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Maternal drought stress induces abiotic stress tolerance to the progeny at the germination stage in sunflower

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Abstract

Climate change produces more frequent and intense drought events during seed development that can affect seed quality. Germination is critical to ensure plant growth and reproductive success but it can be impacted by various abiotic stresses. Here, we studied the effect of drought stress during sunflower seed development on the germination of the progeny. We applied different scenarios of drought stress during seed development in sunflower inbred lines and hybrids and assessed seed germination of the progeny. Drought stress during seed development provided tolerance to water, hypoxic, cold and salt stresses during seed germination and also induced lower dormancy. We established that the induction of these traits was not transgenerational but maternally transmitted and could be reproduced in sunflower hybrids. Drought stress during seed development decreased pericarp thickness and induced higher leakage of soluble electrolytes from pericarp but it also modified embryo metabolism. A metabolomics analysis showed that ABA, oligosaccharides and polyphenols accumulated differently in drought stress seeds and could also participate in the seed tolerance to abiotic stress conditions during germination. Altogether our results reveal an adaptative process that allows sunflower plants exposed to drought stress during their reproductive stage to produce seeds with higher fitness. Besides bringing novel insight on natural adaption of plant populations to climate change, these results may have implications for the seed industry through the production of seeds resilient to higher climatic variability during the establishment of sunflower crop.

Keywords: abiotic stress, development, germination, maternal effect, seed, sunflower

1. Introduction

In the context of global warming, water stress on plants is likely to become more intense and to affect more geographic areas (Pachauri et al., 2014). Climate models predict increased plant evapotranspiration and lower soil moisture levels in a nearby future (IPCC, 2019). For crop plants, as sunflower, water stress, in combination with heat stress, could potentially decrease yields from 5 to 20 % in southern parts of Europe in 2030, according to climatic predictions (Debaeke et al., 2017). Besides this effect on whole plant fitness, climate change is also expected to modify the pattern of seed development on the mother plant, which will in turn modify seed germinative properties (seed vigour) and seed dormancy. Both traits (*i.e.* seed vigour and dormancy) control the germination process and drive seedling emergence in the field. Seed vigour is defined as the properties of seeds to germinate rapidly and homogenously in wide environmental conditions (ISTA, 1999), whereas dormancy is an intrinsic seed property that prevents germination in apparently favourable environmental conditions (Bewley, 1997). The dispersal units of sunflower are achenes and they display a physiological dormancy (Baskin and Baskin, 2004) that results from an inhibitory action of the envelopes (pericarp and seed coat) and from the embryo itself (Corbineau et al., 1990). The pericarp-seed coat imposed dormancy mostly affects germination above 20-25°C while embryo dormancy prevents germination below 15°C (Corbineau et al., 1990). Thus the germination process of sunflower seeds is strongly sensitive to a combination of extrinsic factors, such as water, light, oxygen and temperature and intrinsic factors such as dormancy. For example Saux et al. (2020) have shown that lowering water availability had a marked impact on sunflower seed germination. Gay et al. (1991) demonstrated that germination of whole achenes was strongly inhibited when oxygen tension was below 10 %.

Because plants are sessile organisms exposed to variable environments throughout their life or between generations, their phenotypic plasticity has been widely studied (Galloway, 2005).In particular, phenotype of the progeny can be modified by the environment experienced by the mother plant (Roach and Wulff, 1987). For example, in *Arabidopsis thaliana*, cool temperatures during seed maturation induce strong primary dormancy (Chiang et al., 2011; Donohue et al., 2007) while high temperatures reduce dormancy and negatively affect the final seed yield (Huang et al., 2014). In wheat, higher levels of dormancy were obtained in grains from plants grown at lower temperatures (Walker-Simmons and Sesing, 1990). Several studies have also shown that seed vigour could be positively influenced by abiotic stress during seed development (Finch-Savage and Bassel, 2016). The maternal effect

is defined as the causal influence of the maternal genotype or phenotype on the offspring phenotype (Wolf and Wade, 2009). The mechanisms involved in maternal effect in plants on the offspring are not completely understood because they can be mediated by the seed coat, which is a maternal tissue, by the triploid endosperm, with two-thirds of its genotype of maternal origin, or can result from a maternal effect on embryo provisioning during seed development. In addition to maternal effect, which concerns a single generation, the so-called transgenerational effect can occur on the progeny that did not experience any stress during its development (Heard and Martienssen, 2014). This effect is characterized if a phenotypic memory of an initial environmental stress can persist over multiple generations. It is supposed to result from a pre-programming of offspring phenotypes via epigenetic mechanisms such as histone modification or DNA methylation (Bruce et al., 2007; Wolf and Wade, 2009). Transgenerational effect has been demonstrated in the context of various biotic and abiotic stresses in plants, such as drought stress in *Brassica napus* L. (Hatzig et al., 2018). In Arabidopsis, for example, progeny issued from a warm parental environment (heat stress) showed faster germination rates, growth of root elongation, higher leaf biomass and increased seed production in different temperatures compared to seeds from plants grown in cold environment (cold stress) (Blödner et al., 2007). Recently it also been shown that spaceflight could affect the growth of future generations through changes in epigenetic modifications (Xu et al., 2021). Yin et al. (2019) summarized 139 studies about transgenerational effect and suggested possible transgenerational effects in many cases that can have an impact on the responses of plants to changing environments.

In sunflower, seed developmental conditions have been shown to have an effect on seed dormancy at harvest. Bodrone et al. (2017) showed that high temperatures during seed development decreased embryo dormancy but increased achene dormancy and Lachabrouilli et al. (2021) showed that faster seed desiccation during late maturation phase induced lower achene dormancy. However, the effect of water stress on sunflower mother plant, a highly probable environmental cue in the context of global warming, on seed vigour and dormancy has not been investigated yet. In addition, nothing is known about possible transgenerational effects of stress for this species. The aim of this work was to characterized the effects of a drought stress occurring after flowering of the mother plant on the physiological properties of the progeny and to identify the relative roles of embryo and maternal tissues (*i.e.* pericarp mainly) in this process.

2.Material and methods

2.1. Seed production

Sunflower (*Helianthus annuus* L.) seeds were obtained from hybrid or line plants cultivated on Phenotoul-Heliaphen platform at Auzeville-Tolosane (INRAe Toulouse, France) (Gosseau et al., 2019). All plants were grown in pots (15 l, Sopparco) filled with soil Proveen PAM2. During the plant development 4 fertilizations were applied with 1 l of Peter's Professional (N-K-P 17-07-27 at 0.8 g/l + Hortilon 0.46 g/l) per plant. Several seed production protocols were set-up as follow.

Plants from XRQ genotype were either fully irrigated till harvest [Fraction of Transpirable Soil Water (FTSW) maintained to a value of 1 (Sinclair et al., 2005), watered plants (W)] or were drought-stressed 2 d before flowering till maturity by holding irrigation until a FTSW value of 0.2 (stressed plants, S), as already described by Gosseau et al. (2019). The value of FTSW for drought stress was chosen accordingly to Gosseau et al. (2019) because it resulted in visible drought stress on sunflower plants but it did not prevented seed formation and development. Drought stress was also applied from flowering to the beginning of maturation [10 days after flowering (daf)] (early stress,E), from the beginning (10 daf) to the end (25 daf) of the maturation phase (Middle stress, M) and from the end of maturation phase (25 daf) to harvest (Late stress, L) (Fig. 1). In all cases water stress corresponded to a FSTW value of 0.2. Seeds resulted from self-fertilization and were all harvested at the same time.

Seeds from different genetic crosses were also produced to study the parental origin of acquired tolerance and the potential impact of cytoplasmic sterility genes. Sunflower XRQ/A (sterile cmsPET1 line) plants were crossed with sunflower PSC8 plants. Each parental plant was subjected to either S or W conditions, as previously described (Fig. 1) and resulting seeds were harvested simultaneously.

To determine the paternal and maternal components in response to drought stress, XRQ/A (sterile cmsPET1 line) plants, stressed or not, were crossed with XRQ/B (fertile cmsPET1 maintainer line) plants, also stressed or not, which gave 4 possibilities of crossing. Seeds were harvested simultaneously.

At last, in order to study the transgenerational effect of drought stress on seed physiology, the same growing and self-fertilizing scheme was repeated for 3 generations, obtaining 3W and 3S seeds from XRQ plants grown either in well-watered (W) or in drought stress (S) conditions, respectively. At the last generation (*i.e.* the 4^{th}), we applied drought stress to the mother plants of seeds issued from well-watered plants, thus giving 3WS seeds,

and reciprocally, thus giving 3SW seeds. The same water regime was also maintained in the last generation to 3W and 3S seeds, giving 4W and 4S seeds, respectively.

After harvest, all seeds were dried at 26°C for 48 h. They were then stored in a cold room at 3°C with a hygrometry of 30 % relative humidity (RH). After 6 months of storage, all seeds were non-dormant.

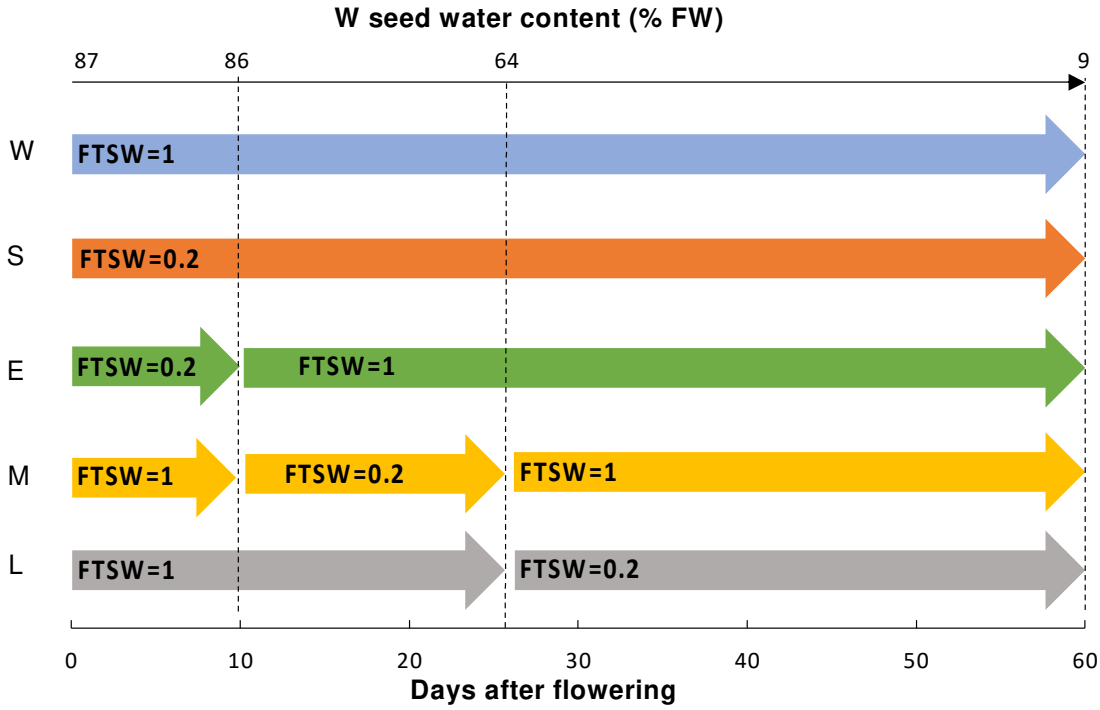


Fig. 1. Schematic representation of the experimental design showing the different drought stress scenarios applied during sunflower seed development. W, well-watered condition (FTSW=1); S, drought stress (FTSW=0.2) during entire seed development; E (Early), M (Middle), and L (Late) applications of drought stress (FTSW=0.2), as indicated by the time scale below the figure. The changes in seed moisture content of W seeds during seed development is indicated at the top of the figure.

