

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

E. Castillo-Lorenzo^{a,b,*}, W.E. Finch-Savage^b, C.E. Seal^a, H.W. Pritchard^a

^a Department of Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, United Kingdom

^b School of Life Sciences, Warwick University, Wellesbourne, Warwick CV35 9EF, United Kingdom

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ABSTRACT

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

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

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

1. Introduction

Global climate change is predicted to increase environmental temperatures, but may have other consequences such as earlier frosts and changes to the frequency of flooding and drought (IPCC, 2013), all of which may affect the outcome of germination and seedling emergence. In particular, warming temperatures and fluctuations in precipitation are known to increase the risk to yield in the most economically important crops, such as wheat, rice, maize and barley (Lobell and Field, 2007; Porter and Semenov, 2005). Moreover, crops bred for predictable and more uniform germination and harvest may contribute to loss of variability in responses to environmental conditions (Gepts, 2010). In contrast, crop wild relatives (CWRs) represent a gene pool that is potentially adapted to a large range of habitats and, therefore, likely

better equipped to survive the challenges associated with climate change (Dempewolf et al., 2014). In addition to possessing high genetic diversity, CWR species may also have greater plasticity in traits enabling them to cope better with rapid environmental change (Nicotra et al., 2010).

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

* Corresponding author at: Department of Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, United Kingdom.

E-mail addresses: e.castillolorenzo@kew.org, e.castillolorenzo@hotmail.com (E. Castillo-Lorenzo), Bill.Finch-Savage@warwick.ac.uk (W.E. Finch-Savage), c.seal@kew.org (C.E. Seal), h.pritchard@kew.org (H.W. Pritchard).

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Table 1

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

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.				Growth conditions	
	Provider	Genotype	Vigour	Day (°C)	Night (°C)
<i>B. oleracea</i> (crop)	B&T World Seeds (France)	CG (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)	RG_L (A12DHD)	Low	22	18

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

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

The convergence of functional traits in species occupying similar environments can be considered an adaptation to environmental filtering (Keddy, 1992). Precipitation and temperature are the main environmental factors that have an impact on plant traits, e.g., leaf mass, yield, height and flowering time; and seed traits, such as seed mass, germination and dormancy, show similar ecological associations (Ackerly et al., 2000; Baskin and Baskin, 1998; Dornbos and Mullen, 1991; Franks et al., 2007; Menzel et al., 2006; Peñuelas et al., 2004; Porter, 2005). Seed germination (i.e., radicle emergence) and seedling establishment are thought to be the most sensitive stage of a plant to environmental changes (Fay and Schultz, 2009; Kimball et al., 2010; Lloret et al., 2004). Consequently, the effects of both precipitation and temperature on germination rate (Céspedes et al., 2012; Clauss and Venable, 2000; Levine et al., 2008; Meyer et al., 1990), final germination (Alexander and Wulff, 1985; Gareca et al., 2012; Gutterman, 2000b) and seedling establishment (Cochrane et al., 2015; Jump et al., 2008; Lloret et al., 2004; Smith et al., 2000) have been widely explored.

Crop domestication has resulted in larger seed size (Preece et al., 2017) and seed mass is also known to be responsive to environmental fluctuations (Donohue et al., 2005; Nicotra et al., 2010; Roach and Wulff, 1987). In particular, seed filling is influenced by environmental conditions and mean seed mass of a population has been positively correlated with annual rainfall (Harel et al., 2011) or with mean annual temperature (Murray et al., 2004). Whilst seed size is thought to affect germination rate (Norden et al., 2009), seed mass is rarely co-analysed with seed physiological traits of germination base temperature (T_b) or

base water potential (Ψ_b). In this context, seed mass and T_b of tree seeds of *Aesculus hippocastanum* from across Europe had no correlation (Daws et al., 2004), and a negative correlation was found between seed mass and Ψ_b of neotropical forest species (Daws et al., 2008).

Our aim was to investigate how the originating environment of *Brassica* CWRs impact on their germination functional traits, testing the assumption that current *Brassica* crops may have been selected for fast germination under managed, irrigated conditions. We used seven geo-referenced seed collections (from across Europe, North Africa and the Middle East) and three commercially available crop seed lots and research genotypes. The genus *Brassica* possesses a wide diversity in morphology, genetics (Arias et al., 2014), environment stress tolerance and is economically important around the world for its edible roots crops, vegetables and oilseeds (Tsunoda et al., 1980). We selected wild taxa from locations with variable mean monthly precipitation (1–94 mm) and temperature (5–26 °C). We show an intimate relationship between *Brassica* seed collection site and germination functional traits.

2. Material and Methods

2.1. Seed material

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

A commercial seed lot of *B. oleracea* (cabbage) was obtained from B & T World Seeds (France) (Table 1B) and seeds of two research genotypes derived from *B. oleracea* crops (A12DHD from kale and AGSL101 from kale and broccoli) were provided by the University of Warwick. Both crop research genotypes have the same genetic

background (A12) but SL101 has introgressions from at least two QTL (RABA1 and SOG1) from a faster germinating genotype (GD33DH, broccoli) that confers higher vigour (Morris et al., 2016). For simplicity, henceforth A12DHd and AGSL101 will be called RG_L (low vigour research genotype) and RG_H (high vigour research genotype). The commercial genotype will be called CG. Both crop research genotypes were grown under the same controlled conditions at 22/18 °C (day/night) in a glasshouse to produce seeds. On receipt at RBG Kew, all seed lots were equilibrated to 15 % RH and 15 °C and stored under these conditions for a few weeks until use.

