

1 **Effects of agroclimatic conditions on sunflower seed dormancy at harvest**

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29 **Abstract**

30 At harvest sunflower seeds (*Helianthus annuus* L.) are dormant and poorly germinate at 10
31 and 25°C. Seed sensitivity to low temperatures (10°C) results from an embryo dormancy
32 whereas the envelopes (pericarp and seed coats) inhibit the germination at 25°C. Removal of
33 pericarp greatly improved seed germination when the germination of whole achenes was
34 higher than the critical value of 20 %. Using 107 seed batches corresponding to 26
35 genotypes cultivated in 2015, 2016 and 2017 in various production areas in France (Center
36 and South) and Spain (South), we investigated the effects of agro-environmental factors on
37 the depth of dormancy at harvest. A principal Component Analysis (PCA) performed with all
38 the seed batches did not show any significant effect of the genotype (variety or female) and
39 year of production on the intensity of dormancy, but it revealed the role of agro-climatic
40 conditions. Experimental field trials performed with plants treated by a chemical desiccant
41 after flowering demonstrated that accelerated desiccation during seed maturation on the
42 mother plant was associated with lower dormancy. Our data also suggest that the longest the
43 duration of the developmental cycle from sowing to harvest, the lowest the dormancy at
44 harvest. We provide new insights into the modulation of dormancy intensity by genetic and
45 environmental factors which might have practical applications in seed production.

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47 **Key words:** agro-climatic conditions; development; germination; dormancy; seed; sunflower

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49 **Abbreviations:**

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51 ABA, abscisic acid; daf, days after flowering; DOG1, DELAY OF GERMINATION1; FW, fresh
52 weight; GAs, gibberellins; LEC1 and 2, leafy cotyledon 1 and 2; MC, moisture content; PCA,
53 Principal Component Analysis; QTL, Quantitative Trait Loci; RH, relative humidity;

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58 1. Introduction

59 Seed dormancy is defined as the inability of a viable seed to germinate under apparently
60 favorable environmental conditions (Bewley, 1997) thereby reducing the seed germination in
61 sub- or supra-optimal conditions of temperature, light or/and oxygen (Finch-Savage and
62 Leubner-Metzger, 2006). In natural ecosystems, dormancy is essential since it inhibits seed
63 germination at the unfavorable season and postpones it until appropriate environmental
64 conditions allow successful survival of the resulting seedlings. The so-called primary
65 dormancy is established during seed development on the mother plant, and generally
66 gradually disappears after harvest during seed storage or cold stratification (Baskin and
67 Baskin, 1998; Finch-Savage and Leubner-Metzger, 2006). The mechanisms involved in
68 either the establishment and the expression of dormancy during seed development are far
69 from being understood. It is widely admitted that dormancy is under the hormonal control of
70 abscisic acid (ABA) and gibberellins (GAs), two plant hormones having antagonistic effects
71 on seed germination (Finch-Savage and Leubner-Metzger, 2006; Holdsworth et al., 2008).
72 ABA accumulates during seed development and inhibits germination whereas GAs have an
73 opposite effect (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006). Seed dormancy
74 has also a genetic component. For some species, like barley or wheat, it is associated with
75 Quantitative Trait Loci (QTL) even though this trait has often been removed by selection
76 (Derera et al., 1977). Among the loci involved in seed dormancy DELAY OF
77 GERMINATION1 (DOG1), which codes for a protein of unknown function, has been identified
78 in Arabidopsis as a major QTL (Bentsink et al., 2006). The occurrence of DOG1 has been
79 reported in several species (Graeber et al., 2010), but not in sunflower (Layat et al., 2014;
80 Xia et al., 2018). The induction of dormancy early during seed maturation is controlled by
81 various major molecular regulators, including abscisic acid insensitive 3 (ABI3), FUSCA3
82 (FUS3), leafy cotyledon 1 and 2 (LEC 1 and 2) (Holdsworth et al., 2008). However, the
83 expression of these genes did not seem to be related with the degree of dormancy
84 (Baumbusch et al., 2004). In addition seed dormancy is also strongly regulated by the
85 environmental conditions that prevail during seed development on the mother plant, *i.e.* by

86 the maternal environment. In Arabidopsis, low temperatures during seed maturation induce
87 higher dormancy at harvest (Chiang et al., 2011; Springthorpe and Penfield, 2014; Kerdaffrec
88 and Nordborg, 2017). Low temperatures during grain development also result in a higher
89 dormancy in wheat and barley (Belderock 1965; Black et al., 1987; Rodriguez et al., 2001;
90 Gualano and Benech-Arnold, 2009; Nakamura et al., 2011). In barley, a positive linear
91 relationship exists between the temperature within a thermal time window during grain
92 development and the germination ability after physiological maturity (Rodriguez et al., 2001;
93 Gualano and Benech-Arnold, 2009). In wheat, seed dormancy on the ear is maintained for
94 longer time when the sum of temperatures (higher than 12.5°C) was lower during the dough
95 stage (Belderock, 1965). This is however not a general trend since in Quinoa, for example,
96 high temperature and extended photoperiod during seed development induce higher
97 dormancy (Ceccato et al., 2011).

98 At harvest sunflower seeds display physiological dormancy (Baskin and Baskin,
99 2004) and they poorly germinate both at low and high temperature (Corbineau et al., 1990).
100 Sunflower seed dormancy results from an inhibitory action of the envelopes (pericarp and
101 seed coat), which affect germination above 20-25°C, and from the embryo itself, which
102 prevents germination below 15°C (Corbineau et al., 1990). Embryo dormancy is rapidly
103 settled after flowering, at the beginning of mid seed filling, and is induced by a marked
104 synthesis of ABA (Le Page-Degivry et al., 1990) after what the embryo ABA content
105 gradually decreases. When seeds reach mass maturity (*i.e.* at the end of seed filling), at
106 about 40 % fresh weight (FW) basis (Rondanini et al., 2007), dormancy is at its maximum
107 (Corbineau et al., 1990). Further seed desiccation on the mother plant is associated with a
108 progressive decrease in seed dormancy, but it does not completely eliminate dormancy
109 which can therefore remain strong at harvest (Corbineau et al., 1990; Le Page-Degivry et al.,
110 1990). Environmental and crop conditions during maturation of sunflower seeds have an
111 effect on depth of dormancy. It has been proposed that warm conditions during seed
112 development could induce higher seed coat dormancy, while cool temperatures would
113 increase embryo dormancy (Bodrone et al., 2017). To date, the influence of cropping

114 practices on sunflower seed dormancy has been scarcely studied. Szemruch et al. (2014)
115 have shown that accelerating seed dehydration by applying a chemical desiccant at the end
116 of seed maturation increased seed coat dormancy at harvest. Sunflower seed dormancy is
117 generally released after few months of dry storage (after-ripening), depending on the storage
118 conditions (Bazin et al., 2011), and non-dormant sunflower seeds become able to fully
119 germinate from 5°C to 35°C, the thermal optimum being between 25 and 30°C (Gay et al.,
120 1991).