2.2. Characterization of seed development

Impact of water stress on plants on yield and biomass traits have been determined as a function of the water stress indicator sFTSW, which integrates the fraction of transpirable soil water over time (Gosseau et al., 2019). Number of seeds was determined for each plant and thousand kernel weight (TKW) was calculated by (seed dry weight / seed number)*1000. Plant biomass (stems, leaves, flower head, seeds) was measured after drying during 48h at 80°C. Seed dry weight was measured after seed cleaning and further drying at 80°C during

24h.drying during 24h at 120°C and. The relative root mean square error (rRMSE) was calculated by R for each graph.

2.3. Germination assays

Germination assays were performed with 3 replicates of 25 seeds (achenes) or naked seeds (*i.e*. seeds without pericarp) obtained from 8 plants. Whole achenes or naked seeds were placed at 20°C in darkness in 9 cm Petri dishes one layer of cotton wool moistened seeds with distilled water, with a solution of polyethylene glycol (PEG 8000) giving a defined water potential value indicated in the text (according to Michel and Kaufmann, 1973) or with 300 mM NaCl. PEG and NaCl concentrations were chosen after preliminary experiments showing that these concentrations were decreasing seed germination speed without decreasing markedly final seed germination rates (i.e. remaining above 80 %). Since seed sensivity to PEG varied among the seed batches we also adapted the PEG concentrations to each seed production, since -1.0 MPa, for example, might in some case induce a too strong inhibitory effect on seed germination. Germination in atmospheres with different oxygen tensions was performed as described by Gay et al. (1991).

Naked seeds were also germinated at 20°C on a PEG solution containing extracts of pericarps (obtained after imbibing 60 pericarps in 15 ml of PEG -0.7 MPa for 24 h) or directly on a bed of pericarps extracted from W seeds and placed between the PEG solution and the seeds (bedding assay).

A seed was considered as germinated when the radicle pierced the envelopes (seed coat and pericarp) (whole seeds) or when embryonic axis elongated (naked seeds)

2.4. Electrolyte leakage measurements

Solute leakage of seed pericarp seed was estimated by measurements of electrolyte leakage with 3 replicates of pericarps obtained with 20 seeds. Conductimetry was measured with a microcomputer conductometer (K220, CONSORT Bioblock) after soaking pericarps during 3 h in 10 ml of distilled water at 20°C. The total electrolyte leakage of pericarps was obtained after boiling pericarps in water at 80°C for 20 min and it was used to express relative conductivity (Bailly et al., 1996). Results are expressed in µS by g of dry pericarp or as percentage of total leakage and correspond to the means of 3 replicates ± SD.

2.5. Measurement of phenolic compounds

Phenolic compounds were extracted from 0.1g of pericarp in 3.5ml of ethanol 70 % (v/v). The extracts were mixed with 1ml of Folin-Ciocalteu reagent and 1ml of Na_2CO_3 20 % (w/v) and heated at 40°C for 30min.(Caboni et al., 1997) Absorbance of samples was read at 760 nm by spectrophotometer (Genesys 10S UV_Vis Thermo Scientific). The total phenolic content in each extract was calculated using a standard curve obtained with gallic acid. Results are expressed as milligrams of total phenolic equivalents per g of pericarp. Measurements are means of 3 replicates± SD.

2.6. Microscopy

Transversal cross sections of pericarps were performed in the equatorial area of the seeds using a cryostat Leica CM3050S and sections were observed with a microscope AXIOZOOM V16 ZEISS apotome 2 at a magnification of 258X. Pericarp thicknesses and area of phytomelamin layer were measured with 10 samples using Fiji Image J 1.53c software.

2.7. Metabolomic analysis

Liquid chromatography (LC) MS–MS was performed with 5 replicates of 50 mg of naked seeds. The extraction was done in 800 µl methanol: water =7:3 and 20 µl of internal standards (d3-Leucine , 13C9-Phenylalanine , d5-Tryptophan , 13C3- Progesterone) . The mixture was ground in a Tissue grinder (50 Hz, 5min) followed by ultrasonic treatment with water bath at 4°C for 30min and keep in the refrigerator at -20°C for 1h. Subsequently, the samples were centrifuged at 4°C for 15 min at 14000 rpm, filtered and placed in a vial (1.5ml) for LC-MS analysis. Twenty µl of each sample was mixed into a Quality Check sample to evaluate the repeatability and stability of LC-MS analysis. A LC-MS system consisting of Waters 2D UPLC (Waters, USA) and Q Exactive high resolution mass spectrometer (Thermo Fisher Scientific, USA) was used for metabolite separation and detection.
A Hypersil GOLD aQ column (100*2.1 mm, 1.9 µm, Thermo Fisher Scientific, USA) was used and the mobile phase consisted of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid). The flow rate was 0.3 ml/min. The column oven was maintained at 40°C. The injection volume was 5 µl. Q Exactive mass spectrometer (Thermo Fisher Scientific, USA) was used to obtain MS1 and MS2 data. The MS scan method was in the range of m/z 150–1500, The MS1 resolution was 70,000, AGC was $1e^6$, and the maximum injection time was 100 ms. According to the precursor ion intensity, Top 3 ions were selected for MS2 analysis, MS2 resolution was 35,000, AGC was 2e5, maximum injection time was

50 ms, and collision energy were set as: 20, 40 and 60 eV. The parameters of ESI were sheath gas of 40 l/min, aux gas of 10 l/min, spray voltage(|KV|) of 3.80 in positive ion mode and of 3.20 in negative ion mode, capillary temperature of 320°C and aux gas heater temperature of 350°C. Raw data collected by LC-MS/MS was imported into Compound Discoverer 3.1 (Thermo Fisher Scientific, USA) for data processing, which mainly included peak extraction, retention time correction within and between groups, adduct ion combination, missing value filling, background peak labeling and metabolite identification. Finally, the molecular weight, retention time, peak area and identification results were derived. Metabolites were identified using BGI Library and mzCloud database. Multivariate statistical analysis and univariate analysis were used to select the groups of metabolites. The clustering analysis was performed for differential metabolites using log2 conversion, zero-mean normalization and euclidian distances.

2.8. Statistics

Statistical analyses were performed using the OriginPro software version 9.1 (Seifert, 2014). Tukey tests with a p.value= 0.05 were used for analyzing germination data, and the number of replicates are indicated within the legends.

3. Results

3. 1. Effect of maternal water stress on the mother plant

Fig. 2 shows the effect of the drought stress scenarios shown Fig. 1 on the pattern of seed development *on planta*. Seed moisture content (MC) decreased continually from flowering to harvest (60 daf). Differences in seed MC caused by the water stress scenarios were visible at the first time-point only, i.e. at 10 daf. At this time-point, seed MC of S (stress during the entire seed development) and E (early stress) seeds was close to 4.5 $gH_2O.gDW^{-1}$ whereas it was close to 6 $gH_2O.gDW^{-1}$ for seeds that developed on non-stressed plants (W), middle- and late-stressed plants (M, L) (Fig. 2a). At the second (25 daf) and last (60 daf) time points, seed MC was roughly similar in all seed lots, i.e. close to 1-2 $gH_2O.gDW^{-1}$ and 0.4 $gH_2O.gDW^{-1}$, respectively (Fig. 2a). Seed fresh weight increased from flowering till ca 30 daf and decreased until harvest in all conditions (Fig. 2b). As expected, during seed development, seed fresh weight was lower in seeds from stressed plants (S and E) than in seeds issued from other categories. At harvest, seed fresh weight was close to 0.8 g for all seed lots. At last, water stress applied on the mother plant at any stage (S, E, M, L) did not modify seed dry weight during development, when compared to well-watered plants (W) (Fig. 2 c).

We confirmed the impact of drought stresses on seed production using the sum average daily water deficit, expressed as sFTSW (Supplementary Fig. S1). Stressed plants had higher values of sFTSW. The S plants had a sFTSW value around 30 whereas W plants had the lower sFTSW value, close to 11. L plants had a sFTSW value close to the one of W plants, and E and M plants displayed intermediate sFTSW values, close to 20 (Supplementary Fig. S1). The major effects of drought stress were observed on the plant biomass, as previously reported (Gosseau *et al.*, 2019) with a negative impact of stress on the plant dry weight (Supplementary Fig. S1c). In contrast, the impact of water stress on the progeny (thousand seed weight, seed number and seed weight) was less important, highly variable and not statistically significant.

3.3. Effect of maternal environment on seed dormancy and vigour

We first assessed the effect of maternal stress on seed dormancy. Immediately after harvest, W and S seeds were dormant, *i.e.* they poorly germinate at 20°C (Fig. 3a). S seeds, however, were less dormant than E, M, L and W seeds, as shown by their higher germination (Fig. 3a). After 6 months of storage in ambient temperature all seeds were no dormant and fully germinated at 20°C (Fig. 3b).

