pd), achenes with a lateral cut affecting half of the pericarp length and towards the embryonic axis end plus a damage of the seed coat and endosperm (i.e., Pd+SCd+Ed), and achenes after the removal of pericarp but no damage to the seed coat neither endosperm (i.e., seeds, S).

Sodium Hypochlorite Treatment of Achenes Performed in the Laboratory

Sodium hypochlorite was tested to determine whether an oxidant could increase the oxygen supply in achenes with pericarp-imposed dormancy. Achenes were immersed for 2 min in a 0.5% sodium hypochlorite solution (commercial concentration 55 g Cl L^{-1}, Ayudín, Clorox Argentina S.A., Aldo Bonfi, Argentina) and blotted with paper towels, without washing them, before incubation (i.e., treated achenes). Germination of four replicates of non-treated (i.e., control) and treated achenes was tested daily in distilled water at 12, 25, and 30°C.

Preparation of Plant Material for Abscisic Acid Analysis

Embryo ABA concentration was measured through three experiments of (i) achenes and seeds incubated at 30°C, (ii) achenes incubated at 12 and 30°C, and (iii) achenes (treated with sodium hypochlorite and non-treated) incubated at 30°C. In all cases three replicates of achenes or seeds were incubated in distilled water.

For ABA determination 15 seeds and/or achenes from each experiment were sampled in different incubation times before the germination of the first seed or achene. These seeds and achenes were dissected to obtain embryos, frozen in liquid nitrogen, and stored at −80°C until processing. Each sample was then lyophilized, powdered, weighed, and stored at −30°C until assayed for embryo ABA concentration. These three experiments from which samples were taken for measurement of embryo ABA concentration were complemented with another experiments not sampled, in which germination percentage of three replicates of each treatment was scored daily for 20 days.

Quantification of Abscisic Acid by Radioimmunoassay

Distilled water was added to each powdered sample (100:1 v/w) and left overnight on a shaker at 4°C. After centrifugation at 10,000 rpm for 4 min, 50 μL of the supernatant per sample was assayed twice with a radioimmunoassay as described by Steinbach et al. (1995) using the mono-clonal antibody AFR MAC 252 (Quarrie et al., 1988), and tritiated ABA (Amersham Biosciences, Little Chalfont, Bucks, UK). The average and standard error of three replicates, each measured twice, was reported.

Seed Sensitivity to Abscisic Acid

Three seed replicates were incubated at 12, 25, and 30°C in distilled water (i.e., control) and ABA solutions (Sigma-Aldrich, Saint Louis, MO) at concentrations of 1, 10, and 100 μmol L^{-1} ABA. The ABA solutions were prepared by dissolving the compound in 1 mol L^{-1} NaOH (according to "Solution preparation" by Sigma-Aldrich).

Effects of Ethylene and Gibberellins

Five replicates of achenes were incubated at 25 and 30°C in distilled water, Tifón solutions (i.e., an ethylene releasing compound, Gleba S.A., La Plata, Argentina) at concentrations of 10, 100, and 500 μmol L^{-1} Tifón and GA_3 gibberellin solutions (Sigma-Aldrich, Saint Louis, MO) at concentrations of 100 and 200 μmol L^{-1} GA_3. GA_3 solutions were prepared by dissolving in EtOH (according to "Solution preparation" by Sigma Aldrich).

Sodium Hypochlorite Treatment of Achenes Performed at Industrial Scale

To investigate sodium hypochlorite treatment at an industrial scale to remove pericarp-imposed dormancy in sunflower achenes twelve treatments (Treatments 1–12, Table 1) were conducted in facilities owned by Dow AgroSciences (Buenos Aires, Argentina) and included the application of proprietary chemicals using commercial seed treatment equipment. The first chemical was an insecticide (Reldan Plus, 2×10^{-5} L kg achenes^{-1}, Dow AgroSciences, Ciudad Autónoma de Buenos Aires, Argentina) that is typically applied before storage. The second chemical was a liquid mix that is typically applied before sowing in the field that contains water (2×10^{-3} L kg achenes^{-1}), a fungicide (Apron Gold, 3×10^{-3} L kg achenes^{-1}, Syngenta Production France S.A.S., Saint Pierre, France), a polymer (Secure, 7×10^{-4} L kg achenes^{-1}), a coating (Blue Solid, 3×10^{-3} L kg achenes^{-1}), and an insecticide (Cruiser 60 FS semillero, 6×10^{-3} L kg achenes^{-1}, Syngenta Crop Protection Inc., Omaha, NE). The same inbred line was used as before, but obtained from a different location and harvest year. Treatments 1–11 used achenes from a seed lot that expressed dormancy at high incubation temperatures and Treatment 12 (i.e., control) used achenes from a non-dormant seed lot. Each treatment was applied to 2 kg of achenes and moisture content was measured after each application and adjusted when necessary. Germination of three replicates was tested in the laboratory in distilled water at 12, 25, and 30°C, and emergence was tested under field conditions.

