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1 **Adaptive significance of functional germination traits in crop wild relatives of**
2 ***Brassica***

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12 **Abstract**

13 Functional germination traits contribute to both niche competitiveness and crop yield
14 outcomes. However, there is little understanding of the adaptive significance of the
15 germination thermal- and hydro-parameters in crop wild relatives (CWRs), yet these
16 species are anticipated to be the source of adaptive traits for future agriculture. Seeds of
17 10 lots of *Brassica* species, sub-species and inbred lines from across Europe, North
18 Africa and the Middle East were subjected to a range of temperature and water potential
19 conditions. The germination progress curves recorded were analysed using repeated
20 probit analysis and the functional trait parameters (thermal- and hydro thresholds and
21 times) determined. Relationships between these seed parameters (and the physical trait,
22 seed mass) and the seed source environment were investigated.

23 The *Brassica* genus was found to have diverse seed germination phenotypes, with
24 thermal (θ_T) and hydro times (θ_H) differing by 3 to 7-fold, base temperatures (T_b) by c. 9
25 °C and base water potentials (Ψ_b) by -1.5 MPa. Crop seed lots of *Brassica oleracea* had
26 shorter θ_H for germination and higher values of Ψ_b , but longer θ_T for germination than
27 their CWR. For the CWRs, the mean monthly precipitation or the precipitation of the
28 predicted month of germination of the seed collection site, was linearly correlated with
29 T_b , θ_T , and Ψ_b . This increases the predictability of identifying valuable brassica
30 germplasm for crop development through regeneration trait screening.

31 In conclusion, the selection of the crop *B. oleracea* for fastest germination under
32 irrigated conditions likely limits the capacity of the current genepool to cope with
33 erratic periods of drought predicted in future European climates.

34 **Keywords:** Seed functional traits; thermal time; hydro time; vigor; precipitation; seed
35 mass.

36 **1. Introduction**

37 Global climate change is predicted to increase environmental temperatures, but may
38 have other consequences such as earlier frosts and changes to the frequency of flooding
39 and drought (IPCC, 2013), all of which may affect the outcome of germination and
40 seedling emergence. In particular, warming temperatures and fluctuations in
41 precipitation are known to increase the risk to yield in the most economically important
42 crops, such as wheat, rice, maize and barley (Lobell and Field, 2007; Porter and
43 Semenov, 2005). Moreover, crops bred for predictable and more uniform germination
44 and harvest may contribute to loss of variability in responses to environmental
45 conditions (Gepts, 2010). In contrast, crop wild relatives (CWRs) represent a genepool
46 that is potentially adapted to a large range of habitats and, therefore, likely better
47 equipped to survive the challenges associated with climate change (Dempewolf et al.,
48 2014). In addition to possessing high genetic diversity, CWR species may also have
49 greater plasticity in traits enabling them to cope better with rapid environmental change
50 (Nicotra et al., 2010).

51 Global plant variability can be explained by just six adult plant-functional traits, only
52 one of which relates to the regenerative phase, specifically diaspore mass (Díaz et al.,
53 2016). This seed physical trait has been shown to be predictive of a light requirement
54 for germination, persistence in the soil and dispersal (Baskin and Baskin, 2014).
55 However, increasing importance is being attributed to germination functional traits such
56 as germination rate, thermal time and hydro time, determined by the quantification of
57 the physiological process of germination as a descriptor of regeneration potential.

58 Germination rate is an important and widely used descriptor of seed vigour (Pollock and
59 Roos 1972). These traits are now recognised as critical components of the multiple
60 environmentally regulated factors that define the ecological niche for population growth
61 (Dürr et al., 2015; Fernández-Pascual et al., 2015; Grubb, 1977; Poschlod et al., 2013)

62 Recent characterisation of germination functional traits (base temperature, T_b ; and
63 thermal time, θ_T) in CWRs of grapevine have identified populations at greater risk of
64 climate change on the basis of insufficient cold to remove seed dormancy (Orru et al.,
65 2012). In consideration of future proofing agriculture in France, 36 cover crops (across
66 six plant families including the *Brassicaceae*) have been characterised for germination
67 functional traits (Tribouillois et al., 2016). Four *Brassicaceae* (*B. juncea*, *B. rapa*, *B. napus*,
68 *B. carinata*) had T_b of c. 7°C and three species had similar base water potentials, Ψ_b , for
69 germination (-0.9 to -1 MPa), the exception being *B. rapa* (-2.2 MPa) (Tribouillois et
70 al., 2016). These findings lend weight to the argument that a species geographical origin
71 defines its ability to germinate (Cochrane et al., 2014; Dürr et al., 2015).

72 The convergence of functional traits in species occupying similar environments can be
73 considered an adaptation to environmental filtering (Keddy, 1992). Precipitation and
74 temperature are the main environmental factors that have an impact on plant traits, e.g.,
75 leaf mass, yield, height and flowering time; and seed traits, such as seed mass,
76 germination and dormancy, show similar ecological associations (Ackerly et al., 2000;
77 Baskin and Baskin, 1998; Dornbos and Mullen, 1991; Franks et al., 2007; Menzel et al.,
78 2006; Peñuelas et al., 2004; Porter, 2005). Seed germination (i.e., radicle emergence)
79 and seedling establishment are thought to be the most sensitive stage of a plant to
80 environmental changes (Fay and Schultz, 2009; Kimball et al., 2010; Lloret et al.,
81 2004). Consequently, the effects of both precipitation and temperature on germination

82 rate (Céspedes et al., 2012; Clauss and Venable, 2000; Levine et al., 2008; Meyer et al.,
83 1990), final germination (Alexander and Wulff, 1985; Gareca et al., 2012; Gutterman,
84 2000b) and seedling establishment (Cochrane et al., 2015; Jump et al., 2008; Lloret et
85 al., 2004; Smith et al., 2000) have been widely explored.