We also investigated the effect of the maternal environment on seed vigour traits, using non-dormant seeds only (*i.e.* stored for 6 months, their germination at 20°C on water is shown in Fig. 3b). When seeds came from well-watered plants (W) their germination was dramatically inhibited by water stress since only *ca* 45 % of W seeds were able to germinate in the presence of PEG -0.7 MPa (Fig. 4a). In contrast, S seeds, produced by drought stressed plants, fully germinated within 5 d in this condition (Fig. 4a). When the drought stress was applied early (E) or lately (L) on the mother plants, it did not significantly modify seed germination when compared to W seeds. However, applying drought stress during seed maturation phase (M) had a beneficial effect on seed germination under water stress, even though it was not as pronounced as for S seeds (Fig. 4a). Because many abiotic stress responses share common physiological and molecular pathways in plants (Ben Rejeb et al., 2014; Koyro et al., 2012), we investigated whether drought stress applied on the mother plant could also improve seed germination in other penalizing conditions, *i.e.* under cold stress, salt stress and hypoxia. When non-dormant seeds were imbibed for 24 h at 5°C and then germinated at 20°C, germination of W seeds decreased to ca 80 % (Fig. 4b). This negative effect of a cold period on seed germination was cancelled in S, E, L and M seeds (Fig. 4b). In the presence of 300 mM NaCl, germination of W, E, M and L seeds decreased to ca 70 %

whereas S seeds germinated to 84 % (Fig. 4c). Finally, we also assessed seed germination in hypoxia by germinating seeds under 3 and 5 % oxygen (Fig. 4d). Germination of S seeds was only partially inhibited in hypoxia since they germinated to 75 % in 3% oxygen and to 82 % in 5 % oxygen (Fig. 4d). In contrast W seeds only germinated to 16 and 58 % in 3 and 5 % oxygen, respectively (Fig. 4d). When water stress on the mother plant was applied at the early (E) or maturation (M) stages it improved seed

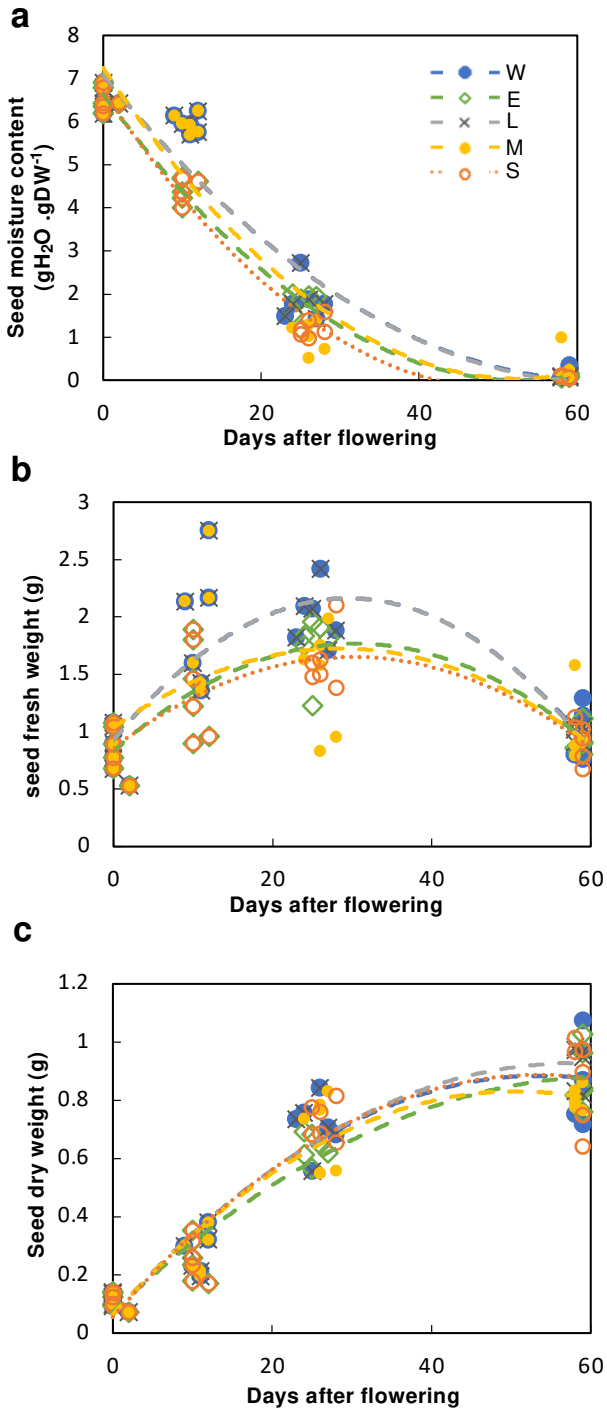


Fig. 2. Changes in water content (a), fresh weight (b), dry weight (c) of seeds produced under the different scenarios of water stress shown Fig. 1. Mother plants (XRQ genotype) were grown in well-watered condition (W, constant FTSW=1) or under drought stress (FTSW=0.2) during the early (E), middle (M) and late (L) phase of seed maturation and from flowering to harvest (S). (d) Germination at 20°C in the presence of a -0.7 MPa PEG solution of W, E, M, L and S seeds. Means ± SD of 3 replicates. Statistics were made using Tukey's test with alpha=0.05.

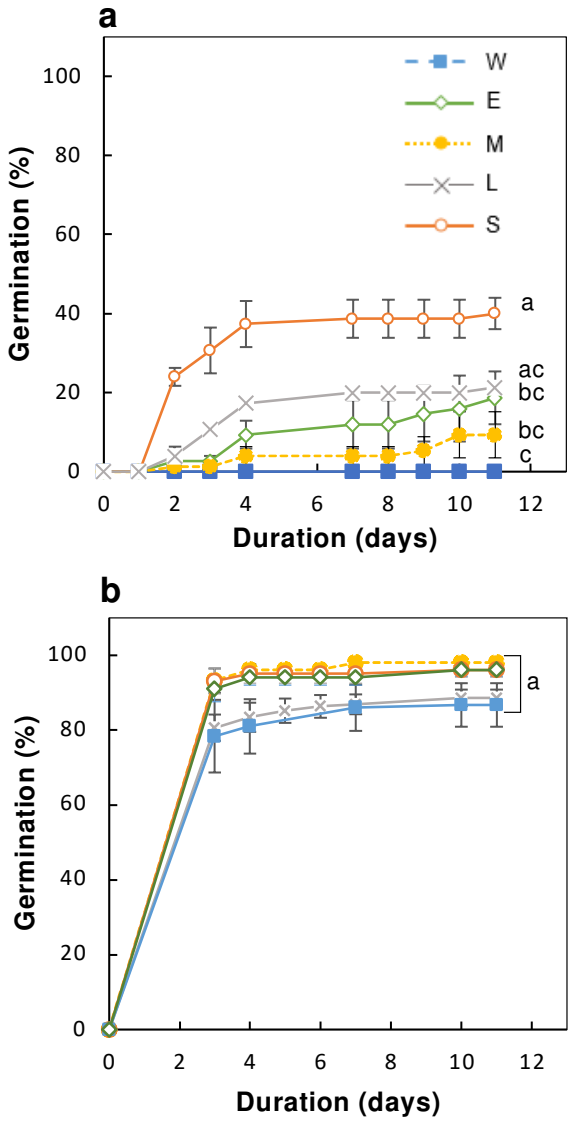


Fig. 3. Germination of XRQ/B dormant whole seeds (a) and non-dormant whole seeds (c) on water at 20°C in darkness. Seeds were obtained from plants grown in well-watered condition (W), in drought stress condition during the early (E), middle (M) and late phase (L) of seed maturation, or from flowering to harvest (S). Data are means of 3 replicates ± SD. Statistics were made using Tukey's test with alpha=0.05.

germination under hypoxia when compared to W seeds. However the late application of water stress on the mother plant did not confer a better tolerance to hypoxia (Fig. 4d).

3.4 Possible involvement of pericarp in seed germination

The effects of hypoxia on seed germination (Fig. 4d) suggested that pericarp could play a role in this response since it is well know that seed envelop structures can modulate oxygen diffusion towards the embryo (Porter and Wareing, 1974; Werker, 1980). We therefore investigated whether the maternal environment could modify seed germinative properties through an effect

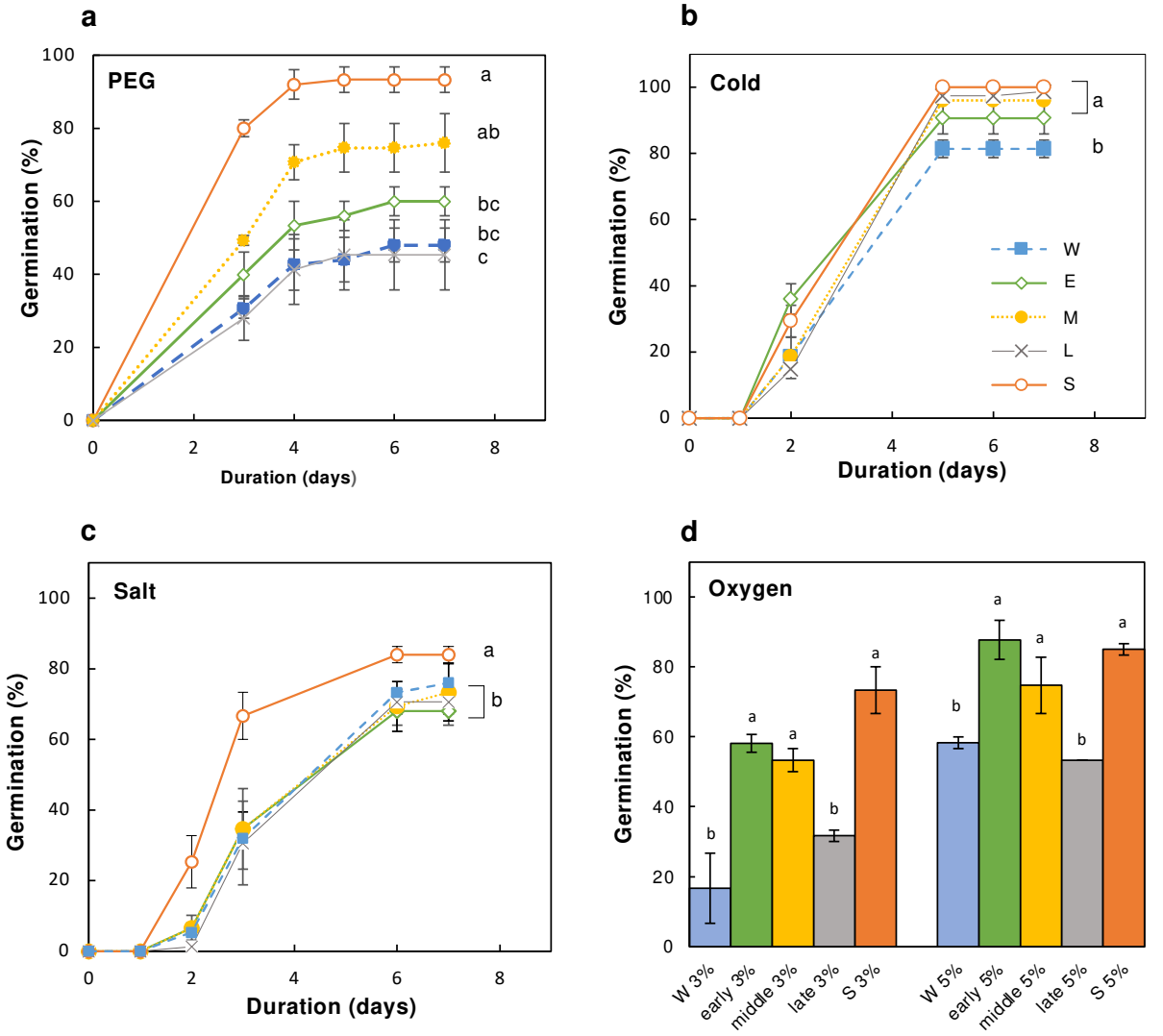


Fig. 4. Germination at 20°C in darkness of non-dormant seeds produced by plants grown in well-watered condition (W) or under drought stress during the early (E), middle (M) and late (L) phase of seed maturation and from flowering to harvest (S). (a) Germination in the presence of a -0.7 MPa PEG solution. (b) Germination after 24h at 5°C on water. (c) Germination on a 300 mM NaCl solution and (d) germination in the presence of 3 or 5 % oxygen. Data are the mean of 3 replicates ± SD. Statistics were made using Tukey's test with alpha=0.05.

on pericarp. We addressed this question by germinating W and S naked seeds in the conditions previously described.