2.2. Seed mass and germination

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

For germination, seeds were sown at constant temperatures from 5 to 45 °C, at 5 °C intervals. Seeds were also sown at a range of water potentials (0, −0.3, −0.5, −0.8 and −1.0 MPa) using water and increasing concentrations of polyethylene glycol 8000 (PEG, Fisher Scientific, UK) solution, but only at constant temperatures of 25 and *B. rapa* subsp. *campestris* at 30 °C. Due to limited seed availability, *B. nigra* seeds were sown under constant temperatures without water stress. Either three replicates of 25 seeds, for the CWRs, or four replicates of 25 seeds for the crops, were used in each treatment. Seeds were sown onto two layers of germination test paper (90 mm diameter; Fisher Scientific, UK) in Petri dishes. The volume of PEG solution used (7 mL) was calculated following the equation of Hardegree and Emmerich (1990) to account for the weight of the dry germination paper. The water potential of the solutions was confirmed with an osmometer (Camlab, UK). For consistency, the same volume (7 mL) of solution was used for all PEG and water treatments. The vented Petri dishes were placed in sealable plastic bags and incubated at the relevant temperature with a 12 hour photoperiod (radiometric flux density of 50–100 W/m²). Germination (radicle emergence of 2 mm) was recorded every hour during the first 24 hours and then every two-three hours until no more germination was observed. Non-germinated seeds that were still firm after PEG treatment were briefly washed with distilled water to remove surface PEG and transferred to a new Petri dish with germination test paper and 7 mL of water. After 15 days in this recovery assessment, if the seeds did not germinate, the seed coat was removed and the viability was tested with 1 % triphenyl tetrazolium chloride (TZ) in darkness at 30 °C (ISTA, 2003) for 18 hours. Stained red embryos were considered to be viable.

2.3. Data analysis

Germination was quantified as final germination percentage and germination rate (GR). Germination progress over time was represented as a sigmoidal curve (fitted using the Boltzmann distribution) from which the GR was estimated for all treatments in each population. The GR at the 50th percentile (t₅₀) is the reciprocal of time the population needs to reach 50 % germination of viable seeds. The seeds were germinated at a range of temperatures and the GR describes a positive (sub-optimal range of temperatures) or negative (supra-optimal range of temperatures) regression line when plotted against temperature. The intercept of both lines is the optimal temperature (T_o) where the GR is estimated to be at a maximum. Base temperature (T_b) and ceiling temperature (T_c) are the intercept of the lines when GR is estimated to be zero, and germination does not proceed to completion when seeds are below T_b and above T_c (García-Huidobro et al., 1982). Thermal time (θ_T) was estimated by repeated probit analysis in GenStat 12.1 software (VSN International Ltd, 2009). This method consisted of varying T_b until the best fit was obtained (minimum residual) (Bradford, 1995; Ellis et al., 1986) based on the following two equations:

$$\text{Sub-optimal } \theta_T \text{ is Probit}(g) = K + (\log \theta_T(g)) / \sigma_{\theta_T} \quad (1)$$

See Refs. Bradford (1995), Ellis et al. (1986)

$$\text{Supra-optimal } \theta_T \text{ is Probit}(g) = K + \theta_{T_{\text{supra}}}(g) / \sigma_{\theta_T} \quad (2)$$

See Refs. Covell et al. (1986), García-Huidobro et al. (1982)

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

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

$$\theta_H \text{ at } T_o \text{ is Probit}(g) = K - (\Psi_b(g)) / \sigma_{\theta_H} \quad (3)$$

See Refs. Bradford (1995), Gummerson (1986)

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

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

2.4. Environmental parameters associated with seed lot provenance

The original, geo-referenced location of the CWR seed lots was used to extract climate data from WordClim with an accuracy of one kilometre for historical data over the period 1960–1990 (Hijmans et al., 2005). Associations between germination parameters and the following environmental factors were assessed: annual mean temperature (minimum, maximum and median in °C), mean monthly precipitation (mm), mean precipitation and temperature of the month of germination and the altitude. The month of germination was the first month after seed collection date that the following assumptions were met: (1) the minimum rainfall required for germination is a monthly mean of 15 mm (Freas and Kemp, 1983; Gutterman, 1993; Gutterman, 2000a) and (2) the temperature exceeded T_b but did not exceed T_c (Fig. S1). The exception was *B. rapa* subsp. *sylvestris* from Algeria where the maximum monthly precipitation did not exceed 2 mm (Fig. S1). For this species, the first period of significant rainfall was taken as the precipitation threshold. In addition, correlations between the environmental factors and the seed germination parameters of the wild species, (θ_T and θ_H time, T_b and Ψ_b) were tested with scatter matrix and linear correlations in Origin 9.0 software (OriginLab Corporation, 2013).

3. Results

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

3.1. Germination trait parameters

Seed germination of the 10 *Brassica* seed lots (CWRs and crops) showed a c. 9 °C range in each of the cardinal temperatures: T_b from 1.7 to 10.5 °C; T_o from 25 to 35 °C; and T_c from 36 to 45 °C (Fig. 1A, C, E and G). Intra-specific variability in trait parameters was observed in two wild seed lots of *B. rapa* from France and Switzerland (Fig. 1C) and two wild seed lots of *B. rapa* subsp. *sylvestris* from Morocco and Algeria (Fig. 1E), with significant differences (P < 0.05) in germination rate (GR) and T_b (Table S1). Thermal time (θ_T) values also differed three-