121 The interactions between genetic background and environmental conditions and its
122 effects on the level of sunflower seed dormancy at harvest is largely unknown. There is a
123 need for the growers to better understand the factors triggering the establishment of seed
124 dormancy, especially in the context of a changing climate, which is likely to alter this trait
125 (Walk et al., 2011; Huang et al., 2017). In this work, using a combination of large-scale
126 analysis of sunflower seed dormancy of numerous commercial seed lots and field
127 experiments we have identified environmental factors that can modulate sunflower seed
128 dormancy during seed development.

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130 **2. Materials and methods**

131 *2.1. Plant material*

132 This study was performed with several subsets of seeds from commercial sunflower
133 (*Helianthus annuus* L.) hybrids produced by Limagrain (France). A first subset of 16 seed
134 lots, harvested in 2015 in France (Center and South) and in Spain (North and South), was
135 used for investigating achene and embryo germination at 10 and 25°C (Table 1). Another
136 subset of 107 seed batches from 26 commercial varieties harvested in 2015, 2016 and 2017
137 in France (Center or South) and Spain (South) was used for investigating depth of dormancy
138 at harvest, as related to area and year of production (Table A). Seeds from both subsets
139 were stored 1 to 5 days after harvest at -18°C in order to maintain their dormancy before the
140 germination assays (Corbineau et al., 1990). The duration between harvest and freezing
141 ranged from 1 to 5 d, so that all seed batches could be considered as being in the same

142 physiological status (*i.e.* freshly harvested) and having not started to after-ripen. Dormancy
143 was alleviated by storing seeds at 20°C and 60 % relative humidity (RH) for at least 3 months
144 (Corbineau et al., 1990).

145 *2.2. Field experiments*

146 Two field experiments were carried out with varieties (12, 29, 30 and 34) chosen for
147 their similar precocity and their adaptation to the local climate (Table B). In experiment 1,
148 seeds of sunflower varieties 30 and 34 (Table B) were sown in South of France (latitude
149 44.58) on April 22th, 2016, in an experimental unit of 4 rows of 10 m each. The sowing plan
150 was randomized in 2 replicates separated by a 2-meter row. A desiccant (Réglone 2[®],
151 Syngenta, 200 g L⁻¹) was applied on August 18th on half of the plants of the experimental unit.
152 The other half, with no treatment, was used as the control. Conditions of culture after the
153 treatment were conventional excepted for irrigation which was extended until the beginning
154 of September with 30 mm of water per week to avoid water stress
155 (<https://www.terresinovia.fr/tournesol>). From July 28th to September 1st (harvest date), 10
156 capitula were harvested once a week for germination assays and 5 capitula were harvested
157 twice a week for determining seed moisture content (MC). Seeds were threshed manually
158 from capitula and used immediately for measuring seed MC or placed in cotton bags to be
159 dried in an outdoor dryer at 40°C. Drying was stopped when seed water MC was less than
160 10 % FW, and dry seeds were stored at -18°C until use.

161 Experiment 2, carried out in the Center of France (latitude 45.87) in 2017, consisted
162 in producing hybrids 12 and 29 (Table B). For each sowing, 4 rows of male lines were sown
163 with a density of 4.5 grains m⁻¹ and 2 rows of female lines at 5.5 grains m⁻¹. Each row
164 measured 80 m in length with 0.7 m inter-row distance. For female lines, we used 4
165 replicates of 20 m each. For early sowing date, male lines were sown on March 30th and on
166 April 11th in order to lengthen the flowering period and ensure efficient pollination. Female
167 lines were sown on April 11th. For standard sowing date, male lines were sown on April 18th
168 and on May 5th while female lines were sown on May 5th. Seeds were harvested on August
169 21th and August 28th for early and standard sowing, respectively. The duration of the

170 developmental cycle from sowing to harvest was 132 d for early sowing and 115 d for
171 standard sowing. Conditions of culture were conventional and cumulative rainfalls and
172 temperatures occurring during seed production are shown Fig. A. Seed germination, seed
173 filling and seed MC were assessed from July 24th to harvest. Seven heads of sunflower were
174 harvested twice a week for germination, water content and seed filling assays. For
175 germination assays, seeds were threshed manually, placed in cotton bags and dried in an
176 indoor dryer under ambient air, as previously described.

177 *2.3. Germination assays*

178 Germination assays were performed in darkness in 9 cm Petri dishes (25 seeds per dish, 4
179 replicates); seeds were placed on a layer of cotton wool moistened with deionized water at
180 10°C and 25°C. A seed was considered as germinated when the radicle protruded through
181 the pericarp. Germination counts were made daily. The results presented are means ± SD of
182 germination percentages obtained with 3 or 4 replicates as indicated in the text.

183 *2.4. Water content*

184 Water content, expressed on a FW basis, was determined by weighing sunflower achenes
185 before and after drying the material at 105°C for 17 h (for experiment 1) or at 103°C for 18 h
186 (for experiment 2).

187 *2.5. Meteorological data*

188 Temperatures and rainfalls were daily recorded in weather stations near to each
189 experimental field. Thermal time (TT, °C.d) was calculated according to the following formula:

$$190 \quad TT = \frac{T_{max} - T_{min}}{2} - T_b \text{ where } T_{max} \text{ is the maximum daily temperature in } ^\circ\text{C}; T_{min}, \text{ the}$$

191 minimum daily temperature in °C and T_b is the base temperature for sunflower growth. Here,
192 as an approximation, we have chosen 5°C as T_b value, in agreement with Gay et al. (1991)
193 and Allinne (2009), because the base temperature for germination for each genotype was not
194 available. Figure A shows the cumulative rainfalls (Fig. A-1), the thermal time and the mean
195 of daily temperature (Fig. A-2) from April 11th (sowing date of early sowing) till September 1st
196 2017 (harvest date for early and standard sowing) in experiment 2. In this trial, seeds issued

197 from the early sowing date had a duration of development cycle of 144 d against 120 d for
198 the standard sowing date. Early sowing received 354 mm of water against 307 mm for the
199 standard sowing. For the thermal time, plants sown earlier accumulated 1788°C.d and the
200 ones issued from standard sowing accumulated 1722°C.d.

201 *2.6. Statistical analysis*

202 Statistical analyses were performed using R (version 3.3.2 Copyright 2010; the R Foundation
203 for Statistical Computing).

204

205 **3. Results**

206 **3.1. Characterization of seed dormancy at harvest**

207 The main characteristics of sunflower seed dormancy were first investigated with 16 seed
208 batches from different varieties harvested in various location in 2015 (Table 1). Germination
209 assays were performed within 5 d after harvest at 10 and 25°C. At 10°C, seed germination
210 was fully prevented for all seed batches (Table 1). At 25°C, the final germination percentages
211 of whole seeds ranged from 2 to 98 %, but it could be improved by pericarp removal (Table
212 1). The relationship between germination of whole and naked (*i.e.* without pericarp) achenes
213 is shown in Figure 1; when whole achene germination was higher than 20 % removal of
214 pericarp allowed naked seeds to fully germinate. Below this critical value, removal of pericarp
215 was not efficient to improve the germination, and there existed a linear relationship between
216 germination of achenes and naked seeds at 25°C. This relationship was well described by
217 the tendency curve shown in Figure 1. Removal of pericarp did not stimulate the germination
218 at 10°C (data not shown). After 3 months of dry storage (20°C and 60% RH), whole achenes
219 of all seed batches germinated to almost 100% at 25°C (Table 1) and 10°C (data not shown).