86 Crop domestication has resulted in larger seed size (Preece et al., 2017) and seed mass
87 is also known to be responsive to environmental fluctuations (Donohue et al., 2005;
88 Nicotra et al., 2010; Roach and Wulff, 1987). In particular, seed filling is influenced by
89 environmental conditions and mean seed mass of a population has been positively
90 correlated with annual rainfall (Harel et al., 2011) or with mean annual temperature
91 (Murray et al., 2004). Whilst seed size is thought to affect germination rate (Norden et
92 al., 2009), seed mass is rarely co-analysed with seed physiological traits of germination
93 base temperature (T_b) or base water potential (Ψ_b). In this context, seed mass and T_b of
94 tree seeds of *Aesculus hippocastanum* from across Europe had no correlation (Daws et
95 al., 2004), and a negative correlation was found between seed mass and Ψ_b of
96 neotropical forest species (Daws et al., 2008).

97 Our aim was to investigate how the originating environment of *Brassica* CWRs impact
98 on their germination functional traits, testing the assumption that current *Brassica*
99 crops may have been selected for fast germination under managed, irrigated conditions.
100 We used seven geo-referenced seed collections (from across Europe, North Africa and
101 the Middle East) and three commercially available crop seed lots and research
102 genotypes. The genus *Brassica* possesses a wide diversity in morphology, genetics
103 (Arias et al., 2014), environment stress tolerance and is economically important around
104 the world for its edible roots crops, vegetables and oilseeds (Tsunoda et al., 1980). We
105 selected wild taxa from locations with variable mean monthly precipitation (1 – 94 mm)

106 and temperature (5 – 25 °C). We show an intimate relationship between *Brassica* seed
107 collection site and germination functional traits.

108 **2. Material and Methods**

109 *2.1 Seed material*

110 Studies were performed on seven seed lots of non-dormant wild brassica: three species
111 (*Brassica rapa*, *B. nigra* and *B. tournefortii*) and two sub-species (*Brassica rapa* subsp.
112 *campestris* and *B. rapa* subsp. *sylvestris*). (Table 1A). These CWR seed lots were
113 obtained from the Millennium Seed Bank, Royal Botanic Gardens, Kew, UK. Seed lots
114 used were from across Europe, North Africa and the Middle East. Selection was based
115 on an environmental cline typical of *Brassica* species: annual mean temperature from 5
116 to 26 °C; and a mean monthly precipitation from 1 mm to 94 mm, in seed lots with
117 sufficient quantity of seeds. The values were calculated as the mean monthly
118 precipitation and annual mean temperature, i.e. the sum of all 12-monthly means,
119 divided by 12. Seeds were handled in a consistent way on receipt at the seed bank:
120 equilibration to 15 % RH and 15 °C, hermetical sealing in containers and then storage at
121 -20 °C (FAO/IPGRI, 1994). On withdrawal from the bank, seeds were re-equilibrated to
122 15 °C in a room operating at c. 15 % RH.

123 A commercial seed lot of *B. oleracea* (cabbage) was obtained from B&T World Seeds
124 (France) (Table 1B) and seeds of two research genotypes derived from *B. oleracea*
125 crops (A12DHd from kale and AGSL101 from kale and broccoli) were provided by the
126 University of Warwick. Both crop research genotypes have the same genetic
127 background (A12) but SL101 has introgressions from at least two QTL (RABA1 and
128 SOG1) from a faster germinating genotype (GD33DH, broccoli) that confers higher

129 vigour (Morris et al., 2016). For simplicity, henceforth A12DHd and AGSL101 will be
 130 called RG_L (low vigour research genotype) and RG_H (high vigour research
 131 genotype). The commercial genotype will be called CG. Both crop research genotypes
 132 were grown under the same controlled conditions at 18/22 °C (night/day) in a
 133 glasshouse to produce seeds. On receipt at RBG Kew, all seed lots were equilibrated to
 134 15 % RH and 15 °C and stored under these conditions for a few weeks until use.

A.		Annual mean temperature			
Species	Collection site	Mean monthly precipitation (mm)	Min T (°C)	Mean T (°C)	Max T (°C)
<i>B. rapa</i> (CWR)	Chur (Switzerland)	94.17	4.8	9.2	13.7
<i>B. nigra</i> (CWR)	Dorset (England)	68.83	6.2	9.9	13.8
<i>B. rapa</i> (CWR)	Memsault (France)	64.08	6.5	10.9	15.4
<i>B. rapa</i> subsp. <i>campestris</i> (CWR)	Göle-Kars (Turkey)	40.84	-1.6	4.8	11.5
<i>B. rapa</i> subsp. <i>sylvestris</i> (CWR)	Ait Marghad (Morocco)	26.8	3.8	12.1	20.4
<i>B. tournefortii</i> (CWR)	Natrun – Alamin (Egypt)	4.83	14.3	20.7	27.1
<i>B. rapa</i> subsp. <i>sylvestris</i> (CWR)	S. Oran (Algeria)	0.83	17.5	25.7	34.0

B.	Provider	Genotype	Vigour	Growth conditions	
				Day (°C)	Night (°C)
		CG			
<i>B. oleracea</i> (crop)	B&T World Seeds (France)	(Commercial seed lot)	Unknown	Unknown	Unknown
<i>B. oleracea</i> (crop research genotype)	University of Warwick (UK)	RG_H (AGSL101)	High	22	18
<i>B. oleracea</i> (crop research genotype)	University of Warwick (UK)	RH_L (A12DHd)	Low	22	18

135 **Table 1.** Details of the 10 *Brassica* seed lots used. (A) The environment information
136 was obtained by combining seed collection geo-locations (GIS coordinates) for the
137 *Brassica* crop wild relatives (CWRs) and WorldClim data, as historical annual mean
138 temperature (T) and mean monthly precipitation since 1960. (B) Plant growth and seed
139 production conditions are shown for the research genotype seed lots, but are unknown
140 for the commercially purchased *B. oleracea* crop seed lot.