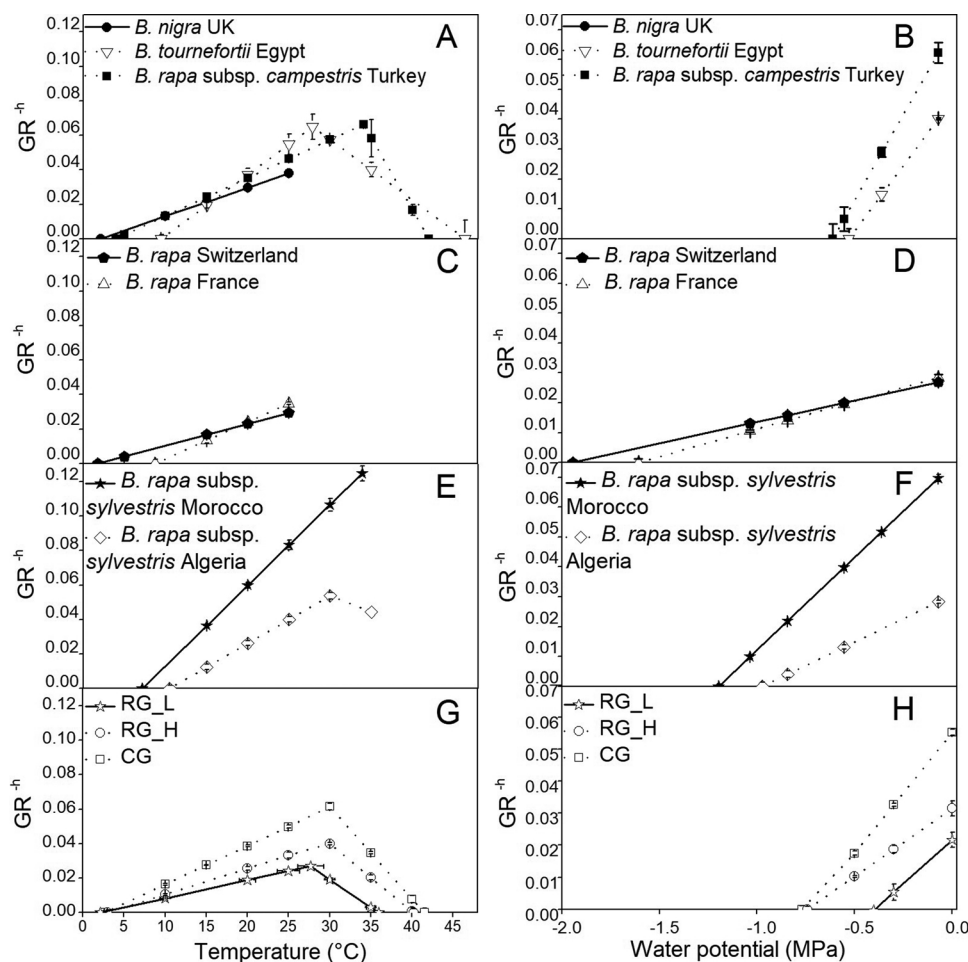


Fig. 1. The germination rate (GR) was plotted against temperature (A, C, E and G) to describe cardinal temperatures and the water potential thresholds (B, D, F and H) of seven wild *Brassica* seed lots, two research genotypes of *B. oleracea* (RG_L and RG_H) and the crop genotype CG. The seeds were germinated under a range of sub and supra optimal temperatures between 5 to 35 °C and on PEG solutions at 0, -0.3, -0.5, -0.8 and -1.0 MPa at one temperature (between 25 and 30 °C). The regression lines were calculated from repeated probit analysis estimations and the error bars are the standard deviation (SD) of three replicates for each treatment in CWRs and four replicates for crops.

fold amongst CWRs (from 214.7 °C h for *B. rapa* subsp. *sylvestris* from Morocco to 775.5 °C h for *B. rapa* from Switzerland) and the crop research genotype *B. oleracea* RG_L had the longest θ_T of 951.6 °C h (Table S1).

In general, seeds of the crop genotypes *B. oleracea* (RG_L, RG_H and CG) were slower to germinate compared with the CWRs in terms of θ_T in the sub-optimal range of temperatures (Table S1). The high vigour crop genotype, RG_H, had faster germination (i.e. shorter θ_T) than the low vigour crop genotype, RG_L ($P < 0.01$); whilst the CG had θ_T very similar to the CWR *B. rapa* subsp. *campestris* (i.e., c. 445 °C h). In contrast, T_b for the *B. oleracea* seed lots did not differ (Fig. 1G). However, T_c was higher in the crop genotype RG_H and CG than the genotype RG_L (40 °C and 41.5 °C vs 36 °C, respectively, $P < 0.001$). In the supra-optimal temperatures, $\theta_{T\text{supra}}$ was shorter in CG, but the other two crop genotypes did not differ.

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

Whilst the θ_H of the crop seed lots was within the range of the CWRs, Ψ_b of the crop seed lots was higher (less negative) than the CWRs, except for *B. tournefortii* and *B. rapa* subsp. *campestris* (Table S1).

Within the crop seed lots, the high vigour genotype, RG_H, had a larger θ_H than RG_L ($P < 0.05$), with CG of *B. oleracea*, having the smallest θ_H (Table S1). In contrast, the Ψ_b was lower in both crop seed lots, RG_H and CG, than in RG_L (-0.74 MPa in RG_H, -0.78 MPa in the CG and -0.40 MPa in RG_L). Therefore, seeds of RG_H and CG could germinate over a wider range of water potentials than RG_L (Fig. 1H).

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

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

3.2. Inter-relations between seed traits

The seed trait parameters of all *Brassica* seed lots (including both crop and wild) were compiled and subjected to linear regression models. A negative relationship was found between T_b and θ_T for all *Brassica* seed lots tested ($P < 0.0001$; $r = -0.72$, Fig. 3A). Ψ_b and θ_H were also negatively correlated ($P < 0.0001$; $r = -0.90$ Fig. 3B). In both cases the lower the base threshold (temperature or water potential) the longer the germination process (thermal or hydro-time). A general trends were found between the mean seed mass and the θ_T and T_b . Such that heavier seeds need to accumulate more θ_T to germinate and had a lower T_b , but these were not significant ($P = 0.1$ and 0.06 , respectively). Neither θ_H nor Ψ_b were correlated with mean seed mass.