220 In order to characterize the contribution of agroclimatic conditions to dormancy level
221 at harvest, we performed a Principal Component Analysis (PCA) with germination data
222 obtained with seeds of 107 batches of 28 genotypes produced in 2015, 2016 and 2017 in 3
223 different locations (South and Center of France, South of Spain) (Figure 2 and Figure B).
224 Seeds from all batches were unable to germinate at 10°C, excepted those from sample 21

225 which only germinated to 26 %, and at 25°C germination ranged from 0 to 95 %, depending
226 on the seed sample (Table A). In consequence, we used the variable “germination at 25°C”
227 at harvest as the indicator of dormancy level. PCA was performed taking into account the
228 germination ability of the 107 seed samples. The correlation circle (Fig. B) indicated that the
229 2 rainfall variables (from sowing to harvest and from flowering to harvest) can be reduced to
230 1 variable since they were highly correlated. Similar comments can be made with the 2
231 thermal time variables. Moreover, the variable "photoperiod from sowing to harvest" was
232 strongly correlated with cycle duration. PCA with one variable for rainfall, thermal time and
233 photoperiod was then performed (Fig. 2). The correlation circle (Fig. 2A) showed that the two
234 principal components explained 67.7 % of the variability. The duration of the developmental
235 cycle seemed to be more correlated with thermal time than with rainfall. The germination at
236 25°C seemed positively correlated with both variables, *i.e.* the duration of the developmental
237 cycle and the thermal time. It was negatively correlated with rainfalls. The variable
238 “germination at 10°C” was also correlated with the previous variables, but it weakly
239 contributed to explain the overall variability, presumably because this value was roughly
240 similar and close to 0 for all seed samples (Table A). We next performed a PCA giving a
241 color-code to variety (Fig. 2B), female (Fig. 2C), year (Fig. 2D) and area of production (Fig.
242 2E) (samples which localize on the left-hand side of Figs 2 B to 2 E displayed a stronger
243 dormancy than samples on the right-hand side). This analysis did not reveal any significant
244 effect of either variety, female or year of production on dormancy intensity at harvest (Figs 2
245 B-D). In contrast we evidenced a clear effect of the area of production on seed dormancy
246 since the 3 areas of production were clearly separated by the PCA (Fig. 2E). Seeds
247 produced in Spain displayed a lower dormancy and this was associated with a longer
248 developmental cycle, high thermal time and few rainfalls.

249 We investigated more closely the possible relationship between seed dormancy and
250 the maternal environment of freshly harvested seeds from 2 representative varieties of the
251 panel (13 and 25) (Table B) cultivated in the Center of France and in the South of Spain in
252 2015. These varieties were chosen because of their adaptation to local climate. Figure 3A

253 shows the climatic conditions in the areas of production of these varieties, from seed sowing
254 to harvest. Varieties cultivated in France received 168 mm (variety 13) and 214 mm (variety
255 25) of rainfalls and accumulated 2394°C.d (variety 13) and 2667°C.d (variety 25) during the
256 developmental cycle. Duration of the cycle was 112 days for variety 13 and 131 days for
257 variety 25 (Fig. 3A). In Spain, rainfall received was 52 mm for variety 13 and 157 mm for
258 variety 25, *i.e.* less than in central France. Variety 13 accumulated 2934°C.d and variety 25
259 3649°C.d. against 2394°C.d and 2667°C.d in central France. Duration of the developmental
260 cycle was 141 days for variety 13 and 154 days for variety 25 (Fig. 3A), *i.e.* slightly higher
261 than in the Center of France. Germination at 25°C of seeds from varieties 25 and 13 reached
262 54 % and 82 %, respectively, when they were produced in Southern Spain (Fig. 3B). In
263 contrast, when they were produced in the Center of France, germination of seeds of both
264 varieties 13 and 25 did not exceed 4 % and 26 %, respectively (Fig. 3B).

265 **3.2. Modulation of depth of dormancy through cultural practices**

266 Our findings suggested that drier and warmer conditions during seed development could
267 result in a lower dormancy at harvest. We hypothesized that these environmental conditions
268 could alter seed desiccation rate *on planta*. In order to confirm this hypothesis, we designed
269 an experimental trial to modulate seed desiccation rate by using a chemical desiccant (field
270 experiment 1). The desiccant was applied 35 days after flowering (daf) when seed water
271 content was 42 % FW for variety 30 and 39.6 % FW for variety 34 (Fig. 4). The treatment
272 accelerated the desiccation rate, but at harvest, *i.e.* at 49 daf, seed moisture content was
273 roughly similar, close to 9.3 % FW and 5.5 % FW respectively, in control and treated seeds
274 in case of variety 30 (Fig. 4A), but significantly higher (15.8 % FW) in control seeds than in
275 treated ones (5.8 % FW) for variety 34 (Fig. 4B). The effect of the desiccant was associated
276 with changes in seed germination at 25°C. At 35 daf, germination of seeds of both hybrids 30
277 and 34 did not exceed 5 % (Fig. 4). One week after the treatment, *i.e.* at 42 daf, germination
278 increased to 22 (variety 34) and 61 % (variety 30) for seeds from treated plants (Fig. 4),
279 whereas it remained close to 5 % for seeds of control plants. Two weeks after the treatment,
280 *i.e.* at 49 daf, germination at 25°C reached 34 % for the control seeds and 98 % for the

281 treated ones for the variety 30 (Fig. 4A), and 46.5 and 80.5 % respectively for the non-treated
282 and treated seeds of the variety 34 (Fig. 4B).

283 At last we also investigated the effect of the duration of the seed developmental cycle on
284 seed dormancy. This was done by performing an early and a standard seed sowing (see
285 material and methods, field experiment 2). Cumulative precipitations and thermal time from
286 sowing to harvest were similar for both sowing dates (Fig. A). Seeds were harvested on
287 august 21th and august 28th for early and standard sowing, respectively. Fig. 5 shows the
288 changes in germination, dry weight and MC during the late phases of seed development for
289 both varieties 29 (Fig. 5A) and 12 (Fig. 5B) For both varieties the patterns of seed
290 desiccation and seed filling were similar, but shifted in time, whatever the date of sowing
291 (Figs 5A and 5B). Seeds from the standard sowing tended to accumulate more dry matter,
292 but less water. At harvest, seeds of variety 29 from early sowing date, *i.e.* having the longest
293 duration of development on the plant, germinated at 65 % at 25°C while seeds from the
294 standard sowing date, have a shorter duration of developmental cycle, germinated at 40 %
295 (Fig. 5A). For variety 12, seeds from early sowing germinated at 10 % and seeds from
296 standard sowing germinated at 4 % (Fig. 5B).

