

Article

Not peer-reviewed version

---

# Mechanisms and Inheritance of Dormancy in Sunflower (*Helianthus annuus* L.) Achenes

---

[Gonzalo Joaquín Arata](#) , Mailén Riveira-Rubin , Diego Batlla , [María Verónica Rodríguez](#) \*

Posted Date: 15 May 2026

doi: 10.20944/preprints202605.0988.v1

Keywords: sunflower; germination; dormancy; thermo-inhibition; hybrid seed; inheritance; reciprocal crosses; abscisic acid; pericarp



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC, OpenAlex.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

# Mechanisms and Inheritance of Dormancy in Sunflower (*Helianthus annuus* L.) Achenes

Gonzalo Joaquín Arata <sup>1,2</sup>, Mailén Riveira-Rubin <sup>1</sup>, Diego Batlla <sup>1,2</sup> and María Verónica Rodríguez <sup>1,3,\*</sup>

<sup>1</sup> Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Universidad de Buenos Aires (UBA), Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA), Av. San Martín 4453, C1417DSE, Ciudad Autónoma de Buenos Aires, Argentina

<sup>2</sup> Cátedra de Cultivos Industriales, Departamento de Producción Vegetal, Facultad de Agronomía de la Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE, Ciudad Autónoma de Buenos Aires, Argentina

<sup>3</sup> Cátedra de Fisiología Vegetal, Departamento de Biología Aplicada y Alimentos, Facultad de Agronomía de la Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE, Ciudad Autónoma de Buenos Aires, Argentina

\* Correspondence: mvr@agro.uba.ar; Tel.: +5411 5287-0111

## Abstract

In dormant sunflower achenes, several structures -pericarp, seed coat and embryo- contribute to repress germination. Achene dormancy varies greatly among cultivated sunflowers, and how dormancy is transmitted to the hybrid progeny is relevant for hybrid seed production, but also to understand the role of these structures in achene dormancy. This work investigated how dormancy is transmitted to the F1 progeny with special focus on inhibition of germination at warm temperatures, or thermo-inhibition. Reciprocal crosses were performed using three oilseed inbred lines with varying dormancy phenotypes. Germination of achenes, seeds, and embryos was tested at 10 and 30 °C at harvest and during postharvest, together with response to hormones (abscisic acid, ethylene and gibberellins) and measurements of endogenous ABA levels. Results show that expression of maternally inherited, pericarp-imposed thermo-inhibition is conditional to the level of dormancy displayed by the hybrid embryo, which follows a zygotic with incomplete dominance pattern. While embryo sensitivity to ABA related positively with thermo-inhibition, surprisingly, embryonic ABA content was inversely related to dormancy level across genotypes. Our results provide novel insight into physiological control of achene dormancy in sunflower while contributing to improve breeding for high quality hybrid seed.

**Keywords:** sunflower; germination; dormancy; thermo-inhibition; hybrid seed; inheritance; reciprocal crosses; abscisic acid; pericarp

## 1. Introduction

Germination is a critical event in the plant life cycle. Seed dormancy is defined as an internal block to germination in a viable seed (or other unit) imbibed under otherwise favorable conditions [1]. Dormancy mechanisms enable seeds to integrate spatial and temporal environmental cues, thereby preventing germination until conditions that maximize population survival are met (reviewed in [2]). Among the dormancy classes defined by Baskin and Baskin [3], non-deep physiological dormancy is the most prevalent in temperate species, including both wild and cultivated sunflowers. Physiological dormancy is imposed during seed development and is regulated primarily by the balance between the antagonistic hormones abscisic acid (ABA), which promotes dormancy, and gibberellins (GA), which promote germination. Other hormones, such as ethylene, can modulate this ABA–GA balance and participate in the developmental and environmental

regulation of dormancy depth [2,4]. Although the central role of the ABA–GA balance in dormancy control is well established, the underlying mechanisms—specifically where and how hormonal regulation operates and which seed structures are involved—vary substantially among species.

The sunflower dispersal unit is a dry, one-seeded fruit derived from an inferior ovary [5]; although it is strictly a cypsel, it is commonly referred to as “achene” as in the present text. At maturity, the dry, quiescent embryo is surrounded by a thin layer of endosperm adhered to the multilayered testa, forming the “true” seed which is further enclosed within a hard pericarp. In sunflower, as in many species producing orthodox seeds, dormancy levels gradually decline during dry after-ripening. This process results in faster and more complete germination at a given imbibition temperature and broadens the temperature range permissive for germination [4,6]. Once rendered non-dormant, sunflower achenes can germinate across a wide thermal range (approximately 5 to 40 °C; [7]), a trait that is highly desirable for commercial seed quality. Although domestication and breeding have generally reduced dormancy levels in crop species, strong dormancy phenotypes remain problematic in cultivated sunflower [6].

In cultivated sunflower, dormancy expression has been reported at cool imbibition temperatures (e.g., 10 °C; [8]), at warm temperatures (above 20–25 °C; [9,10]), or at both extremes of the thermal range [7]. These contrasting responses generate positive, negative, or bimodal germination patterns along the temperature gradient. Dormancy expressed at low temperatures is associated with embryo dormancy [7] and is typically alleviated after several weeks of dry storage. In contrast, dormancy expressed at high temperatures (25–30 °C) is imposed by the pericarp, as isolated (naked) embryos readily germinate under these conditions. This pericarp-mediated inhibition can persist indefinitely in some genotypes [9,10] and meets the definition of thermo-inhibition (TI) as proposed by [11].

Variability in dormancy mechanisms was investigated by our group in 22 sunflower genotypes representing oilseed, edible, and ruderal types [6]. Low-temperature-expressed dormancy (LTED), characterized by increased germination at warmer incubation temperatures, was observed in all genotypes examined and was closely associated with changes in embryo dormancy and/or sensitivity to ABA. Thermo-inhibition, while most pronounced in ruderal types, was also present to varying degrees in cultivated material and established an upper thermal limit for germination, typically between 25 and 30 °C. Notably, embryo responsiveness to ABA at 30 °C was also associated with TI, indicating that TI cannot be attributed solely to constraints imposed by the pericarp [6].

In the context of hybrid seed production, understanding how parental genotypes influence the dormancy phenotype of F<sub>1</sub> progeny is of particular importance. Seed dormancy inheritance is inherently complex because it involves multiple tissues with distinct genetic origins [1]. In sunflower, the achene is composed of structurally and genetically distinct components: the embryo is diploid and biparental (1♀:1♂), the endosperm is triploid (2♀:1♂), and both the testa and the pericarp are diploid and maternally derived (2♀), originating from the ovary and the floral receptacle, respectively [12]. The relative contribution of these tissues to dormancy determines whether inheritance is predominantly maternal or nuclear (zygotic).

Maternal inheritance may arise from the predominance of maternally derived tissues, such as the seed and fruit coats, or from cytoplasmic inheritance via organellar genomes, which are also maternally transmitted and may influence embryo phenotype [13–15]. In addition, maternal effects on endosperm-associated traits may result not only from the chromosomal imbalance favoring the maternal parent (2♀:1♂) but also from epigenetic regulation. Genomic imprinting occurs in the Arabidopsis endosperm, resulting in exclusive expression of maternal alleles and a dormancy phenotype resembling that of the maternal parent [16]. These genetic mechanisms must be clearly distinguished from maternal environmental effects, which arise from environmental conditions experienced by the mother plant during seed development and can also modify progeny traits [17–19]. Conversely, embryo-associated dormancy may depend primarily on the zygotic genotype (nuclear inheritance), with equal genetic contributions from both parents. In such cases, dormancy expression may follow complete dominance (progeny resembles one parent), incomplete dominance

(intermediate phenotype), or codominance (simultaneous expression of both parental phenotypes; [20]).

Several studies have documented maternal inheritance of seed dormancy in species where dormancy is imposed by seed or fruit coverings, including *Arabidopsis* [16,21,22], and wild sunflower [23–26]. By contrast, embryo-associated dormancy governed by the zygotic genotype has been reported in several crop species, including wheat [27], rice [28], and lettuce [29], where dormancy generally exhibits a pattern of incomplete dominance.

To investigate how LTED and TI relate to specific achene components and how these mechanisms are inherited in  $F_1$  progeny, reciprocal crosses were performed among three sunflower lines exhibiting contrasting dormancy phenotypes [6]. Parents and hybrids were characterized using a combination of physiological approaches—germination of intact achenes, seeds, and isolated embryos under different media and incubation temperatures—and morphological analyses, including measurements of achene size and pericarp anatomy. This integrative approach enabled the association of embryo- and pericarp-related traits with whole-achene dormancy phenotypes and their patterns of inheritance. In parallel, parental lines were examined for physiological traits such as embryo ABA content and sensitivity to hypoxia, providing further insight into the mechanisms underlying dormancy variation and thermo-inhibition.