Emergence under Field Conditions

The industrially-treated achenes were hand sown in the field to a depth of 3 cm in two locations: Faculty of Agronomy (Exp. 1) (University of Buenos Aires, Buenos Aires, Argentina; 34°25′ S, 58°25′ W) on 13 Dec. 2012 and at Dow AgroSciences (Exp. 2) (Ferré, Buenos Aires, Argentina; 34°7′ S, 61°7′ W) on 26 Dec. 2012. In both experiments, the treatments were distributed in a randomized complete block design with three replicates because of soil heterogeneity. Within each block the twelve treatments were sown in two rows of 2.5 m length, with 50 achenes per row and 20 cm between rows. Plots were irrigated for adequate germination. Emergence was recorded when the cotyledons were visible. For Exp. 1 emergence was scored daily for 20 d, and for Exp. 2 final emergence was determined 20 d after sowing. Soil temperature was registered every 30 min with a thermometer buried at the depth of sowing and coupled to a data logger.

Additional Laboratory Experiments

Industrially-treated achenes (i.e., Treatments 1–12) were also washed for 15 min with distilled water. This was done for testing any washing experienced in the field due to irrigation, which may have leached away germination inhibitors. Three replicates of washed and non-washed achenes were incubated in distilled water under a single temperature (i.e., 25°C) and under alternating temperatures (i.e., 15°C for 12 h and then 35°C for 12 h) to emulate the thermal environment achenes experienced in the field.

Statistical Analyses

Analysis of variance (i.e., one-way ANOVA and two-way ANOVA), Tukey, and correlation matrix (at the 0.05 probability level) were performed using InfoStat Profesional 2012 (Di Rienzo et al., 2012) and GraphPad Prism 4.0 (Motulsky, 2003).

Table 1. Treatments applied on an industrial scale. Treatments 1 to 11 were applied to dormant achenes; Treatment 12 was the non-dormant control. The first chemical was a liquid insecticide (Reldan Plus, 2×10^{-5} L kg achenes^{-1}, Dow AgroSciences, Ciudad Autónoma de Buenos Aires, Argentina) that Dow AgroSciences applies to achenes before storage. The second chemical was a liquid mix applied before sowing in the field that contains water (2×10^{-3} L kg achenes^{-1}), a fungicide (Apron Gold, 3×10^{-3} L kg achenes^{-1}, Syngenta Production France S.A.S., Saint Pierre, France), a polymer (Secure, 7×10^{-4} L kg achenes^{-1}), a coating (Blue Solid, 3×10^{-3} L kg achenes^{-1}), and an insecticide (Cruiser 60 FS semillero, 6×10^{-3} L kg achenes^{-1}, Syngenta Crop Protection Inc., Omaha, NE).

Treatment	Sequence of applications
1	1st chemical only
2	2nd chemical only
3	1st chemical – washing at 1% sodium hypochlorite – drying – 2nd chemical
4	1st chemical – washing at 3% sodium hypochlorite – drying – 2nd chemical
5	1st chemical – washing at 7% sodium hypochlorite – drying – 2nd chemical – drying
6	1st chemical – 2nd chemical together with 3% sodium hypochlorite
7	1st chemical – 2nd chemical together with 7% sodium hypochlorite – drying
8	1st chemical – 2nd chemical – washing at 3% sodium hypochlorite – drying
9	1st chemical – 2nd chemical – washing at 7% sodium hypochlorite – drying
10	1st chemical – 2nd chemical – sprinkling at 3% sodium hypochlorite – drying
11	1st chemical – 2nd chemical – sprinkling at 7% sodium hypochlorite – drying
12	1st chemical only

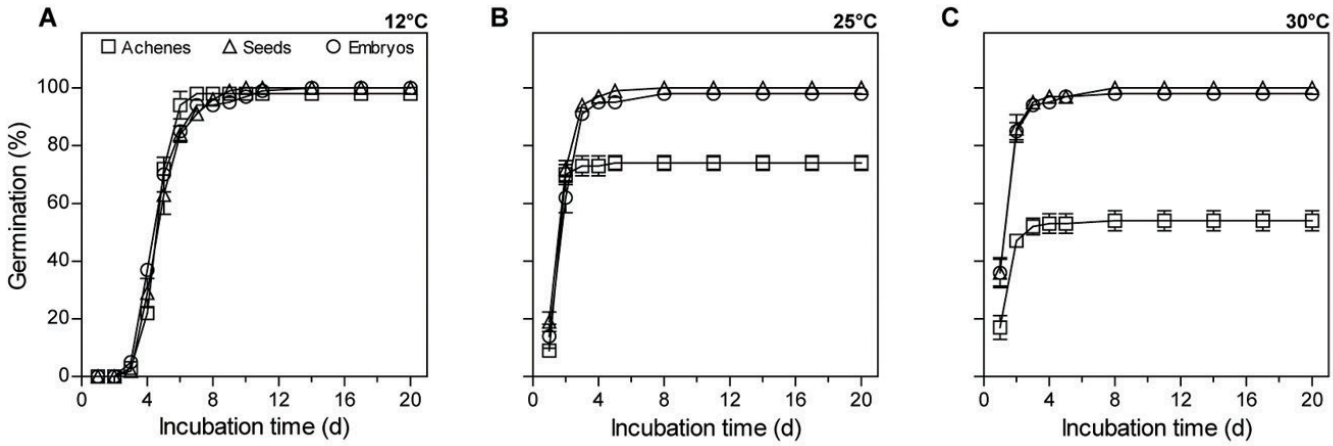

Figure 1. Germination percentage of achenes, seeds, and embryos incubated in distilled water at 12°C (A), 25°C (B), and 30°C (C). Error bars represent standard error of the mean for four replicates. Where no bars are shown the value of standard error of the mean is less than the size of the symbol.