297

298 **4. Discussion and conclusion**

299 At harvest, sunflower seeds germinated poorly at 10 and 25°C and they were then
300 considered as dormant. The present work was carried out with a large range of batches
301 produced in France and in the South of Spain in 2015, 2016 and 2017, and we paid attention
302 to perform seed germination as soon as possible after harvest, in order to rule out any bias
303 which could result from variable durations of after-ripening (Bazin et al., 2011), so that our
304 data are very reliable. In accordance with Corbineau et al. (1990), we show that sunflower
305 seed dormancy has 2 origins, the embryo itself and the seed envelopes (pericarp + seed
306 coats). At 10°C, the inability to germinate results mainly from the embryo since pericarp
307 removal had almost no effect on the germination. At 25°C, when whole achene germination
308 was below *ca.* 20 % at harvest, seed dormancy resulted from the additive effect of both the

309 embryo dormancy and the seed coat imposed dormancy (Fig. 1, Table1). When initial
310 germination was higher than this critical value (20 %), the dormancy at 25°C only resulted
311 from the inhibitory effect of the pericarp, as shown by the high germination rate of naked
312 seeds (Fig. 1). These data reveal that the origin and expression of sunflower seed dormancy
313 is a complex trait. They also show that embryo dormancy can be expressed at high
314 temperature, although it is generally reported to occur at low temperature of imbibition
315 (Corbineau et al., 1990; Bodrone et al., 2017).

316 At 25°C, germination of freshly harvested seeds of the 107 samples from 28 varieties
317 ranged from 0 to 95 %, with 80 seed batches having a germination below 20 %. In
318 agreement with our previous statement, this suggests that a large majority of the seed lots
319 obtained in agricultural conditions across different years and environments exhibited both an
320 embryo and a pericarp-imposed dormancy. The PCA carried out here allowed ruling out any
321 significant effect of the year of production, genetics or parent plant interference on seed
322 dormancy (Figs 2 B, C and D). This nevertheless does not mean that dormancy was not
323 related to any genetic issue, as already demonstrated by studies which led to the
324 identification of quantitative trait loci (QTL) controlling seed dormancy (Alonso-Blanco et al.,
325 2003; Bentsink et al., 2006; Bentsink et al., 2010; Sugimoto et al., 2010), including in
326 sunflower (Gandhi et al., 2005). The varietal effect on seed dormancy in sunflower has also
327 been demonstrated (Vujakovic et al., 2012; Rosello et al., 2016; Vigliocco et al., 2017).
328 However, the use of a large panel of seed lots clearly demonstrates that the environmental
329 imprints has much stronger effect than the genetic one in regulating the depth of sunflower
330 seed dormancy at harvest. In particular we demonstrate that the duration of the
331 developmental cycle, the thermal time and the rainfalls from sowing until harvest or from
332 flowering to harvest, which are all directly related to the environment of production, control
333 seed dormancy (Fig. 2A). The positive correlation between the length of the developmental
334 cycle and the ability to germinate at harvest was also demonstrated by one of our field
335 experiments (Fig. 3). The impact of the environmental conditions on the establishment of
336 dormancy during seed development on the mother plant has already been reported in

337 numerous species (Fenner, 1991; Baskin and Baskin, 1998; Donohue et al., 2007; Footitt et
338 al., 2013). Using a field trial Bodrone et al. (2017) found that high temperatures during
339 sunflower seed development decreased embryo dormancy intensity but increased achene
340 dormancy. In contrast, we show here that seeds that were produced in South of Spain, *i.e.*
341 theoretically in warmer and drier conditions, displayed a lower achene dormancy. Although
342 this may appear as contradictory, we also demonstrate in the present work that germination
343 of achenes at 25°C could be prevented by an embryo dormancy, which has been not
344 reported yet. This could explain the apparent discrepancy between the results of Bodrone et
345 al. (2017) and our results.

346 The findings brought by the PCA were confirmed by the experimental trials we
347 conducted with 2 varieties in the Center of France and in the South of Spain (Fig. 3). Using 2
348 different genotypes grown in both locations we clearly demonstrated the effect of
349 temperature and rainfalls (Fig. 3A) on seed dormancy at harvest (Fig. 3B). The fact that less
350 precipitations and higher temperatures were associated with lower dormancy led us to
351 hypothesize that seed desiccation pattern *on planta*, modulated by rainfalls and
352 temperatures, could play a role in the establishment of seed dormancy during seed
353 development. We verified this hypothesis by applying a desiccant on the plants during the
354 seed maturation phase of 2 genotypes when seed MC was close to 40 % FW (varieties 30
355 and 34), *i.e.* at the end of seed filling (Fig. 4). This treatment resulted in a faster loss of water
356 and in an increase in the germination at 25°C for both varieties, *i.e.* in a reduced dormancy
357 intensity (Fig. 4 C, D). These data suggest that seed desiccation rate plays a major role in
358 dormancy level at harvest. To our knowledge only few studies have directly addressed the
359 possible relationship between seed desiccation on the mother plant and seed dormancy at
360 harvest. In sunflower, chemical desiccation is generally used for practical purposes in order
361 to anticipate harvest (Szemruch et al., 2014). In this context, and in contrast to our results, it
362 has been proposed that chemical desiccation would increase seed dormancy, as assessed
363 by germination at 25°C (Szemruch et al., 2014). These authors proposed that desiccants
364 could directly modify seed coat anatomy thus explaining the phenotype of dormancy.

365 Sunflower seed dormancy also depends on ABA metabolism, and Le Page-Degivry et al.
366 (1990), using fluridone (an inhibitor of ABA synthesis) applied on the capitula, suggested that
367 sunflower seed dormancy was mostly correlated with a peak of ABA synthesis about 2
368 weeks after flowering, *i.e.* well before the maturation phase. Here we show that the
369 modulation of seed desiccation in the later stage of seed development, *i.e.* after 25 daf, had
370 a marked effect on dormancy (Fig. 4). These data indicate that the ABA synthesis in
371 developing seeds does alone explain the sunflower seed dormancy at harvest. Regulation of
372 dormancy by ABA must also take into account the sensitivity of the embryo to ABA; for
373 example embryo responsiveness to ABA is highly correlated to dormancy intensity in seeds
374 of numerous species such as wheat, oat and barley (Morris et al., 1989; Corbineau et al.
375 1993; Corbineau et al. 2000; Rodriguez et al., 2015) and sunflower (Le Page-Degivry et al.,
376 1996). Bodrone et al. (2017) also suggested that changes in ABA sensitivity of sunflower
377 achenes could participate to the regulation of seed dormancy, but this remains to be
378 confirmed in various agro-climatic conditions. The role of ABA metabolism and signaling
379 pathways in the 2 components of sunflower seed dormancy, and their putative role in the
380 response of developing seeds to agroclimatic conditions will have to be addressed properly.
381 Additionally, it would be also important to determine whether known players of desiccation
382 programs in seeds such as LEAs and HSPs (Holdsworth et al. 2008), and which have been
383 shown to play a role in the expression of sunflower seed dormancy (Zinsmeister et al. 2020),
384 could be involved in seed dormancy establishment.

