Running title: ERECTA- and mucilage-mediated control of seed germination

Timing seed germination under changing salinity: a key role of the ERECTA receptor-kinases

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Highlight

The ERECTA family of receptor-kinases regulates seed germination under salinity, through mucilage-mediated sensing of conditions at the seed surface, and interaction with secondary dormancy mechanisms.

Abstract

Appropriate timing of seed germination is crucial for the survival and propagation of plants, and for crop yield, especially in environments prone to salinity or drought. Yet, how exactly seeds perceive changes in soil conditions and integrate them to trigger germination remains elusive, especially once non-dormant. Here we report that the Arabidopsis ERECTA (ER), ERECTA-LIKE1 (ERL1) and ERECTA-LIKE2 (ERL2) leucine-rich-repeat receptor-like kinases synergistically regulate germination and its sensitivity to salinity and osmotic stress. Loss of ER alone, or in combination with ERL1 and/or ERL2 slows down the initiation of germination and its progression to completion, or arrests it altogether until better conditions return. That function is maternally controlled via the embryo surrounding tissues, primarily the properties of the seed coat determined during seed development on the mother plant, that relate to both seed coat expansion and subsequent differentiation, particularly the formation of its mucilage. Salt-hypersensitive er, er erl1, er erl2 and triple mutant seeds also exhibit increased sensitivity to ABA during germination, and under salinity show an enhanced upregulation of the germination repressors and inducers of dormancy ABA-insensitive-3, ABA-insensitive-5, DELLA encoding RGL2 and Delay-Of-Germination-1. These findings reveal a novel role of the ERECTA kinases in the sensing of conditions at the seed surface and the integration of developmental and stress signalling pathways in seeds. They also open novel avenues for the genetic improvement of plant adaptation to harsh soils.

Key-words

Seed germination; salinity; osmotic stress; drought; ERECTA genes; receptor-kinases; mucilage; environmental sensing; abiotic stress signalling; seed dormancy; seed size
Introduction

Seed germination is a vital life-cycle transition in plants. When and under which conditions it occurs, largely determine survival, reproductive success, yield and ability to expand. To maximise chances of timely germination under favourable conditions, seeds have evolved mechanisms for dormancy, a state that prescribes the environmental conditions that need to occur before germination can take place (Bewley, 1997; Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). As dormancy fades over time, or is lifted by appropriate cues, water becomes the most critical requirement for successful germination, in interaction with temperature (Alvarado and Bradford, 2002). As soon as moisture contacts it, the highly desiccated seed imbibes like a sponge. Seed imbibition reactivates metabolism and enables embryo expansion and rupture of its protective tissues. In *Arabidopsis thaliana*, this occurs in two separate steps, the rupture of the testa, or seed coat, a dead tissue, and then endosperm rupture (Liu et al., 2005; Müller et al., 2006). Embryo reactivation and weakening of surrounding tissues are tightly coordinated, through complex biochemical and hormonal pathways, with a prominent role of abscisic acid (ABA) and gibberellins (GAs), in interaction with ethylene, brassinosteroids and reactive oxygen species (ROS) (Koornneef and Van der Veen, 1980; Steber and McCourt, 2001; Bailly, 2004; Finch-Savage and Leubner-Metzger, 2006; Kucera et al. 2005; Finkelstein et al., 2008; Weibrecht et al., 2011; Rajjou et al., 2012; Yu et al., 2016). ABA inhibits germination whereas GAs promote it, through regulation of inter-signalling between seed coat, endosperm, and embryo, in a feedback loop involving DELLA proteins and interactions with cell-wall remodelling enzymes (Müller et al., 2006; Stamm et al., 2012; Graeber et al., 2014; Nonogaki, 2014).

Drought and salinity stress are two inter-related and widespread conditions in natural environments, and major causes of germination failure, poor crop establishment and yield loss (Boyer, 1982; Bradford K.J., 1990; Finch-Savage and Leubner-Metzger, 2006; Yamaguchi and Blumwald, 2005; Munns and Tester, 2008). The high vulnerability of seeds to these stresses has long-been recognised. Yet, the molecular controls remain poorly understood, apart from evidence for a deregulation of ABA-GA homeostasis and an impairment of ethylene and ROS signalling (Lopez-Molina et al., 2001; Kim et al., 2008; Yuan et al. 2010; Yu et al., 2016). Natural genetic variation in seed germination under optimal conditions, drought or salinity has been widely documented, and numerous QTLs identified (e.g. Quesada et al., 2002; Clerkx et al., 2004; Galpaz and Reymond, 2010; Wang et al., 2010; DeRose-Wilson and Gaut, 2011; Yuan et al., 2016). This demonstrates the
potential for genetic improvement, but also the complexity of the underlying molecular pathways. While the genetic dissection of seed dormancy has received much attention, very few genes have been demonstrated to control the germination of non-dormant seeds to tune it to prevailing soil conditions (Kim et al., 2008; Ren et al., 2010; Yu et al., 2016). How seeds monitor their surroundings, how this information is communicated to their inner compartments and modulates the intricate communication between them and the environment that timely germination requires, remains little known (Donohue et al., 2010).

Receptor-like protein kinases (RLKs) at the cell plasma membrane play major roles in signal perception and transduction to downstream intra- and inter-cellular signalling networks. A vast array of RLKs are encoded by plant genomes (Shiu et al., 2004). Among them are Leucine-Rich-Repeat Receptor-Like Kinases (LRR-RLKs) which form a large family of receptor proteins characterised by an extra-cellular receptor domain, a trans-membrane domain and an intra-cellular kinase domain for signal transduction through phosphorylation cascades. The few that have been characterised provide evidence for central functions in integrating developmental, hormonal, and abiotic stress or defence signalling pathways (Becraft, 2002; Osakabe et al., 2013). Scant information is available on RLKs in seeds, even though developing seeds show high abundance of secreted peptides and recent studies point to the importance of peptide-mediated signalling in inter-compartmental coordination during seed development (Ingram and Gutierrez-Marcos, 2015).