141 2.2 Seed mass and germination

142 To characterise the seed mass of the *Brassica* seed lots, 100 seeds were individually
143 weighed to 0.0001 mg accuracy. The distribution of seed mass at 0.5 mg intervals was
144 analysed and the mean and coefficient of variation (CV) calculated.

145 For germination, seeds were sown at constant temperatures from 5 to 45 °C, at 5 °C
146 intervals. Seeds were also sown at a range of water potentials (0, -0.3, -0.5, -0.8 and -1.0

147 MPa) using water and increasing concentrations of polyethylene glycol 8000 (PEG,
148 Fisher Scientific, UK) solution, but only at constant temperatures of 25 and 30 °C. Due
149 to limited seed availability, *B. nigra* seeds were sown under constant temperatures
150 without water stress. Either three replicates of 25 seeds, for the CWRs, or four replicates
151 of 25 seeds for the crops, were used in each treatment. Seeds were sown onto two layers
152 of germination test paper (90 mm diameter; Fisher Scientific, UK) in Petri dishes. The
153 volume of PEG solution used (7 mL) was calculated following the equation of
154 Hardegree and Emmerich (1990) to account for the weight of the dry germination paper.
155 The water potential of the solutions was confirmed with an osmometer (Camlab, UK).
156 For consistency, the same volume (7 mL) of solution was used for all PEG and water
157 treatments. The vented Petri dishes were placed in sealable plastic bags and incubated at
158 the relevant temperature with a 12 hour photoperiod (radiometric flux density of 50-100
159 W/m²). Germination (radicle emergence of 2 mm) was recorded every hour during the
160 first 24 hours and then every two-three hours until no more germination was observed.
161 Non-germinated seeds that were still firm after PEG treatment were briefly washed with
162 distilled water to remove the PEG and transferred to a new Petri dish with germination
163 test paper and 7 mL of water. After 15 days in this recovery assessment, if the seeds did
164 not germinate, the seed coat was removed and the viability was tested with 1 %
165 triphenyl tetrazolium chloride (TZ) in the darkness at 30 °C (ISTA, 2003) for 18 hours.
166 Stained red embryos were considered to be viable.

167 2.3 Data analysis

168 Germination was quantified as final germination percentage and germination rate (GR).
169 Germination progress over time was represented as a sigmoidal curve (fitted using the
170 Boltzmann distribution) from which the GR was estimated for all treatments in each

171 population. The GR at the 50th percentile (t₅₀) is the reciprocal of time the population
172 needs to reach 50 % germination of viable seeds. The seeds were germinated at a range
173 of temperatures and the GR describes a positive (sub-optimal range of temperatures) or
174 negative (supra-optimal range of temperatures) regression line when plotted against
175 temperature. The intercept of both lines is the optimal temperature (T_o) where the GR is
176 estimated to be at a maximum. Base temperature (T_b) and ceiling temperature (T_c) are
177 the intercept of the lines when GR is estimated to be zero, and germination does not
178 proceed to completion when seeds are below T_b and above T_c (García-Huidobro et al.,
179 1982). Thermal time (θ_T) was estimated by repeated probit analysis in GenStat 12.1
180 software (VSN International Ltd, 2009). This method consisted of varying T_b until the
181 best fit was obtained (minimum residual) (Bradford, 1995; Ellis et al., 1986) based on
182 the following two equations:

183 Sub-optimal θ_T is $\text{Probit}(g) = K + (\log \theta_T(g)) / \sigma_{\theta_T}$ (Bradford, 1995; Ellis et al., 1986) (1)

184 Supra-optimal θ_T is $\text{Probit}(g) = K + \theta_{T\text{supra}}(g) / \sigma_{\theta_T}$ (Covell et al., 1986; García-Huidobro
185 et al., 1982). (2)

186 In both equations, K is the intercept constant when θ_{Tsupra} is zero. θ_T is the heat units the
187 seed needs to accumulate for a given percentile (g) to complete germination in
188 chronological time and σ is the standard deviation of the seed population response.

189 The basis of estimating hydro-time is the positive regression line between the GR and
190 water potentials. The base water potential (Ψ_b) is calculated as the intercept of the
191 regression line of GR on water potential. Hydro time (θ_H) was also estimated by
192 repeated probit analysis. In this case, the method consisted of varying θ_H until the best
193 fit was obtained (minimum residual) (Bradford, 1995) using equation 3:

194 θ_H at T_o is $\text{Probit}(g) = K - (\Psi_b(g) / \sigma_{\theta_H})$ (Bradford, 1995; Gummerson, 1986) (3)

195 where K is the intercept constant, Ψ_b is the base water potential (below this water
196 potential no germination is predicted to occur) for a given percentile (g) and σ is the
197 standard deviation.

198 Additionally, analysis of variance (ANOVA) and t-tests were used to compare the seed
199 mass and seed germination parameters between wild seed lots and between crop seed
200 lots.

201 2.4. *Environmental parameters associated with seed lot provenance*

202 The original, geo-referenced location of the CWR seed lots was used to extract climate
203 data from WordClim with an accuracy of one kilometre for historical data over the
204 period 1960 – 1990 (Hijmans et al., 2005). Associations between germination
205 parameters and the following environmental factors were assessed: annual mean
206 temperature (minimum, maximum and median in °C), mean monthly precipitation
207 (mm), mean precipitation and temperature of the month of germination and the altitude.
208 The month of germination was the first month after seed collection date that the
209 following assumptions were met: (1) the minimum rainfall required for germination is a
210 monthly mean of 15 mm (Freas and Kemp, 1983; Gutterman, 1993; Gutterman, 2000a)
211 and (2) the temperature exceeded T_b but did not exceed T_c (Figure S1). The exception
212 was *B. rapa* subsp. *sylvestris* from Algeria where the maximum monthly precipitation
213 did not exceed 2 mm (Figure S1). For this species, the first period of significant rainfall
214 was taken as the precipitation threshold. In addition, correlations between the
215 environmental factors and the seed germination parameters of the wild species, θ_T , θ_H

216 time, T_b and Ψ_b were tested with scatter matrix and linear correlations in Origin 9.0
217 software (OriginLab Corporation, 2013).