Pericarp removal strongly stimulated germination of dormant W seeds at 20°C on water (Fig. 5a), since it reached almost 55 %, vs 0 % for whole seeds (see Fig. 3a). Germination of naked dormant S seeds increased from 40 to 68 % (Fig. 3a and Fig. 5a). Non-dormant naked W and S seeds germinated similarly under water stress (Fig. 5b), after cold stress (Fig. 5c), on a NaCl solution (Fig. 5d) or in 3 % oxygen (Fig. 5e), and in all cases at higher levels than whole

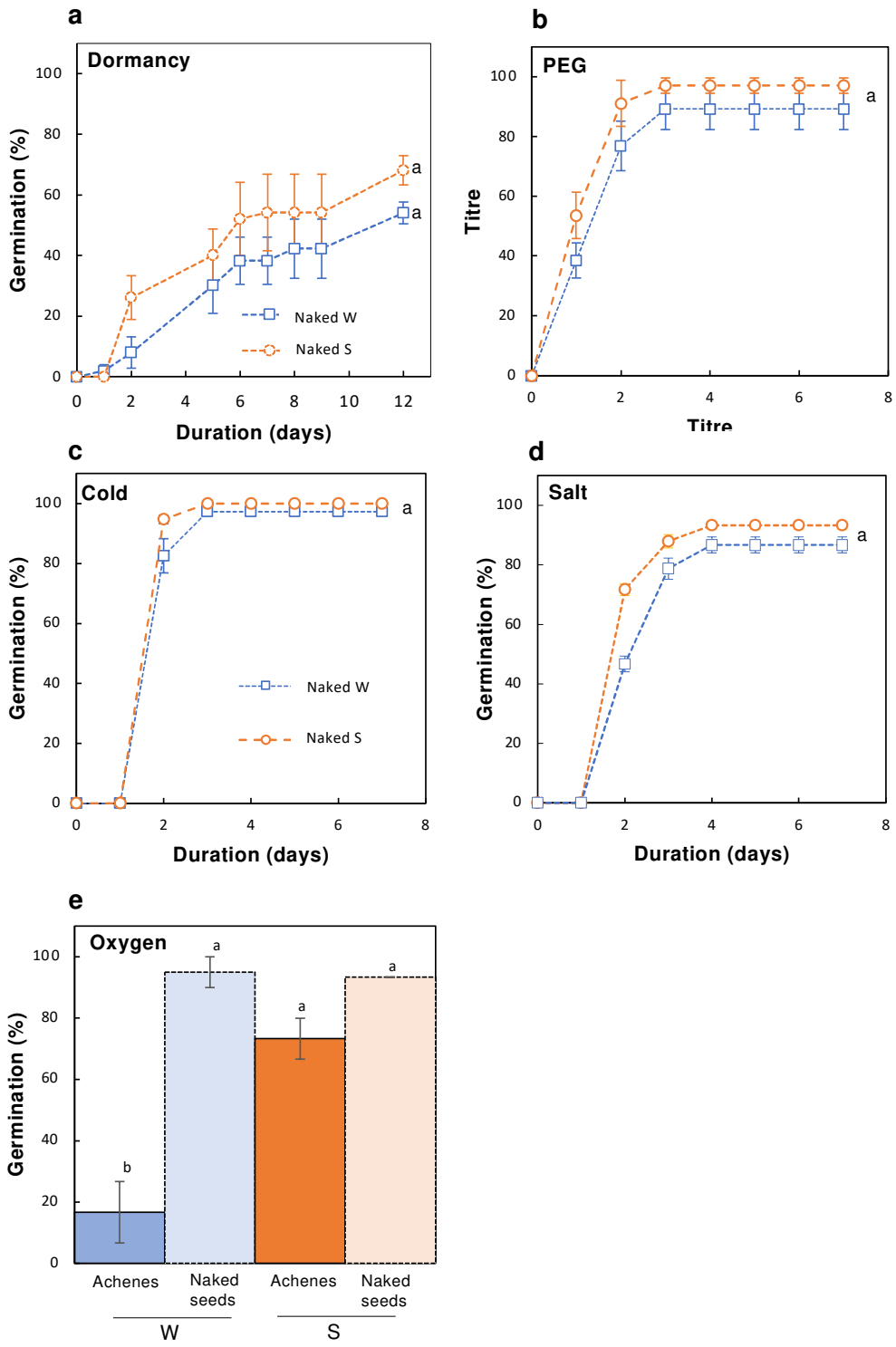


Fig. 5. Germination at 20°C in darkness of naked seeds (*i.e.* without pericarp) produced by plants grown in well-watered condition (W) or under drought stress from flowering to harvest (S). Germination of dormant naked seeds (a) and of non-dormant naked seeds in the presence of a -0.7 MPa PEG solution (b), after 24h at 5°C on water (c), on a 300 mM NaCl solution and in the presence of 3 or 5 % oxygen (d) obtained from plants grown in well-watered condition (W), in drought stress condition during the early (E), middle (M) and late phase (L) of seed maturation, or from flowering to harvest (S). Data are the mean of 3 replicates ± SD or of 2 replicates of 30 seeds ± SE (hypoxia experiment). Statistics were made using Tukey's test with alpha=0.05.

achenes (cf. Fig. 4). This was particularly spectacular for W seeds, which indicated that their pericarp played an inhibitory role on seed germination.

To understand the possible role of pericarp in seed germination, we investigated the structure of pericarps from S and W seeds by microscopy (Fig. 6a and 6b). Thickness of S pericarp (Fig. 6a) was lower than that of W pericarp (Fig. 6b), i.e. 0.99 µm vs 1.44 µm although the number of cell strata was roughly the same (4-5). The area of the pigmented sub-epidermal layer containing phytoalexin was similar in W and S pericarps but sclerenchyma cells of S pericarps were smaller with thicker cell walls (Fig. 6a and 6b). Further, we measured conductivity of pericarps isolated from S and W seeds (Fig. 6c). Electrolyte leakage of pericarps from W and S increased similarly during their imbibition, from ca 1000 to 1300 $\mu S.g^{-1}$ (Fig. 6c). However, when expressed as a function of total electrolytes, conductivity of pericarps from S seeds was significantly higher than the one of W seeds (Fig. 6c), although total electrolyte leakage was similar in both pericarps and close to 1600 $\mu S.g^{-1}$ (data not shown). After 3 h of imbibition, almost 83 % of electrolytes had leaked from S pericarps when only 75 % leaked from W pericarps (Fig. 6c). Since seed conductivity often results from phenolic compounds we measured total phenol content in pericarps (Fig. 6d). It was significantly higher in S pericarps than in W pericarps, with 5.43 mg vs 2.88 mg of total phenol per g of pericarp, respectively (Fig. 6d). At last we tested whether soluble compounds from pericarp could directly influence germination and therefore play a role of pericarp in tolerance to abiotic stresses. For this, we germinated W naked seeds (in the presence of a -0.7 MPa PEG solution) with macerate of pericarps or using a pericarp bedding assay (see material and methods). As already shown (see Fig. 6b), removing of pericarp allowed W naked seeds to fully germinate under water stress conditions, in contrast to whole W seeds whose germination did not exceed 40 %. However, when naked W seeds were imbibed on a PEG solution containing a macerate of pericarp or when placed on extracted pericarps (pericarp beding assay) then germination declined (Fig. 6d).

We also performed a non-targeted metabolomic analysis with dry naked S and W seeds. The objective of this study was to determine whether drought stress during seed development had only an effect on pericarp properties or could also have an effect on embryo per itself (Fig. 7). This analysis allowed the identification of 474 metabolites which clustered in 4 groups with 48 and 31 classes (Supplementary Fig. S2 and S3). Out of this, 21 metabolites accumulated differently between S and W seeds. The Heat map Fig. 7 shows metabolites that were specifically accumulated in either S or W seeds. In S seeds, complex sugars such as stachyose, tracheloside, intermediates such as malic, isopropylmalic, quinic

and glutamic acids and ω9 insaturated fatty acids like palmitoleic (C16 ω9) and linoleic acid (C18 ω9,12) accumulated. On the contrary, we observed a reduction of some aromatic amino acids (thryptophan, phenylalanine) and precursors (shikimic acid), of furoic, isochlorogenic C acids, of methylcytidine and interestingly of abscisic acid (ABA) in W seeds.

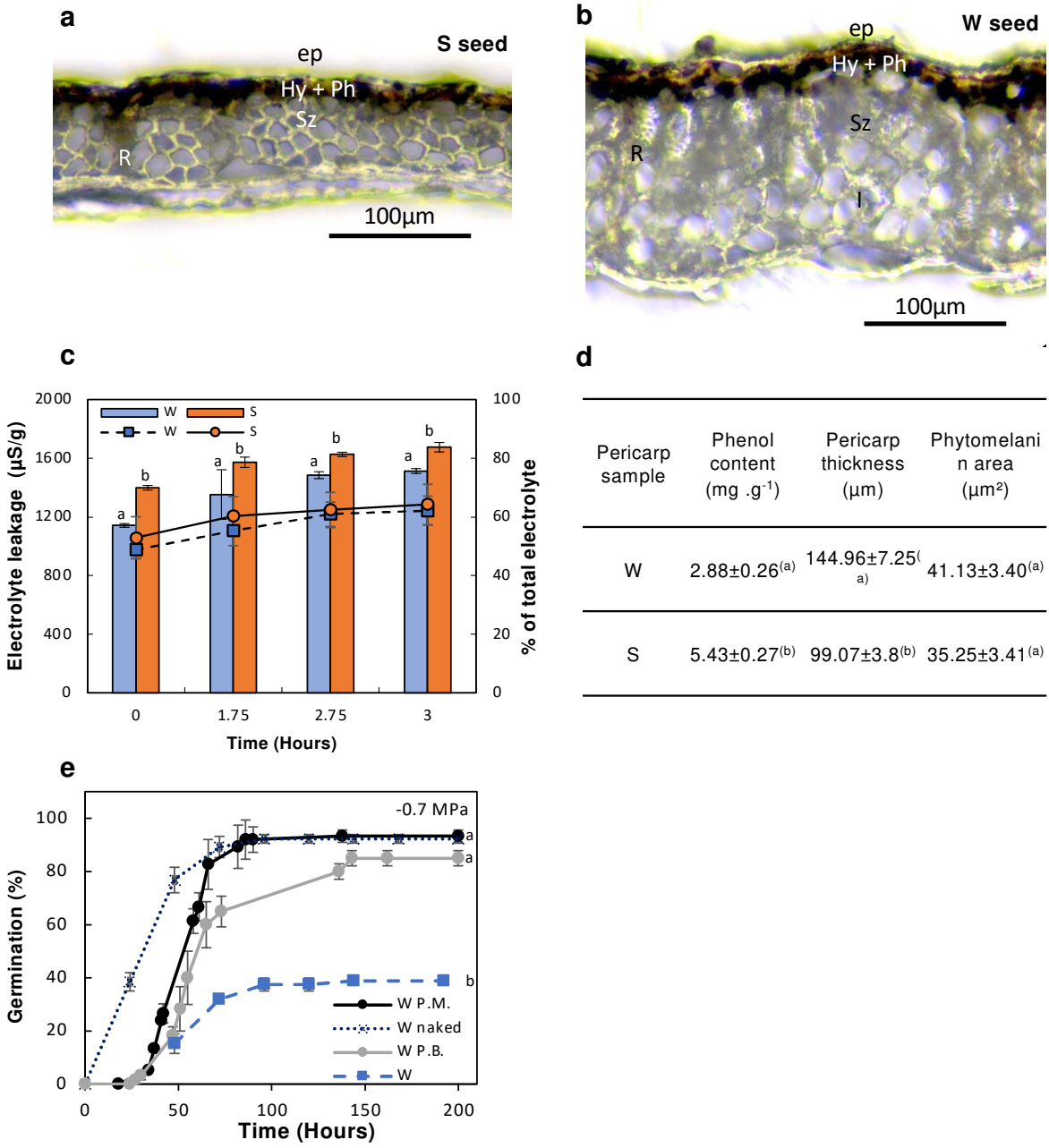
3.2. Characteristics of stress tolerance inheritance