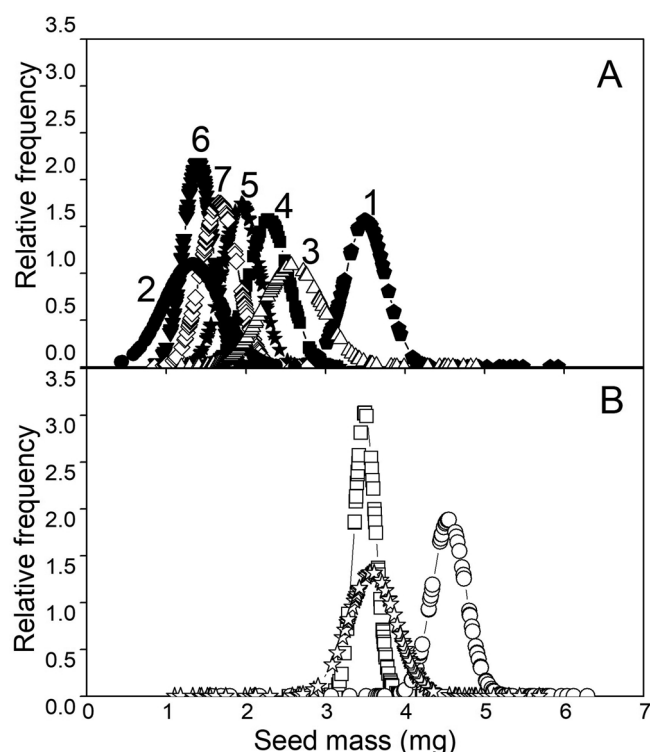


Fig. 2. Seed mass of 100 individual seeds were shown as normal distribution for A) CWRs, 1) *Brassica rapa* (Switzerland), 2) *B. nigra* (England), 3) *B. rapa* (France), 4) *B. rapa* subsp. *campestris* (Turkey), 5) *B. rapa* subsp. *sylvestris* (Morocco), 6) *B. tournefortii* (Egypt) and 7) *B. rapa*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

3.3. Relations between environment and CWR seed traits

The dependencies of CWR functional seed traits on the collection site environment were assessed for the seven wild *Brassica* seed lots (Table S2). T_b was lower when the monthly precipitation, and the

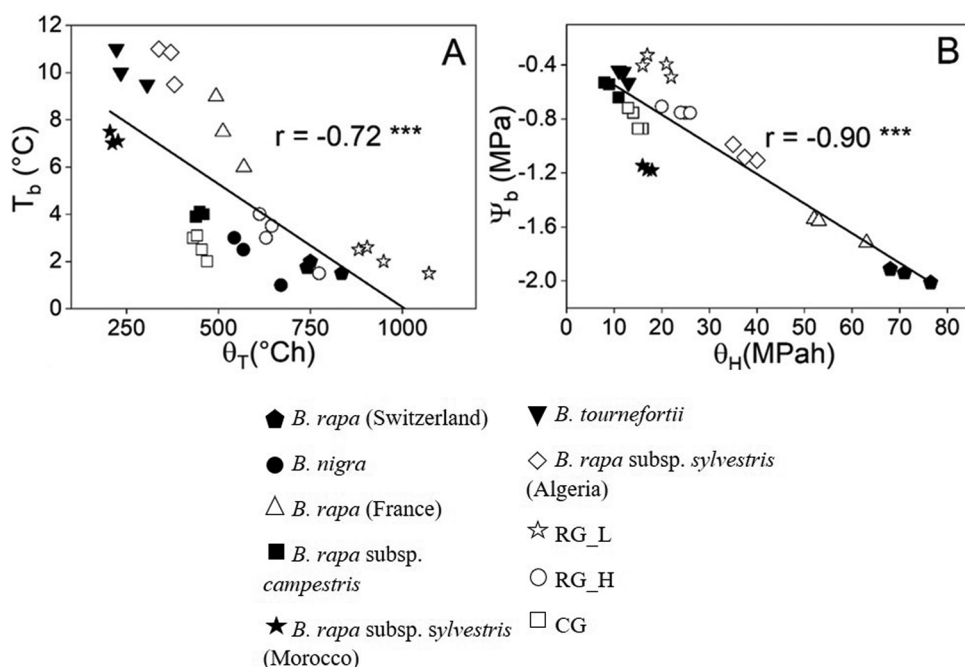


Fig. 3. Correlations between seed traits of 10 *Brassica* seed lots (seven CWRs and three crops). A) Base temperature (T_b) and thermal time (θ_T); B) base water potential (Ψ_b) and hydro time (θ_H). The values of θ_T , T_b , θ_H and Ψ_b were calculated on the 50th percentile. Each point represents a replicate of each population, three replicates for CWRs and four replicates for crops. Asterisks indicate the significance at $P < 0.001^{***}$ DF = 30 in A, 27 in B.

precipitation of the predicted month of germination based on the quantified responses, were higher (Fig. 4A). Seed lots of species from wetter environments also had slower germination (i.e., longer θ_T ; Fig. 4B). The mean monthly precipitation was negatively correlated with Ψ_b (Fig. 4C). However, the precipitation was not significantly correlated with θ_H (Fig. 4D). With regard to temperature, T_b and the annual mean temperature (mean and maximum) were correlated (Table S2). Finally, altitude was not correlated ($P > 0.05$) with any germination parameter; and seed functional traits did not correlate with the temperatures at other times of the year when plant developmental events such as flowering would have occurred.

4. Discussion

4.1. Comparative seed germination thresholds

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

The range of Ψ_b for the 50th percentile of germination was also wide (-1.54 MPa) for species in the *Brassica* genus, i.e., from -0.40 to -1.94 MPa. This range is similar to that for other crop seeds from different families [such as, *Daucus carota* (Apiaceae), *Helianthus annuus* (Asteraceae), *Hordeum vulgare* and *Zea mays* (Poaceae)], that have a range extending to -2.1 MPa (Dürr et al., 2015). Interestingly, *B. rapa* has been estimated to have a Ψ_b as low as -2.2 MPa (Tribouillois et al., 2016). However, that study used data for only the 20th and 30th percentiles of germination as viability was poor. We also found an average Ψ_b for the same percentiles of germination to be -2.2 and -1.99 MPa for wild *B. rapa* from Switzerland and France, respectively. However, the