The Arabidopsis ERECTA gene family (ERf) encodes three closely related LRR-RLKs - ER, ERL1 and ERL2 - known to synergistically regulate many aspects of plant development and morphogenesis with prominent roles in organ shape, stomatal patterning, cell proliferation and meristematic activity (Torii et al., 1996; Shpak et al., 2004; 2005; Pillitteri et al., 2007; Uchida et al., 2012; Bemis et al., 2013; Etchells et al., 2013; Ikematsu et al., 2016), as well as being involved in some pathogenic responses (Godiard et al., 2003; Llorente et al., 2005; Jordá et al., 2016). In contrast, little is known of its function in abiotic stress responses, beyond a role in leaf heat tolerance (Shen et al. 2015). We earlier reported a role of ERECTA as a major controller of water use efficiency, under both well watered and drought conditions (Masle et al., 2005). That function appears to be broadly conserved in diverse species (Xing et al., 2011; Zheng et al., 2015), and is suggestive of an important adaptive role of the ERf to abiotic stress. Here we probe the ERf function during germination, a key switch that is extremely sensitive to variations in osmotic and ionic soil conditions, both of which vary widely in nature.
Material and Methods

Plant material and growth conditions

Arabidopsis thaliana Columbia (Col-0, CS1093) was used as wild type (WT), alongside two independent sets of single, double and whole ERECTA family loss-of-function mutants: one carrying the previously characterised mutations erl105, erl1-2, erl2-1, in the ER (At2g26330), ERL1 (At5g62230) and ERL2 (At5g07180) genes, respectively (Torii et al., 1996; Shpak et al., 2004; Masle et al., 2005; Bundy et al., 2012; Bemis et al., 2013) and here coded er, erl1 and erl2 for simplicity; the other carrying the er2 (C3401) mutation (Rédei, 1992; Lease et al., 2001; Masle et al., 2005; Hall et al., 2007), and the erl1-5 (SALK_019567) and erl2-2 (SALK_015275C) insertional mutations from the SALK Institute collection (Alonso et al. 2007). Absence of residual target gene expression in the latter two lines was confirmed, and T-DNA insertion sites verified (insertion located 4605 bp and 2775 bp from ERL1 and ERL2 start codon in erl1-5 and erl2-2, respectively).

Double and triple erf mutants were generated through crosses. As the triple mutants are sterile, the segregating progeny of er erl1/+ erl2, or er2 erl1-5+/− erl2-2 was used to investigate germination of triple mutant seeds, and is referred to in text and figures as er erl1/seg erl2 or er2 erl1-5/seg erl2-2, respectively.

All seeds in any given experiment were of the same age and harvested from spaced plants grown together, under the same conditions (21°C constant temperature; 12 or 16 h day length, depending on experiment; 120-130 μmol quanta m⁻² s⁻¹ light intensity). For investigation of parent-of-origin effects on seed germination and seed size, seeds were manually excised from tagged mature siliques, of the same age and same position on the primary inflorescence.

Germination assays

All assays were done using seeds stratified by moist chilling at 4°C to remove residual dormancy. Seeds were surface-sterilised and sown on 0.7% agar media supplemented with Hoagland’s nutrient solution (2 mM KNO₃, 5 mM Ca[NΟ₃]₂4H₂O, 2 mM MgSO₄7H₂O, 2 mM KH₂PO₄, 0.09 mM Fe-EDTA and micronutrients) pH 5.8, and NaCl or KCl in desired concentrations. For germination assays under iso-osmotic conditions generated by PEG8000 or NaCl, seeds were plated on filter paper imbibed with solutions of NaCl or PEG8000
dissolved in water. The osmotic pressure ($\pi_e$) of the basal medium or NaCl- or KCl-containing media was calculated using the classic van’t Hoff equation and verified experimentally using a VAPRO vapour pressure osmometer (Wescor Inc.). The concentrations of PEG8000 required to obtain a given $\pi_e$ were determined from a calibration curve of $\pi_e$ as a function of [PEG] using the same instrument. Seeds WT and all erf mutant combinations were sown in equal number ($n \geq 33$) within each of 3 to 4 plates (total $n = 100$ to 120 seeds per line per treatment and experiment). After stratification at 4°C, in the dark for 2-3 days, plates were exposed to continuous light (100-115 μmol quanta m$^{-2}$ s$^{-1}$) and a constant 21°C temperature. “Demucilaged” seeds were sown straight after mucilage removal (see protocol below), and kept at 4°C in darkness for an additional day, so as to keep total stratification time to 48 h, as for control intact seeds.

Seeds were individually scored for both testa and endosperm rupture (germination sensu stricto) under a binocular microscope, within the growth chamber, and at 3-4 h intervals until all seeds on control plates (0 mM NaCl) had germinated (i.e. 30 hours at most), or three times to once daily, as appropriate on NaCl, KCl, or PEG plates, until no change in scores was observed. Data are represented either as percentages of seeds exhibiting testa or endosperm rupture as a function of incubation time post-stratification, or as $T_{50}$ values, corresponding to the times (h post-stratification) when 50% of seeds showed testa or endosperm rupture (Bewley et al., 2013).

**Embryo culture**

Mature embryos were excised from dry seeds pre-imbibed with water for 1-2 h, briefly rinsed twice in water to remove endosperm debris and plated on either 0 or 150 mM NaCl media, placed in the dark at 4°C for 3 d, before transfer to the growth chamber. Embryos were individually imaged at the time of transfer and again 72h later using a LEICA M205 FA microscope fitted with a DFC 550 camera (LEICA Instruments). Relative embryo expansion rates over that 72 h interval were calculated from measurements of projected areas using *ImageJ* software.