Lastly it was important to decipher the genetic bases of the transmission of germinative traits by the mother plant to the offspring. This was achieved by studying the effect of different schemes of crosses on the induction of water stress tolerance only. Following the first observations obtained with seeds of XRQ/B line produced by self-fertilization, we checked whether the maternal effect on inherited tolerance to water stress (Fig. 8a) could also be observed in the context of hydrid production. To test this hypothesis, we crossed male-sterile plants (XRQ/A) with restorer plants (PSC8/R) that were well-watered (W) or stressed (S) or. from flowering to harvest (S). When each parent was stressed, hybrid seeds germinated to 80 % in the presence of -1.0 MPa PEG solution, when only 43 % of seeds produced by well-watered parent plants germinated (Fig. 8a).

We next determined whether the better tolerance to water stress in S seeds was transmitted by the maternal gamete and/or by the paternal gamete. For this, we combined water stresses in parent plants using cmsPET1 XRQ/A female plants and XRQ/B maintainer male plants (Fig. 8b). As observed previously, seeds obtained from stressed male and female plants (XRQ/A S x XRQ/B S) had the highest germination rate on a solution of -1.0 MPa PEG solution (58 %) whereas when parent plants were well watered (XRQ/A W x XRQ/B W) seed germination was lower (Fig. 8b). When male plants only were stressed (XRQ/A W x XRQ/B S) seed germination was the same as for seeds obtained from non-stressed plants (XRQ/A W x XRQ/B W). In contrast, when female plants only were stressed (XRQ/A S x XRQ/B W), resulting seeds displayed a faster germination and an intermediate final germination (Fig. 8b).

Finally, to determine if the induction of water stress tolerance was transgenerational or only resulting from a maternal effect, we grew plants for 3 successive years with or without water stress (at each generation plants issued from W or S seeds were grown without or with water stress, respectively, giving 3W and 3S seeds). The last year the same experimental protocol was applied, giving 4S and 4W seeds but in addition 3W plants were stressed (giving 3WS seeds) and 3S plants were well watered (giving 3SW seeds). Germination of the resulting seeds was assessed on a PEG solution and it showed that seeds were able to fully

germinate under water stress only when they were produced by a plant that had been stressed during seed development at the last generation (Fig. 8c).



Pericarp sample	Phenol content (mg .g^{-1})	Pericarp thickness (µm)	Phytomelanin area (µm²)
W	2.88±0.26$^{(a)}$	144.96±7.25$^{(a)}$	41.13±3.40$^{(a)}$
S	5.43±0.27$^{(b)}$	99.07±3.8$^{(b)}$	35.25±3.41$^{(a)}$

Fig. 6 Composition and role of pericarp in seed germination. (a,b) Cross sections of pericarps of S (a) and W (b) seeds imaged by optical microscopy. Ep: epidermis, HY+PH: hypodermis plus phytomelanin layer, Sz: Sclerenchyma zone, I: internal parenchyma, R : parenchyma ray. (c) Electrolyte leakage in µS.g^{-1} (curves) and in percentage of total electrolytes (bars) of pericarps of seeds issued from plants grown in well-watered conditions (W) or under drought stress (S). Data are means of 3 replicates of 25 seeds ± SD. (d) Phenol content and morphological characteristics of pericarps from W and S. Data are the mean of 3 measurements ± SE (phenolics) or 10 measurements ± SE (morphology). (e) Germination of whole (W) and naked W seeds and of naked W seeds in the presence of a pericarp macerate (W P.M.) or on a pericarp bed (W P.B.) at 20°C under water stress (PEG -0.7 MPa). Statistics in c, d and e were made using Tukey's test with alpha=0.05.

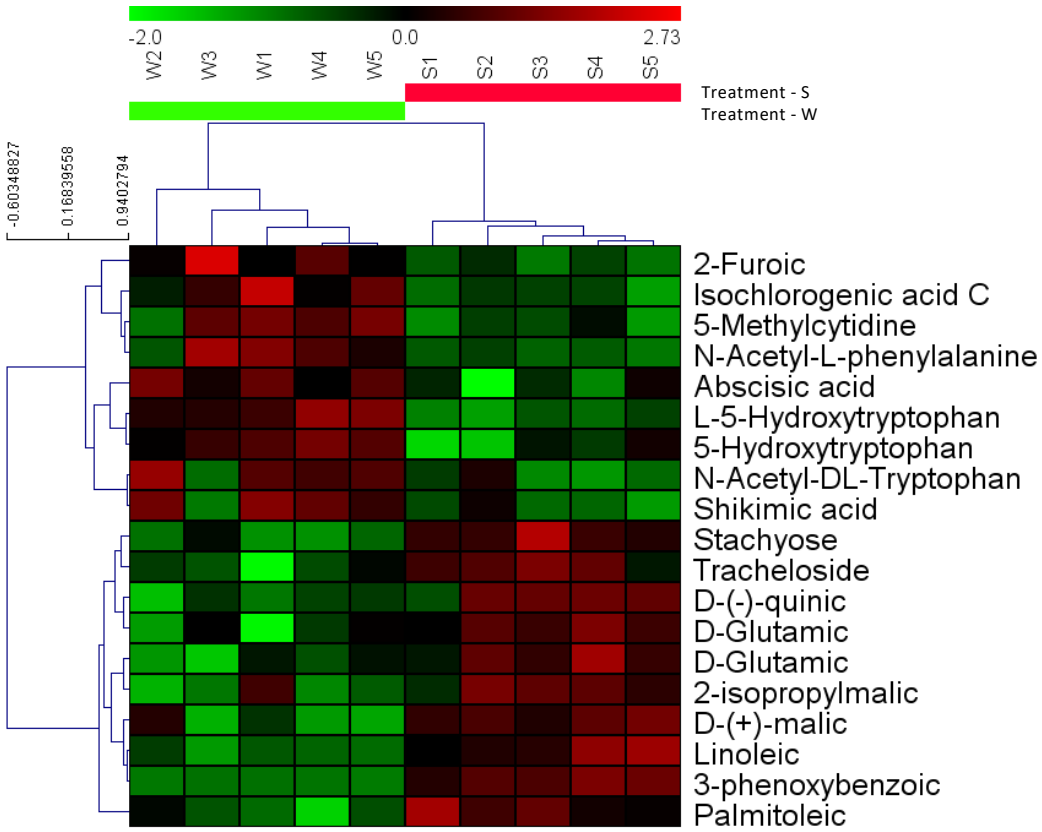


Fig. 7. Heatmap showing the differencially accumulated metabolites in dry seeds obtained from plants grown in well-watered condition (W) or in drought stress condition from flowering to harvest (S). Each row in the figure represents a metabolite and each column represents a sample. Colors ranging from green to red indicate metabolite abundance.

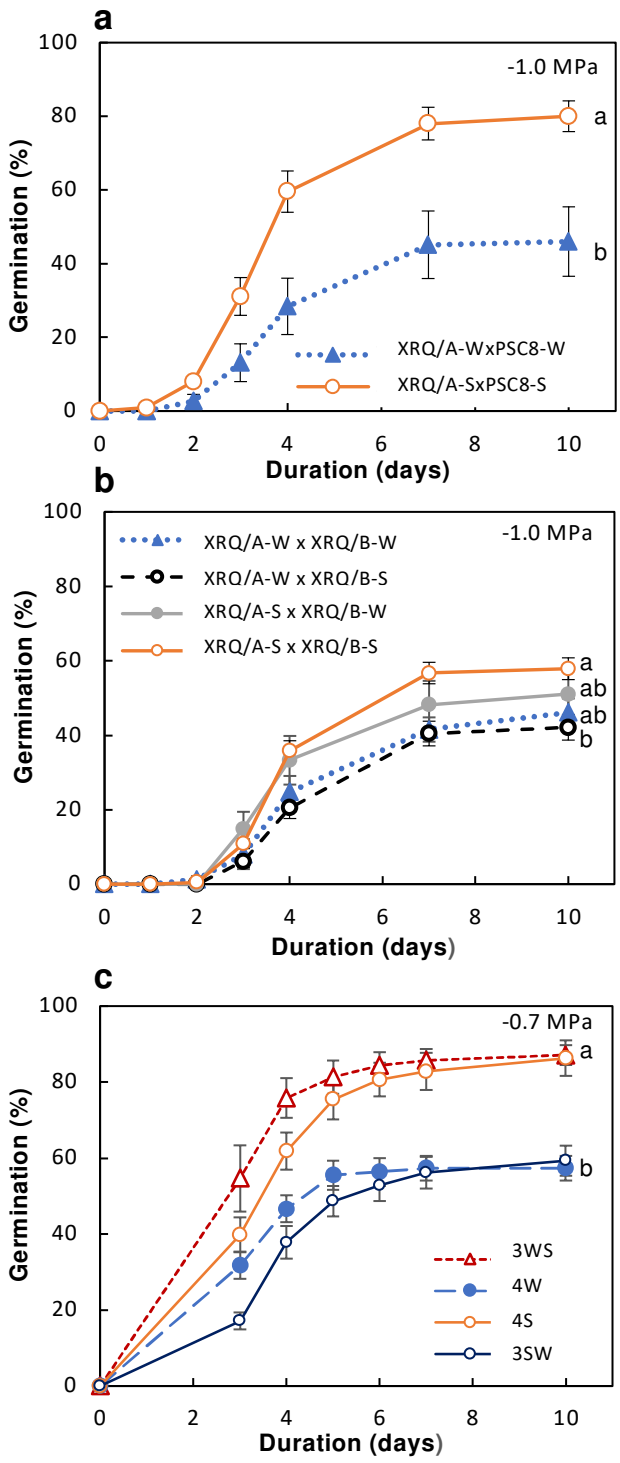


Fig. 8. Germination at 20°C on a PEG solution of seeds issued from plants grown in well-watered conditions (W) or under drought stress (S). (a) Seeds from the INEDI hybrid obtained from the cross of parental lines XRQ/A and PSC8. (b), seeds issued from different crosses female (XRQ/A) and male (XRQ/B) parental lines. (c), seeds from plants stressed or not for 3 generations (3S and 3W, respectively) before being produced in well-watered condition (3SW and 4W) or not (3WS and 4S) at the fourth generation. Concentrations of PEG solutions

are indicated within the figures. Data are the mean of 3 replicates of 25 seeds ± SE. Statistics were made using Tukey's test with alpha=0.05.