## 2. Materials and Methods

### *Cultivation of Contrasting Parental Lines and Reciprocal Crosses*

Sunflower genotypes displaying different dormancy phenotypes previously described in [6] were selected to perform reciprocal crosses. Field trials were conducted at the experimental field of the Faculty of Agronomy, University of Buenos Aires, during the 2017–2018 and 2018–2019 growing seasons. Each genotype was cultivated in a single plot (randomly assigned within the field) comprising eight rows of 10–12 m (0.7 m between rows, and 0.25 m between plants). The genotypes cultivated in both experiments were lines 609 (low–intermediate dormancy, without TI), 1579 (intermediate–high dormancy, with TI), and HA342 (high dormancy with TI). Sowing dates were staggered to synchronize flowering time (R5.1) among genotypes. Phenology of individual plants was recorded according to [30]. At stage R4, each capitulum was covered with a semi-heavy polyamide bag to prevent cross-pollination between stages R5.1 and R5.9 (from onset of flowering to 90% anthesis). Once plants reached R5.1, emasculation of mother plants was performed daily by removing anthers at dawn, immediately upon anther emergence. Residual pollen was removed with a fine water spray. Given that stigma receptivity is low on the first day of exposure, contamination by self-pollen was considered negligible. After approximately 60% of the capitulum was emasculated, central florets were removed to prevent contaminating previously emasculated florets. This treatment was applied to pure parental plants ( $\text{♀A} \times \text{♂A}$  and  $\text{♀B} \times \text{♂B}$ ) to ensure comparable source–sink dynamics that might otherwise influence seed dormancy levels. Pollen was manually collected from selected paternal plants, stored in plastic bags, and kept at 4 °C. Manual pollination was conducted twice: once when the “mother” capitulum reached 30% emasculation and again at 60%, to avoid loss of stigma viability and receptivity. After fruit set, capitula from pure parental plants were monitored to determine physiological maturity and harvest time (Figure S1).

Plants selected for germination assays (parentals and hybrids) were those whose phenology and flowering dates matched (R5.1 occurring within a 4–5 d window). For each genotype, between 6–10 mature capitula were harvested, manually threshed, and achenes from the middle third were retained and pooled together. Drying was performed in experimental seed dryer for 6–8 h at 35 °C until reaching 6–7% moisture content on a dry basis. Achenes were stored dry in sealed plastic bags at 25 °C in a temperature-controlled chamber.

### *Germination Assays*

Germination assays involved incubating 25 achenes/seeds or 20 embryos per Petri dish on double filter paper moistened with 6 mL distilled water (three replicates per treatment) for 15 days in temperature-controlled chambers set constant at 10 °C and 30 °C. Germination (visible radicle protruding and curving slightly) was recorded every 2–3 days; germinated units were removed, and water was replenished as needed. Assays were conducted at harvest (after conditioning), and during storage at 30, 75 and 150 days after harvest (DAH). Achenes and embryos were incubated in distilled water (control) or in solutions containing plant hormones (ABA, GA<sub>3</sub>, ethephon) at various concentrations. These treatments were also tested at 10 °C and 30 °C.

### *Determination of Endogenous ABA by Radioimmunoassay*

Achenes were sampled at harvest, and after storage (150 days after conditioning, DAH) to quantify endogenous ABA levels in dry or imbibed achenes (in distilled water, 24 h at 30 °C). Each treatment was assessed in 3 biological replicates of 25 embryos, with 2 technical duplicates. Coverings were removed, embryo axes were dissected with scalpel and immediately frozen in liquid nitrogen and stored at –80 °C until processing. Samples were subsequently lyophilized (vacuum cryodesiccation), weighed, and ground for aqueous ABA extraction. Quantification was performed by radioimmunoassay following [30], and results were expressed as picograms (pg) ABA per gram of dry weight of the original sample.

### *Evaluation of Embryo Sensitivity to Hypoxia*

The germination capacity of naked embryos was evaluated under hypoxic and normoxic conditions (3% and 21% O<sub>2</sub>, respectively) to simulate the gas-exchange restriction imposed by the pericarp and seed coat. Incubations were carried out inside a semi-hermetic plastic chamber (Model C-274, BioSpherix, New York) placed within a temperature-controlled incubator and connected to an O<sub>2</sub> controller (PRO-OX Model 110, BioSpherix, New York). The controller was supplied with N<sub>2</sub> gas, which was injected into the chamber to maintain a stable O<sub>2</sub> concentration of 3%. Use of this O<sub>2</sub> concentration was based on previous results [9]. An incubation temperature of 30 °C was selected because thermo-inhibition (TI) is enhanced under these conditions in cultivated sunflower varieties [6]. Embryo sensitivity to ABA was also evaluated at 30 °C by incubating embryos in water and ABA solutions (0.5, 5 and 25 μM) at both 3 and 21% oxygen.

Embryos were placed on filter paper layered over approximately 2 cm of cotton inside plastic trays (9 cm wide × 6 cm deep × 4 cm high). Cotton and filter paper were moistened with 55 mL of either distilled water or ABA solutions (0.5, 1, 5, and 25 μM). Each tray contained 20 units (fruits or embryos), with three replicates per treatment (n = 3). After a 7-day incubation period, germination was recorded.

### *Anatomical Characterization and Morphological Assessment of Achene Components*

To identify and describe structural differences in the fruit pericarp (e.g., thickness, number of cell layers, presence of sclerenchymatic tissues; [32]) in parental lines (609, 1579, HA342) and their reciprocal hybrids at harvest, histological preparations were performed. Samples were fixed in FAA for 48 h, dehydrated through an ascending alcohol series, cleared with xylene, and embedded in pure paraffin. Serial sections of 10–15 μm were obtained using a Minot rotary microtome. Sections were stained with safranin–fast green, which differentiates primary and secondary cell walls, and permanently mounted using Canada balsam. Histological preparations were examined and photographed using a fluorescence microscope (Zeiss Axioplan). The number of pericarp cell layers, including sclerenchymatic cells within the median parenchyma, was recorded. Pericarp thickness was measured from digital micrographs using Image Tool software [33]. Tissue characterization protocols were based on studies of Asteraceae fruit anatomy [32–36]. Additionally, the morphological structure of achene components in each genotype (parental lines and their hybrids) was described.

Achene dimensions at harvest (length, width, and thickness) were measured using a caliper on a subsample of 10 achenes. The weight of 100 achenes was determined in triplicate, followed by separation of seed and pericarp to calculate the pericarp-to-seed ratio.

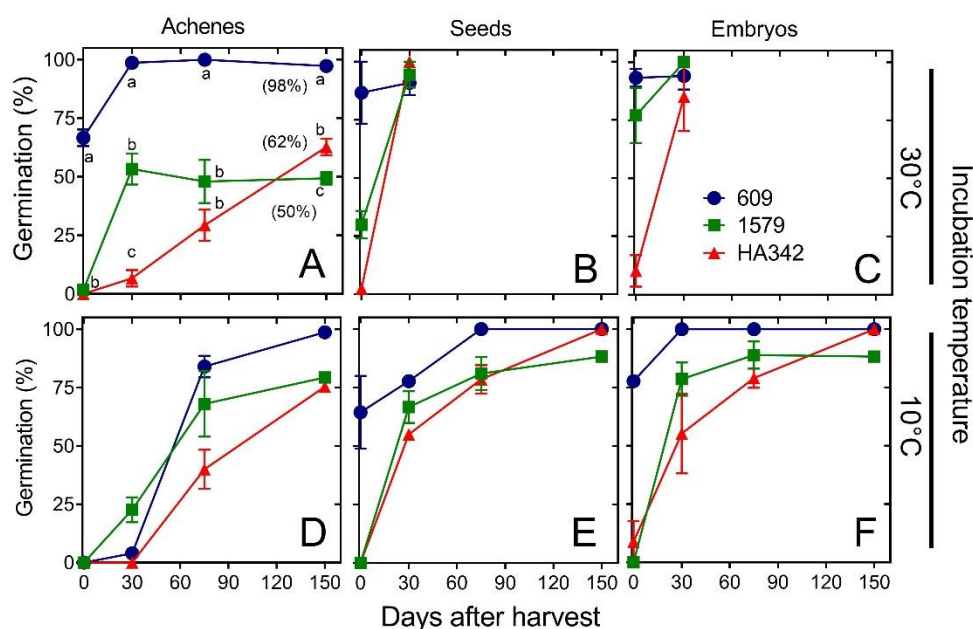
### Statistical Analysis

Germination data and other variables (including endogenous ABA levels, anatomical measurements) were subject to one or two way analysis of variance (ANOVA) and Tukey tests ( $p \leq 0.05$ ) or Kruskal-Wallis followed by multiple comparisons when assumptions for ANOVA were not met. All analyses were conducted using InfoStat 2018 supported by R Studio Statistics (Central Core R, 2017). Figures were produced using GraphPad Prism 7.0 [39].

## 3. Results

### 3.1. Dormancy Release Dynamics and Structural Components in Parent Lines

Dormancy release dynamics for sunflower lines 609, 1579 and HA342 are shown in Figure 1. Final germination percentage values obtained at harvest and different postharvest times are shown for achenes, seeds, and embryos tested at two incubation temperatures (30 and 10 °C). The temporal changes in achene germination percent at 30 °C indicate that dormancy release occurred rapidly in line 609 as compared to 1579 and HA342; this is reflected in the storage period (in days) required to reach 50% germination, which was 0, 31 and 122, for 609, 1579 and HA342, respectively; Table S1). Lines 1579 and HA342 also displayed thermo-inhibition (TI) at 30 °C, otherwise absent in 609. TI was imposed by the pericarp at 30 °C and limited achene germination to 50-60% (Figures 1A and S2). This maximum was reached earlier in 1579 as compared to HA342 (i.e., 30 and 150 DAH, respectively) because of faster dormancy release rate in 1579.