RESULTS

Role of Embryo-Covering Tissues to Sunflower Dormancy

Seeds and embryos reached 100% germination regardless of incubation temperature (Fig. 1) indicating that embryo dormancy did not exist in this seed lot at this stage, and that the seed coat and endosperm did not inhibit germination. In contrast, achenes only reached 100% germination when incubated at 12°C (Fig. 1A); total germination of achenes at 25 and 30°C was significantly reduced to 75 and 50%, respectively, as compared to germination of seeds and embryos ($P < 0.05$; Fig. 1B and C). Hence, pericarp-imposed dormancy was expressed when achenes were imbibed at 25 and 30°C, but not at 12°C.

To further explore the contribution of embryo-covering tissues to the expression of dormancy at high temperatures, other treatments were applied, which consisted of the removal or damage of these tissues. At 25°C, germination of Pd and Pd+SCd+Ed was significantly improved as compared to A ($P < 0.05$; Table 2) and similar to S. At 30°C, the two damaging treatments and the removal of pericarp significantly increased germination percentage as compared to A ($P < 0.05$; Table 2); however, at 30°C the lowest damage intensity (Pd) was not enough to produce germination similar to that of S. These results indicate that the imposition of dormancy is due mostly to the pericarp, though the seed coat and endosperm may have some effect since Pd differed significantly from S at 30°C ($P < 0.05$; Table 2); nevertheless, the latter was only observed at the highest temperature tested.

Sodium Hypochlorite Treatment of Achenes Performed in the Laboratory

Germination of treated achenes with sodium hypochlorite (0.5%, 2 min) increased significantly by 23 and 25% when incubation was performed at 25 and 30°C, respectively, as compared to non-treated achenes ($P < 0.05$; Fig. 2B and C). These results suggest that pericarp-imposed dormancy could be removed, at least partially, by proper achene conditioning with sodium hypochlorite.

Abscisic Acid Concentration in Achenes and Seeds Incubated at Different Temperatures

Embryo ABA concentration from achenes remained fairly constant during incubation at 30°C (Fig. 3). Embryo ABA concentration from seeds at the same incubation temperature remained constant until 4 h, but thereafter it began to increase and reached 4000 pg mg DW^{-1} at 16 h after the start of incubation (Fig. 3A).

Embryo ABA concentration from achenes incubated at 12 and 30°C were similar (Fig. 3B) in spite of the markedly different final germination percentages attained

Table 2. Final germination percentage of achenes (A), achenes with a lateral cut affecting half of the pericarp length and towards the embryonic axis end (i.e., pericarp damage, Pd), achenes with a lateral cut affecting half of the pericarp length and towards the embryonic axis end plus a damage of the seed coat and endosperm (Pd+SCd+Ed), and achenes after the removal of pericarp but no damage to the seed coat neither endosperm (S), after 20 d of incubation at 25°C and 30°C in distilled water. Values are the means (for four replicates) ± the standard error of the mean.

Treatment	Germination†	
	At 25°C	At 30°C
	%	
A	75.0 ± 4.4 b	47.0 ± 5.7 c
Pd	97.0 ± 1.0 a	80.0 ± 5.4 b
Pd+SCd+Ed	90.0 ± 4.2 a	87.0 ± 3.0 ab
S	96.0 ± 0.0 a	98.0 ± 1.2 a

† Treatment means within each column followed by the same letter are not significantly different at the 0.05 probability level.