4. Discussion

On the Heliaphen Phenotyping platform, pattern of sunflower seed development reproduced the dynamics of water content and seed dry weight changes already observed for seeds of this species (Rondanini et al., 2006; Lachabrouilli et al., 2021). The time from flowering to harvest lasted ca 60 days and was associated with a relatively constant rate of water loss (Fig 2a). Achene fresh weight increased rapidly during early grain-filling and peaked at ca 35 daf when seed mass maturity was reached at ca 40 daf (Fig. 2), what is similar to what was observed by Lachabrouilli et al. (2021). Application of water stress on the mother plant had a limited and transient effect on these characteristics and was only visible for E and S seeds at the first time point (10 daf). Water stress (FSTW=20) applied continuously after flowering (i.e. S condition) had a unpredictable effect on the components of the yield at harvest, as estimated by TSW, seed dry weight and seed number per plant when compared to control plants, that was considered as being not significant (Supplementary Fig. S1). In contrast, as already shown by Gosseau et al. (2019), drought stress had a negative effect on whole plant biomass.

As already reported (Donohue, 2009; Fernández Farnocchia et al., 2019; Penfield, 2017), the maternal environment had an effect on seed dormancy. At 20°C, a temperature at which sunflower seed dormancy can be expressed (Gay et al., 1991; Lachabrouilli et al., 2021), germination was not complete, which revealed dormancy (Corbineau et al., 1990). Seeds issued from constantly drought stressed plants (S) were clearly less dormant than seeds from the other stress regimes (Fig. 3a). In particular, early, middle or late drought stress did not induce a phenotype of dormancy compared to well-watered plants (W). This suggests that faster seed desiccation during seed filling, as shown Fig. 2a, is likely to play an important role in the establishment of sunflower seed dormancy. Interestingly, Lachabrouilli et al. (2021) also demonstrated that rapid sunflower seed desiccation on the mother plant was associated with lower dormancy, which is in agreement with our findings. In *Lolium perenne*, whose seeds also display a physiological dormancy, high temperature during seed filling reduced dormancy (Fernández et al., 2021). In our conditions, after 6 months of dry storage, all seed batches became non-dormant as shown by their full germination at 20°C (Fig. 3b).

A moderate drought stress continuously applied to the mother plants from flowering to harvest (S seeds) induced a dramatic stimulation of germination of non-dormant seeds under

water stress, when compared to seeds obtained from well-watered plants (Fig. 4a). The beneficial effect of the drought stress required that it was applied within a proper time window to induce water stress tolerance at the germination stage. This time window, which ran from 10 daf to 25 daf, roughly corresponded to the beginning of the seed filling phase. Earlier or later stresses had no significant effect on the induction of water stress tolerance, which suggests that this trait was acquired during seed filling but not during the late maturation phase, in contrast to what was shown for seed longevity (Leprince et al., 2017). Interestingly, drought stress on the mother plant also induced tolerance to other abiotic stresses to the offspring. In particular it induced a better germination after cold stress, under salt stress and in hypoxia, if it was applied continuously after flowering (Fig. 4). Indeed, the time-window of drought stress application that induced stress tolerance depended on the nature of the stress. Tolerance to cold stress was acquired during any regime of drought stress, tolerance to salt stress required a constant water stress during seed filling (S seeds) and tolerance to hypoxia was not induced if drought stress was applied lately (L condition). This demonstrates that the mechanisms and signaling pathways involved in the tolerance to different stresses are specific and are timely induced during the seed developmental program. The relationship between pre-germination cues with post-germination environments has already been shown in various contexts (D'Aguillo et al., 2019). For example, the quantity and quality of light during seed maturation can influence seed germination of the next generation (Vayda et al., 2018). Heat stress, often concomitant to drought stress, can induce thermotolerance in the next generation in wheat seeds (Wang et al., 2016). The effect of drought stress during seed development on seed vigour is already documented but in some cases it has been shown to be detrimental for the progeny (Hatzig et al., 2018; Wijewardana et al., 2019) whereas some other studies have shown an opposite effect (Matzrafi et al., 2021; Van Dooren et al., 2020). Drought stress was also shown to improve germination of the progeny under water stress conditions in rice (Zheng et al., 2017) and *Brassica napus* L (Hatzig et al., 2018). The induction of tolerance to various stresses by a single stress applied on the mother plant, as evidenced here, has already been shown by Lui et al. (2017) in *Brassica rapa* where cold acclimation conferred tolerance to heat stress to the progeny. Similarly, in wheat, seeds collected from drought stressed plants had better tolerance against salt stress (Tabassum et al., 2017). To our knowledge, the effect of maternal environment on seed sensivity to hypoxia has not been demonstrated yet.

Pericarp removal had a dramatic effect on seed germination properties (Fig. 5), and it is worth noting that it generally conferred the phenotype of whole S seeds, *i.e.* lower

dormancy and higher vigour, to naked W seeds. Sunflower seeds display a physiological dormancy (Baskin and Baskin, 2004) that includes an inhibitory action of the envelopes (pericarp and seed coat), which is mostly expressed at 20-25°C, and an embryo dormancy, which generally prevents germination below 15°C (Corbineau et al., 1990). Removal of pericarp increased germination of dormant seeds but it did not exceed 70 %, which suggests that the observed dormancy resulted from the additive effect of both embryo dormancy and pericarp coat imposed dormancy (Lachabrouilli et al., 2021). This also demonstrates that embryo dormancy can be expressed at high temperature, even though it is generally reported to be expressed at low temperature (Corbineau et al., 1990; Bodrone et al., 2017). The specular increase in W seeds germination after pericarp removal (Fig. 3a and Fig. 6a) suggests that the inhibitory action of the pericarp was much higher in W seeds than in S seeds, thus bringing a first line of evidence of a direct effect of the maternal environment on pericarp properties. Here we also show that pericarp strongly repressed germination of non-dormant W seeds under stress conditions (water stress, cold, salt stress, hypoxia) and that the maternal environment suppressed this inhibitory effect, as shown by the germination of whole S seeds (Fig. 6). The role of seed covering structures on non-dormant seed germination is also well documented, mostly with regards to water and oxygen diffusion from the imbibition medium to the embryo (Grafi, 2020; Magnée et al., 2020; Huss and Gierlinger, 2021). However the effect of pericarp on seed germination under cold or salt stress is not documented to our knowledge.

Sunflower seed pericarp is a maternal tissue formed by the fusion of the ovary tissues and part of the receptacle (Schneiter et al., 1997). Our results show that the acquisition of stress tolerance and low dormancy in the offspring of drought stressed plants involved the pericarp. Transverse sections of pericarps allowed identifying their histological structures (Fig. 6). Pericarps from S seeds were much thinner than pericarps from W seeds, which resulted from smaller sclerenchyma cells with thick cell walls, but the general organization appeared to be the same, including a phytomelanin pigmented layer, and sclerenchyma areas in between parenchyma rays (Denis et al., 1994; Lindström et al., 2021). This suggested however that drought stress during seed development had a significant effect on sclerenchyma cells enlargement and cell wall deposition. Thickness of seed coats are known to be environmentally responsive in many species (Baskin and Baskin, 1998; Mousseau and Fox, 1998) and the effect of growing environment conditions on sunflower pericarp anatomy has already been demonstrated elsewhere (Lindström et al., 2021). Conductivity measurements revealed that the level of electrolyte leakage was timely similar in both S and W seeds but that

a more important fraction of electrolytes leaked from S pericarps during their imbibition (Fig. 6c). This suggests that a rapid removal of chemicals from pericarp of whole S seeds could help their faster germination. Phenolic content of S pericarps was nevertheless higher than the one of W pericarps, when expressed per g FW^{-1} (Fig. 6). However, if one considers the difference of thickness between S and W pericarps (Fig. 6), then the amount of soluble phenolics surrounding the embryo was roughly similar in pericarps of both categories of seeds. Interestingly the pericarp bedding assay and germination of naked seeds with pericarp extracts (Fig. 6d) confirmed that pericarps of W seeds, in particular, contained compounds that inhibited germination. We therefore propose that pericarps of S seeds would release more rapidly inhibitors of germination in the imbibition media, which would in turn allow a better germination of dormant seeds at 20°C and of non-dormant seeds under various stresses. Alternatively the chemical composition of pericarps of S and W seeds could also differ, S seeds containing lower amounts of inhibitory compounds, and this will have to be further investigated. This is in agreement with the studies of Khadka et al. (2020) and Swetha et al. (2021) who showed that the maternal environment had an effect on thechemical composition of pericarps of *Anastatica hierochuntica* and *Brassica juncea*, respectively. Phenolic compounds, present in the pericarp of sunflower seeds, have been frequently cited as being good candidates for inhibiting seed germination (Reigosa Roger et al., 1999). Phenolics are easily oxidized (Devlin and Harris, 1984) and can in turn cause oxygen limitation to the embryo, thus decreasing germination. Other inhibitory compounds cannot be excluded, such as ABA, which was the major component of the pericarp of dry seeds from sunflower plants grown under drought (Andrade et al. 2009).

The balance of maternal versus embryonic control of germination is up to date poorly characterized. If our results unambiguously showed the role of pericarp in the induction of low dormancy and stress tolerance to the offspring, one could not exclude that this maternal effect might also include a role of maternal provisioning on metabolite and hormone contents, or proteins and transcripts within the embryo. We indeed identified metabolites that specifically accumulated in either W or S embryos during seed development, which demonstrates that water stress on the mother plant had also an effect on the embryo characteristics *per se*. Even though it is too speculative to attribute a direct role to the accumulated metabolites in the germination phenotypes of S and W seeds, the accumulation of some compounds may have a biological sense. For example, stachyose, which accumulated in S seeds, belongs to raffinose-family oligosaccharides (RFOs) which are known to accumulate during seed late maturation (Baud et al., 2002) and have the role to protect

biological from desiccation-related damage (Hincha et al., 2003) and in seed longevity (Angelovici et al., 2010). Stachyose has been shown to accumulate in *Coffea arabica* seeds under water deficit, high salt, and heat stress (dos Santos et al., 2011; Santos et al., 2015). The fatty acids palmitoleic and linoleic also accumulated in embryos of S seeds. Specific accumulation of fatty acids in oil crops such as sunflower could be interesting specific food or industrial usages and will require further investigation. A set of metabolites was also specifically found in embryos of W seeds and among them we noticeably identified ABA (Fig. 7). Sunflower seed dormancy depends on ABA metabolism (Bianco et al., 1996; Bodrone et al., 2017), which is synthetized ca 2 weeks after flowering (LePage-Degivry et al., 1990), and the higher content of ABA in W seeds is in agreement with the higher dormancy phenotype highlighted for these seeds (Fig. 3).