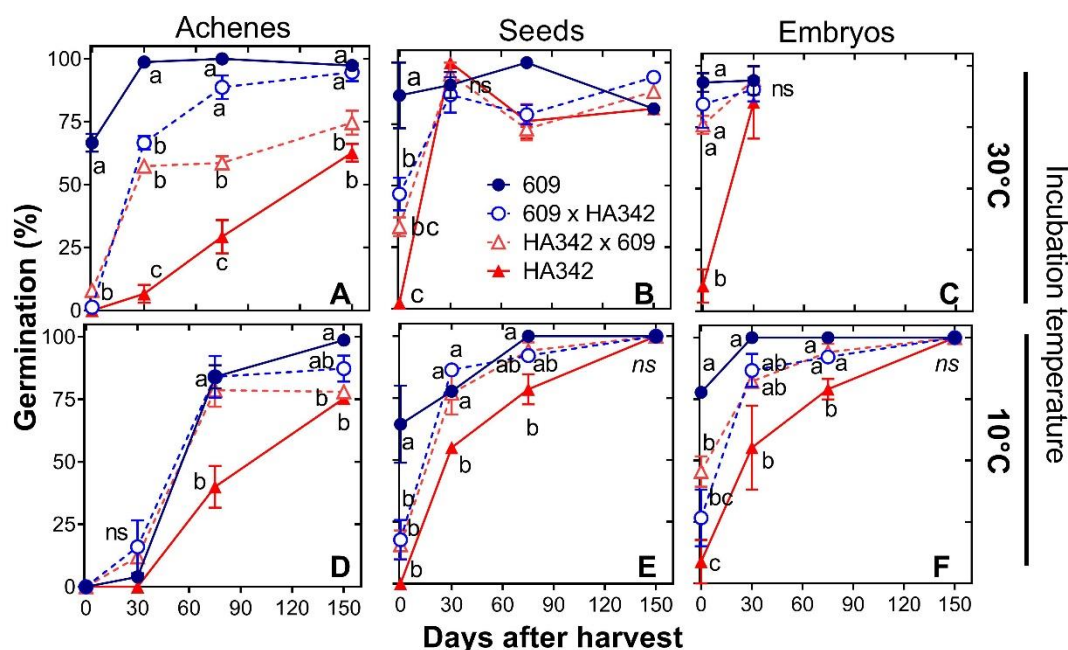
**Figure 1. Dormancy release dynamics for different achene parts in parent lines.** Final germination (%) of achenes (A,D), seeds (without pericarp; (B,E)), and naked embryos (C,F) tested at different times after harvest (0-150 DAH), for parent lines “609”, “1579” and “HA342”. Germination tests were conducted at 30 °C (A–C) and 10 °C (D–F). Final achene germination percentage is also shown in parentheses in A. Each data point is the mean and bars are SEM (n=3 replicate Petri dishes).

Initial germination at 30 °C of isolated embryos and seeds was high in 609, intermediate in 1579 and very low in HA342 (Figure 1B,C), indicating different levels of embryo and seed coat-imposed dormancy (Figure S2). Nevertheless, by 30 DAH, seed and embryo germination values at 30 °C were similar and close to maximum in all genotypes, indicating that embryo and seed coat-imposed dormancy decayed rapidly between 0-30 DAH (Figures 1B,C and S2). Therefore, differences in achene germination at 30 DAH (100, 50 and 5% for 609, 1579 and HA342, respectively) were related to differences in the restrictive effect of the pericarp. None of the genotypes showed a meaningful seed-coat effect during the postharvest period; seed and embryo germination were generally similar (Figures 1B,C and S2).

At 10 °C incubation, dormancy release dynamics of achenes were more similar among parents, and 609 displayed null germination at 0-30 DAH. By 30 DAH, inhibition of achene germination in 609 at 10 °C was imposed mostly by the pericarp (and to a lesser extent by the seed coat; Figures 1 and S2B). Instead, achene germination in 1579 and HA342 closely followed changes in embryo germination, with a minor contribution of the pericarp at 30 DAH (Figure S2D,F).

### 3.2. Achene Dormancy in HA342:609 Hybrids is Influenced by Zygote and Maternal Genotypes

In the 609:HA342 crossing system (Figure 2), during early dormancy release (0-30 DAH) both reciprocal hybrids behaved similarly and differed from their parents displaying intermediate behavior. This applied to both seeds and embryos, and for achenes until 30 DAH. This pattern is compatible with zygotic control of embryo (and achene) dormancy. Nevertheless, as storage progressed to 70 DAH, achene germination increased only for 609xHA342 (reaching values like its female parent, 609), while in the case of HA342x609, germination stabilized at ca 65% (and was gradually approached by its corresponding female parent, HA342) (Figure 2A). In the case of HA342 and HA342x609, thermo-inhibition (TI) imposed by the pericarp was evident as a 30-35% inhibition of achene germination at 30 °C which persisted beyond the 5-month storage period. Seeds and embryos germinated fully at 30 °C after 30 d of storage, regardless of their genotype, supporting that TI expressed at 30 °C depends on the pericarp, maternally inherited from HA342.



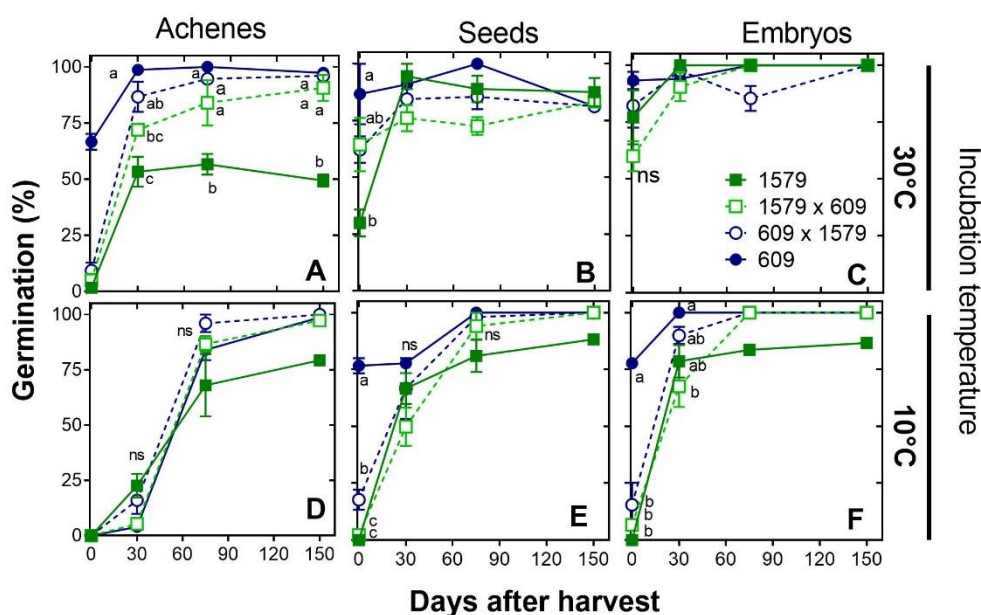
**Figure 2.** Dormancy release during post-harvest in parent lines 609 and HA342 and reciprocal F1 progenies. Final germination (%) of achenes (A,D), seeds (B,E), and embryos (C,F) tested at different times after harvest (0-150 DAH), for parent lines and F1 hybrids obtained from reciprocal crosses between less-dormant line “609” and more dormant line “HA342”. Germination tests were conducted at 30 °C (A–C) and 10 °C (D–F). Each data point

is the mean of 3 replicate Petri dishes  $\pm$  SEM (n=3) with 25 units each. ANOVA followed by multiple comparisons (Dunnett's test) was performed on each time (0, 30, 75 or 150 DAH).

### 3.3. Achene Dormancy in 1579:609 Hybrids is Defined by the Zygote

In the 609:1579 crossing system (Figure 3) achenes from both reciprocal hybrids behaved similarly and followed dormancy release dynamics intermediate to their parents. Different from the previous crosses (involving HA342), no maternal effects were observed; achenes from 1579 $\times$ 609 did not display TI and eventually reached maximum germination values like 609 and 609 $\times$ 1579, significantly higher than 1579 (Figure 3A). A zygotic control of dormancy with incomplete dominance is supported by the intermediate dormancy release dynamics observed in both hybrids as compared to their parents (Figure 3A; Table S1) but also by seed germination data (reciprocal crosses were similar and intermediate to both parents). Embryo germination in water at 30 °C (Figure 3C) was high and similar for both parents and hybrids since harvest. By 30 DAH, seed and embryo germination had reached values above 90% in both parents and hybrids indicating a differential effect of the pericarp on achene germination. Thermo-inhibition was observed in 1579 since 30 DAH but not in the F1 progeny.