218 **3. Results**

219 The seeds of all *Brassica* seed lots had high viability. Seed germination of the CWRs
220 varied from 93 % (*B. rapa*) to 100 % (*B. tournefortii*) and seeds of the crop genotypes
221 of *B. oleracea* reached 100 % germination.

222 *3.1 Germination trait parameters*

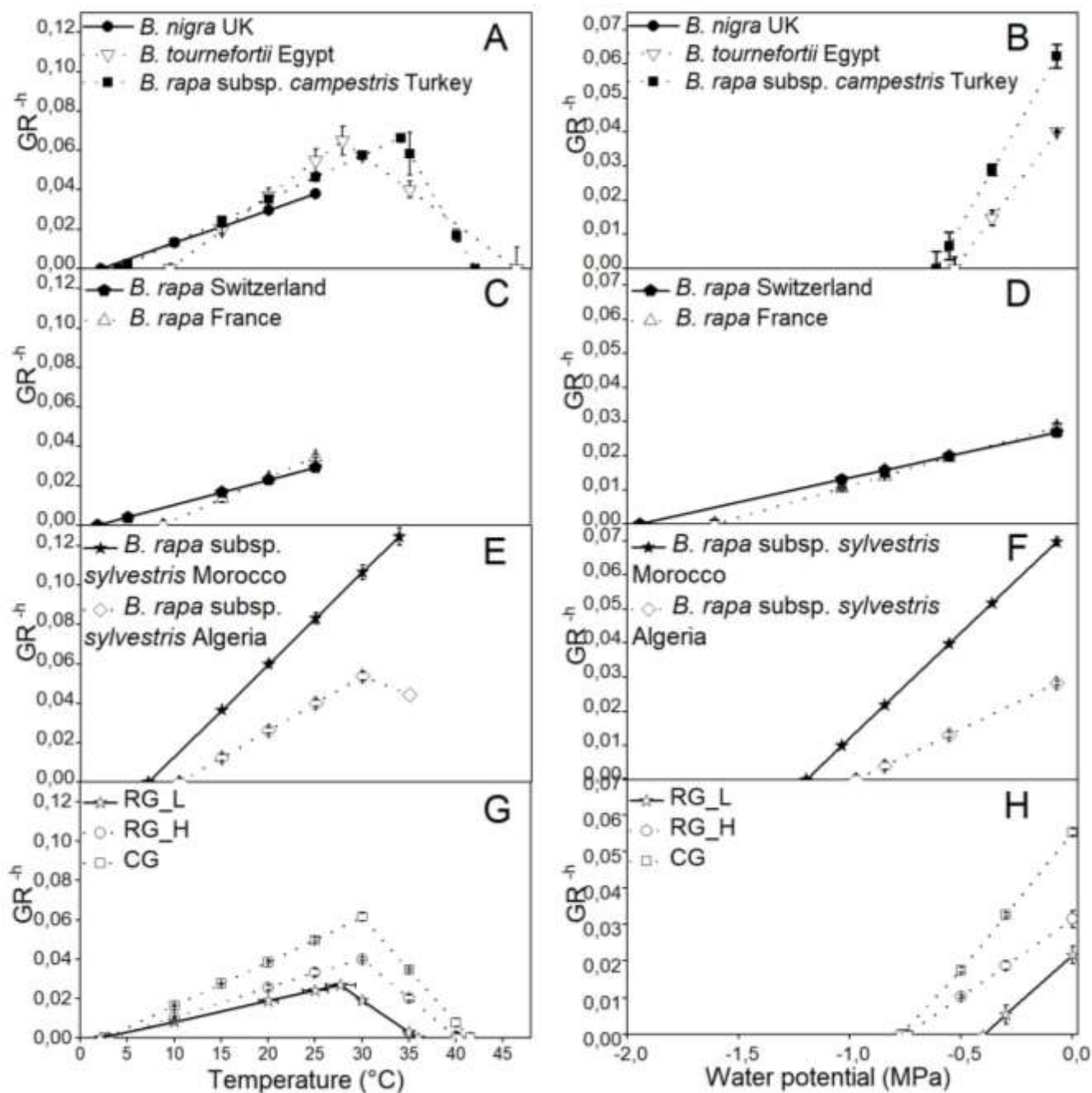
223 Seed germination of the 10 *Brassica* seed lots (CWRs and crops) showed a *c.* 9 °C
224 range in each of the cardinal temperatures: T_b from 1.7 to 10.5 °C; T_o from 25 to 35 °C;
225 and T_c from 36 to 45 °C (Figures 1A, C, E and G). Intra-specific variability in trait
226 parameters was observed in two wild seed lots of *B. rapa* from France and Switzerland
227 (Figure 1C) and two wild seed lots of *B. rapa* subsp. *sylvestris* from Morocco and
228 Algeria (Figure 1E), with significant differences ($P < 0.05$) in germination rate (GR)
229 and T_b (Table S1). Thermal time (θ_T) values also differed three-fold amongst CWRs
230 (from 214.7 °Ch for *B. rapa* subsp. *sylvestris* from Morocco to 775.5 °Ch for *B. rapa*
231 from Switzerland) and the crop research genotype *B. oleracea* RG_L had the longest θ_T
232 of 951.6 °Ch (Table S1).

233 In general, seeds of the crop genotypes *B. oleracea* (RG_L, RG_H and CG) were slower
234 to germinate compared with the CWRs in terms of θ_T in the sub-optimal range of
235 temperatures (Table S1). The high vigour crop genotype, RG_H, had faster germination
236 (i.e. shorter θ_T) than the low vigour crop genotype, RG_L ($P < 0.01$); whilst the CG had
237 θ_T very similar to the CWR *B. rapa* subsp. *campestris* (i.e., *c.* 445 °Ch). In contrast, T_b

238 for the *B. oleracea* seed lots did not differ (Figure 1G). However, T_c was higher in the
239 crop genotype RG_H and CG than the genotype RG_L (40 °C and 41.5 °C vs 36 °C,
240 respectively, $P < 0.001$). In the supra-optimal temperatures, $\theta_{T_{supra}}$ was shorter in CG,
241 but the other two crop genotypes did not differ.