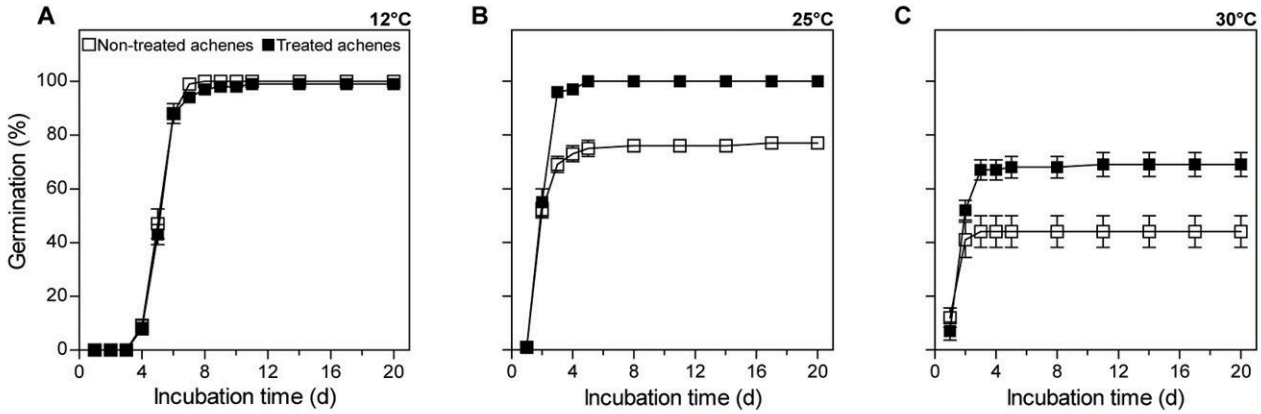

Figure 2. Germination percentage of non-treated and treated achenes with 0.5% sodium hypochlorite incubated in distilled water at 12°C (A), 25°C (B), and 30°C (C). Error bars represent standard error of the mean for four replicates. Where no bars are shown the value of standard error of the mean is less than the size of the symbol.

at these two incubation temperatures (inset of Fig. 3B). This suggests that embryo ABA concentration from achenes was not affected by incubation temperature and showed no relationship with the different dormancy expression observed at 12 and 30°C.

Abscisic Acid Concentration in Sodium Hypochlorite Treatment

The promotion of germination at 30°C in treated achenes with sodium hypochlorite (inset of Fig. 3C) was accompanied by a significant decrease in embryo ABA concentration from these achenes observed 16 h after the beginning of incubation ($P < 0.05$; Fig. 3C) and a few hours before the germination of the first achene.

Taken together these results indicate that neither pericarp-imposed dormancy nor its expression at 30°C appear to be caused by alterations in ABA concentration. However, they do suggest that the stimulatory effect of sodium hypochlorite on germination of dormant achenes at high incubation temperature may be associated with an alteration in ABA concentration, via lower ABA synthesis and/or a higher ABA catabolism, during imbibition and before germination.

Seed Sensitivity to Abscisic Acid at Different Incubation Temperatures

At 12°C 100 μmol L^{-1} ABA nearly completely suppressed seed germination, while at this temperature 10 μmol L^{-1} ABA only affected it slightly (Table 3). In contrast, 10 μmol L^{-1} ABA was enough to nearly suppress germination when incubation was performed at 25 and 30°C (Table 3). These results suggest that incubation at high temperatures increase ABA seed sensitivity.

Effects of Ethylene and Gibberellins

Addition of Tifón or GA_3 at different concentrations lead to a loss of pericarp-imposed dormancy in achenes at 25 and 30°C (Table 4).

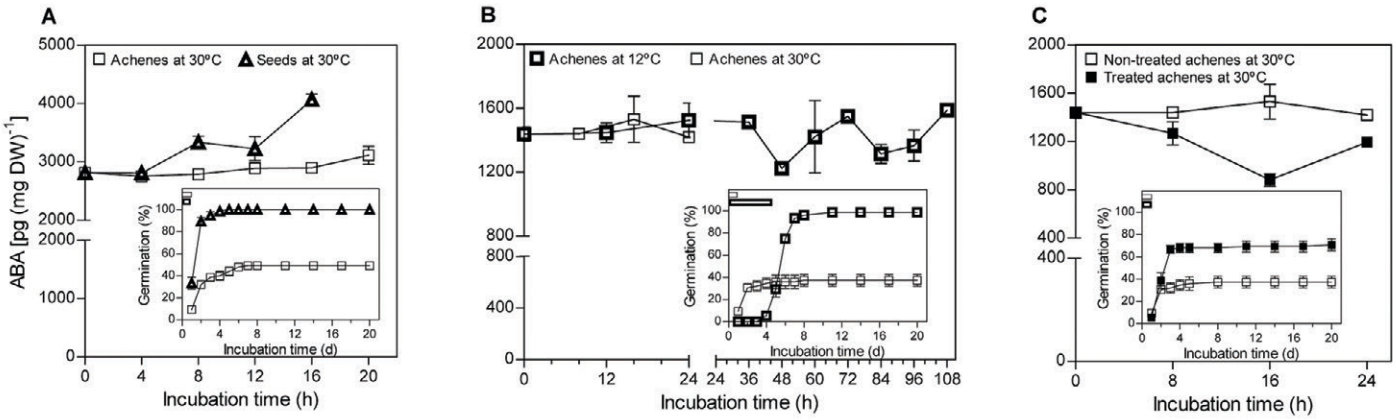

Figure 3. Embryo abscisic acid (ABA) concentration during incubation in distilled water. Achenes and seeds incubated at 30°C (A), achenes incubated at 12°C and 30°C (B), and non-treated and treated achenes (i.e., 0.5% sodium hypochlorite) incubated at 30°C (C). Bars represent standard error of the mean for three replicates, each measured twice. Error bars represent standard error of the mean for three replicates. Where no bars are shown the value of standard error of the mean is less than the size of the symbol. Insets show the germination percentages in distilled water. Horizontal bars of insets indicate the sampling period for embryo ABA concentration.