**Staining procedures**
GUS histochemical staining of seeds from *proERf:GUS* reporter lines (Shpak et al., 2004) was performed on embryos dissected from dry and germinating seeds sampled from 0 and 150 mM NaCl plates. Staining was done as described (Sessions et al., 1999).

For tetrazolium permeability assays (Debeaujon and Koornneef, 2000) dry seeds were incubated in the dark in an aqueous solution of 1% (w/v) tetrazolium red (2,3,5-triphenyltetrazolium chloride, Sigma-Aldrich) at 30°C for 4, 24, 48, 72 and 120 h, and then rinsed twice with deionised water, resuspended in 95% ethanol and quickly ground to extract formazans. The final volume was adjusted to 2 ml with 95% ethanol, followed by centrifugation at 15000 g and measurement of supernatant’s absorbance at 485 nm, using a Tecan Infinite M1000 Pro spectrophotometer (Tecan Trading AG, 2008). Each sample was assayed in triplicates.

Mucilage ruthenium red staining was performed as described (http://www.bio-protocol.org/e1096). Ruthenium red stains acidic pectins (Hanke and Northcote, 1975) and is widely used to stain *Arabidopsis* seed mucilage (Western et al., 2000; Penfield et al., 2001).

**Profiling fatty acid methyl esters derived from lipids stored in the embryo**

Fifty mature embryos were dissected from dry seeds after 1 h imbibition in water, in 4 replicates per genotype. Fatty acid methyl esters (FAMEs) were prepared by direct transesterification as described by James et al. (2011). Embryos were placed in a reacti-vial (1.5mL) fitted with a Teflon-lined cap. To this was added CHCl₃ (50 µL) followed by the internal standard, heptadecanoic acid (C₁₇:0, 15 µL, 9.66 mg in 25mL CHCl₃), and methanolic HCl (3M, 500 µL). The samples were mixed and heated at 90 °C for 60 min, and then allowed to cool before being washed into glass tubes with CHCl₃. Water (1 mL) was added to each tube and the FAMEs extracted (hexane:chloroform, 4:1 v/v, 3 x 1 mL). The extracts were combined and washed with water (200 µL). The organic phase was then dried with anhydrous Na₂SO₄, decanted and evaporated under nitrogen. The residue was dissolved in CH₂Cl₂ (150 µL) and transferred to GC/MS auto-sampler vials for analysis.

**Mucilage extraction and analysis**
Mucilage extraction was performed on aliquots of 40 mg dry seeds. Each aliquot (n=4 per genotype per experiment) was suspended in 1ml milliQ water, followed by shaking at 500 rpm for 24 h at 4°C, vortex for 5 s, and centrifugation at 8000 g for 3 mins. 600 µl supernatant was recovered. Seeds were rinsed twice with 200 µl water, and 200 µl supernatant was recovered after vortexing and centrifugation. The pooled supernatants (1ml total volume) was snap-frozen in liquid nitrogen and immediately lyophilised. The mucilage thus recovered was weighed on a 10⁻⁶ g high precision micro-balance. Given the observed genetic variation in seed size (see Text), sub-aliquots of a known number of seeds (at least 500) were weighed, imaged at high resolution and analysed for size with ImageJ prior to mucilage extraction, allowing derivation of average mucilage amount per seed. The reductions of uronic acid methyl-esters and free uronic acids in the extracted mucilage were carried out following established protocols (Kim and Carpita, 1992; Pettolino et al., 2012). The reduced polysaccharides were then hydrolysed, reduced, acetylated and subjected to GC/MS analysis as described (Peng et al., 2000).

**Analysis of seed sodium content**

Dry seeds (3 biological replicates of 10 mg seeds each were per genotype and treatment) were imbibed and stratified at 4°C in the dark in a 0 or 150 mM NaCl solution for two days followed by 24 h at room temperature with shaking. Seeds were rinsed 3 times with 2 ml water, freeze-dried, weighed and microwave-digested for 2 h in 4 ml of 20% nitric acid at 175°C (USAP Method 3051). Digest volumes were diluted to a final volume of 5 ml. Sodium ions were measured by ICP-OES (Varian Vista-Pro CCD Simultaneous).

**Quantitative RT-PCR**

Total RNA was extracted from dry, imbibed or germinating seeds using TRIzol reagent (Invitrogen). mRNA isolation and reverse transcription were done as described (Chen et al., 2018). Primer sequences are given in Supplementary Table 1. The analysis was done on four biological replicates per genotype, time point and treatment, of 300 seeds each, sampled from 4 plates where all genotypes compared were represented. Target gene expression levels were normalised to the geometric mean of expression levels of four reference genes, *APT1* (At1g27450), *PDF2* (At1g13320), *bHLH* (At4g38070), and *PPR* (At5g55840). Gene
expression was measured just before sowing (“Dry” seeds) and then at: the end of seed
imbibition and stratification (germination stage I); 20 h later (stage II, testa rupture); and 52 h
later (stage III-G, endosperm rupture; seeds non-germinated yet, III-NG, were analysed
separately). Seeds were sampled within the cold room or growth room (dry seeds and stage I
to III, respectively), within 5 minutes from start to finish for each plate, and immediately
snap-frozen in liquid nitrogen. The experiment was repeated three times.

Statistical analysis
Statistical significance of results was analysed using the Statistix 9 software (Analytical
Software, Tallahassee, USA). For multivariate comparison of mucilage composition profiles,
discriminant Orthogonal Projected Latent Structure (OPLS) analysis was carried out using
the SIMCA software (Umetrics, www.umetrics.com) with salinity as a quantitative variable.