242 Seeds were also germinated under five water potentials. GR slowed and final
243 germination decreased with more negative water potentials, and Ψ_b values varied
244 between -0.4 MPa and -2 MPa (Figure 1B, D, F and H). Both seed lots of the CWR *B.*
245 *rapa* (France, Switzerland) had the lowest Ψ_b of -1.59 MPa and -1.94 MPa respectively
246 (Figure 1D). The hydro time (θ_H) values also differed among all CWRs from 9.0 MPah
247 for *B. rapa* subsp. *campestris* to 72 MPah of *B. rapa* Switzerland (Table S1). At the end
248 of the experiment, all non-germinated seeds were transferred to water and subsequently
249 germinated within 48 hours, except for *B. rapa* subsp. *sylvestris* (Algeria) which only
250 achieved 40 % of germination after 15 days. However, a tetrazolium test showed that all
251 the seeds of *B. rapa* subsp. *sylvestris* (Algeria) were viable.

252 Whilst the θ_H of the crop seed lots was within the range of the CWRs, Ψ_b of the crop
253 seed lots was higher (less negative) than the CWRs, except for *B. tournefortii* and *B.*
254 *rapa* subsp. *campestris* (Table S1). Within the crop seed lots, the high vigour genotype,
255 RG_H, had a larger θ_H than RG_L ($P < 0.05$), with CG of *B. oleracea*, having the
256 smallest θ_H (Table S1). In contrast, the Ψ_b was lower in both crop seed lots, RG_H and
257 CG, than in RG_L (-0.74 MPa in RG_H, -0.78 MPa in the CG and -0.40 MPa in
258 RG_L). Therefore, seeds of RG_H and CG could germinate over a wider range of water
259 potentials than RG_L (Figure 1H).

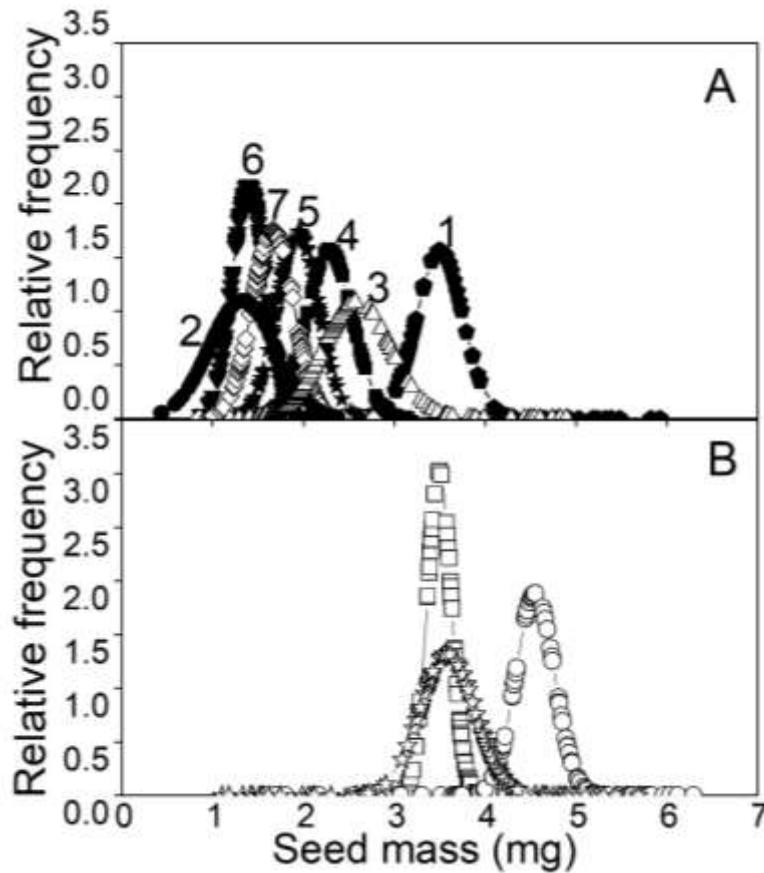


260 **Figure 1:** The germination rate (GR) was plotted against temperature (A, C, E and G)
 261 to describe cardinal temperatures and the water potential thresholds (B, D, F and H) of
 262 seven wild *Brassica* seed lots, two research genotypes of *B. oleracea* (RG_L and
 263 RG_H) and the crop genotype CG. The seeds were germinated under a range of sub and
 264 supra optimal temperatures between 5 to 35 °C and on PEG solutions at 0, -0.3, -0.5, -
 265 0.8 and -1.0 MPa at one temperature (between 20 and 25 °C). The regression lines were
 266 calculated from repeated probit analysis estimations and the error bars are the standard

267 deviation (SD) of three replicates for each treatment in CWRs and four replicates for
268 crops.

269 Variation in seed mass of the CWRs was normally distributed, based on a normality test
270 (D'Agostino's test, $P < 0.05$) (Figure 2A). Two CWRs (*B. rapa* from France and *B.*
271 *nigra* from UK) had the highest variability (CV 36 %) and *B. tournefortii* had the
272 smallest variability in seed mass (CV 18.3 %). The crop seed lot variability in mass was
273 similar to that of the CWRs from CV 13% for CG to CV 30% for RG_L.

274 Of the CWRs, mean seed mass of *B. rapa* from Switzerland was the largest ($P < 0.05$)
275 (Figure 2A). In general, the crop seed lots had heavier seeds than the CWRs, with the
276 seeds of RG_H heavier than those of RG_L and CG of *B. oleracea* (Figure 2B).