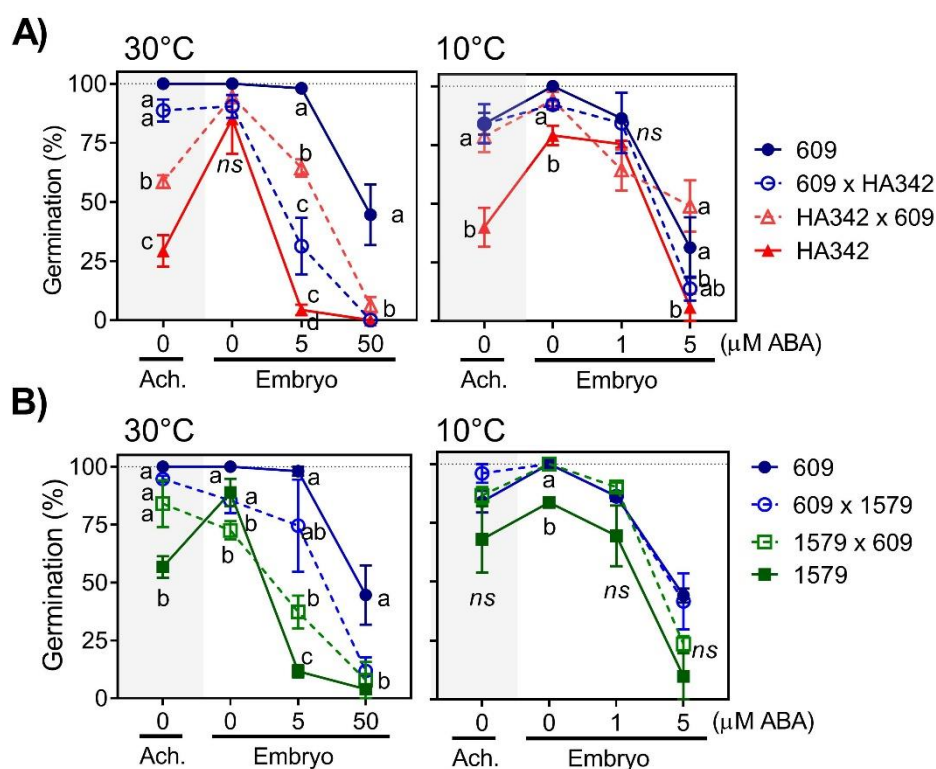
At 10 °C imbibition, achene germination increase was delayed as compared to 30 °C, and all four genotypes displayed similar dynamics (Figure 3D). Notably, seed/embryo dormancy was significantly lower in 609 as compared to hybrids and 1579, but this lower embryo dormancy was compensated with a stronger inhibition by the pericarp (Figures 3E–F and S2). Seeds/embryos in both hybrids behaved like dormant parent 1579, suggesting dominance by dormant allele/s from 1579 or incomplete dominance. These two possibilities were further investigated in the following experiments.



**Figure 3. Dormancy release during post-harvest in parent lines and reciprocal F1 progenies.** Final germination (%) of achenes (A,D), seeds (B,E), and embryos (C,F) tested at different times after harvest (0-150 DAH), for parent lines and F1 hybrids obtained from reciprocal crosses between less-dormant line “609” and more dormant line “1579”. Germination tests were conducted at 30 °C (A–C) and 10 °C (D–F). Each data point is the mean of 3 replicate Petri dishes  $\pm$  SEM (n=3) with 25 units each. ANOVA followed by multiple comparisons (Dunnett's test) was performed on each time (0, 30, 75 or 150 DAH).

### 3.4. Embryo Sensitivity to Exogenous ABA Supports a Zygotic Control with Codominance Pattern

To further characterize physiological responses in embryos from reciprocal crosses and parents, embryos were incubated in ABA solutions at both 30 °C and 10 °C (Figure 4). Results for HA342:609 crossing system (Figure 4A) at 30 °C support that both hybrids had a similar sensitivity to ABA which was intermediate to their parents. At 10 °C, sensitivity to ABA was similar among genotypes. Similarly, in the 1579:609 crossing system (Figure 4B), at 30 °C, hybrids showed an intermediate behavior to their parents, while differences were negligible when incubated at 10 °C.



**Figure 4. Embryo sensitivity to ABA.** Germination data for parent lines and reciprocal crosses is shown for 609 vs HA342 (A) and for 609 vs 1579 (B) after incubation at 30 °C (left) and 10 °C (right). Each panel shows the final germination of achenes in water (shaded area) and embryos in different ABA solutions (0-50 μM ABA at 30 °C, and 0-5 μM ABA at 10 °C). Achenes had been after-ripened for 75 d (75 DAH). Each data point is the mean of 3 replicate Petri dishes ± SEM (n=3) with 25 embryos each. ANOVA was performed for each incubation medium and significant differences are shown in different letters (Dunnett's multiple comparisons test).

### 3.5. Achene Response to Germination Promoters GA and Ethylene

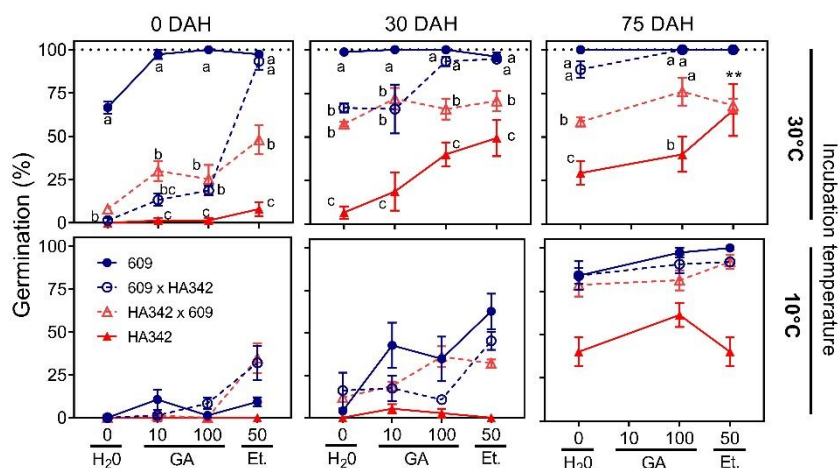
Achene germination was also assessed at 0, 30 and 75 DAH in presence of gibberellic acid (GA) and Etephon (Et, an ethylene donor). Hybrids from the HA342:609 system (Figure 5A) responded differently and resembled their maternal parent when exposed to most effective germination promoting doses at 30 °C (e.g., 50 μM Et at 0 DAH, or 100 μM GA and 50 μM Et at 30 DAH). Treatment with Et at harvest anticipated the pattern otherwise observed after 75 DAH under control conditions (water). Notably, even after 75 DAH, Et could not overcome TI and promote full germination of HA342 and HA342x609 achenes (germination values with 50 μM Et were 65.3± 14.8 and 68±4 for HA342 and HA342x609, respectively, and 100% for both 609 and 609xHA342; Kruskal-Wallis test p=0.0058\*\*).

In the 1579:609 crossing system incubated at 30 °C (Figure 5B), GA and Et effectively promoted germination since 0 DAH in 609 and in both hybrids (50 μM Et increased germination from 0 to over

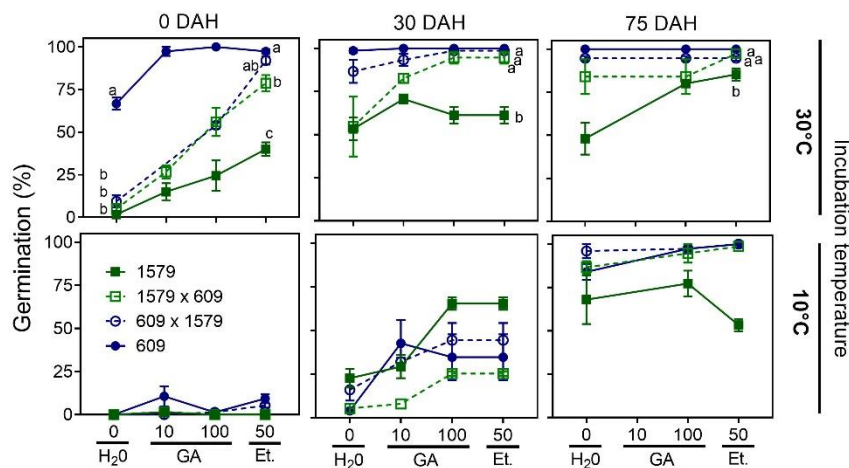
75%). In this case, both hybrids behaved similarly and intermediate to their parents, compatible with a zygotic control with incomplete dominance inheritance pattern. Incubation in 100  $\mu$ M GA or 50  $\mu$ M Et at 30 °C was able to partially revert TI in 1579 at 75 DAH (from 48% in water to 85% in Et).