Since a genetic component has already been shown to be involved in inheritance of seed traits (Alvarez et al., 2021; Donohue, 2009), we studied the relative importance of gametes using male sterile, maintainer, and restorer plants. Our results showed that the acquisition of water stress tolerance at the germination stage could be obtained for a commercial hybrid and was not dependent of the cytoplasmic origin (Fig. 8). Beside genetics, we studied whether paternal and/or maternal environments contribute to the acquisition of this tolerance. We carried out reciprocal crosses with male and female plants that were either stressed or not and concluded that the maternal environment was solely controlling the acquired tolerance as unstressed plants never produced water stress tolerant seeds. This is in accordance with the literature since most studies have demonstrated that environment elicits maternal effects (Donohue, 2009; Dyer et al., 2010; Galloway, 2005; Mousseau and Fox, 1998) and that the paternal effect on seed germination was often reported as being not statistically significant (Baskin and Baskin, 2019). We also performed a multigenerational experiment, in which drought stress, or none, was repeated over three generations before plant growing conditions were inverted for an additional generation. This confirmed our previous results and showed that growing plants under drought stress during the last generation was the prerequisite to provide seed tolerance to water stress. This shows that the offspring phenotype resulted from a maternal effect and not from a transgenerational effect, similarly to results obtained in Arabidopsis (Ganguly et al., 2017; Van Dooren et al., 2020).

In conclusion, we demonstrate that water stress during sunflower seed development induced seed tolerance to various abiotic stresses and reduced dormancy in the progeny at the germination stage. Our results show that these phenotypes result from the maternal environment but are not transgenerational because they did not persist over subsequent

generations. This mechanism could be an adaptation to improve the offspring fitness and permit seed germination in sub-optimal conditions thus ensuring successful establishment of the next generation. Our data also bring new insights about the relative roles of maternal tissues and embryo physiology in germination tolerance to abiotic stresses. Future studies will aim to determine how these both components interact together to drive the germination process.

Authors contribution

NL and CB designed and supervised the research. BV performed experiments and analyzed data. NB produced plants and seeds. BV NL and CB wrote the article.

Conflict of interest

The authors declare that they have no conflict of interest

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References

Alvarez, M., Bleich, A., Donohue, K., 2021. Genetic differences in the temporal and environmental stability of transgenerational environmental effects. Evolution 75, 2773–2790. https://doi.org/10.1111/evo.14367

Andrade, A., Vigliocco, A., Alemano, S., Alvarez, D., Abdala, G., 2009. Differential accumulation of abscisic acid and its catabolites in drought-sensitive and drought-tolerant sunflower seeds. Seed Sci. Res. 19, 201–211. https://doi.org/10.1017/S096025850999016X

Angelovici, R., Galili, G., Fernie, A.R., Fait, A., 2010. Seed desiccation: a bridge between maturation and germination. Trends Plant Sci. 15, 211–218. https://doi.org/10.1016/j.tplants.2010.01.003

Bailly, C., Benamar, A., Corbineau, F., Come, D., 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiol. Plant. 97, 104–110. https://doi.org/10.1111/j.1399-3054.1996.tb00485.x

Baskin, C.C., Baskin, J.M., 1998. Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination. Elsevier.

Baskin, J.M., Baskin, C.C., 2019. How much influence does the paternal parent have on seed germination? Seed Sci. Res. 29, 1–11. https://doi.org/10.1017/S0960258518000417

Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy. Seed Sci. Res. 14, 1–16. https://doi.org/10.1079/SSR2003150

Baud, S., Boutin, J.-P., Miquel, M., Lepiniec, L., Rochat, C., 2002. An integrated overview of seed development in Arabidopsis thaliana ecotype WS. Plant Physiol. Biochem. 40, 151–160. https://doi.org/10.1016/S0981-9428(01)01350-X

Ben Rejeb, I., Pastor, V., Mauch-Mani, B., 2014. Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms. Plants 3, 458–475. https://doi.org/10.3390/plants3040458

Bewley, J., 1997. Seed Germination and Dormancy. Plant Cell 9, 1055–1066.

Bianco, J., Daymond, J., Page-Degivry, M.-T., 1996. Regulation of germination and seedling root growth by manipulations of embryo GA levels in sunflower. Acta Physiol. Plant. 18, 59–66.

Blödner, C., Goebel, C., Feussner, I., Gatz, C., Polle, A., 2007. Warm and cold parental reproductive environments affect seed properties, fitness, and cold responsiveness in Arabidopsis thaliana progenies. Plant Cell Environ. 30, 165–175. https://doi.org/10.1111/j.1365-3040.2006.01615.x

Bodrone, M.P., Rodríguez, M.V., Arisnabarreta, S., Batlla, D., 2017. Maternal environment and dormancy in sunflower: The effect of temperature during fruit development. Eur. J. Agron. 82, 93–103. https://doi.org/10.1016/j.eja.2016.10.007

Bruce, T.J.A., Matthes, M.C., Napier, J.A., Pickett, J.A., 2007. Stressful “memories” of plants: Evidence and possible mechanisms. Plant Sci. 173, 603–608. https://doi.org/10.1016/j.plantsci.2007.09.002

Caboni, E., Tonelli, M.G., Lauri, P., Iacovacci, P., Kevers, C., Damiano, C., Gaspar, T., 1997. Biochemical aspects of almond microcuttings related to in vitro rooting ability. Biol. Plant. 39, 91–97. https://doi.org/10.1023/A:1000365224324

Chiang, G.C.K., Bartsch, M., Barua, D., Nakabayashi, K., Debieu, M., Kronholm, I., Koornneef, M., Soppe, W.J.J., Donohue, K., De Meaux, J., 2011. DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in Arabidopsis thaliana. Mol. Ecol. 20, 3336–3349. https://doi.org/10.1111/j.1365-294X.2011.05181.x

Corbineau, Bagniol, S., Côme, D., 1990. Sunflower (helianthus Annuus L.) Seed Dormancy and Its Regulation by Ethylene. Isr. J. Bot. 39, 313–325. https://doi.org/10.1080/0021213X.1990.10677156

Corbineau, Francoise, Bagniol, S., Côme, D., 1990. Sunflower (Helianthus annuus L.) seed dormancy and its regulation by ethylene. Isr. J. Bot. 39, 313–325. https://doi.org/10.1080/0021213X.1990.10677156

Corbineau, Françoise, Bagniol, S., Côme, D., 1990. Sunflower (helianthus Annuus L.) Seed Dormancy and Its Regulation by Ethylene. Isr. J. Bot. 39, 313–325. https://doi.org/10.1080/0021213X.1990.10677156

D'Aguillo, M.C., Edwards, B.R., Donohue, K., 2019. Can the Environment have a Genetic Basis? A Case Study of Seedling Establishment in Arabidopsis thaliana. J. Hered. 110, 467–478. https://doi.org/10.1093/jhered/esz019

Debaeke, P., Casadebaig, P., Flenet, F., Langlade, N., 2017. Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe. OCL 24, D102. https://doi.org/10.1051/ocl/2016052

Denis, L., Coelho, V., Vear, F., 1994. Pericarp structure and hullability in sunflower inbred lines and hybrids. Agronomie 14, 453–461.

Devlin, H.R., Harris, I.J., 1984. Mechanism of the oxidation of aqueous phenol with dissolved oxygen. Ind. Eng. Chem. Fundam. 23, 387–392. https://doi.org/10.1021/i100016a002

Donohue, K., 2009. Completing the cycle: maternal effects as the missing link in plant life histories. Philos. Trans. R. Soc. B Biol. Sci. 364, 1059–1074. https://doi.org/10.1098/rstb.2008.0291

Donohue, K., Heschel, M.S., Chiang, G.C.K., Butler, C.M., Barua, D., 2007. Phytochrome mediates germination responses to multiple seasonal cues. Plant Cell Environ. 30, 202–212. https://doi.org/10.1111/j.1365-3040.2006.01619.x

dos Santos, T.B., Budzinski, I.G.F., Marur, C.J., Petkowicz, C.L.O., Pereira, L.F.P., Vieira, L.G.E., 2011. Expression of three galactinol synthase isoforms in Coffea arabica L. and accumulation of raffinose and stachyose in response to abiotic stresses. Plant Physiol. Biochem. 49, 441–448. https://doi.org/10.1016/j.plaphy.2011.01.023

Dyer, A.R., Brown, C.S., Espeland, E.K., McKay, J.K., Meimberg, H., Rice, K.J., 2010. The role of adaptive trans-generational plasticity in biological invasions of plants. Evol. Appl. 3, 179–192. https://doi.org/10.1111/j.1752-4571.2010.00118.x

Fernández Farnocchia, R.B., Benech-Arnold, R.L., Batlla, D., 2019. Regulation of seed dormancy by the maternal environment is instrumental for maximizing plant fitness in Polygonum aviculare. J. Exp. Bot. 70, 4793–4806. https://doi.org/10.1093/jxb/erz269

Fernández, R., Chantre, G.R., Renzi, J.P., 2021. Seed dormancy of Lolium perenne L. related to the maternal environment during seed filling. Seed Sci. Res. 31, 217–223. https://doi.org/10.1017/S0960258521000155

Finch-Savage, W.E., Bassel, G.W., 2016. Seed vigour and crop establishment: extending performance beyond adaptation. J. Exp. Bot. 67, 567–591. https://doi.org/10.1093/jxb/erv490

Galloway, L.F., 2005. Maternal effects provide phenotypic adaptation to local environmental conditions. New Phytol. 166, 93–100. https://doi.org/10.1111/j.1469-8137.2004.01314.x

Ganguly, D.R., Crisp, P.A., Eichten, S.R., Pogson, B.J., 2017. The Arabidopsis DNA Methylome Is Stable under Transgenerational Drought Stress1[OPEN]. Plant Physiol. 175, 1893–1912. https://doi.org/10.1104/pp.17.00744

Gay, C., Corbineau, F., Côme, D., 1991. Effects of temperature and oxygen on seed germination and seedling growth in sunflower (Helianthus annuus L.). Environ. Exp. Bot. 31, 193–200. https://doi.org/10.1016/0098-8472(91)90070-5

Gosseau, F., Blanchet, N., Varès, D., Burger, P., Campergue, D., Colombet, C., Gody, L., Liévin, J.-F., Mangin, B., Tison, G., Vincourt, P., Casadebaig, P., Langlade, N., 2019. Heliaphen, an Outdoor High-Throughput Phenotyping Platform for Genetic Studies and Crop Modeling. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01908