Table 3. Final germination percentage of seeds after 20 d of incubation at 12°C, 25°C, and 30°C in distilled water (control) and abscisic acid (ABA) at 1, 10, and 100 µmol L^{-1}. Values are means (for three replicates) ± standard error of the mean.

Treatment	Germination†		
	At 12°C	At 25°C	At 30°C
	%		
Control	98.7 ± 1.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
1 µmol L^{-1} ABA	100.0 ± 0.0 a	97.3 ± 1.3 a	100.0 ± 0.0 a
10 µmol L^{-1} ABA	84.0 ± 4.0 b	14.7 ± 4.8 b	26.7 ± 1.3 b
100 µmol L^{-1} ABA	14.7 ± 3.5 c	8.0 ± 2.3 b	16.0 ± 0.0 c

† Treatment means within each column followed by the same letter are not significantly different at the 0.05 probability level.

Table 4. Final germination percentage of achenes after 20 d of incubation at 25°C and 30°C in distilled water (control), Tifón solutions at 10, 100, and 500 µmol L^{-1} and GA_3 solutions at 100 and 200 µmol L^{-1}. Values are means (for five replicates) ± the standard error of the mean.

Treatment	Germination†	
	At 25°C	At 30°C
	%	
Control	74.4 ± 3.2 c	47.2 ± 4.6 b
10 µmol L^{-1} Tifón	88.8 ± 2.7 ab	84.0 ± 2.8 a
100 µmol L^{-1} Tifón	86.4 ± 1.6 ab	88.0 ± 3.1 a
500 µmol L^{-1} Tifón	84.8 ± 2.7 bc	88.0 ± 1.8 a
100 µmol L^{-1} GA_3	96.8 ± 1.5 a	83.2 ± 4.3 a
200 µmol L^{-1} GA_3	93.6 ± 2.7 ab	89.6 ± 2.7 a

† Treatment means within each column followed by the same letter are not significantly different at the 0.05 probability level.

Industrial Scale Treatments Assessed under Laboratory and Field Conditions

Treatments performed at the industry facilities (see Table 1) were tested in the laboratory. Most treatments resulted in high germination percentages at 12°C (Table 5). However, at 25 and 30°C dormancy was expressed differentially depending on the treatment and the incubation temperature. Germination was low in Treatments 1 and 2 (i.e., non-treated, dormant controls) at 25 and 30°C. In general, treatments that included washing with sodium hypochlorite 3 and 7% (i.e., Treatments 4, 5, 8, and 9) promoted germination at 25 and 30°C as compared to the dormant controls, but the effectiveness of this treatment was enhanced when the oxidant was applied after the second chemical (i.e., Treatments 8 and 9) (Table 5).

To evaluate if the improved germination observed under laboratory conditions also results in improved performance in the field, achenes belonging to the twelve treatments were sown in the field (Exp. 1 and 2). Results from Treatments 3 to 11 (i.e., treatments including the application of sodium hypochlorite) were pooled together and compared against Treatments 1 and 2 (i.e., non-treated controls) for each field experiment, separately: treated achenes with sodium hypochlorite produced significantly higher emergence in the field than non-treated controls in Exp. 1 and 2 ($P < 0.05$; Fig. 4A and Fig. 5A). Also, results from Treatments 8 and 9 (i.e., washing with sodium hypochlorite at 3 and 7%, respectively) were pooled together and compared against Treatments 1 and 2 for each field experiment, separately: in Exp. 1 achenes from Treatments 8 and 9 produced significantly

Table 5. Final germination percentage of achenes from Treatments 1 to 12 after incubation for 20 d at 12°C, 25°C, and 30°C in distilled water. Treatments are described in Table 1. Values are means (for three replicates) ± the standard error of the mean.

Treatment	Germination†		
	At 12°C	At 25°C	At 30°C
	%		
1	96.0 ± 2.3 ab	48.0 ± 4.0 cde	10.7 ± 1.3 ef
2	90.7 ± 2.7 abc	26.7 ± 5.3 de	2.7 ± 1.3 f
3	86.7 ± 2.7 abc	64.0 ± 2.3 bc	18.7 ± 3.5 def
4	92.0 ± 0.0 abc	52.0 ± 4.0 cd	28.0 ± 2.3 cde
5	96.0 ± 2.3 ab	65.3 ± 5.3 bc	38.7 ± 9.6 bcd
6	88.0 ± 2.3 abc	20.0 ± 8.3 e	2.7 ± 2.7 f
7	81.3 ± 4.8 bc	29.3 ± 9.3 de	10.7 ± 5.8 ef
8	97.3 ± 1.3 ab	82.7 ± 3.5 ab	49.3 ± 5.3 bc
9	98.7 ± 1.3 a	69.3 ± 5.8 abc	57.3 ± 2.7 ab
10	85.3 ± 6.7 abc	21.3 ± 5.3 e	6.7 ± 1.3 ef
11	78.7 ± 5.8 c	26.7 ± 5.3 de	2.7 ± 1.3 f
12	100.0 ± 0.0 a	94.7 ± 3.5 a	76.0 ± 4.6 a

† Treatment means within each column followed by the same letter are not significantly different at the 0.05 probability level.

higher emergence in the field than non-treated controls ($P < 0.05$; Fig. 4B); in Exp. 2 the differences were not significant ($P > 0.05$; Fig. 5B), though there was a trend of higher germination in achenes from Treatments 8 and 9. These results indicate that pericarp-imposed dormancy in this inbred line can be removed with sodium hypochlorite at an industrial scale, with measurable improvement under field conditions.