Consistent with stronger expression of embryo (and achene) dormancy at low incubation temperature, achene responses to GA and Et were attenuated as compared to 30 °C. When imbibed at 10 °C, and for both crossing systems (Figure 5 A,B), responses to GA and Et were absent at harvest or moderate at 30 DAH (as dormancy had decreased). Reciprocal hybrids in both crosses behaved similarly to parent 609 confirming that achene behavior is defined by the zygote (embryo) regardless of the differences in the maternal pericarp. While 1579 responded partially to GA and Et (at 30 DAH), HA342 was unresponsive to GA and Et even after 75 DAH, in agreement with deeper embryo dormancy in the latter.

(A)



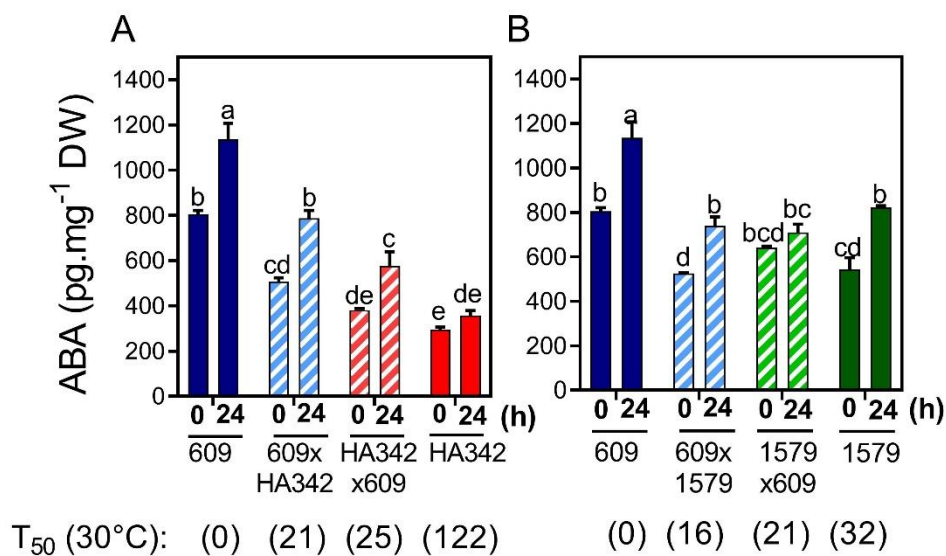
(B)



**Figure 5. Achene sensitivity to GA<sub>3</sub> and ethephon in the parent lines and their reciprocal crosses.** Final germination (%) of achenes incubated in distilled water, GA<sub>3</sub> (10 and 100  $\mu$ M) and ethephon (50  $\mu$ M) at 30 and 10 °C, at three different times (0, 30 and 75 DAH) for crossing systems HA342:609 (A) and 1579:609 (B). Parent lines are shown in solid symbols and lines (609, blue circles and lines; HA342, red triangles and lines; 1579, green squares and lines), and their respective hybrids are shown in open symbols and dotted lines of the same shape and color as the mother. Each point is the mean  $\pm$  SEM (n=3 replicate Petri dishes). Significant differences within an incubation medium are shown in letters (ANOVA and multiple comparisons). Asterisks in (A, 75 DAH) indicate significant genotype effect in ANOVA, but not multiple comparisons (Dunnett's).

### 3.6. Endogenous ABA Content in Parents and Hybrids

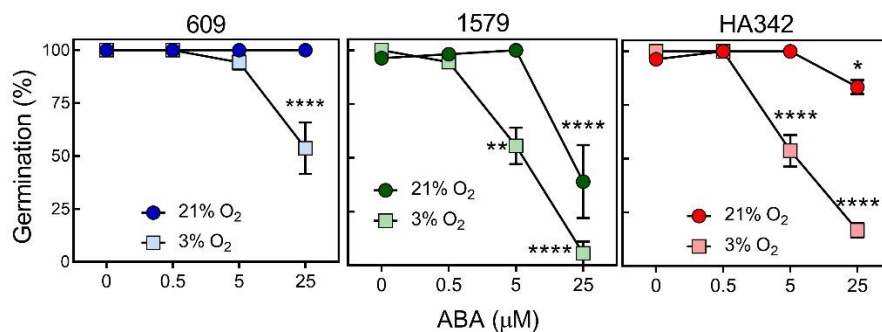
ABA content in embryonic axes from dry and imbibed achenes (for 24 h, 30 °C; 0 DAH) in both crossing systems is shown in Figure 6. Strikingly, when comparing parent lines, initial ABA levels (in the dry state) were highest in the less dormant line, 609, decreasing in 1579 and HA342. Within each crossing system, ABA levels in both hybrids were similar and intermediate to their parents'. This pattern was observed in both dry (800, 500 and 300 pg ABA.mg<sup>-1</sup> DW) and imbibed achenes (1100, 800 and 400 pg ABA.mg<sup>-1</sup> DW). Increases in ABA levels upon 24 h imbibition ranged between 10 and 50% as compared to the dry state (Table S2). These measurements were repeated after 150 DAH. No relevant changes in ABA content occurred between 0 and 150 DAH, either when comparing dry or imbibed achenes (Figure 6, Table S2).



**Figure 6. Endogenous ABA content in embryonic axes of dry and imbibed achenes.** (A) Crossing system HA342:609 and (B) 1579:609. For each condition, ABA was measured in axes of dry achenes (-) and after 24 h imbibition in water (+) at 30 °C. Data points are the mean  $\pm$  SEM (n=3). Different letters represent statistically significant differences ( $p < 0.05$ ) within each data set (2-way ANOVA followed by Šídák's multiple comparisons test, N=3). Genotype  $\times$  imbibition time (0, 24h) was significant ( $p < 0.05$ ). Storage time to reach 50% achene germination at 30 °C (T<sub>50</sub>) is shown for each genotype between parentheses (data from Table S1).

### 3.7. TI is Related to Increased Embryo Sensitivity to ABA Under Hypoxia

A possible, differential reinforcement of embryo sensitivity to ABA by hypoxia was explored in the parent lines by incubating embryos in water or 5, 25 and 50  $\mu$ M ABA, under normal air (21% O<sub>2</sub>) or hypoxia (3% O<sub>2</sub>) at 30 °C (Figure 7). In agreement with data in Figure 3, line 609 was less sensitive to 25  $\mu$ M ABA than 1579 and HA342 when incubated at 21% oxygen. In all cases, incubation at 3% O<sub>2</sub> enhanced inhibition by ABA, but this effect was stronger in both 1579 and HA342 as compared to 609 (which required 25  $\mu$ M ABA to achieve similar inhibition by 5  $\mu$ M ABA in 1579 and HA342).



**Figure 7.** Effect of hypoxia on embryo sensitivity to ABA at 30 °C. Different panels show final germination (%) of embryos incubated at 30 °C under ambient air (21% O<sub>2</sub>, circles) or hypoxia (3% O<sub>2</sub>, squares) obtained for inbred lines 609, 1579 and HA342. Embryos were isolated from achenes after-ripened for 100 d. Each data point n=3 replicate Petri dishes. Data was analyzed by 2-way ANOVA followed by Dunnett's multiple comparisons test. Within each Oxygen level (21 or 3%), significant differences for each ABA concentration (0-25 μM) as compared to the control (0 μM) are shown with asterisks.

### 3.8. Differences in Pericarp Structure are Related to TI in Parent Lines and Crosses

Measurements of pericarp cross sections were performed in the parent lines and hybrids. Total pericarp thickness, mid parenchyma thickness (μm), and number of mid parenchyma cell layers are shown in Table 1. Lines 609 and 1579 showed thinner pericarp and mid-parenchyma than HA342. As expected, hybrids resembled their maternal parent. In the HA342:609 system, HA342 and HA342x609 displayed greater total and mid-parenchyma thickness, and a slightly higher number of parenchyma cells compared to 609 and 609xHA342. The most pronounced differences were observed in mid-parenchyma, with visibly thicker, more sclerified cell walls. No differences in the density of the phytomelanin layer were found. Mid-parenchyma in HA342 (and HA342x609) was ~80% thicker than 609 and 609xHA342. Although HA342 had a slightly higher cell number than 609, no significant differences were found between parentals and their hybrids, suggesting differences in mid-parenchyma thickness may be attributed to larger cell size. In the 1579:609 system, there were no differences between parentals in pericarp thickness, mid-parenchyma thickness, or cell layer number. No visual differences were observed in cell wall thickening, degree of sclerification, or phytomelanin layer density. These results point at a more restrictive pericarp in HA342 as compared to 1579 and 609.

The maternal parent not only defined pericarp characteristics but overall achene dimensions (length, width, thickness; Table S3, Figure S4). For example, HA342 and HA342x609 had similar, larger achene length and thickness than 609 and 609xHA342; this trend was consistent in 1579:609 system. Thousand-achene weight and the pericarp/kernel ratio of hybrids also resembled the maternal parent. The 609 parental line and its hybrids 609xHA342 and 609x1579 had smaller size, lower thousand-achene weight, and pericarp/kernel ratio, followed by 1579, and finally HA342.