Results
The ERf controls the timing and pace of germination in response to changing salinity
and osmotic conditions
Loss of ER/ERL function had no effect on testa nor endosperm rupture on 0 mM NaCl
media, except in er erl1 seeds which showed a small but systematic lag in testa rupture (Fig.
1 and Supplementary Fig. S1A-B). That lag carried through to the next germination phase
leading to radicle protrusion. Salinity delayed germination in a dose-dependent manner
(Supplementary Fig. S2A, B), as expected, but with striking differences among lines (Fig. 1
and Supplementary Fig. S1C-D). Wild type (WT), erl1, erl2 and erl1 erl2 seeds germinated
first, ahead of er, er erl2, er erl1 and finally er erl1/seg erl2 seeds, due to both delayed testa
rupture and slower progression to endosperm rupture. Similar results were obtained with an
independent set of erf knock-out mutants carrying different erf null alleles (Supplementary
Fig. S3). This demonstrates that the observed genetic variations in seed germination are
causally related to disruption of the ERf genes. Similar germination kinetics were also
obtained regardless of whether seeds were challenged with salinity stress post-stratification or
directly from sowing (Fig. 2A-D). Strikingly, when exposed to 150 mM NaCl once
germinated, all genotypes displayed similar sensitivity to salinity stress (Fig. 2E). Together,
these data demonstrate a germination-specific function of the ERf in the sensing and
signaling of salinity stress. That function requires ER but involves the three family members,
in a non-totally redundant manner.

ERf expression during seed germination has not been reported. To investigate it, we
examined ERf promoter activity in transgenic seeds expressing proERf:GUS constructs
(Supplementary Fig. S4). ERf expression patterns did not appear to be influenced by salinity,
but differed among family members, with ERL2 expression seen only in the cotyledons and
the shoot apical meristem, while ER and ERL1 promoter activities were also detected in the
hypocotyl. Measurements of transcript abundance by RT-qPCR (Fig. 3) confirmed the
presence of ERf transcripts in dry seeds and showed a strong and early induction of ER and
ERL1 expression during stratification and imbibition (germination phase I), and the next
phase (stage II) leading to testa rupture, while ERL2 remained lowly expressed. Salinity
induced ER expression, especially during germination phase III, leading to radicle protrusion,
but had little influence on ERL1 or ERL2 expression. These results support a role of the ERf
throughout germination, with specificity among family members.

Salinity induces both osmotic and ionic stress (Munns and Tester, 2008). To investigate the
contributions of these two components, we next examined germination responses to
Polyethylene Glycol (PEG)8000 - a high molecular weight non-permeating osmoticum
mimicking drought-induced osmotic stress-, in the salinity hyper-sensitive mutants, er, er
erl1, er erl2 and er erl1/seg erl2. Under iso-osmotic external conditions (media osmotic
pressure, $\pi_e$), seed germination was significantly less inhibited by PEG than NaCl. Up to
0.50 MPa $\pi_e$ (equivalent to 100 mM NaCl), PEG was innocuous (Fig. 4A). When, however,
PEG was provided at higher concentrations raising $\pi_e$ to 0.74 and 0.99 MPa (iso-osmotic
conditions with 150 and 200 mM NaCl, respectively), germination was slowed down but to a
greater extent in the double and triple mutants than WT. Nevertheless, the germination delay
was mild, of the order of 1 day. By d3 post-stratification, germination was complete (WT, er,
er erl1 and er erl2 seeds) or near complete (er erl1/seg erl2 seeds, 90% germination), even
under 0.99 MPa (Fig. 4A-B), in contrast to the strong to total germination inhibition observed
under 200 mM NaCl, at the same $\pi_e$ (Fig. 4C, Supplementary Fig. S2A, B). Only at much
higher PEG concentrations was as severe an inhibition observed, but some seeds still
germinated (Fig. 4B). Taken together, these data indicate that 1) in the germination-
permissive range of NaCl concentrations, the ERf modulates seed germination sensitivity to
salinity mostly via interactions with NaCl ionic effects; 2) however, the ER is also involved in the control of germination sensitivity to osmotic and hyper-osmotic stress. The NaCl-hypersensitive erf mutants also exhibited increased sensitivity to KCl, but to a much lower extent than to NaCl under iso-osmotic conditions (Fig. 5). This result indicates that the ERf function in seed germination under salinity predominantly relates to effects of the sodium ion.

Notably, while all or the vast majority of WT, erl1, erl2, erl1 erl2 seeds plated on NaCl medium eventually germinated (90 to 100%, similar to salt-free media), a significant proportion of er, er erl1, er erl2 and er erl1/seg erl2 seeds failed to do so, even after a lengthy incubation period (Fig. 6; Supplementary Fig. S1). Among those, a majority (up to 70%) did not even exhibit testa rupture. To test whether these were damaged or dead seeds, we transferred them to NaCl-free media. Most germinated readily, within 20-25 h (Fig. 6), bringing the final percentage of germinated seeds to similar levels as those observed for seeds never exposed to salt. Failure to germinate on saline media was thus not due to irreversible cellular damage and loss of seed viability, but rather to a slower or halted progression of the germination process. Consistent with their maintained viability and fast germination upon salinity stress release, seeds with arrested germination on salty media showed similar ERf expression levels as germinated seeds (ERL1 and ERL2 genes) or even higher (ER), (Fig. 3, comparison of III-NG to III-G seeds).