385 In summary, using a large dataset of sunflower seed lots we clearly identified the
386 pivotal role of environmental conditions on establishment of seed dormancy during seed
387 development. These results were confirmed by experimental trials, highlighting the role of
388 seed desiccation on the mother plant in dormancy establishment, and open novel
389 perspectives of research in this topic. On a practical point of view, we provide new insights
390 on agricultural practices which should help the stakeholders to better cope with the problem
391 of seed dormancy at harvest.

392

393 **Acknowledgements**

394 The authors would like to thank Marine Blondeau (Limagrain) for their help with germination
395 assays and Christelle Esquerdo (Limagrain) for analyzing and discussing data.

396

397 **Funding source**

398 This work was supported by Limagrain and Association Nationale de la Recherche
399 Technique (ANRT, grant CIFRE n°2016/136).

400

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563 **Table 1.** Influence of the variety, the area of cultivation and the date of harvest on the
564 germination percentages obtained after 11 days at 10°C with achenes and 7 days at 25°C
565 with achenes and embryos at harvest and germination percentages obtained after 7 days at
566 25°C with achenes after-ripened for 3 months of dry storage at 20°C and 60% RH. Means
567 of 4 replicates \pm SD.

Variety	Location of cultivation	Date of harvest (dd/mm/yy)	Germination (%) at harvest at 10°C of	Germination (%) at harvest at 25°C of		Germination after 3 months of dry storage at 25°C of
			Achenes	Achenes	embryos	Achenes
13	Center of France	31/08/2015	6 \pm 2	2 \pm 2	32 \pm 0	100 \pm 0
13	Center of France	31/08/2015	0 \pm 0	3 \pm 2	10 \pm 5	97 \pm 1
13	Center of France	28/08/2015	0 \pm 0	5 \pm 3	25 \pm 2	98 \pm 1
29	North of Spain	02/09/2015	1 \pm 2	11 \pm 13	44 \pm 2	100 \pm 0
16	North of Spain	10/09/2015	0 \pm 0	11 \pm 6	62 \pm 14	100 \pm 0
30	North of Spain	10/09/2015	1 \pm 2	11 \pm 6	82 \pm 14	100 \pm 0
13	South of France	16/09/2015	0 \pm 0	14 \pm 8	80 \pm 0	95 \pm 2
13	Center of France	31/08/2015	1 \pm 2	15 \pm 4	96 \pm 0	97 \pm 1
13	Center of France	11/09/2015	1 \pm 2	19 \pm 6	45 \pm 11	100 \pm 0
11	South of Spain	25/08/2015	8 \pm 8	51 \pm 8	98 \pm 2	100 \pm 0
16	South of France	07/09/2015	4 \pm 5	53 \pm 16	100 \pm 0	98 \pm 2
11	South of Spain	19/08/2015	3 \pm 3	60 \pm 10	92 \pm 0	100 \pm 0
13	Center of France	10/09/2015	0 \pm 0	64 \pm 8	78 \pm 2	100 \pm 0
13	Center of France-	11/09/2015	2 \pm 2	69 \pm 11	100 \pm 0	100 \pm 0
7	South of France-	28/08/2015	4 \pm 3	70 \pm 13	98 \pm 2	100 \pm 0
19	South of France	02/09/2015	0 \pm 0	82 \pm 12	100 \pm 0	97 \pm 1

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572 **Legends for figures**

573 **Fig. 1.** Relationship between germination after 4 days at 25°C of freshly harvested wholes
574 achenes and achenes without pericarp (●) of various seed batches (one dot corresponds to
575 one seed batch, see table 1). The germination at 25°C of non-dormant achenes of the same
576 seed batches is also shown (○). Results were obtained with 3 replicates of 25 seeds for each
577 condition.

578
579 **Fig. 2.** Principal component analysis (PCA) of dormancy intensity, estimated by germination
580 rate after 11 d at 10°C and 4 d at 25° C, thermal time (°C.d, 5°C base temperature) and
581 rainfalls (mm) from sowing to harvest or from flowering to harvest, cycle duration (days) from
582 sowing to harvest, of 107 samples of sunflower seeds (see details in Table S1). A,
583 correlation circle of the repartition of variables in dimension 1 (38.7 % of explanation) and
584 dimension 2 (27.4 % of explanation). PCA analysis with samples color-coded by variety (B),
585 by female (C), by harvest year (D) and by production area (E).

586 The variable “germination at 10°C” was also correlated with the previous variables, but it
587 weakly contributed to explain the overall variability, presumably because this value was
588 roughly similar and close to 0 for all seed samples (Table A).

589
590 **Fig. 3.** Influence of environmental conditions of seed development on seed dormancy at
591 harvest. A, cycle duration (number of days between sowing and harvest) and cumulative
592 rainfalls from sowing to harvest for varieties 13 and 25 produced in Center of France and
593 South of Spain. B, thermal time (°C.d) from sowing to harvest for varieties 13 and 25
594 produced in Center of France and South of Spain. C, germination curve obtained at 25°C
595 with freshly harvested seeds of the variety 13 produced in Center of France (white symbols)
596 and South of Spain (black symbols). D, germination curve obtained at 25°C with freshly
597 harvested seeds of the variety 25 produced in Center of France (white symbols) and South of
598 Spain (black symbols). Mean of results obtained with four replicates of 25 seeds ± SE.

599

600 **Fig. 4.** Changes in seed water content and germination during seed development of varieties
601 30 (A) and 34 (B). Germination was obtained after 4 days at 25°C. Mother plants were
602 treated (T) or not (NT) with a chemical desiccant applied 35 d after flowering. Germination
603 results are the mean of 4 replicates of 25 seeds \pm SD and water content is the mean of 2
604 replicates of 500 seeds with average deviation (*, significant according to Tuckey's test).

605

606 **Fig. 5.** Changes in germination, water content (fresh weight basis) and thousand seed dry
607 weight during seed development of varieties 29 (A) and 12 (B) cultivated in Center of France
608 and sown on April 11, 2017 (early sowing, dotted line and white bar) or may 5, 2017
609 (standard sowing, full line, black bar). Seeds were harvested at the last time point of each
610 experiment. Germination was obtained after 4 d at 25°C (results are the means of 4
611 replicates of 25 seeds \pm SD). Water contents are the means of 3 replicates of 100 seeds \pm
612 SD and expressed as percent of fresh weight. Dry weight was calculated for 1000 seeds.