**Table 1.** Anatomical measurements of the pericarp in parents and hybrids. Measurements of pericarp cross sections performed in the parental lines (HA342, 1579, 609) and reciprocal crosses with 609. Total pericarp thickness, mid parenchyma thickness (μm), and number of mid parenchyma cell layers are shown. Values are means ± SE (n = 3). Different letters indicate statistically significant differences (p < 0.05).

| Genotype  | Pericarp width (μm)       | Mid parenchyma width (μm) | N° cell layers mid parenchyma |
|-----------|---------------------------|---------------------------|-------------------------------|
| 609       | 175,2 ± 12,3 <sup>A</sup> | 135,4 ± 14,5 <sup>A</sup> | 7 ± 0,5 <sup>A</sup>          |
| 609xHA342 | 179,4 ± 18,3 <sup>A</sup> | 140,3 ± 19,0 <sup>A</sup> | 6 ± 0,4 <sup>A</sup>          |
| HA342x609 | 315,9 ± 19,6 <sup>B</sup> | 270,2 ± 14,8 <sup>B</sup> | 9 ± 0,5 <sup>A</sup>          |
| HA342     | 287,0 ± 28,8 <sup>B</sup> | 245,8 ± 26,5 <sup>B</sup> | 8 ± 0,5 <sup>A</sup>          |

|          |                          |                          |                      |
|----------|--------------------------|--------------------------|----------------------|
| 609x1579 | 185,0 ± 5,9 <sup>A</sup> | 154,6 ± 6,3 <sup>A</sup> | 6 ± 0,4 <sup>A</sup> |
| 1579x609 | 166,8 ± 5,8 <sup>A</sup> | 139,9 ± 3,5 <sup>A</sup> | 6 ± 0,4 <sup>A</sup> |
| 1579     | 176,8 ± 5,7 <sup>A</sup> | 145,1 ± 5,7 <sup>A</sup> | 6 ± 0,6 <sup>A</sup> |

#### 4. Discussion

The aim of this study was to elucidate how achene structures contribute to dormancy and thermo-inhibition, as well as the inheritance patterns of these traits in cultivated sunflower. We analyzed embryo, seed, and achene germination in three inbred lines and their reciprocal crosses. Embryo abscisic acid (ABA) content, sensitivity to ABA and hypoxia, and pericarp morphology were also assessed. These data were integrated to explain how interactions between the pericarp and the embryo impose low-temperature-expressed dormancy (LTED) during early after-ripening and, at later stages, may lead to thermo-inhibition (TI).

##### *Inheritance Patterns of Dormancy and Thermo-Inhibition*

Results in this study show that early postharvest dormancy release of achenes is determined by changes in embryo dormancy, which is governed by the embryo nuclear genotype, as observed in both crossing systems (609xHA342 and 609x1579; Figures 2 and 3 and Table S1). Incomplete dominance of dormancy alleles from each parent pair is also inferred, as reciprocal hybrids (achenes, seeds and embryos) behaved similarly and intermediate to their parents and irrespective of their different (maternally inherited) pericarp (Table 1; Figure S3).

In contrast, thermo-inhibition (TI), when transmitted to the F1 progeny, showed a maternal inheritance pattern, consistent with reports in wild-cultivated crosses [24–26]. Nevertheless, transmission of TI from a maternal parent to the F1 was conditional on pericarp characteristics in combination with the embryo genetics. The different capacity of the pericarp to impose TI became evident when comparing the HA342x609 (with TI) and 609xHA342 (no TI) hybrids, which shared the same embryo genetics and physiology (Figures 2,4 and 5) but different pericarps (Table 1; Figure S3), being the HA342 pericarp capable of imposing TI in contrast to the pericarp from 609. The absence of maternal transmission of TI from parent 1579 to hybrid 1579x609, indicates that TI expression depends on the combined effect of the zygote and the maternal pericarp, which in the case of 1579, was structurally similar to 609 (Table 1, Figure S3). In other words, a less restrictive pericarp (like 609 or 1579) only leads to TI when combined with a more sensitive embryo (as in 1579), but not in less sensitive embryos (hybrids and 609).

##### *ABA content, ABA Sensitivity, and Their Relationship with Dormancy*

A possible association between ABA content and dormancy was explored. Previous work [39] comparing two sunflower inbred lines with contrasting dormancy reported a significantly higher ABA content in the (dry) embryo of the high dormancy line. Nevertheless, in our study, ABA levels (in embryo axes of dry achenes) across parents and hybrids, were inversely related to dormancy. These values did not change with after-ripening (Figure 6; Table S2). Although ABA content increased upon imbibition at 30 °C, these changes did not correlate with TI expression or with differences in dormancy between genotypes. This agrees with previous studies in sunflower showing that ABA accumulation in the embryo axis during incubation is not linked to germination responses under contrasting temperatures or oxygen levels [9,10,41].

In Arabidopsis, maternal control of dormancy is mediated by ABA synthesized in the endosperm, secreted to -and imported by- the embryo [42,43]. While increased ABA synthesis is instrumental to impose TI in Arabidopsis seeds [44], this is not the case in sunflower. Whether the sunflower endosperm also secretes ABA towards the embryo is not known, but its contribution to germination control in the post-harvest period appears to be minor compared with differences in embryo sensitivity to ABA. In our study, the seed coat (which includes the living endosperm layer) had a significant effect only in 1579 at harvest and was small or negligible in 609 and HA342 (Figures

1–3 and S2). In contrast, differences in ABA sensitivity evident among parents (higher in high-dormancy genotypes) were also reflected in the hybrids, which showed intermediate responses to ABA. This supports that embryo sensitivity to ABA —not ABA content— is the key determinant of dormancy and TI during post-harvest period, consistent with findings in wheat and sorghum [45–48]. Thus, although *de novo* ABA synthesis contributes to dormancy induction in developing sunflower embryos [48,49], final dormancy levels at harvest and changes afterwards depend on embryo sensitivity, not ABA content [41,51,52]. Indeed, the inverse relationship between embryo ABA content and embryo sensitivity to ABA observed in the present study suggests a possible feedback regulation of ABA metabolism.

#### *Structural Traits of the Pericarp and Their Role in TI*

Thermo-inhibition in sunflower is known to be imposed mainly by the pericarp, potentially aided by the seed coat, at least in part through restriction of oxygen diffusion [9]. Structural traits such as mesocarp sclerification, phytomelanin density, cuticular waxes, and pericarp thickness [53,54] limit permeability to oxygen and can cause hypoxia, especially at high temperatures where embryo oxygen demand increases and oxygen solubility decreases [55,56]. Our results with reciprocal hybrids indicated that pericarp traits did not explain early dormancy release behavior, which was dominated by the zygote (Figures 2 and 3). However, at more advanced stages of after-ripening, transmission of TI to the hybrid was only possible when the maternal pericarp showed greater thickness and sclerification (Figures 2,3 and S3 and Table 1). This is consistent with observations in wild–cultivated hybrids where the thick and highly sclerified pericarp inherited from the wild maternal parent strongly inhibited germination at warm temperatures [23,26,57–59]. Conversely, lines 1579 and 609 pericarps showed no detectable histological differences, matching the absence of TI transmission in the 1579×609 hybrid, and its similarity with its reciprocal 609×1579 along the entire after-ripening period. To establish a causal relationship between hypoxia and pericarp structure, direct measurements of permeability to oxygen are still needed.

#### *Integration of Physiological and Structural Determinants*

The combined evidence indicates that dormancy and TI arise from distinct but interacting mechanisms. Low temperature expressed dormancy (LTED) is largely controlled by the zygotic genotype, through variation in embryo sensitivity to ABA. On the other hand, TI depends on the interaction between embryo physiology and maternal coverings. As suggested by earlier work comparing different sunflower genotypes [6], our results with reciprocal crosses confirm that the zygote also contributes to the expression of TI. While all genotypes in the present study displayed similar sensitivity to ABA at low incubation temperature (10 °C), TI was related to higher embryo sensitivity to ABA at 30 °C and to increased embryo responsiveness to ABA under hypoxia (Figure 7).

The effect of high temperature on pericarp-imposed TI can be explained by the imposition of hypoxia to the embryo by a thicker, more restrictive pericarp (e.g., as in HA342), which enhances embryo responsiveness to endogenous ABA, as supported by experiment under hypoxia (Figure 7). Therefore, in addition to being modulated by pericarp-derived hypoxia, embryo responsiveness to ABA at 30 °C was found to be determined by the zygote. This dual control explains why some high-dormancy parents transmit TI maternally while others do not, and why TI becomes more evident after storage, when embryo dormancy has dissipated and the relative contribution of coverings increases. In this regard, it is important to notice that achene response to an ethylene donor (Et) at 30 °C tested at harvest anticipated achene behavior at later stages (compare 50 µM Et—0 DAH with water—75 DAH; Figure 5A,B). This implies that incubation in Et at 30 °C at harvest may serve as an early predictor of TI.