The ERf affects the ABA and GA regulation of seed germination

Salinity and osmotic stress promote ABA signalling and biosynthesis during germination (Seo et al., 2006; Piskurewicz et al., 2008; Yuan et al., 2010). ABA is a strong inhibitor of seed germination. To test whether the ERF-mediated differences in germination sensitivity to salinity stress are ABA-related, we compared germination kinetics of WT and erf seeds in the presence of ABA. ABA treatment consistently had a mild delaying effect on testa rupture, which was most pronounced for er erl1 seeds (Fig. 7A). ABA strongly inhibited endosperm rupture also in an ERF-dependent manner (Fig. 7B). er erl1 seed germination was the most sensitive to ABA, lagging behind WT even in the 1µM ABA range. Under higher ABA concentrations, seeds of the other two salt-hypersensitive mutants, er erl2 and er erl1/seg erl2, but not er, also separated from WT, showing enhanced ABA sensitivity. Interestingly,
so did the salinity non-hypersensitive erl1 erl2 seeds (Fig. 7B). These data indicate the involvement of both ABA-dependent and ABA-independent pathways in the ERf-mediated sensitivity of seed germination to salinity.

The germination inhibiting effect of ABA is antagonised by GAs (Koornneef et al., 1982; Holdsworth et al., 2008; Weitbrecht et al., 2011; Liu et al., 2016). Rather than the absolute levels of these hormones, the ABA/GA balance is key to the commitment of seeds to germinate. The DELLA RGL2 protein plays a pivotal role in the cross-talk between ABA and GA signalling in the imbibed seed. RGL2 acts as the main GA signalling repressor through activation of a number of transcriptional regulators, including ABI3 and ABI5, the central effectors of ABA signalling, establishment of dormancy, and repression of seed germination (Lopez-Molina et al. 2001, 2002; Lee et al., 2002; Piskurewicz et al. 2008, 2009; Liu et al. 2016). ABI3 and ABI5 are also involved in the regulation of early seedling growth arrest under water stress in Arabidopsis (Lopez-Molina et al. 2001; 2002), and in the reversible inhibition of germination in related E. salsugineum under salinity (Kazachkova et al., 2016).

To better understand the interaction of the ERf with the ABA regulation of seed germination we monitored the expression of ABI3, ABI5 and RGL2 in WT and er erl1/seg erl2 seeds during germination, and also of DELAY OF GERMINATION1 (DOG1), a pivotal seed dormancy gene which genetically interacts with ABI3 and with a central type 2C protein phosphatase of the ABA signalling pathway during germination, and also regulates ABI5 expression (Dekkers et al. 2016; Née et al., 2017; Nishimura et al. 2018). Constitutive gene expression levels were similar in WT and mutant seeds. Salinity systematically caused an up-regulation of gene expression, but that was stronger in er erl1/seg erl2 seeds than WT (Fig. 7C). This result indicates that the ERf-mediated signalling cascade of salinity interacts with the ABA-GA signalling network of germination and dormancy. We also examined the expression of ABA and GA biosynthetic genes - ABA2 and NCDE4; GA3OX1 and GAOX2- respectively. None showed a differential response to salinity between mutant and WT (Supplementary Fig. S5).

The role of the ERf in seed germination partly overlaps with a role in seed size and is primarily maternally controlled

Seed germination occurs when the pressure exerted by the turgid expanding embryo radicle overcomes the mechanical resistance of the surrounding testa and micropylar endosperm.
(Linkies et al., 2009; Nonogaki, 2014). As er erl1 erl2 mature embryos have smaller
cotyledons (Uchida et al., 2013), we reasoned that reduced growth potential could be a factor
in the delayed radicle emergence observed in that mutant and possibly the other salt-
hypersensitive er, er erl1, er erl2 mutants under salinity and osmotic stress. As a first step to
examine this, we measured seed size as a surrogate for embryo size, since the Arabidopsis
embryo occupies most of the seed volume. er, er erl1, er erl2 and er erl1/seg erl2 seeds, i.e.
all salt-hypersensitive seeds, were significantly smaller than WT or erl1 and erl2 seeds, and
even smaller than erl1 erl2 seeds which were larger than WT (Fig. 8A). These data uncover a
function of the ERf in seed size determination. They also suggest a link between the ERf
function in germination sensitivity to salinity and its influence on seed size. However, the fact
that erl1 erl2 seeds germinate simultaneously with WT seeds in the presence or absence of
salt despite their significant seed size difference, indicates that the link is not absolute.

We next considered the possibility of developmental defects in the smaller, salinity
hypersensitive erf seeds. As expected, homozygous er erl1 erl2 segregants displayed
reduced, rounder cotyledons and a broader shoot apical meristem, as previously reported
(Uchida et al. 2013). However, their hypocotyl and embryonic root were similar to WT, in
length, number and size of constitutive cells (Supplementary Fig. S6).

Seeds reserves are essential for successful germination, and in Arabidopsis are mostly stored
in cotyledons. Smaller seeds and cotyledons suggest less reserves, which could be responsible
for hypersensitivity to salinity and osmotic stress. To examine this, we quantified fatty acid
methyl esters (FAMES) derived from embryo lipids, which constitute the major fraction of
Arabidopsis seed reserves (Penfield et al., 2004; Lionen and Schwender, 2009). There was no
significant genetic difference across the range of genotypes, except for er erl1/seg erl2 seeds
(15% decrease) and thus, apart from that genotype, no correlation with germination
sensitivity to salt (Supplementary Fig. S7A). The relative proportions of FAMES species
were also similar across genotypes (Supplementary Fig. S7B-C). Taken together, these results
indicate that the delayed or arrested germination of er, er erl1, er erl2 and er erl1/seg erl2
seeds on saline media was not likely due to reduced embryo size and growth potential per se.