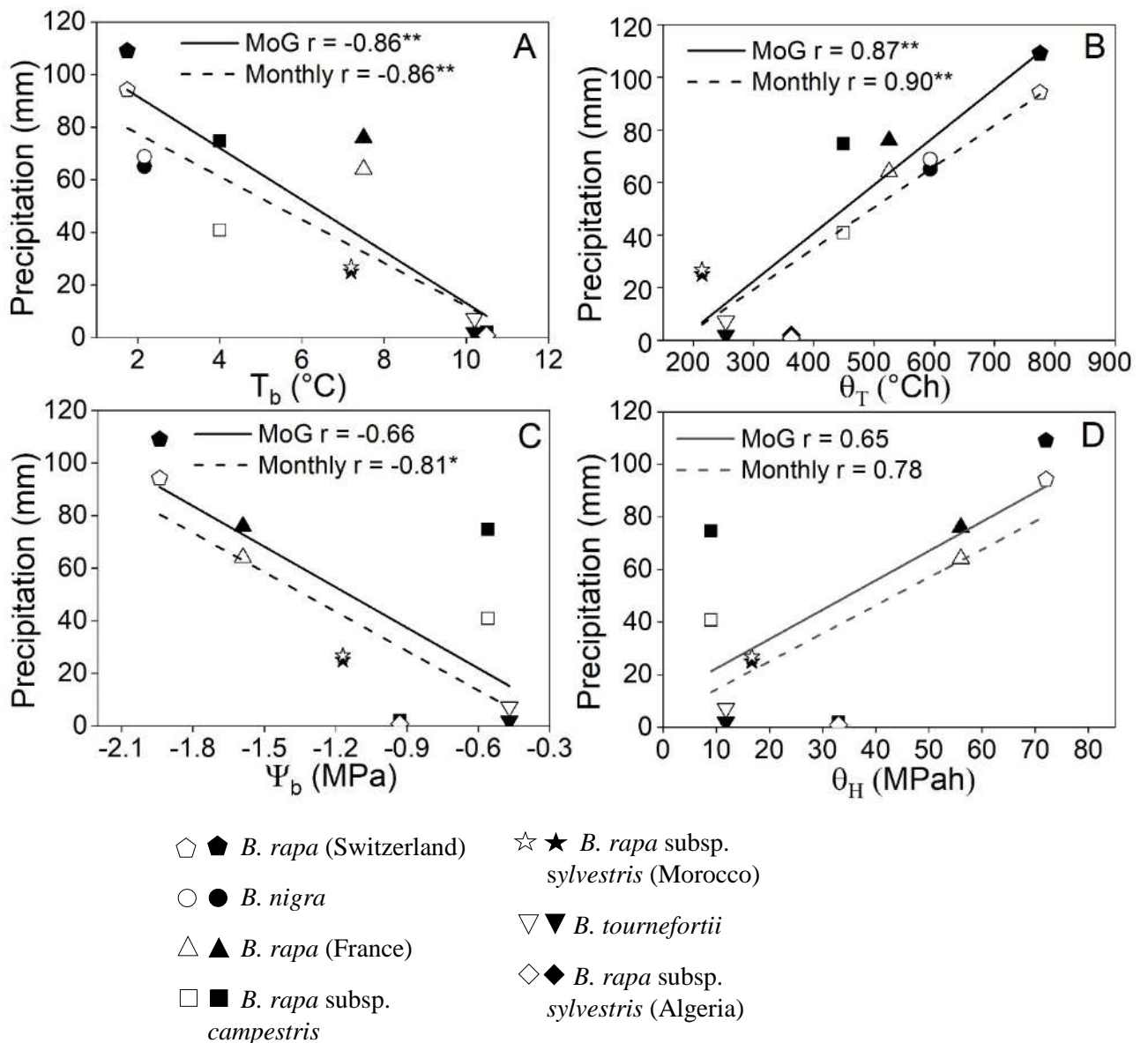
277 **Figure 2.** Seed mass of 100 individual seeds were shown as normal distribution for **A)**
 278 CWRs, 1) *Brassica rapa* (Switzerland), 2) *B. nigra* (England), 3) *B. rapa* (France), 4) *B.*
 279 *rapa* subsp. *campestris* (Turkey), 5) *B. rapa* subsp. *sylvestris* (Morocco), 6) *B.*
 280 *tournefortii* (Egypt) and 7) *B. rapa* subsp. *sylvestris* (Algeria); and **B)** two research
 281 genotypes of *B. oleracea*, RG_L (open stars) and RG_H (open circles) and one CG
 282 (open squares). Each symbol is the mass of an individual seed.

283 3.2 Inter-relations between seed traits

284 The seed traits of all *Brassica* seed lots (including both crop and wild) were compiled
 285 and subjected to linear regression models. A negative relationship was found between
 286 T_b and θ_T for all *Brassica* seed lots tested ($P < 0.0001$; $r = -0.72$, Figure 3A). Ψ_b and θ_H
 287 were also negatively correlated ($P < 0.0001$; $r = -0.90$ Figure 3B). In both cases the
 288 lower the base threshold (temperature or water potential) the longer the germination

289 process (thermal or hydro-time). A trend was found between the mean seed mass and
290 the θ_T and T_b ($r = 0.53$ and -0.61 , respectively) so that heavier seeds need to accumulate
291 more θ_T to germinate and had a lower T_b , but it was not significant ($P = 0.1$ and 0.06 ,
292 respectively). Furthermore, the CV of the seed mass was positively correlated with θ_T (P
293 < 0.05 ; $r = 0.40$), but not with T_b . Neither θ_H nor Ψ_b were correlated with mean seed
294 mass, however, θ_H was positively correlated with the CV of seed mass ($r = 0.48$; $P <$
295 0.01).

308 monthly precipitation was negatively correlated with Ψ_b (Figure 4C). However, the
 309 precipitation was not significantly correlated with θ_H (Figure 4D). With regard to
 310 temperature, T_b and the annual mean temperature (minimum, mean and maximum) were
 311 correlated (Table S2). Finally, the altitude was not correlated ($P > 0.05$) with any
 312 germination parameter; and seed functional traits did not correlate with the temperatures
 313 at other times of the year when plant developmental events such as flowering would
 314 have occurred.