Although emergence in the field of sodium hypochlorite-treated achenes was higher than that from non-treated ones, the difference between treatments was lower than expected from laboratory experiments performed before field trials (Fig. 4A and B, Fig. 5A and B, and Table 5). This could be explained by the fact that the environmental conditions experienced by the achenes in the field are different from those used in the laboratory. Laboratory experiments were performed under constant incubation temperatures while, in the field, a large thermal amplitude was observed (Fig. 4C and Fig. 5C). Furthermore, leaching of possible inhibitors from the pericarp would also vary under both experimental conditions (i.e., petri dishes versus field). Therefore, because of these possibilities and in an attempt to reproduce those conditions in the laboratory, achenes from the twelve treatments were incubated in petri dishes with distilled water in four incubation conditions: non-washed achenes and washed achenes (i.e., washed with distilled water for 15 min before incubation) at 25°C and also at an alternating temperature, that is, achenes incubated at 15°C for 12 h and then at 35°C for 12 h.

The final germination percentage of achenes was measured after the incubation period of 20 days (Table 6). A significant interaction was found between treatments and incubation conditions ($P < 0.01$; Table 6). A correlation matrix was completed to relate germination percentages under each incubation condition to the field

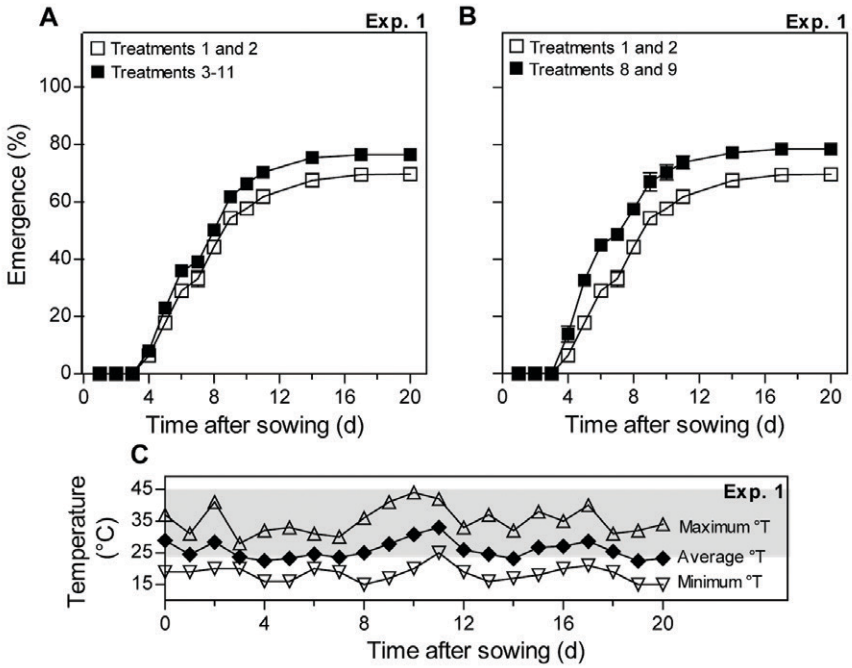

Figure 4. Emergence percentage dynamics for Exp. 1: Treatments 1 and 2 plotted with Treatments 3 to 11 (A), Treatments 1 and 2 plotted with Treatments 8 and 9 (B), and the maximum, average, and minimum soil temperatures; grey shading indicates temperatures greater than 25°C (C). Treatments are described in Table 1. Error bars represent standard error of the mean. Where no bars are shown the value of standard error of the mean is less than the size of the symbol.