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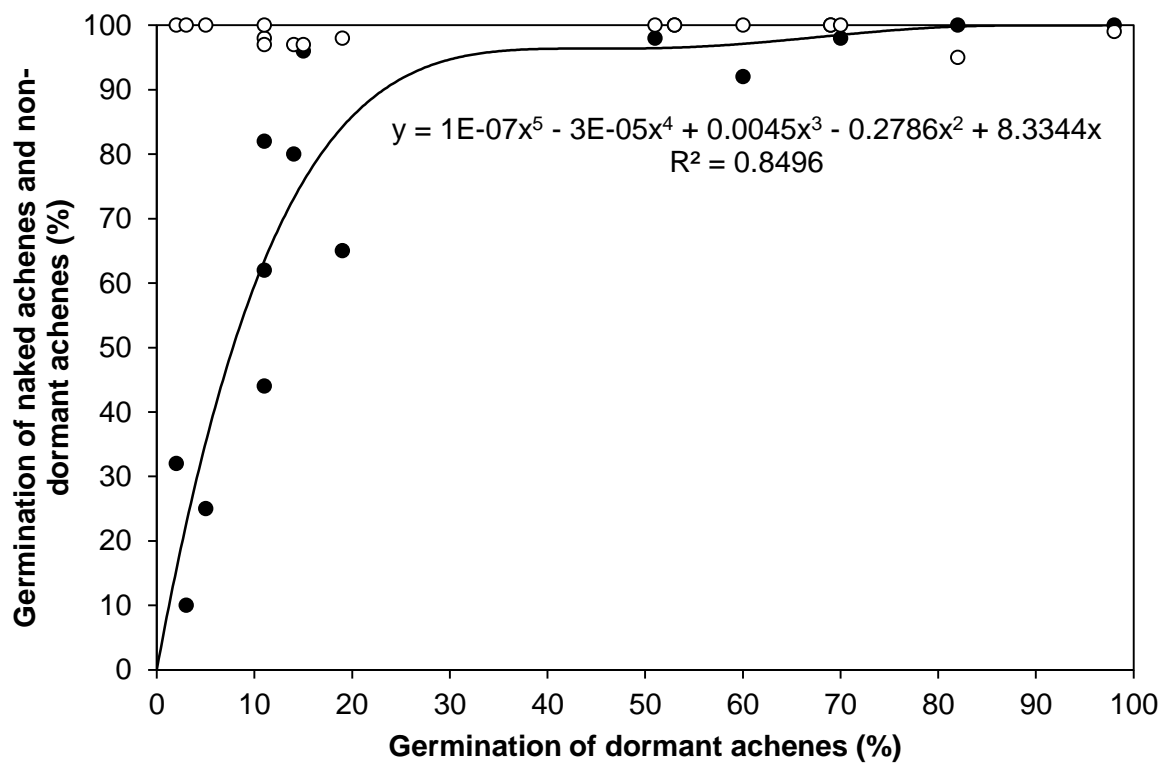


Fig. 1. Relationship between germination after 4 days at 25°C of freshly harvested whole achenes and achenes without pericarp (●) of various seed batches (one dot corresponds to a seed batch, see table S1). The germination at 25°C of non-dormant achenes of the same seed batches is also shown (○). Results were obtained with 3 replicates of 25 seeds for each condition.

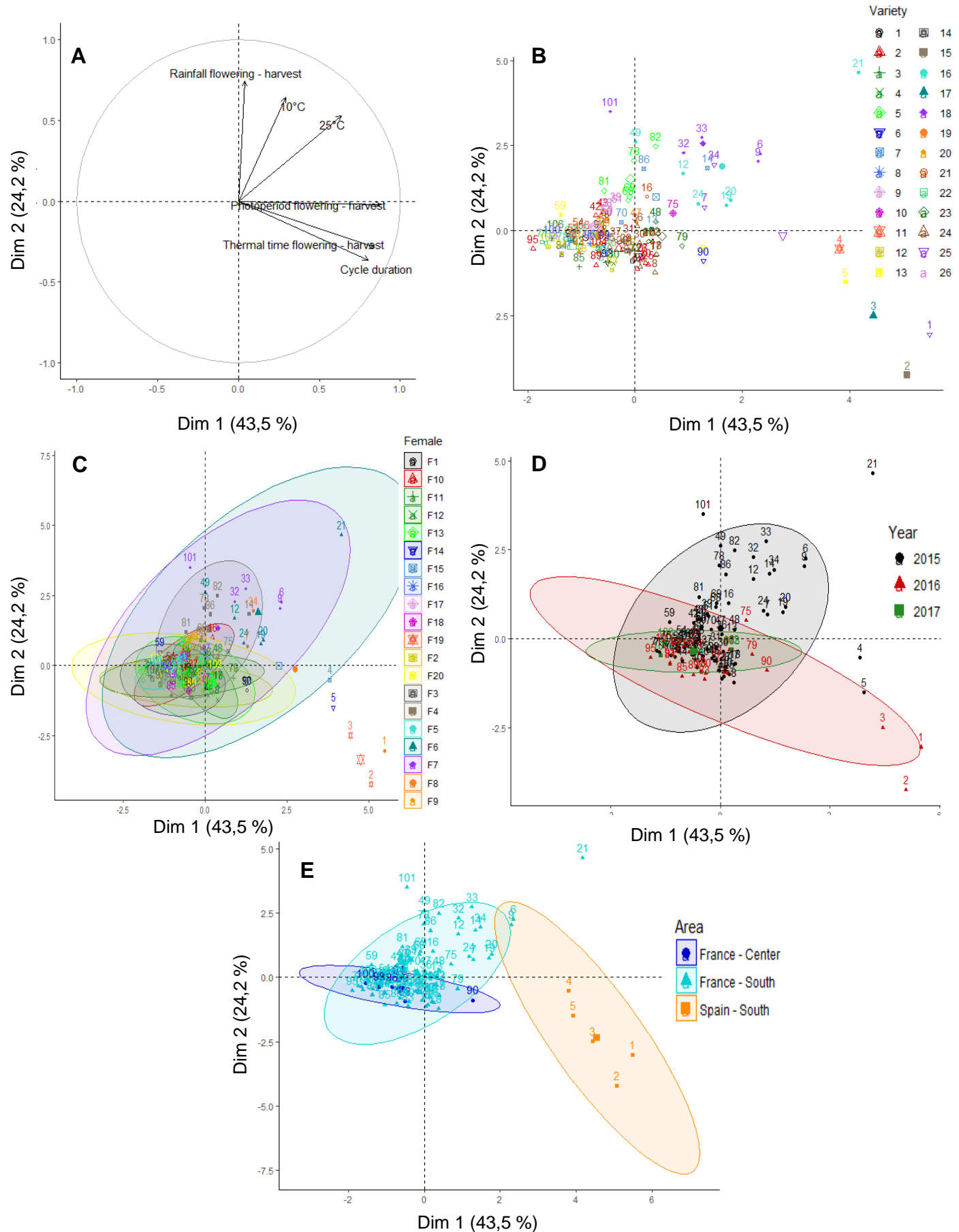


Fig. 2. Principal component analysis (PCA) of dormancy intensity, estimated by germination rate after 11 d at 10°C and 4 d at 25° C, thermal time (°C.d, 5°C base temperature), rainfalls (mm) and photoperiod (cumulative daily photoperiod) from flowering to harvest, cycle duration (days) from sowing to harvest, of 107 samples of sunflower seeds (see details in Table A). A, correlation circle of the repartition of variables in dimension 1 (43,5 % of explanation) and dimension 2 (24,2 % of explanation). PCA analysis with samples color-coded by variety (B), by female (C), by harvest year (D) and by production area (E). The variable “germination at 10°C” was also correlated with the previous variables, but it weakly contributed to explain the overall variability, presumably because this value was roughly similar and close to 0 for all seed samples (Table A).