315 **Figure 4.** Correlation of the seed germination traits of seven CWRs and the
 316 environment of the seed collection site. Mean monthly precipitation (open symbols and

317 dashed line) and the precipitation of the month of germination (MoG) (solid symbols
318 and solid line) were plotted against the base temperature (T_b **A**), the thermal time (θ_T ,
319 **B**), the base water potential (Ψ_b , **C**) and the hydro time (θ_H , **D**). The values of θ_T , T_b , θ_H
320 and Ψ_b were calculated on the 50th percentile. Each point is one of the three replicates of
321 each population. Asterisks indicating the significance at $P < 0.05^*$ and $P < 0.01^{**}$ DF =
322 5 in A and B, and 4 in C and D

323 **4. Discussion**

324 4.1. *Comparative seed germination thresholds*

325 The variation in *Brassica* germination thresholds (c. 9 °C) amongst 10 seed lots is wider
326 than that observed for other comparative studies based on taxonomy or habitat. For
327 example, in cold and warm season grasses, T_b ranges by 2.6 °C to 5.9 °C, respectively
328 (Jordan and Haferkamp, 1989). Pasture species (e.g., clover, ryegrass and chicory) also
329 display a relatively narrow T_b range of 3.5 °C (Moot et al., 2000). As the local
330 environment is known to influence the expression of this trait, such variation in CWRs
331 of *Brassica* can be explained by the selection of taxa from seven countries with widely
332 differing environments, having annual mean temperatures varying from 5 to 26 °C,
333 across a latitudinal span of c. 20°. In more than 50 species of Cactaceae, sampled across
334 an environmental envelope that covers 70° of latitude, seed T_b varies by c. 20 °C (Seal
335 et al., 2017).

336 The range of Ψ_b for the 50th percentile of germination was also wide for species in the
337 *Brassica* genus (i.e., -1.54 MPa) from -0.40 to -1.94 MPa. This range is similar to that
338 for other crop seeds from different families [such as, *Daucus carota* (Apiaceae),
339 *Helianthus annuus* (Asteraceae), *Hordeum vulgare* and *Zea mays* (Poaceae)], that have

340 a range extending to -2.1 MPa (Dürr et al., 2015). Interestingly, *B. rapa* has been
341 estimated to have a Ψ_b as low as -2.2 MPa (Tribouillois et al., 2016). However, this
342 study used data for only the 20th and 30th percentiles of germination as viability was
343 poor. We also found an average Ψ_b for the same percentiles of germination to be -2.2
344 and -1.99 MPa for wild *B. rapa* from Switzerland and France, respectively. However,
345 the 50th percentile of the population is a better descriptor of germination performance if
346 seed viability is above 50 % (Soltani et al., 2016).

347 The diversity of germination performance in terms of the thermal and hydro traits
348 observed in the *Brassica* genus is due to both, their genetic variability (Arias et al.,
349 2014; Arias and Pires, 2012) and the influence of the environment (phenotypic
350 plasticity) (Mousseau and Fox, 1998; Schmid and Dolt, 1994; Van Kleunen et al.,
351 2007). In this study, we cannot definitively distinguish between genetic or phenotypic
352 variability in the CWRs. However, by studying two *Brassica* seed lots, each of two
353 CWRs from different environments, along with different species from across a broad
354 environmental range, we were able to assess the influence on seed functional traits of
355 the collection site abiotic features. Moreover, studying two *B. oleracea* research crop
356 genotypes selected for differences in vigour (RG_L and RG_H), and grown under
357 identical glasshouse conditions, enabled us to consider the impact of high vigour alleles
358 in these research lines on germination traits.

359 4.2. *Selecting for seed performance in Brassica*

360 Seed quality is an essential trait for crop production and food security (Finch-Savage
361 and Bassel, 2015). As a consequence, the seed industry strives to produce seed lots with
362 enhanced performance, particularly vigour which is often assessed under non-optimal
363 conditions. Vigour is a property of the seed that determines performance in a wide range

364 of environments (ISTA, 2017). Amongst 10 *Brassica* seed lots assessed over many
365 temperature and water potential conditions we show strong correlations between T_b and
366 θ_T and Ψ_b and θ_H (Figure 3) in addition to the correlations with the precipitation of the
367 climate of seed collection site of the CWRs (Figure 4). A relationship between Ψ_b and
368 θ_H is anticipated, as the hydro time model shows that germination rate is inversely
369 proportional to the difference between the actual water potential (Ψ) and the Ψ_b
370 (Bradford, 1995). Thus, seeds with lower Ψ_b (more negative water potentials) will
371 require more hydro time to germinate, i.e., have longer θ_H (Bradford, 1995). On the
372 other hand, the correlation between T_b and θ_T has been reported previously for a range
373 of different species (Dürr et al., 2015; Gardarin et al., 2011; Seal et al., 2017; Trudgill et
374 al., 2005; Trudgill et al., 2000). This might reflect ecological adaptation such that the
375 seeds with a high threshold then proceed to germinate faster, i.e., shorter thermal times
376 (Gardarin et al., 2011; Trudgill et al., 2005). As there is intra- and inter-specific
377 variation in the thresholds for seed germination progress under a wide range of (thermal
378 and water potential) environments (Dürr et al., 2015), it is critical that these parameters
379 are determined for each seed lot so that vigour can be more accurately described.

380 In general, the CWRs had a lower Ψ_b , hence a wider window for water potential
381 tolerance than the crops. Whereas the seed selection of the crop *B. oleracea* for growth
382 under optimal, irrigated monoculture agricultural conditions may have led to a higher
383 Ψ_b that increases the risk of crop failure in a future climates of variable water
384 availability. The low Ψ_b are a greater ability to cope with variable water conditions
385 indicated in the CWRs may provide a means to improve crop genotypes in the future.