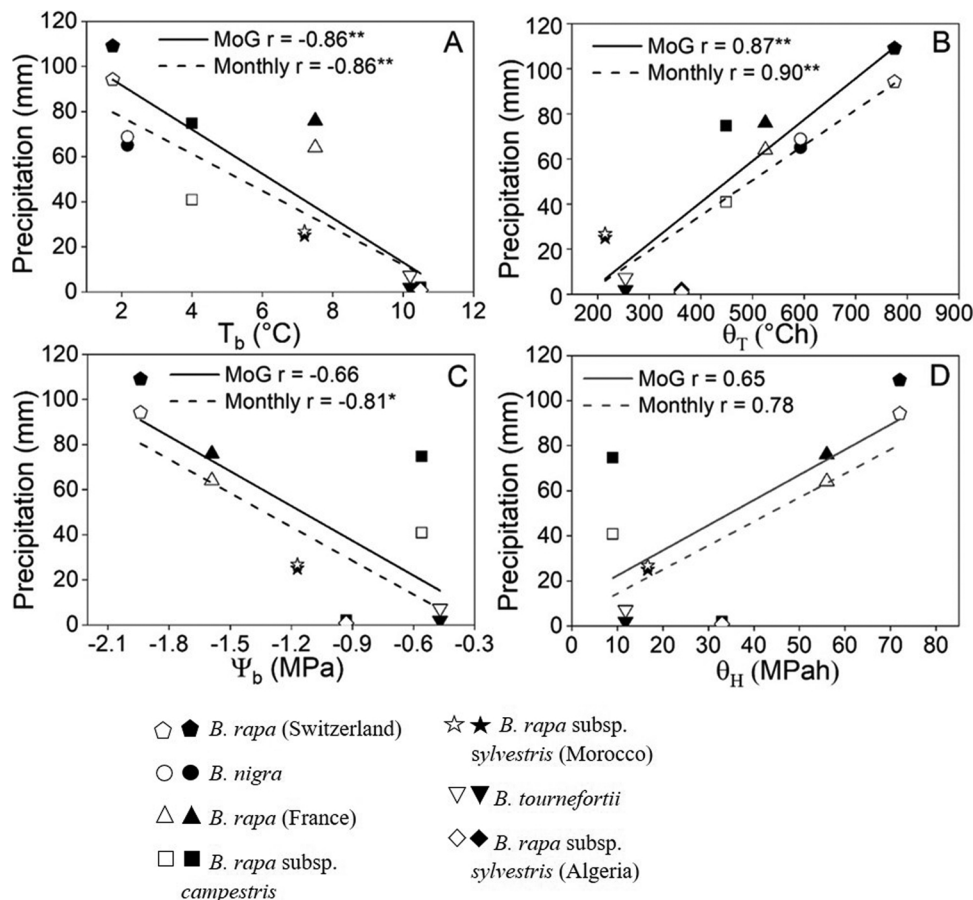


Fig. 4. Correlation of the seed germination traits of seven CWRs and the environment of the seed collection site. Mean monthly precipitation (open symbols and dashed line) and the precipitation of the month of germination (MoG) (solid symbols and solid line) were plotted against the base temperature (T_b , A), the thermal time (θ_T , B), the base water potential (Ψ_b , C) and the hydro time (θ_H , D). The values of θ_T , T_b , θ_H and Ψ_b were calculated for the 50th percentile. Each point is the mean of three replicates of each population. Asterisks indicate the significance at $P < 0.05^*$ and $P < 0.01^{**}$ DF = 5 in A and B, and 4 in C and D.

50th percentile of the population is a better descriptor of germination performance if seed viability is above 50 % (Soltani et al., 2016).

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

4.2. Selecting for seed performance in *Brassica*

Seed quality is an essential trait for crop production and food security (Finch-Savage and Bassel, 2015). As a consequence, the seed industry strives to produce seed lots with enhanced performance, particularly vigour which is often assessed under non-optimal conditions. Vigour is a property of the seed that determines performance in a wide range of environments (ISTA, 2017). Amongst 10 *Brassica* seed lots assessed over many temperature and water potential conditions we show strong correlations between both T_b and θ_T and Ψ_b and θ_H (Fig. 3), in addition to the correlations with the precipitation of the CWR seed collection site (Fig. 4). A relationship between Ψ_b and θ_H is anticipated, as the hydro time model shows that germination rate is inversely proportional to the difference between the actual water potential (Ψ) and the Ψ_b (Bradford, 1995). Thus, seeds with lower Ψ_b

(more negative water potentials) will, in general require more hydro time to germinate, i.e., have longer θ_H (Bradford, 1995). However, an exception was observed in the crop genotypes compared; the RG_L had higher Ψ_b than CG but θ_H was slightly longer (18 MPa and 14.3 MPa, respectively).

On the other hand, the correlation between T_b and θ_T has been reported previously for a range of different species (Dürr et al., 2015; Gardarin et al., 2011; Seal et al., 2017; Trudgill et al., 2005; Trudgill et al., 2000). This might reflect ecological adaptation such that the seeds with a high threshold then proceed to germinate faster, i.e., shorter thermal times (Gardarin et al., 2011; Trudgill et al., 2005). As there is intra- and inter-specific variation in the thresholds for seed germination progress under a wide range of (thermal and water potential) environments (Dürr et al., 2015), it is critical that these parameters are determined for each seed lot so that vigour can be more accurately described.

In general, the CWRs had a lower Ψ_b , hence a wider window of water potential tolerance than the crops. Whereas the seed selection of the crop *B. oleracea* for growth under optimal, irrigated monoculture agricultural conditions may have led to a higher Ψ_b but faster germination in hydro time.

The CWRs tended to have similar θ_T compared to the crops. Therefore, the selection and breeding of *B. oleracea* has not resulted in particularly faster germination in terms of thermal time, based on the seed lots characterised here. Even though crop genotype RG_H was the product of the introgression of two high vigour alleles, the impact of this on thermal time was not beneficial compared with CWRs, but it was beneficial compared with the low vigour genotype. RG_L had the longest θ_T compared with the other crops and CWRs, i.e. germination is slower, which would increase the risk of inclement drought or seedbed deterioration impacting on the more slowly emerging seedlings. Such subtle differences in thermal and hydro-time characteristics amongst a

range of *Brassica* seed lots (Fig. 3, Dürr et al., 2015; Tribouillois et al., 2016) tends to suggest a continuum of responses within the genus.