### *Implications for Hybrid Seed Production and Future Research*

Thermo-Inhibition poses a challenge for hybrid sunflower seed production because, unlike low temperature expressed dormancy (LTED), it does not decline completely during dry after-ripening. Understanding its inheritance is therefore essential for selecting parental combinations that minimize TI expression in the hybrid progeny. Our results show that maternal coverings can impose TI only when combined with a zygotic genotype predisposed to express it, highlighting the need to evaluate both parents for structural and physiological traits. In parallel, our results suggest that the degree of TI could be exacerbated by any conditions that further limit oxygen availability to the imbibed embryo, such as seed-coating treatments. Future work should focus on quantifying oxygen diffusion properties of pericarps from contrasting genotypes, and on identifying genetic determinants of ABA sensitivity underlying LTED and TI. Together, our findings contribute to a more mechanistic understanding of dormancy inheritance in sunflower and provide actionable insights for breeding programs aimed at reducing TI in hybrid seed production.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1. Dormancy release rates in parents and hybrids. Table S2. ABA content in embryos. Table S3: Achene morphological measurements. Figure S1. Grain filling and moisture content dynamics. Figure S2. Evolution of pericarp, seed coat and embryo dormancy. Figure S3. Pericarp histological preparations.

**Author Contributions:** Conceptualization, M.V.R. and G.J.A.; methodology, G.J.A., M.R-R., M.V.R., and D.B.; formal analysis, M.V.R. and G.J.A.; investigation, M.V.R., G.J.A. and D.B.; resources, M.V.R. and D.B.; data curation, M.V.R. and G.J.A.; writing—original draft preparation, M.V.R. and G.J.A.; writing—review and editing, M.V.R. and G.J.A.; visualization, M.V.R. and G.J.A.; supervision, M.V.R. and D.B.; project administration, M.V.R. and D.B.; funding acquisition, M.V.R. and D.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Universidad de Buenos Aires, Argentina (grants UBACYT (2014-2017) N° 20020130100653 BA and (2018-2021) N°20020170100599BA), and by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) of Argentina (PIP 2015-2018, no. 11220130100669). G.J. Arata and M. Riveira-Rubin were supported by doctoral scholarships from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina This work is part of the doctoral thesis of G.J.A. supervised by M.V.R.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data supporting reported results will be proved upon request.

**Acknowledgments:** We would like to thank technical staff (Mirta Tinaro, Maximiliano Rodríguez and Cristian Escudero) and undergraduate student María Victoria Rambaud for their valuable support during the field and laboratory experiments.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

### **Abbreviations**

The following abbreviations are used in this manuscript:

|      |                                    |
|------|------------------------------------|
| LTED | Low Temperature Expressed Dormancy |
| TI   | Thermo Inhibition                  |
| ABA  | Absciscic Acid                     |
| Et   | Etephon                            |
| GA   | Gibberellic Acid                   |

## References

1. Hilhorst, H.W.M. (1995). A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research*, 5: 61-73. doi:10.1017/S096025850002634
2. Finch-Savage, W. E. and Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist* 171: 501-523. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>
3. Baskin, J.M. and Baskin, C.C. (2004). A classification system for seed dormancy. *Seed Science Research* 14(1), 1-16.
4. Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M. and Nonogaki, H. (2013). *Seeds: Physiology of Development, Germination and Dormancy*. 3rd Edition, Springer, New York.
5. Marzinek J, De-Paula OC and Oliveira DMT (2008) Cypselas or achenes? Refining terminology by considering anatomical and historical factors. *Revista Brasileira de Botânica* 31, 549–553
6. Arata, G.J., Riveira-Rubin, M.A., Batlla, D. and Rodríguez, M. V. (2021). Dormancy attributes in Sunflower achenes (*Helianthus annuus* L.): I. Intraspecific variability. *Crop Science*, (February), 1–16. <https://doi.org/10.1002/csc2.20610>
7. Corbineau, F., Bagniol, S., and Côme, D. (1990). Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. *Israel Journal of Botany*, 39(4–6), 313–325. <https://doi.org/10.1080/0021213X.1990.10677156>
8. Bodrone, M. P., Rodríguez, M. V., Arisnabarreta, S., y Batlla, D. (2017). Maternal environment and dormancy in sunflower: The effect of temperature during fruit development. *European Journal of Agronomy*, 82, 93–103. <https://doi.org/10.1016/j.eja.2016.10.007>
9. Dominguez, C., Batlla, D., Rodríguez, M.V., Windauer, L.B., Gerbaldo, M. and Benech-Arnold R.L. (2016). Pericarp-Imposed Dormancy in Sunflower: Physiological Basis, Impact on Crop Emergence, and Removal at an Industrial Scale. *Crop Sci.* 56:716–726. <https://doi.org/10.2135/cropsci2015.06.0335>
10. Dominguez, C.P., Rodríguez, M.V., Batlla, D., García de Salamone, I., Andreani, A. and R.L. Benech-Arnold (2019). Sensitivity to hypoxia and microbial activity are instrumental for pericarp-imposed dormancy expression in sunflower (*Helianthus annuus* L.). *Seed Science Research* 29(2), 85–96. doi:10.1017/S0960258519000060
11. Hills, P. N., and Van Staden, J. (2003). Thermoinhibition of seed germination. *South African Journal of Botany*, 69(4), 455–461. [https://doi.org/10.1016/S0254-6299\(15\)30281-7](https://doi.org/10.1016/S0254-6299(15)30281-7)
12. Boesewinkel, F.D. and Bouman, F. (1995) *The Seed: Structure and Function*. In: Kigel, J. and Galili, G., Eds., *Seed Development and Germination*, Marcel Dekker, Inc., New York, 1-24.
13. Foley, M. E., and Fennimore, S. A. (2008). Genetic basis for seed dormancy. *Seed Science Research*, 8(2), 173–182. <https://doi.org/10.1017/s0960258500004086>
14. Roach, D. A., and R. D. Wulff 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18:209–235.
15. Wolf, J. B., and Wade, M. J. (2009). What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1520), 1107–1115. <https://doi.org/10.1098/rstb.2008.0238>
16. Piskurewicz, U., Iwasaki, M., Susaki, D., Megies, C., Kinoshita, T., and Lopez-Molina, L. (2016). Dormancy-specific imprinting underlies maternal inheritance of seed dormancy in *Arabidopsis thaliana*. *eLife*, 5, e19573
17. Penfield, S., and MacGregor, D. R. (2017). Effects of environmental variation during seed production on seed dormancy and germination. *Journal of Experimental Botany*, 68(4), 819–825. <https://doi.org/10.1093/jxb/erw436>
18. Riveira-Rubin, M., Arata, G. J., López, E. D., Rodríguez, M. V., y Batlla, D. (2021). Dormancy attributes in sunflower achenes (*Helianthus annuus* L.): II. Sowing date effects. *Crop Science*, (February), 1–16. <https://doi.org/10.1002/csc2.20612>
19. Lachabrouilli, A. S., Rigal, K., Corbineau, F., and Bailly, C. (2021). Effects of agroclimatic conditions on sunflower seed dormancy at harvest. *European Journal of Agronomy*, 124, 126209. <https://doi.org/10.1016/j.eja.2020.126209>