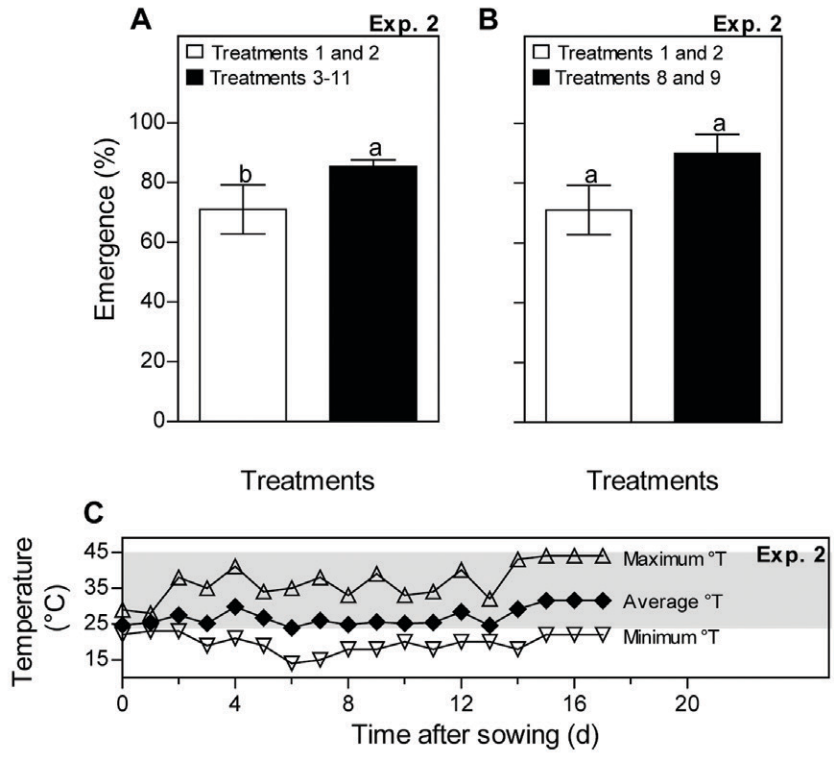

Figure 5. Final emergence percentage 20 d after sowing for Exp. 2: Treatments 1 and 2 plotted with Treatments 3 to 11 (A), Treatments 1 and 2 plotted with Treatments 8 and 9 (B), and the maximum, average, and minimum soil temperatures; grey shading indicates temperatures greater than 25°C (C). Treatments are described in Table 1. Error bars represent standard error of the mean. Different lowercase letters indicate significance at the 0.05 probability level.

Table 6. Final germination percentage of achenes from Treatments 1 to 12 after 20 d of incubation in distilled water for four IC (i.e., incubation conditions): non-washed achenes (NW) and washed achenes (W) using distilled water for 15 min before incubation at 25°C or the alternating temperature (15°C for 12 h and then 35°C for 12 h). Treatments are described in Table 1. Values are the means (for three replicates) ± the standard error of the mean. Results of the two-way ANOVA test are also shown.

	Germination			
Treatment	N-W achenes at 25°C	N-W achenes at 15/35°C	W achenes at 25°C	W achenes at 15/35°C
	%			
1	34.7 ± 2.7	78.7 ± 3.5	52.0 ± 4.6	74.7 ± 1.3
2	32.0 ± 2.3	38.7 ± 9.6	68.0 ± 0.0	58.7 ± 5.8
3	36.0 ± 4.0	54.7 ± 6.7	78.7 ± 1.3	80.0 ± 4.6
4	46.7 ± 1.3	64.0 ± 14.4	81.3 ± 3.5	86.7 ± 5.8
5	50.7 ± 3.5	66.7 ± 2.7	89.3 ± 4.8	88.0 ± 0.0
6	25.3 ± 11.9	36.0 ± 4.6	58.7 ± 5.8	61.3 ± 5.8
7	29.3 ± 4.8	40.0 ± 6.1	70.7 ± 4.8	60.0 ± 6.1
8	66.7 ± 12.7	84.0 ± 4.6	84.0 ± 4.0	80.0 ± 4.6
9	77.3 ± 2.7	70.7 ± 8.1	88.0 ± 4.6	84.0 ± 4.0
10	26.7 ± 8.1	48.0 ± 12.2	60.0 ± 4.6	61.3 ± 5.3
11	37.3 ± 11.9	36.0 ± 2.3	61.3 ± 1.3	57.3 ± 8.1
12	85.3 ± 2.7	97.3 ± 1.3	94.7 ± 3.5	98.7 ± 1.3
Effect:		*p*-value:		
Treatment (T)		<0.001		
Incubation conditions (IC)		<0.001		
T x IC		0.002		

emergence of industrially-treated achenes of Exp. 1. It was shown in the field of Exp. 1 that there was a significantly higher correlation with the incubation conditions of washed achenes at 25°C ($r = 0.66$; $P < 0.05$; data not shown) and washed achenes at the alternating temperatures ($r = 0.66$; $P < 0.05$; data not shown) as compared to the rest.