4.3. Seed mass

Crops were compared to CWRs to observe the effect of breeding and domestication on the *Brassica* genus, one of the main effects of which can be greater seed mass (Doganlar et al., 2000). Research crop genotype RG_H had the heaviest seeds compared to the other *B. oleracea* seed lots and the CWRs (Fig. 2). Moreover, less population variation in seed mass (CV) would be expected for crops and this was generally the case. A trend was found where heavier seeds tended to germinate slower than lighter seeds on a thermal basis (longer θ_T). This finding was contrary to that of Huang et al. (2016) who found that larger seeds germinated faster in wild desert species. Smaller seeds germinating faster than larger seeds has been found in other species. For example, Grime et al., 1981, who studied 400 species and reported a decrease of germination rate with increased seed weight. Norden et al. (2009) found a similar correlation between the mean seed mass and the mean time to germination (MTG) in more than 1000 tropical forest trees. Kikuzawa and Koyama (1999) determined that small seeds had the capacity to imbibe water faster than larger seeds, thus the germination process could start earlier. There was also a tendency for heavier seeds to have lower T_b . Thus, small seeds should require less time to germinate because they need to accumulate less heat units above a higher T_b . Counter-intuitively, it seems that breeding to increase seed mass in *Brassica* crops is likely to have unintended consequences on germination rate based on thermal time characteristics. However, further investigation using a higher number of *Brassica* accessions and/or species is needed to confirm these trends.

4.4. Ecological correlates of seed performance

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

In *Brassica* CWRs, θ_T was positively correlated with precipitation, which is in agreement with the suggestion that seeds from drier environments might be adapted to germinate faster to avoid drought periods during seedling establishment (Evans and Etherington, 1990; Fenner and Thompson, 2004; Gardarin et al., 2011; Moles and Westoby, 2004). At the same time, T_b and Ψ_b were negatively correlated with precipitation which means that: 1) the T_b might be higher in drier environments; and 2) in drier environments the seeds might be adapted to germinate rapidly in response to sporadic rainfall events that increase soil water potential. According to these findings, the germination of wild *Brassica* seed lots, especially thermal time parameters (θ_T and T_b) seem to be closely adapted to the precipitation of the maternal environment.

There are many reports of correlations between the mean seed mass and the maternal environment (mainly precipitation) including species such as *B. campestris* (Sinniah et al., 1998), 15 herbaceous species from UK (Evans and Etherington, 1990), annual species (e.g. *Glycine* genus) from a Mediterranean climate (Murray et al., 2004) and two wild species of barley and oat (Volis, 2012). Pakeman et al. (2008) found the annual temperature across several locations within Europe was positively correlated with seed mass. In this study, the mean seed mass was

only correlated with the precipitation of the month of germination (Table S2; $P < 0.05$). This finding is in agreement with that on wheat, soya and walnut (Brocklehurst et al., 1978; Meckel et al., 1984; Stromberg and Patten, 1990). Since the size of the seed and number of seeds is limited by the availability of resources (Baskin and Baskin, 1998; Wulff, 1986), in this case, mother plants from environments with low precipitation are likely to produce fewer and smaller seeds.

5. Conclusions

Based on the thermal and hydro-time characteristics (thresholds and rates) of seed germination of *Brassica* CWRs appear better equipped to adapt to changes in the environment than the crop *B. oleracea*. CWRs tended to have lower Ψ_b than crops which suggest greater tolerance to low water potentials. These traits are somewhat predictable as the interspecific variation in germination functional traits (T_b , Ψ_b and θ_T) correlate strongly to the climate at the seed collection site, particularly to precipitation (the mean monthly and the mean of the month of cumulative thermal time germination). Our results reinforce the need to characterise the functional traits of each seed lot, so that seed vigour can be defined more precisely under a wide range of environments. Moreover, these findings have implications for the selection of crop genetic resources to cope with germination under specific environments anticipated from climatic change.

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References

- Ackerly, D.D., et al., 2000. The evolution of plant ecophysiological traits: recent advances and future directions: new research addresses natural selection, genetic constraints, and the adaptive evolution of plant ecophysiological traits. *AIBS Bulletin* 50, 979–995.
- Alexander, H.M., Wulff, R.D., 1985. Experimental ecological genetics in *Plantago*. X. The effects of maternal temperature on seed and seedling characters in *P. lanceolata*. *J. Ecol.* 73, 271–282.
- Arias, T., Beilstein, M.A., Tang, M., McKain, M.R., Pires, C.J., 2014. Diversification times among *Brassica* (Brassicaceae) crops suggest hybrid formation after 20 million years of divergence. *Am. J. Bot.* 101, 86–91.
- Arias, T., Pires, C.J., 2012. A fully resolved chloroplast phylogeny of the brassica crops and wild relatives (Brassicaceae): novel clades and potential taxonomic implications. *Taxon* 61, 980–988.
- Baskin, C.C., Baskin, J.M., 2014. Second Edition. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego.
- Bradford, K.J., 1995. Water Relations in Seed Germination. In: Galili, J.K.A.G. (Ed.), Seed development and germination, pp. 351–396 New York.
- Brocklehurst, P., Moss, J., Williams, W., 1978. Effects of irradiance and water supply on grain development in wheat. *Ann. Appl. Bot.* 90, 265–276.
- Céspedes, B., Torres, I., Urbiet, I.R., Moreno, J.M., 2012. Effects of changes in the timing and duration of the wet season on the germination of the soil seed bank of a seeder-dominated Mediterranean shrubland. *Plant Ecol.* 213, 919–931.
- Clauss, M.J., Venable, D.L., 2000. Seed germination in desert annuals: an empirical test of adaptive bet hedging. *Am. Nat.* 155, 168–186.
- Cochrane, A., Hoyle, G.L., Yates, C.J., Wood, J., Nicotra, A.B., 2014. Predicting the impact of increasing temperatures on seed germination among populations of Western Australian *Banksia* (Proteaceae). *Seed Sci. Res.* 24, 195–205.
- Cochrane, J.A., Hoyle, G.L., Yates, C.J., Wood, J., Nicotra, A.B., 2015. Climate warming delays and decreases seedling emergence in a Mediterranean ecosystem. *Oikos* 124, 150–160.
- Covell, S., Ellis, R.H., Roberts, E.H., Summerfield, R.J., 1986. The influence of temperatures on seed germination rate in grain legumes. A comparison of chickpea, lentil, soybean and cowpea at constant temperatures. *Exp. Bot.* 37, 705–715.
- Daws, M.I., Crabtree, L.M., Dalling, J.W., Mullins, C.E., Burslem, D.F.R.P., 2008. Germination responses to water potential in neotropical pioneers suggest large-seeded species take more risks. *Ann. Bot.* 102, 945–951.