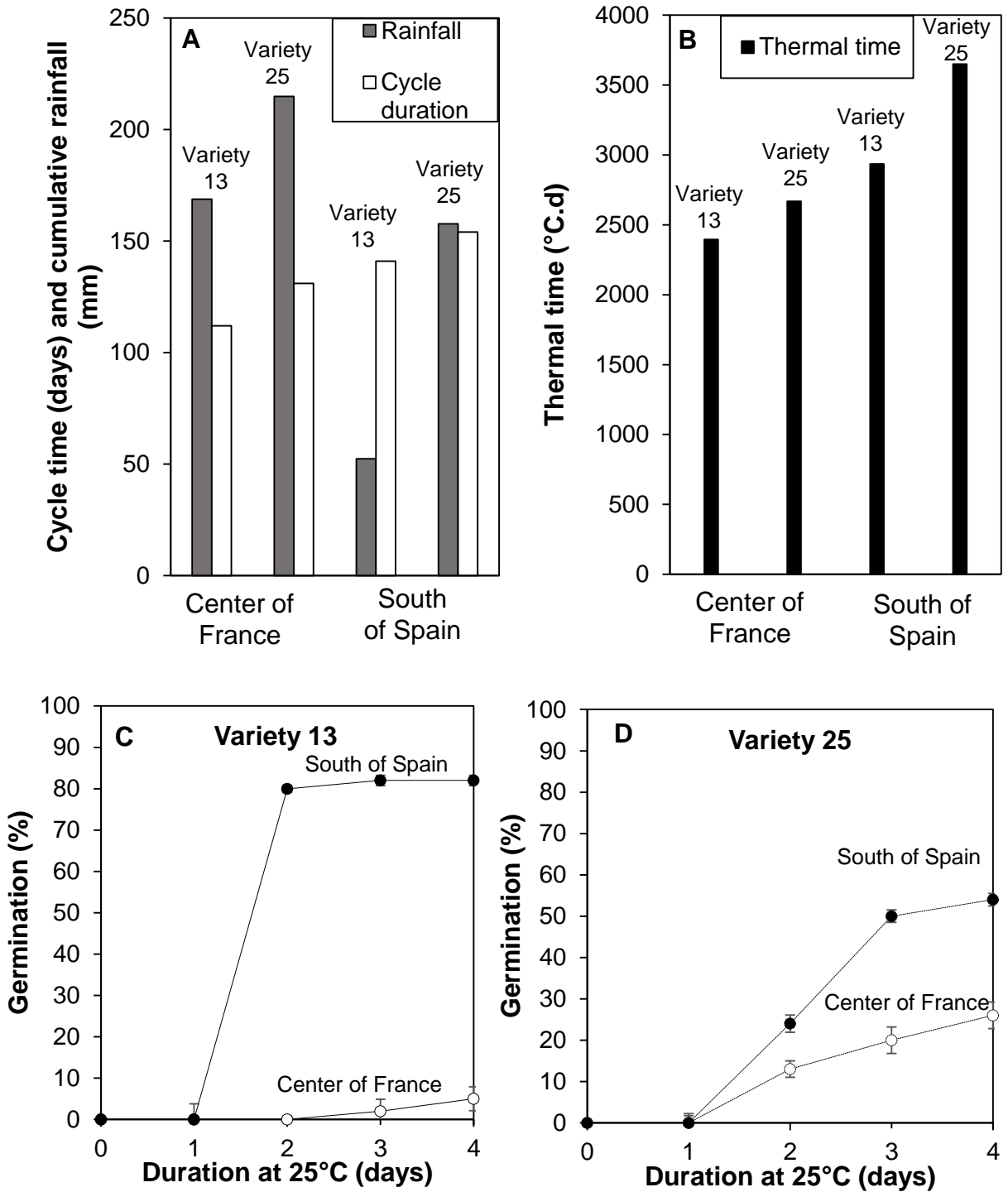


Fig. 3. Influence of environmental conditions of seed development on seed dormancy at harvest. A, cycle duration (number of days between sowing and harvest), and cumulative rainfalls (mm) from sowing to harvest for varieties 13 and 25 produced in Center of France and South of Spain. B, thermal time (°C.d) from sowing to harvest for varieties 13 and 25 produced in Center of France and South of Spain. C, D germination after 4 days at 25°C of freshly harvested seeds of the varieties 13 (C) and 25 (D) produced in Center of France (white symbols) and South of Spain (black symbols) (full lines). Mean of results obtained with four replicates of 25 seeds ± SE.