DISCUSSION

One objective of the seed industry is to obtain a seedling from each seed, and dormancy impairs this outcome, leading to important economic losses (Snow et al., 1998; Nasreen et al., 2015). Past research was largely devoted to study sunflower embryo dormancy (Bazin et al., 2011; El-Maarouf-Bouteau et al., 2015). However, the literature is scarce in terms of studies on pericarp-imposed dormancy. In this work we assessed a sunflower inbred line with long-lasting dormancy whose expression is evident at high incubation temperatures (i.e., 25 to 30°C), which was determined to be imposed by the pericarp (Fig. 1 and Table 2). At high temperatures, substances in the pericarp of sunflower are prone to be oxidized and bind more oxygen than at low temperatures, thus exacerbating oxygen deprivation to the embryo (Benech-Arnold et al., 2006). This oxygen deprivation may have altered ABA metabolism in favor of its accumulation, though our experiments did not detect any consistent difference in ABA concentration during incubation at different temperatures when comparing seeds versus achenes (Fig. 3A and B). Therefore, the physiological mechanism responsible for inhibition of germination remains undetermined, whose alteration may be caused by an induced hypoxia condition imposed by the pericarp. In contrast, seed sensitivity to ABA was increased several-fold when incubated at high temperatures (Table 3) suggesting that lower germination at 25 and 30°C may be explained in part by an increase in ABA sensitivity. An enhanced responsiveness to ABA exists in dormant barley embryos exposed to high incubation temperature and low oxygen tensions (Benech-Arnold et al., 2006). A mechanism relating oxygen levels and ABA signaling was recently described in *Arabidopsis* and barley, and it involves a targeted proteolysis pathway (N-end rule pathway). Reduced activity of this pathway under low oxygen levels leads to an increased ABA response through stabilization of the ethylene response factor ERFVII (Holman et al., 2009; Mendiondo et al., 2015). A similar pathway may operate in sunflower, and furthermore, may be a common mechanism involved in the enhanced ABA response observed at high incubation temperatures in many different species and may promote low oxygen tensions within the seed.

Washing the achenes with sodium hypochlorite, an oxidizer, was sufficient to largely overcome dormancy (Fig. 2B and C) either by reducing hypoxia to the embryo or ABA concentration, although the second possibility might be a consequence of the first one (Fig. 3C). Similar results were obtained in barley by Wang et al. (1998) using hydrogen peroxide (H_2O_2) as an oxidant that also resulted in a reduction of the level of endogenous ABA. As an alternative explanation to the effect of sodium hypochlorite it could be argued that it is through a weakening of the pericarp as it was reported to occur in thermo-inhibited lettuce seeds (Drew and Brocklehurst, 1984). Ethylene and gibberellins also overcame pericarp-imposed dormancy suggesting that the germination response is affected by the balance of multiple hormones (Table 4). In view of the

success of sodium hypochlorite treatments under laboratory conditions, and considering that its implementation should be much less expensive than other tested methods of removing dormancy (i.e., ethylene and gibberellins), the effect of sodium hypochlorite was investigated at an industrial scale throughout the procedure used by the seed industry to prepare achenes for sowing, followed by evaluating seed performance under field conditions. Laboratory assessments (Table 5) performed immediately after industrial applications showed that, within each temperature tested, the treatments consisting in washing the achenes with 3 and 7% of sodium hypochlorite after application of the second chemical (i.e., Treatments 8 and 9, respectively) produced higher germination percentage as compared to the other treatments, particularly at 30°C (Table 5). Therefore, these two treatments were effective in counteracting pericarp-imposed dormancy. Under field conditions these two treatments together had close to 20% higher emergence than the non-treated controls (Fig. 4B and Fig. 5B). In addition, Treatments 3 to 11 (i.e., treatments including the application of sodium hypochlorite) together had close to 15% higher emergence than the non-treated controls (Fig. 4A and Fig. 5A). However, the improvement observed in field emergence was much less than that measured between treated and non-treated achenes under high temperatures in laboratory conditions performed before field trials (Fig. 4A and B, Fig. 5A and B, and Table 5). The lower difference between treatments under field conditions was not due to a poorer performance of treated achenes, but to a higher than expected performance of non-treated achenes. Environmental conditions in the field are different from those under which germination experiments were performed in the laboratory. For this reason, the industrially-treated achenes were further incubated in the laboratory under incubation conditions that were designed to mimic field conditions possibly encountered by the achenes, namely, rain or irrigation leaching inhibitors away and fluctuating temperatures. Hence, treatments included washing of achenes for 15 min with distilled water before incubation in petri dishes and incubation at 15°C for 12 h and then 35°C for 12 h (Table 6). A significant strong correlation was found between emergence in the field of Exp. 1 and the germination under incubation conditions of washed achenes at 25°C and washed achenes at the alternating temperature (15°C for 12 h and then 35°C for 12 h), suggesting that under field conditions, consequences of pericarp-imposed dormancy may be attenuated due to the prevalence of a fluctuating soil thermal regime and leaching of possible inhibitors.

In this work we provided insight into pericarp-imposed dormancy in sunflower. This dormancy may represent a limitation for crop establishment under field conditions, although less than can be expected from laboratory experiments. We found that this problem can be overcome with sodium hypochlorite acting as an oxidizing agent that can be applied even at industrial scale, with measurable field benefits.

CONCLUSIONS

The present study provides a greater understanding of the physiological basis of pericarp-imposed dormancy in sunflower and its impact on crop emergence. The results reported suggest that sodium hypochlorite can be applied on an industrial scale to remove pericarp-imposed dormancy. Future research is needed to assess how embryo-covering tissues control oxygen levels in the embryo and how oxygen availability to the embryo affects germination.

Acknowledgments

This work was supported by Dow AgroSciences and CONICET. We thank Silvina Enciso for excellent technical assistance with ABA determinations and Dr. Déborah Rondanini for assistance with statistical analysis.

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