386 The CWRs tended to have similar θ_T compared to the crops. Therefore, the selection and
387 breeding of *B. oleracea* has not resulted in particularly faster germination in terms of

388 thermal time, based on the seed lots characterised here. Even though crop genotype
389 RG_H was the product of the introgression of two high vigour alleles, the impact of this
390 on thermal time was not beneficial compared with CWRs, but it was beneficial
391 compared with the low vigour genotype. RG_L had the longest θ_T compared with the
392 other crops and CWRs, i.e. germination is slower, which would increase the risk of
393 inclement drought or seedbed deterioration impacting on the more slowly emerging
394 seedlings. Such subtle differences in thermal and hydro-time characteristics amongst a
395 range of *Brassica* seed lots (Figure 3, Dürr et al., 2015; Tribouillois et al., 2016) tends
396 to suggest a continuum of responses within the genus.

397 4.3. *Seed mass*

398 Crops were compared to CWRs to observe the effect of breeding and domestication on
399 the *Brassica* genus, one of the main effects of which can be greater seed mass (Doganlar
400 et al., 2000). Research crop genotype RG_H had the heaviest seeds compared to the
401 other *B. oleracea* seed lots and the CWRs (Figure 2). Moreover, less population
402 variation in seed mass (CV) would be expected for crops and this was generally the
403 case. A trend was found where heavier seeds tended to germinate slower than lighter
404 seeds on a thermal basis (longer θ_T). This finding was contrary to that of Huang et al.
405 (2016) who found that larger seeds germinated faster in wild desert species. Smaller
406 seeds germinating faster than larger seeds has been found in other species. For example,
407 Grime et al., 1981, who studied 400 species and reported a decrease of germination rate
408 with increased seed weight. Norden et al. (2009) found a similar correlation between the
409 mean seed mass and the mean time to germination (MTG) in more than 1000 tropical
410 forest trees. Kikuzawa and Koyama (1999) determined that small seeds had the capacity
411 to imbibe water faster than larger seeds, thus the germination process could start earlier.

412 There was also a tendency for heavier seeds to have lower T_b . Thus, small seeds should
413 require less time to germinate because they need to accumulate less heat units above a
414 higher T_b . Counterintuitively, it seems that breeding to increase seed mass in *Brassica*
415 crops is likely to have unintended consequences on germination rate based on thermal
416 time characteristics. However, further investigation using a higher number of *Brassica*
417 accessions and/or species is needed to confirm these trends.

418 4.4. *Ecological correlates of seed performance*

419 In addition to temperature, precipitation controls the timing of germination in the wild.
420 One general assumption is that germination occurs only if the monthly precipitation is
421 >15 mm (Freas and Kemp, 1983; Gutterman, 1993; Gutterman, 2000a). On this basis,
422 we predicted in which month the seeds will germinate. However, that assumption was
423 not true for *B. rapa* subsp. *sylvestris* from Algeria, where the maximum monthly
424 precipitation was not above 2 mm (Figure S1). The behaviour of *B. rapa* subsp.
425 *sylvestris* from Algeria is similar to that of annual plants in an extreme desert climate
426 (Gutterman, 1993) and also coincides with one of the fastest seed lots to germinate.
427 When water is regularly available, temperature becomes the major influence of
428 germination timing.

429 In *Brassica* CWRs, θ_T was positively correlated with precipitation, which is in
430 agreement with the suggestion that seeds from drier environments might be adapted to
431 germinate faster to avoid drought periods during seedling establishment (Evans and
432 Etherington, 1990; Fenner and Thompson, 2004; Gardarin et al., 2011; Moles and
433 Westoby, 2004). At the same time, T_b and Ψ_b were negatively correlated with
434 precipitation which means that: 1) the T_b might be higher in drier environments; and 2)
435 in drier environments the seeds might be adapted to germinate rapidly in response to

436 sporadic rainfall events that increase soil water potential. According to these findings,
437 the germination of wild *Brassica* seed lots, especially thermal time parameters (θ_T and
438 T_b) seem to be closely adapted to the precipitation of the maternal environment.

439 There are many reports of correlations between the mean seed mass and the maternal
440 environment (mainly precipitation) including species such as *B. campestris* (Sinniah et
441 al., 1998), 15 herbaceous species from UK (Evans and Etherington, 1990), annual
442 species (e.g. *Glycine* genus) from a Mediterranean climate (Murray et al., 2004) and two
443 wild species of barley and oat (Volis, 2012). Pakeman et al. (2008) found the
444 temperature of several vegetation types across Europe was positively correlated with
445 seed mass. In this study, the mean seed mass was only correlated with the precipitation
446 of the month of germination (Table S2; $P < 0.05$). This finding is in agreement with that
447 on wheat, soya and walnut (Brocklehurst et al., 1978; Meckel et al., 1984; Stromberg
448 and Patten, 1990). Since the size of the seed and number of seeds is limited by the
449 availability of resources (Baskin and Baskin, 1998; Wulff, 1986), in this case, mother
450 plants from environments with low precipitation are likely to produce fewer and small
451 seeds.

452 **5. Conclusions**

453 Based on the thermal and hydro-time characteristics (thresholds and rates) *Brassica*
454 CWRs appear better equipped to adapt to changes in the environment than the crop *B.*
455 *oleracea*. CWRs tended to have lower Ψ_b than crops with suggest more tolerance to low
456 water potentials. These adapted traits are somewhat predictable as the interspecific
457 variation in germination functional traits (T_b , Ψ_b , θ_T) correlate strongly to the climate at
458 the seed collection site, particularly to precipitation (the mean monthly and the mean of
459 the month of cumulative thermal time germination). Our findings reinforce the need to

460 characterise each seed lot, with the additional benefit of more precisely defining seed
461 vigour ‘under a wide range of environments’.

462

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470

471 **Supplementary data**

472 Supplementary data is associated with this manuscript.

473

474 **References**

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