20. Miko, I. (2008). Genetic dominance: genotype-phenotype relationships. *Nature Education* 1(1):140 <https://www.nature.com/scitable/topicpage/genetic-dominance-genotype-phenotype-relationships-489/>
21. Fedi, F., O'Neill, C. M., Menard, G., Trick, M., Dechirico, S., Corbineau, F., Bailly, C., Eastmond, P. J., & Penfield, S. (2017). Awake1, an ABC-Type Transporter, Reveals an Essential Role for Suberin in the Control of Seed Dormancy. *Plant physiology*, 174(1), 276–283. <https://doi.org/10.1104/pp.16.01556>
22. Tamura, N., Yoshida, T., Tanaka, A., Sasaki, R., Bando, A., Toh, S., Lepiniec, L., & Kawakami, N. (2006). Isolation and characterization of high temperature-resistant germination mutants of *Arabidopsis thaliana*. *Plant & cell physiology*, 47(8), 1081–1094. <https://doi.org/10.1093/pcp/pcj078>
23. Presotto A., Poverene M., Cantamutto M. (2014) Seed dormancy and hybridization effect of the invasive species, *Helianthus annuus*. *Annals of Applied Biology*, 164, 373–383. <https://doi.org/10.1111/aab.12104>
24. Hernández, F., Lindström, L. I., Parodi, E., Poverene, M., and Presotto, A. (2017). The role of domestication and maternal effects on seed traits of crop–wild sunflower hybrids (*Helianthus annuus*). *Annals of Applied Biology*, 171(2), 237–251. <https://doi.org/10.1111/aab.12368>
25. Pace, B. A., Alexander, H. M., Emry, J. D., and Mercer, K. L. (2015). Seed fates in crop-wild hybrid sunflower: Crop allele and maternal effects. *Evolutionary Applications*, 8(2), 121–132. <https://doi.org/10.1111/eva.12236>
26. Weiss A.N., Primer S.B., Pace B.A. and Mercer K.L. (2013) Maternal effects and embryo genetics: germination and dormancy of crop–wild sunflower hybrids. *Seed Science Research*, 23(4):241-255. doi:10.1017/S0960258513000226
27. Lan, X. J., Wei, Y. M., Liu, D. C., Yan, Z. H., and Zheng, Y. L. (2005). Inheritance of seed dormancy in Tibetan semi-wild wheat accession Q1028. *Journal of Applied Genetics*, 46(2), 133–138.
28. Gu, X. Y., Chen, Z. X., y Foley, M. E. (2003). Inheritance of seed dormancy in weedy rice. *Crop Science*, 43(3), 835–843.
29. Eenink, A.H. (1981). Seed dormancy in lettuce: Influence of temperature on induction and expression of seed dormancy and studies on the inheritance. *Acta Horticulturae*, 111, 41-46. <https://doi.org/10.17660/ActaHortic.1981.111.4>
30. Schneiter, A.A. and Miller, J.F. (1981). Description of Sunflower Growth Stages. *Crop Science*, 21, 901-903. <https://doi.org/10.2135/cropsci1981>.
31. Steinbach, H.S., Benech-Arnold, R.L., Kristof, G., Sanchez, R.A., and Marcucci-Poltri, S. (1995). Physiological basis of pre-harvest sprouting resistance in *Sorghum bicolor* (L.) Moench. ABA levels and sensitivity in developing embryos of sprouting-resistant and -susceptible varieties. *Journal of Experimental Botany*, 46: 701-709. <https://doi.org/10.1093/jxb/46.6.701>
32. Lindström, L. I., Pellegrini, C. N., and Hernández, L. F. (2007). Histological development of the sunflower fruit pericarp as affected by pre- and early post-anthesis canopy shading. *Field Crops Research*, 103(3): 229–238. <https://doi.org/10.1016/j.fcr.2007.06.005>
33. Wilcox, D., Done, A., McDavid, P. and Greer, P. (2002). Image Tool v. 3.0, University of Texas, Health Science Center, San Antonio, TX.
34. Lindström, L. I. and Hernández, L. F. (2015). Developmental morphology and anatomy of the reproductive structures in sunflower (*Helianthus annuus*): a unified temporal scale. *Botany*, 93(5), 307–316. <https://doi.org/10.1139/cjb-2014-0245>
35. Mantese, A. I., Medan, D., and Hall, A. J. (2006). Achene structure, development and lipid accumulation in sunflower cultivars differing in oil content at maturity. *Handbook of Environmental Chemistry, Volume 5: Water Pollution*, 97(6), 999–1010. <https://doi.org/10.1093/aob/mcl046>
36. Marzinek, J., De-Paula, O. C. and Oliveira, D. M. T. (2008). Cypsela or achene? Refining terminology by considering anatomical and historical factors. *Revista Brasileira de Botânica*, 31(3): 549–553.
37. Pandey, A.K., and Dhakal, M.R. (2001). Phytomelanin in Compositae. *Current Science*. 80: 933-940
38. Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2018). InfoStat. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL <http://www.infostat.com.ar>
39. Motulsky, HJ. (2003). *GraphPad Prism Statistics Guide-Statistical Analyses for Laboratory and Clinical Researchers*. GraphPad Software Inc., San Diego, CA, USA. [www.graphpad.com](http://www.graphpad.com)

40. Bel, Z. D., Andrade, A., Maddalena, M. D., Vigliocco, A., & Alemano, S. (2023). The interplay of specific hormonal profile in fruit parts of sunflower inbred lines with contrasting dormancy levels during germination and dormancy breaking by exogenous application of plant growth regulators. *Crop Science*, 63, 852–866. <https://doi.org/10.1002/csc2.20912>
41. Arata, G.J., Batlla, D., Riveira-Rubin, M., Demkura, P.V., Mazzobre, F., Rolandelli, G., Buera, P., Rodríguez, M.V. (2025) Biophysical boundaries for dormancy release in sunflower achenes. *Journal of Experimental Botany* 76, 7105–7122. <https://doi.org/10.1093/jxb/eraf389>
42. Kang, J., Yim, S., Choi, H., Kim, A., Lee, K. P., Lopez-Molina, L., Martinois, E. and Lee, Y. (2015). Abscisic acid transporters cooperate to control seed germination. *Nature Communications*, 6, 1–10. <https://doi.org/10.1038/ncomms9113>
43. Lee KP, Piskurewicz U, Turecková V, Strnad M, y Lopez-Molina L (2010). A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. *PNAS* 107(44):19108-19113. <https://pmc.ncbi.nlm.nih.gov/articles/PMC4569717/>
44. Ali-Rachedi, S., Bouinot, D., Wagner, M. H., Bonnet, M., Sotta, B., Grappin, P., and Jullien, M. (2004). Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: Studies with the Cape Verde Islands ecotype, the dormant model of Arabidopsis thaliana. *Planta*, 219(3), 479–488. <https://doi.org/10.1007/s00425-004-1251-4>
45. Walker-Simmons, M. (1987). ABA Levels and Sensitivity in Developing Wheat Embryos of Sprouting Resistant and Susceptible Cultivars. *Plant Physiology*, 84(1), 61–66. <https://doi.org/10.1104/pp.84.1.61>
46. Walker-Simmons, M. (1988). Enhancement of ABA responsiveness in wheat embryos by high temperature. *Plant, Cell y Environment*, 11(8), 769–775. <https://doi.org/10.1111/j.1365-3040.1988.tb01161.x>
47. Gualano N, Carrari F, Rodríguez MV, Pérez-Flores L, Sánchez RA, Iusem ND and Benech-Arnold RL (2007). Reduced embryo sensitivity to ABA in sprouting susceptible sorghum (*Sorghum bicolor*) variety is associated with an altered ABA signalling. *Seed Sci. Res.* 17(2):81-90. DOI: <https://doi.org/10.1017/S0960258507708115>
48. Rodríguez MV, Mendiondo GM, Maskin L, Gudesblat GE, Iusem ND and Benech-Arnold RL (2009) Expression of ABA signalling genes and ABI5 protein levels in imbibed *Sorghum bicolor* caryopses with contrasting dormancy and at different developmental stages. *Annals of Botany*. 104: 975-985. <https://doi.org/10.1093/aob/mcp184>
49. Le Page-Degivry, M. T. y Garello, G. (1992). In situ abscisic acid synthesis. A requirement for induction of embryo dormancy in *Helianthus annuus*. *Plant Physiology* 98: 1386-1390. <https://doi.org/10.1104/pp.98.4.1386>
50. Le Page-Degivry, M. T., Barthe, P. y Garello, G. (1990). Involvement of endogenous abscisic acid in onset and release of *Helianthus annuus*. *Plant Physiology* 92: 1164-1168. DOI: 10.1104/pp.92.4.1164
51. Bodrone, M. P., Rodríguez, M. V., Arisnabarreta, S., and Batlla, D. (2017). Maternal environment and dormancy in sunflower: The effect of temperature during fruit development. *European Journal of Agronomy*, 82, 93–103. <https://doi.org/10.1016/j.eja.2016.10.007>
52. Xia, Q., Ponnaiah, M., Thanikathansubramanian, K., Corbineau, F., Bailly, C., Nambara, E., ... ElMaarouf-Bouteau, H. (2019). Re-localization of hormone effectors is associated with dormancy alleviation by temperature and after-ripening in sunflower seeds. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-40494-w>
53. López, F. B., y Barclay, G. F. (2017). Plant Anatomy and Physiology. In *Pharmacognosy: Fundamentals, Applications and Strategy*. <https://doi.org/10.1016/B978-0-12-802104-0.00004-4>
54. Jocković, J., Rajčević, N., Terzić, S., Zorić, L., Jocković, M., Miladinović, D., y Luković, J. (2020). Pericarp features of wild perennial *Helianthus* L. species as a potential source for improvement of technical and technological properties of cultivated sunflower. *Industrial Crops and Products*, 144: 112030. <https://doi.org/10.1016/j.indcrop.2019.112030>
55. Rolletschek, H., Stangelmayer, A., & Borisjuk, L. (2009). Methodology and Significance of Microsensor-based Oxygen Mapping in Plant Seeds—an Overview. *Sensors (Basel, Switzerland)*, 9(5), 3218–3227. <https://doi.org/10.3390/s90503218>

56. Rolletschek, H., Borisjuk, L., Sánchez-García, A., Gotor, C., Romero, L. C., Martínez-Rivas, J. M., & Mancha, M. (2007). Temperature-dependent endogenous oxygen concentration regulates microsomal oleate desaturase in developing sunflower seeds. *Journal of experimental botany*, 58(12), 3171–3181. <https://doi.org/10.1093/jxb/erm154>
57. Presotto, A., Hernández, F., Casquero, M., Vercellino, R., Pandolfo, C., Poverene, M., and Cantamutto, M. (2020). Seed bank dynamics of an invasive alien species, *Helianthus annuus* L. *Journal of Plant Ecology*, 1–40. <https://doi.org/10.1093/jpe/rtaa016>
58. Seiler, G.J. (1998), Seed Maturity, Storage Time and Temperature, and Media Treatment Effects on Germination of Two Wild Sunflowers. *Agron. J.*, 90: 221-226. <https://doi.org/10.2134/agronj1998.00021962009000020018x>
59. Seiler, G.J. (1997). Anatomy and morphology of sunflower, pp. 67–111 in Schneiter AA (ed), *Sunflower Technology and Production*. Madison, Wisconsin, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.