Grafi, G., 2020. Dead but Not Dead End: Multifunctional Role of Dead Organs Enclosing Embryos in Seed Biology. Int. J. Mol. Sci. 21, 8024. https://doi.org/10.3390/ijms21218024

Hatzig, S.V., Nuppenau, J.-N., Snowdon, R.J., Schießl, S.V., 2018. Drought stress has transgenerational effects on seeds and seedlings in winter oilseed rape (Brassica napus L.). BMC Plant Biol. 18, 297. https://doi.org/10.1186/s12870-018-1531-y

Heard, E., Martienssen, R.A., 2014. Transgenerational Epigenetic Inheritance: Myths and Mechanisms. Cell 157, 95–109. https://doi.org/10.1016/j.cell.2014.02.045

Hincha, D.K., Zuther, E., Heyer, A.G., 2003. The preservation of liposomes by raffinose family oligosaccharides during drying is mediated by effects on fusion and lipid phase transitions. Biochim. Biophys. Acta BBA - Biomembr. 1612, 172–177. https://doi.org/10.1016/S0005-2736(03)00116-0

Huang, Z., Footitt, S., Finch-Savage, W.E., 2014. The effect of temperature on reproduction in the summer and winter annual Arabidopsis thaliana ecotypes Bur and Cvi. Ann. Bot. 113, 921–929. https://doi.org/10.1093/aob/mcu014

Huss, J.C., Gierlinger, N., 2021. Functional packaging of seeds. New Phytol. 230, 2154–2163. https://doi.org/10.1111/nph.17299

IPCC — Intergovernmental Panel on Climate Change, 2019. URL https://www.ipcc.ch/ (accessed 2.17.21).

ISTA, 1999. International rules for seed testing. Rules 1999. Seed Sci. Technol.

Khadka, J., Raviv, B., Swetha, B., Grandhi, R., Singiri, J.R., Novoplansky, N., Gutterman, Y., Galis, I., Huang, Z., Grafi, G., 2020. Maternal environment alters dead pericarp biochemical properties of the desert annual plant Anastatica hierochuntica L. PLOS ONE 15, e0237045. https://doi.org/10.1371/journal.pone.0237045

Koyro, H.-W., Ahmad, P., Geissler, N., 2012. Abiotic Stress Responses in Plants: An Overview, in: Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. pp. 1–28. https://doi.org/10.1007/978-1-4614-0815-4_1

Lachabrouilli, A.-S., Rigal, K., Corbineau, F., Bailly, C., 2021. Effects of agroclimatic conditions on sunflower seed dormancy at harvest. Eur. J. Agron. 124, 126209. https://doi.org/10.1016/j.eja.2020.126209

Le Page-Degivry, M., Barthe, P., Garello, G., 1990. Involvement of Endogenous Abscisic Acid in Onset and Release of Helianthus annuus Embryo Dormancy. Plant Physiol. 92, 1164–8. https://doi.org/10.1104/pp.92.4.1164

Leprince, O., Pellizzaro, A., Berriri, S., Buitink, J., 2017. Late seed maturation: drying without dying. J. Exp. Bot. 68, 827–841. https://doi.org/10.1093/jxb/erw363

Lindström, L.I., Franchini, M.C., Nolasco, S.M., 2021. Sunflower fruit hullability and structure as affected by genotype, environment and canopy shading. Ann. Appl. Biol. https://doi.org/10.1111/aab.12735

Magnée, K.J.H., Scholten, O.E., Postma, J., Lammerts van Bueren, E.T., Groot, S.P.C., 2020. Sensitivity of spinach seed germination to moisture is driven by oxygen availability and influenced by seed size and pericarp. Seed Sci. Technol. 48, 117–131. https://doi.org/10.15258/sst.2020.48.1.13

Matzrafi, M., Osipitan, O.A., Ohadi, S., Mesgaran, M.B., 2021. Under pressure: maternal effects promote drought tolerance in progeny seed of Palmer amaranth (*Amaranthus palmeri*). Weed Sci. 69, 31–38. https://doi.org/10.1017/wsc.2020.75

Michel, B.E., Kaufmann, M.R., 1973. The Osmotic Potential of Polyethylene Glycol 6000. PLANT Physiol. 51, 914–916. https://doi.org/10.1104/pp.51.5.914

Mousseau, T.A., Fox, C.W., 1998. Maternal Effects As Adaptations. Oxford University Press.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., Church, J.A., Clarke, L., Dahe, Q., Dasgupta, P., Dubash, N.K., Edenhofer, O., Elgizouli, I., Field, C.B., Forster, P., Friedlingstein, P., Fuglestvedt, J., Gomez-Echeverri, L., Hallegatte, S., Hegerl, G., Howden, M., Jiang, K., Jimenez Cisneroz, B., Kattsov, V., Lee, H., Mach, K.J., Marotzke, J., Mastrandrea, M.D., Meyer, L., Minx, J., Mulugetta, Y., O'Brien, K., Oppenheimer, M., Pereira, J.J., Pichs-Madruga, R., Plattner, G.-K., Pörtner, H.-O., Power, S.B., Preston, B., Ravindranath, N.H., Reisinger, A., Riahi, K., Rusticucci, M., Scholes, R., Seyboth, K., Sokona, Y., Stavins, R., Stocker, T.F., Tschakert, P., van Vuuren, D., van Ypserle, J.-P., 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, EPIC3Geneva, Switzerland, IPCC, 151 p., pp. 151, ISBN: 978-92-9169-143-2. IPCC, Geneva, Switzerland.

Penfield, S., 2017. Seed dormancy and germination. Curr. Biol. 27, R874–R878. https://doi.org/10.1016/j.cub.2017.05.050

Porter, N.G., Wareing, P.F., 1974. The Role of the Oxygen Permeability of the Seed Coat in the Dormancy of Seed of Xanthium pennsylvanicum Wallr. J. Exp. Bot. 25, 583–594.

Reigosa Roger, M., Souto, C., González, L., 1999. Effect of phenolic compounds on the germination of six weed species. Plant Growth Regul. 28, 83–88. https://doi.org/10.1023/A:1006269716762

Roach, D.A., Wulff, R.D., 1987. Maternal Effects in Plants. Annu. Rev. Ecol. Syst. 18, 209–235. https://doi.org/10.1146/annurev.es.18.110187.001233

Rondanini, D., Mantese, A., Savin, R., Hall, A.J., 2006. Responses of sunflower yield and grain quality to alternating day/night high temperature regimes during grain filling: Effects of timing, duration and intensity of exposure to stress. Field Crops Res. 96, 48–62. https://doi.org/10.1016/j.fcr.2005.05.006

Santos, T., Lima, R., Nagashima, G., Petkowicz, C., Carpentieri-PÃ-polo, V., Pereira, L., Domingues, D., Vieira, L., 2015. Galactinol synthase transcriptional profile in two genotypes of Coffea canephora with contrasting tolerance to drought. Genet. Mol. Biol. 38, 182–190.

Saux, M., Bleys, B., André, T., Bailly, C., El-Maarouf-Bouteau, H., 2020. A Correlative Study of Sunflower Seed Vigor Components as Related to Genetic Background. Plants 9, 386. https://doi.org/10.3390/plants9030386

Schneiter, A.A., 1940-, Seiler, G.J., 1949-, Bartels, J.M., 1997. Sunflower technology and production. American Society of Agronomy.

Seifert, E., 2014. OriginPro 9.1: scientific data analysis and graphing software-software review. J. Chem. Inf. Model. 54, 1552. https://doi.org/10.1021/ci500161d

Sinclair, T.R., Hammer, G.L., Oosterom, E.J. van, 2005. Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Funct. Plant Biol. 32, 945–952. https://doi.org/10.1071/FP05047

Swetha, B., Singiri, J.R., Novoplansky, N., Grandhi, R., Srinivasan, J., Khadka, J., Galis, I., Grafi, G., 2021. Single and Combined Salinity and Heat Stresses Impact Yield and Dead Pericarp Priming Activity. Plants 10, 1627. https://doi.org/10.3390/plants10081627

Tabassum, T., Farooq, M., Ahmad, R., Zohaib, A., Wahid, A., 2017. Seed priming and transgenerational drought memory improves tolerance against salt stress in bread wheat. Plant Physiol. Biochem. PPB 118, 362–369. https://doi.org/10.1016/j.plaphy.2017.07.007

Van Dooren, T.J.M., Silveira, A.B., Gilbault, E., Jiménez-Gómez, J.M., Martin, A., Bach, L., Tisné, S., Quadrana, L., Loudet, O., Colot, V., 2020. Mild drought in the vegetative stage induces phenotypic, gene expression, and DNA methylation plasticity in Arabidopsis but no transgenerational effects. J. Exp. Bot. 71, 3588–3602. https://doi.org/10.1093/jxb/eraa132

Vayda, K., Donohue, K., Alejandra Auge, G., 2018. Within- and trans-generational plasticity: seed germination responses to light quantity and quality. Aob Plants 10, ply023. https://doi.org/10.1093/aobpla/ply023

Walker-Simmons, M., Sesing, J., 1990. Temperature effects on embryonic abscisic acid levels during development of wheat grain dormancy. J. Plant Growth Regul. 9, 51. https://doi.org/10.1007/BF02041941

Wang, X., Xin, C., Cai, J., Zhou, Q., Dai, T., Cao, W., Jiang, D., 2016. Heat Priming Induces Trans-generational Tolerance to High Temperature Stress in Wheat. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.00501

Werker, E., 1980. Seed Dormancy as Explained by the Anatomy of Embryo Envelopes. Isr. J. Bot. 29, 22–44. https://doi.org/10.1080/0021213X.1980.10676874

Wijewardana, C., Reddy, K.R., Krutz, L.J., Gao, W., Bellaloui, N., 2019. Drought stress has transgenerational effects on soybean seed germination and seedling vigor. PloS One 14, e0214977. https://doi.org/10.1371/journal.pone.0214977

Wolf, J.B., Wade, M.J., 2009. What are maternal effects (and what are they not)? Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 1107–1115. https://doi.org/10.1098/rstb.2008.0238

Xu, P., Chen, H., Hu, J., Cai, W., 2021. Potential evidence for transgenerational epigenetic memory in Arabidopsis thaliana following spaceflight. Commun. Biol. 4, 1–14. https://doi.org/10.1038/s42003-021-02342-4

Yin, J., Zhou, M., Lin, Z., Li, Q.Q., Zhang, Y.Y., 2019. Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. Ecol. Lett. 22, 1976–1986. https://doi.org/10.1111/ele.13373

Zheng, X., Chen, L., Xia, H., Wei, H., Lou, Q., Li, M., Li, T., Luo, L., 2017. Transgenerational epimutations induced by multi-generation drought imposition mediate rice plant's adaptation to drought condition. Sci. Rep. 7, 39843. https://doi.org/10.1038/srep39843