- Daws, M.I., et al., 2004. Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytol.* 162, 157–166.
- Dempewolf, H., et al., 2014. Adapting agriculture to climate change: a global initiative to collect, conserve and use crop wild relatives. *Agroecol. Sust. Food* 38, 369–377.
- Díaz, S., et al., 2016. The global spectrum of plant form and function. *Nature* 529, 167–171.
- Doganlar, S., Frary, A., Tanksley, S., 2000. The genetic basis of seed-weight variation: tomato as a model system. *TAG 100*, 1267–1273.
- Donohue, K., et al., 2005. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution* 59, 740–757.
- Dornbos, D.L., Mullen, R.E., 1991. Influence of stress during soybean seed fill on seed weight, germination and seedling growth rate. *Can. J. Plant. Sci.* 71, 373–383.
- Dürr, C., Dickie, J.B., Yang, X.Y., Pritchard, H.W., 2015. Ranges of critical temperature and water potential values for the germination of species worldwide: contribution to a seed trait database. *Agric. Forest Meteorol.* 200, 222–232.
- Ellis, R.H., Covell, S., Roberts, E.H., Summerfield, R.J., 1986. The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *J. Exp. Bot.* 37, 1503–1515.
- Evans, C.E., Etherington, J.R., 1990. The effect of soil-water potential on seed-germination of some British plants. *New Phytol.* 115, 539–548.
- FAO/IPGRI, 1994. Genebank Standards for Plant Genetic Resources for Food and Agriculture. International Plant Genetic Resources Institute, Rome.
- Fay, P.A., Schultz, M.J., 2009. Germination, survival and growth of grass and forb seedlings: effects of soil moisture variability. *Acta Oecol.* 35, 679–684.
- Fenner, M., Thompson, K., 2004. The ecology of seeds. Cambridge University Press.
- Fernández-Pascual, E., Jiménez-Alfaro, B., Hájek, M., Díaz, T.E., Pritchard, H.W., 2015. Soil thermal buffer and regeneration niche may favour calcareous fen resilience to climate change. *Folia Geobot.* 50, 293–301.
- Finch-Savage, W.E., Bassel, G.W., 2015. Seed vigour and crop establishment: extending performance beyond adaptation. *J. Exp. Bot.* 67, 567–591.
- Franks, S.J., Sim, S., Weis, A.E., 2007. Rapid evolution of flowering time by an annual plant in response to climate fluctuation. *PNAS* 104, 1278–1282.
- Freas, K.E., Kemp, P.R., 1983. Some relationships between environmental reliability and seed dormancy in desert annual plants. *J. Ecol.* 71, 211–217.
- García-Huidobro, J., Monteith, J.L., Squire, G.R., 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S&H). I. Constant temperature. *J. Exp. Bot.* 33, 288–296.
- Gardarin, A., Dürr, C., Colbach, N., 2011. Prediction of germination rates of weed species: relationship between germination speed parameters and species traits. *Ecol. Modell.* 222, 626–636.
- García, E.E., Vandeloof, F., Fernandez, M., Hermy, M., Honnay, O., 2012. Seed germination, hydrothermal time models and the effects of global warming on a threatened high Andean tree species. *Seed Sci. Res.* 22, 287–298.
- Gepts, P., 2010. Crop domestication as a long-term selection experiment. *Plant Breed. Rev.* 24, 1–44.
- Grime, J., Mason, G., Curtis, A., Rodman, J., Band, S., 1981. A comparative study of germination characteristics in a local flora. *J. Ecol.* 69, 1017–1059.
- Grubb, P.J., 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol. Rev.* 52, 107–145.
- Gummerson, R.J., 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. *J. Exp. Bot.* 37, 729–741.
- Guterman, Y., 1993. Seed germination in desert plants. Springer-Verlag, Berlin, Germany.
- Guterman, Y., 2000a. Environmental factors and survival strategies of annual plant species in the Negev Desert, Israel. *Plant Species Biol.* 15, 113–125.
- Guterman, Y., 2000b. Maternal effects on seeds during development. In: Fenner, M. (Ed.), *Seeds: the ecology of regeneration in plant communities*. CAB International, pp. 27–59.
- Hardege, S.P., Emmerich, W.E., 1990. Effect of polyethylene glycol exclusion on the water potential of solution-saturated filter paper. *Plant Physiol.* 92, 462–466.
- Harel, D., Holzapfel, C., Sternberg, M., 2011. Seed mass and dormancy of annual plant populations and communities decreases with aridity and rainfall predictability. *Basic Appl. Ecol.* 12, 674–684.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Clim.* 25, 1965–1978.
- Huang, Z., Liu, S., Bradford, K.J., Huxman, T.E., Venable, D.L., 2016. The contribution of germination functional traits to population dynamics of a desert plant community. *Ecology* 97, 250–261.
- IPCC, 2013. Intergovernmental Panel on Climate Change. Climate change. Cambridge University.
- ISTA, 2003. Working sheets on tetrazolium testing, I and II, Bassersdorf, Switzerland.
- ISTA, 2017. International rules for seed testing. International Seed Testing Association, Bassersdorf, Switzerland.
- Jordan, G.L., Haferkamp, M.R., 1989. Temperature responses and calculated heat units for germination of several range grasses and shrubs. *J. Range Manage.* 42, 41–45.
- Jump, A.S., et al., 2008. Simulated climate change provokes rapid genetic change in the Mediterranean shrub *Fumana thymifolia*. *Global Change Biol.* 14, 637–643.
- Keddy, P.A., 1992. Assembly and response rules: two goals for predictive community ecology. *J. Veg. Sci.* 3, 157–164.
- Kikuzawa, K., Koyama, H., 1999. Scaling of soil water absorption by seeds: an experiment using seed analogues. *Seed Sci. Res.* 9, 171–178.
- Kimball, S., Angert, A.L., Huxman, T.E., Venable, D.L., 2010. Contemporary climate change in the Sonoran Desert favors cold-adapted species. *Global Change Biol.* 16, 1555–1565.