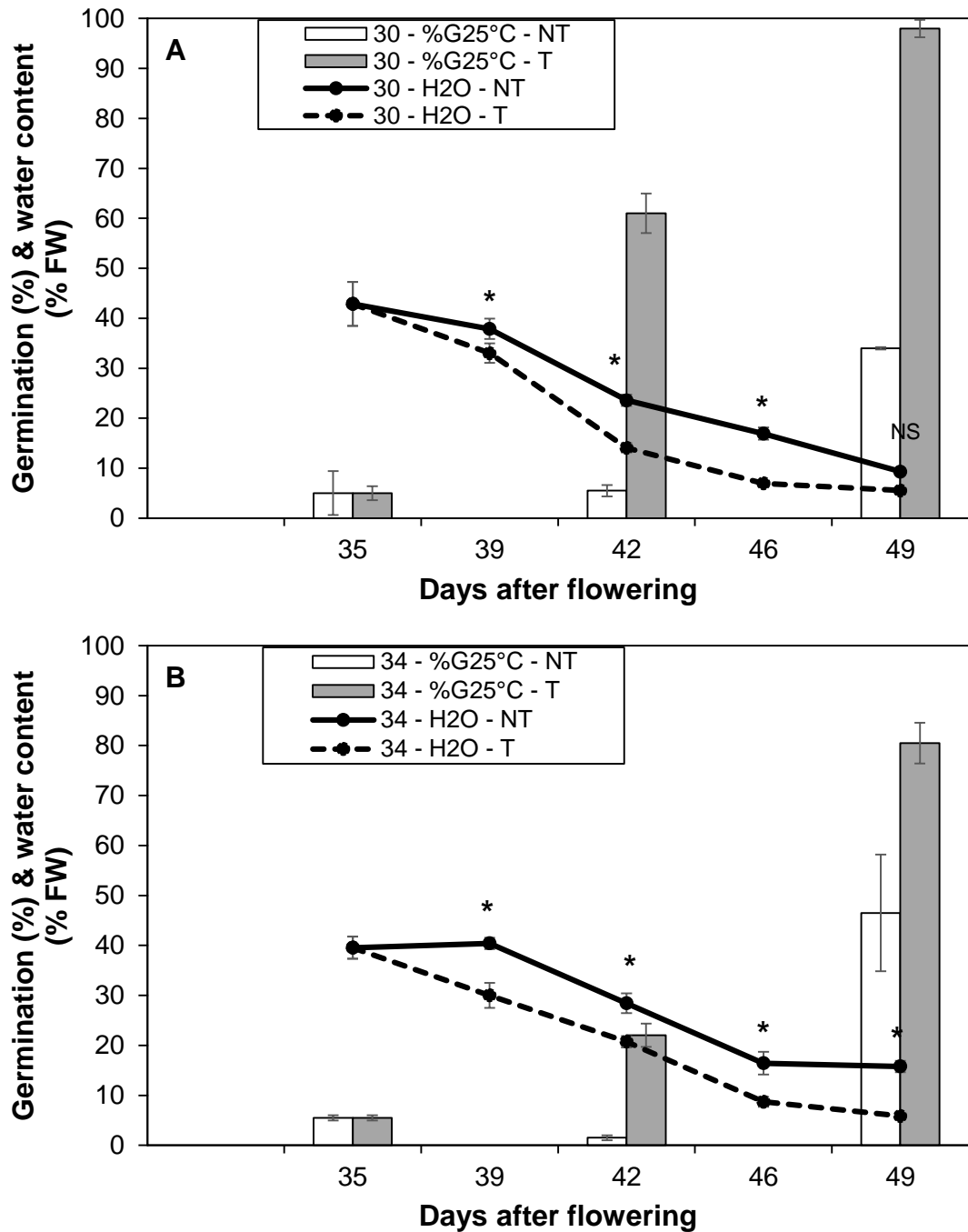


Fig. 4. Changes in seed water content and germination during seed development of varieties 30 (A) and 34 (B). Germination was obtained after 4 days at 25°C. Mother plants were treated (T) or not (NT) with a chemical desiccant applied 35 d after flowering. Germination results are the mean of 4 replicates of 25 seeds \pm SD and water content is the mean of 2 replicates of 500 seeds with average deviation (*, significant according to Tuckey's test).

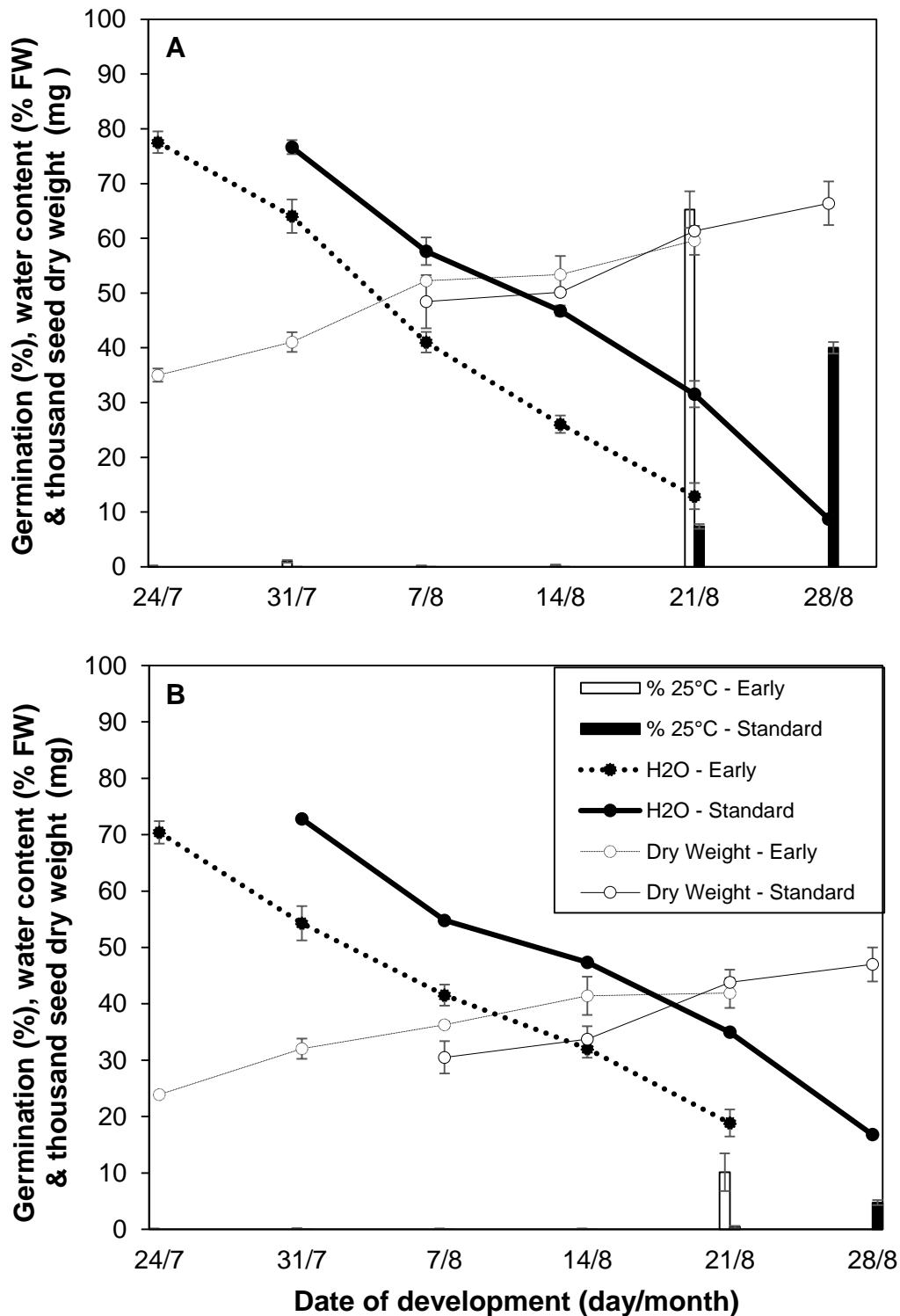


Fig. 5. Changes in germination, water content (fresh weight basis) and thousand seed dry weight during seed development of varieties 29 (A) and 12 (B) cultivated in Center of France and sown on April 11, 2017 (early sowing, dotted line and white bar) or may 5, 2017 (standard sowing, full line, black bar). Seeds were harvested at the last time point of each experiment. Germination was obtained after 4 d at 25°C (results are the means of 4 replicates of 25 seeds \pm SD). Water contents are the means of 3 replicates of 100 seeds \pm SD and expressed as percent of fresh weight. Dry weight was calculated for 1000 seeds.