Germination involves complex communication between embryo, seed coat, and intermediate
devosperm—a one cell thin layer in the Arabidopsis seed. We therefore next considered a role
of the ERf on seed germination via effects on the embryo surrounding tissues. To investigate
that, we took advantage of the different contributions of the maternal and paternal genomes to
the genetic make-ups of the three seed compartments (seed coat ♀♀, endosperm ♀♀♂, embryo ♀♂♂) and performed reciprocal crosses between WT and the salt-hypersensitive er
erl1 or er erl2 mutants. These generated F1 seeds with same embryo genotype, but either WT
or mutant seed coat, and predominantly WT or mutant endosperm. The two groups of F1
seeds germinated synchronously on NaCl-free media, but according to significantly different
kinetics when challenged with salinity stress (Fig. 8C). Remarkably, for each cross, F1 seed
germination occurred synchronously with seeds of the maternal parent. This result
demonstrates that the function of the ERf in the regulation of germination sensitivity to
salinity is primarily maternally controlled and mediated by the embryo-surrounding tissues,
in particular the seed coat. Supporting this, when excised from their covering layers, “naked”
er erl1, er erl2 and er erl1 erl2 mature embryos grew at similar rates as WT embryos,
whether cultured with or without salt (Fig. 8B). F1 seeds also clustered with their maternal
parent on seed size (Fig. 8D), showing the ERf effect on seed size is of maternal origin too,
and strengthening the case for overlap of the ERf-dependent controls of seed size and
germination response to salinity.

The ERf-mediated regulation of seed germination involves the seed coat mucilage

Considering what properties of the seed coat the ERf might control to influence germination
in a salinity-dependent manner we first tested for a role in seed coat permeability. To that
end, seeds were incubated in tetrazolium red, a cationic dye classically used to detect seed
coat defects and abnormal permeability (Wharton, 1955; Molina et al., 2008). Similar
staining and tetrazolium salt reduction rates were observed across lines, except for significant
increases in er erl1 and to a small extent in erl1 seeds (Fig. 9A), suggestive of increased seed
coat permeability or NADPH-dependent reductase activity in these two mutants. We thus
next measured seed sodium contents after 24 h stratification with or without salt. They
showed no significant genetic variation (Fig. 9B). These results indicate that the observed
differential germination response to salt among erf seeds cannot be ascribed to differences in
seed coat permeability and accumulation of sodium ions per se.

During seed coat differentiation on the mother plant, the specialised epidermal cells secrete
mucilage polysaccharides that line their inner walls and build a central volcano-shaped
columella (Beeckman et al., 2000; Western et al., 2000; Haughn and Western, 2012). Upon
hydration, the desiccated, highly hydrophilic mucilage rapidly swells and ruptures the
cellulotic rays radiating from the columella. Mutant seeds affected in mucilage synthesis or
extrusion have been reported to be more sensitive to low water potential during germination
(Penfield et al., 2001; Yang et al., 2010). This prompted us to next examine mucilage release
by WT and erf seeds upon imbibition. We collected the loosely adhering mucilage which can
easily be detached from the seed surface, as opposed to the inner, cell wall-bound fraction.
Large genetic variation was observed in the amounts recovered, but that scaled with genetic
variation in seed size (Fig. 9C). Salinity caused a large increase in mucilage extrusion, but of
similar proportion in all genotypes, resulting in a simple translation of the relationship
observed on control media. Consistently, mucilage staining with ruthenium red, a classic dye
that binds pectins, showed a thicker and often darker mucilage halo under saline than control
conditions, but with no indication of variation among genotypes within each treatment
(Supplementary Fig. S8A, B).

Recent studies suggest the importance for germination of the mucilage physico-chemical
properties and attachment to the seed rather than simply its amount (Rautengarten et al.,
2008; Saez-Aguayo et al., 2013). We thus next analysed mucilage composition. The expected
sugars were detected, mostly rhamnose and galacturonic acid (GalUA) derived from
rhamogalacturonans type I (RG I) - the major pectin of the Arabidopsis seed mucilage
(Macquet et al., 2007; Arsovsky, 2009) - and low amounts of other neutral and acidic sugars,
derived from RGI-s side chains (Supplementary Fig. 8C). When analysed individually, these
sugars showed no statistically significant variations among genotypes. However, examination
of compositional profiles by multivariate analysis suggested genetic variation in the relative
abundance of backbone sugars (Rhm and GalUA) and some side-chain sugars (Xyl and Gal),
leading us to compare their ratios across the full spectrum of lines (Fig. 9D). This revealed
dramatically increased GalUA/Gal ratios in er erl1, er erl2 and er erl1/seg erl2 mucilage
compared to WT and other lines (P=0.027), and a trend for higher rhamnose to xylose ratios
in mutant mucilage other than erl1 erl2, especially in er erl1 and er erl1/seg erl2 mucilage
(P=0.08). These results suggest that the ERf plays a role in the control of mucilage
composition and architecture via interactions with the mechanisms controlling the abundance
of carboxyl sites - i.e. of potential sites for pectin cross-linking - and perhaps also pectin
branching. Moreover, they indicate a link between such a role and the ERf function in the
regulation of seed germination.
To test this and probe causality, we took an indirect, holistic approach, and compared the germination kinetics of intact seeds and demucilaged seeds, deprived of the shell of loosely adherent mucilage extruded during imbibition. Demucilaged seeds systematically germinated more slowly than intact seeds on salt-free media (Fig. 9E), as is common. Under saline conditions, this was also the case for WT, erl1, erl2 and erl1 erl2 seeds but, strikingly, in er erl1, er erl2 and er erl1/seg erl2 seeds, mucilage removal had the opposite effect: germination delay behind WT seeds was reduced, with radicle protrusion lagging by 23 h, 13 h and 39 h behind WT, respectively, instead of 68 h, 39 h, and 153 h, respectively, for intact seeds (Fig. 9E). This reflected faster progression from testa rupture to endosperm rupture. These data demonstrate a critical role of the seed water soluble mucilage in mediating the salinity-dependent function of the ERf in controlling the completion of seed germination.