- Levine, J.M., McEachern, A.K., Cowan, C., 2008. Rainfall effects on rare annual plants. *J. Ecol.* 96, 795–806.
- Lobell, D.B., Field, C., 2007. Global scale climate-crop yield relationships and the impacts of recent warming. *Environ. Res. Lett.* 2, 014002.
- Lloret, F., Peñuelas, J., Estiarte, M., 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Global Change Biol.* 10, 248–258.
- Meckel, L., Egli, D., Phillips, R., Radcliffe, D., Leggett, J., 1984. Effect of moisture stress on seed growth in soybeans. *Agron. J.* 76, 647–650.
- Menzel, A., et al., 2006. European phenological response to climate change matches the warming pattern. *Global Change Biol.* 12, 1969–1976.
- Meyer, S.E., Monsen, S.B., McArthur, E.D., 1990. Germination response of *Artemisia tridentata* (Asteraceae) to light and chill - patterns of between-population variation. *Bot. Gaz.* 151, 176–183.
- Molesl, A.T., Westoby, M., 2004. Seedling survival and seed size: a synthesis of the literature. *J. Ecol.* 92, 372–383.
- Moot, D.J., Scott, W.R., Roy, A.M., Nicholls, A.C., 2000. Base temperature and thermal time requirements for germination and emergence of temperate pasture species. *N. Z. J. Agric. Res.* 43, 15–25.
- Morris, K., Barker, G.C., Walley, P.G., Lynn, J.R., Finch-Savage, W.E., 2016. Trait to gene analysis reveals that allelic variation in three genes determines seed vigour. *New Phytol.* 212, 964–976.
- Mousseau, T., Fox, C.W., 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.* 13, 403–407.
- Murray, B.R., Brown, A.H.D., Dickman, C.R., Crowther, M.S., 2004. Geographical gradients in seed mass in relation to climate. *J. Biogeogr.* 31, 379–388.
- Nicotra, A.B., et al., 2010. Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–692.
- Norden, N., et al., 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. *Funct. Ecol.* 23, 203–210.
- Orru, M., Mattana, E., Pritchard, H.W., Bacchetta, G., 2012. Thermal threshold as predictors of seed dormancy release and germination timing: altitude-related risks from climate warming for the wild grapevine *Vitis vinifera* subsp. *sylvestris*. *Ann. Bot.* 110, 1651–1660.
- Pakeman, R.J., et al., 2008. Impact of abundance weighting on the response of seed traits to climate and land use. *J. Ecol.* 96, 355–366.
- Peñuelas, J., et al., 2004. Complex spatiotemporal phenological shifts as a response to rainfall changes. *New Phytol.* 161, 837–846.
- Pollock, B.M., Roos, E.E., 1972. Seed and seedling vigor. In: Kozłowski, T.T., Gunn, C.R. (Eds.), *Seed Biology, I. Importance, development and germination*. Academic press, London, pp. 314–387.
- Porter, J.R., 2005. Rising temperatures are likely to reduce crop yields. *Nature* 436 174–174.
- Porter, J.R., Semenov, M.A., 2005. Crop responses to climatic variation. *Phil. Trans. Royal Soc. B.* 360, 2021–2035.
- Poschlod, P., 2013. Seed ecology and assembly rules in plant communities. In: Van der Maarel, E., Franklin, J. (Eds.), *Vegetation Ecology, Second Edition*. Wiley-Blackwell, UK, pp. 164–202.
- Preece, C., et al., 2017. How did the domestication of Fertile Crescent grain crops increase their yields? *Funct. Ecol.* 31, 387–397.
- Roach, D.A., Wulff, R.D., 1987. Maternal effects in plants. *Annu. Rev. Ecol. Syst.* 18, 209–235.
- Schmid, B., Dolt, C., 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution* 48, 1525–1549.
- Seal, C.E., et al., 2017. Thermal buffering capacity of the germination phenotype across the environmental envelope of the Cactaceae. *Global Change Biol.* 23, 5309–5317.
- Sinniah, U.R., Ellis, R.H., John, P., 1998. Irrigation and seed quality development in rapid-cycling Brassica: Seed germination and longevity. *Ann. Bot.* 82, 309–314.
- Smith, S.E., Riley, E., Tiss, J.L., Fendenheim, D.M., 2000. Geographical variation in predictive seedling emergence in a perennial desert grass. *J. Ecol.* 88, 139–149.
- Soltani, E., Ghaderi-Far, F., Baskin, C.C., Baskin, J.M., 2016. Problems with using mean germination time to calculate rate of seed germination. *Austral. J. Bot.* 63, 631–635.
- Stromberg, J.C., Patten, D.T., 1990. Variation in seed size of a southwestern riparian tree, Arizona walnut (*Juglans major*). *Am. Midland Nat.* 124, 269–277.
- Tribouillois, H., Dürr, C., Demilly, D., Wagner, M.-H., Justes, E., 2016. Determination of germination response to temperature and water potential for a wide range of cover crop species and related functional groups. *PLoS One* 11, e0161185.
- Trudgill, D.L., Honek, A., Li, D., Van Straalen, N.M., 2005. Thermal time- concepts and utility. *Ann. Appl. Biol.* 146, 1–14.
- Trudgill, D.L., Squire, G.R., Thompson, K., 2000. A thermal time basis for comparing the germination requirements of some British herbaceous plants. *New Phytol.* 145, 107–114.
- Tsunoda, S., Hinata, K., Gómez-Campo, C., 1980. *Brassica* crops and wild allies. Biology and breeding, Japan Scientific Societies Press, Tokyo.
- Van Kleunen, M., Lenssen, P.M., Fischer, M., de Kroon, H., 2007. Selection on phenotypic plasticity of morphological traits in response to flooding and competition in the clonal shore plant *Ranunculus reptans*. *J. Evol. Biol.* 20, 2126–2137.
- Volis, S., 2012. Demographic consequences of delayed germination in two annual grasses from two locations of contrasting aridity. *PPEES*. 14, 335–340.
- Wulff, R.D., 1986. Seed size variation in *Desmodium paniculatum*: I. Factors affecting seed size. *J. Ecol.* 74, 87–97.