Although appearing as distinct layers upon imbibition, mucilage and cell walls are tightly bound. The suberised seed coat and underlying endosperm constitute a mechanically strong barrier that needs to be weakened to enable radicle emergence. The micropylar endosperm that surrounds the radicle tip is thought to be the major source of mechanical resistance to radicle protrusion (Linkies et al., 2009; Dekkers et al., 2013). Endosperm weakening is effected by cell wall-modifying enzymes, in interaction with ROS and hormonal signals from the embryo, GA especially (Finch-Savage and Leubner-Metzger 2006; Muller et al., 2006; Penfield et al., 2006). We thus hypothesised that the importance of the mucilage and seed coat in mediating delayed or arrested germination in the er, er erl1, er erl2 and er el1/seg erl2 mutants on saline media, could in part be related to ERf-dependent differences in endosperm and seed coat mechanical properties. The Arabidopsis seed is too small for direct measurements of testa and endosperm rupture forces, as is possible in other species (Linkies et al., 2009), leading us to instead examine the expression of the Arabidopsis TOUCH (TCH) gene TCH3, which encodes a calmodulin-like protein and is greatly up-regulated in response to a range of mechanical signals in other tissues (Braam and Davis, 1990). Comparison of TCH3 expression in WT and er erl1/seg erl2 seeds (Fig. 9F) showed the presence of transcripts in dry seeds, at similar, low levels. Imbibion triggered de novo TCH3 transcription on 150 mM NaCl media in both WT and erf mutant seeds, consistent with the known role of calcium in salinity signalling (Munns and Tester, 2008). Remarkably, that induction was significantly enhanced in er erl1/seg erl2 seeds, and was transient, preceding testa and then disappearing. These data are suggestive of enhanced mechanical constraint imposed on er erl1/seg erl2 than WT embryos before endosperm rupture. On control media,
de novo TCH3 transcription did not occur before the final phase of germination phase; again it was enhanced in er erl1/seg erl2 seeds compared to WT.

Discussion

Plant propagation, dispersion, ability to compete and yield all ultimately rely on viable seeds being produced and able to germinate at a time favourable to autotrophic growth and establishment of a new seedling. During development on the mother plant, following embryogenesis and acquisition of dormancy during maturation, the seed undergoes intense dehydration and the embryo becomes quiescent. Germination brings that embryo from a highly resilient to a highly vulnerable state, in direct contact with the outer environment, and to a point of no return. How seeds monitor conditions in their immediate surrounding to optimise the timing of germination initiation and its completion is mostly unknown. In this study, we show that the Arabidopsis ERECTA family acts to control the timing of seed germination according to external salinity and osmotic levels (Fig. 1; Fig. 4). Loss of ER, or of ER and its paralogs slows down germination or even prevents it under increasing salinity and osmotic stress, while not compromising seed viability, as germination readily resumes upon the return of favourable conditions (Fig. 6). The ERf-mediated sensing of changing salinity levels involves interactions with the ABA-GA signalling network of germination and dormancy, and is primarily controlled by the embryo surrounding endosperm and testa, with a critical role of the latter and its mucilage (Fig. 8; Fig. 9). These findings reveal unsuspected regulators of the interactions between the seed and its environment, and a novel function of the three ERf receptor-like kinases in controlling these interactions, and cryptic genetic variation in seed germination.

The ERECTA gene family regulates seed germination on salt, via maternally controlled effects on seed coat enlargement and mucilage properties

The seed coat derives from the maternal ovule integuments which, following fertilisation, expand and undergo profound developmental and biochemical transformations, resulting in a highly differentiated, impermeable and mechanically strong tissue (Beeckmann et al. 2000; Western et al. 2000). The mucilage is secreted and deposited in its outer, epidermal layer, concomitantly with embryo morphogenesis, following cessation of integument expansion.
(reviewed by Haughn et al., 2012; North et al., 2014). Its physiological roles have remained elusive. Apart from anchoring the imbibed seed to its physical substrate, the gelatinous mucilage is generally thought to facilitate germination, especially under osmotic stress, through sequestering water and keeping the seed hydrated (Penfield et al., 2001; Arsovski et al., 2010; Yang et al., 2010). However, several studies suggest mucilage can also inhibit germination under unsuitable conditions, perhaps through limiting water and oxygen diffusion to the embryo (Western et al., 2012 and references herein). This study sheds some light on the ill-understood genetic control of the context-dependent role of the seed mucilage on germination, revealing that the ERf are key players. We observed a promoting role of the seed mucilage on germination speed in WT, erl1, erl2 and erl1 erl2 seeds, under both saline and non-saline conditions (Fig. 9E). However, in the salt-hypersensitive er erl1, er erl2, and er erl1/seg erl2 seeds, that role was only expressed under control conditions. Under salinity, it was lost (er erl1, er erl2 seeds) or even reversed (triple mutant). These results link, for the first time, the seed mucilage and the ER pathway in the germination response to environmental variations at the seed surface.

How could the ERf control salinity-dependent properties of the seed mucilage regulating the germination process? The seed mucilage is alike a pectin-rich secondary cell wall (Haughn and Western, 2012). The degrees of pectin branching and cross-linking, with calcium ions in particular, are known to greatly influence pectins’ hydrophilicity, adsorption to cellulose microfibrils and partitioning between loose outer mucilage and adherent inner mucilage (Willats et al., 2006; North et al., 2014; Ralet et al., 2016). It is also well-established that the small monovalent Na⁺ ions have the capacity to easily displace the larger divalent Ca²⁺ ions that cross-link carboxyl residues of adjacent pectin molecules (Fry, 1986; Willats et al., 2006; Ghanem et al., 2010), thus leading to a looser, more hydrophilic mucilage upon imbibition with saline than salt-free water, and also more abundant (Fig. 9C; Ghanem et al. 2010) due to increased release of pectin molecules from the cellular matrix. We propose that the enrichment of er erl1, er erl2, and er erl1/seg erl2 seed mucilage in uronic acids - hence potential sites for Ca²⁺.Na⁺ exchange- and trend to reduced xylose content relative to backbone rhamnose suggestive of altered branching (Fig.9D), thus have the potential to significantly modify a) the seed mucilage and sub-tending wall swelling properties and changes in osmotic potential, conformation and rigidity upon imbibition with a saline or high osmolarity solution (Willats et al., 2006; Ghanem et al., 2010; Ralet et al., 2016); b) the rearrangement of mucilage and wall components as pectin molecules get released.
(Rautengarten et al., 2008); and c) perhaps also free Ca$^{2+}$ influx to the adjoining inner endosperm and embryo; so d) as a whole, the chemical and mechanical interactions between the seed environment, seed coat and interior compartments.

The erf seeds with enhanced sensitivity to salt and hyperosmotic stress during germination are also smaller (Fig. 8A). Arabidopsis seed size is controlled by complex interactions of zygotic and maternal factors, and seed integuments-endosperm inter-signalling (Garcia et al., 2003; Luo et al., 2005; Day et al., 2008; Dilkes et al., 2008; Zhou et al., 2009; Wang et al., 2010; Jiang et al., 2013). Here, our reciprocal crosses show that variation in final seed size among erf mutants and WT is of maternal origin (Fig. 8D). Final seed size is reached early in seed development, through a first phase of active cell proliferation triggered by fertilisation, in both the integuments and the endosperm, followed by a period of mostly cell expansion. Expansion ceases five to six days post-anthesis, concomitantly with the endosperm switching from syncitial development to cellularisation (Garcia et al. 2005), and the start of starch and mucilage synthesis. Variation in maximum cell elongation appears to be the main driver of maternal variations in final seed cavity and seed size as observed here, through a so-called ‘compensatory” growth mechanism (Garcia 2005). The ERECTA gene has been implicated in such compensatory mechanism between cell number and cell size in leaves (Ferjani et al. 2007), and comparison of the seed epidermis of the Ler and Columbia accessions suggests “compensation” may take place in the seed integuments too (Garcia et al. 2005).

Interestingly, the progression and completion of integument growth during ovule development was previously reported to require a minimum ERf signalling (Pillitteri et al., 2007). That requirement was ascribed to a role of the ERf in cellular proliferative activity through interactions with cell cycle regulators. However, the final cell number in the mature ovule was unchanged making it unlikely that the reduced seed size cavity and less expanded seed coat observed here in the er, er erl1, er erl2 and er erl1/seg erl2 seeds (Fig. 8A) are predetermined prior to fertilisation. Moreover, no ovule integument growth defect was reported in ovule integments other than er erl1 erl2+/. Here, loss of ER alone was sufficient to cause reduced seed size, and further loss of ERL1 or ERL2 had only a small or no significant additional inhibitory effect; and, when occurring in an ER background, loss of ERL1 and ERL2 instead caused an increase of seed size beyond that in WT (Fig. 8A). This supports the idea that partly different mechanisms are involve in ERF-mediated control of seed size and germination sensitivity to salinity. Given the role of the ERf in the composition of the mucilage (Fig. 9D) and the reported increases in uronic acids and cellulose in leaves of two
er mutants (Sánchez-Rodríguez et al., 2009), a tentalising hypothesis is that the ERf may regulate cell wall formation and assembly, not only during mucilage and secondary cell wall deposition, but also prior to that, during seed coat enlargement and formation of the seed cavity. That proposition would provide a unifying explanation for a link between the ERf-mediated regulation of seed size, salinity-dependent mucilage properties and germination speed, as uncovered by this study.

**ERf-mediated salt signalling in germinating seeds involves a complex regulatory network**

The *Arabidopsis* seed coat is in immediate contact with the one-cell thin endosperm, itself in direct contact with the embryo. Although less well-documented than in humans, there are demonstrated cases of plant membrane receptors or mechano-sensitive channels’ ability to monitor cell wall integrity, membrane and wall physical interactions, deformation and rheology (Hamann et al., 2012; Monshausen and Haswell, 2013; Hamilton et al., 2015; Haswell and Verslues, 2015). The ERf proteins belong to XIII Leucine-Rich Repeats Receptor-Kinases. Most interestingly, among its other four members, that class includes FEI1 and FEI2 (Shiu et al., 2004) which were recently shown to interact with an arabinogalactan protein in mediating a salt-overly sensitive root and seed adherence mucilage phenotype (Harpaz-Saad et al., 2011; Griffiths et al., 2014). In addition, based on the analysis of disease resistance in two *er* mutants, the ER protein has been suspected of interacting with wall-associated kinases (WAKs) during defence against some pathogens, via effects on cell wall composition (Sánchez-Rodríguez et al., 2009). WAKs are known to be tightly bound to pectins, galacturonic acids especially, in a Ca²⁺-dependent manner (Wagner and Kohorn, 2001; Decreux and Messiaen, 2005), and several WAK/WAK-Like proteins have been implicated in responses to mineral ions, including Na⁺ (Sivaguru et al., 2003; Hou et al., 2005; de Lorenzo et al., 2009) and to osmotic stress (SOS6/AtCSLD5, Zhu et al., 2010), through unknown mechanisms. ERf-mediated modifications of mucilage and bound cell walls may thus be perceived and signalled to the seed interior by the *ERf* proteins themselves either directly or through modified interactions with cell wall-associated proteins, osmo-sensors or mechano-sensors (Dekkers, et al. 2013; Nonogaki, 2014). The induction of *TCH3* in the *er erl1/seg erl2* seed supports this hypothesis.
It will be intriguing to unravel the downstream cascade. The salt-hypersensitive er erl1, er erl2, er erl1/seg erl2 seeds show enhanced sensitivity to exogenous ABA, and enhanced upregulation of ABI3, ABI5 and RGL2 under saline conditions compared to wild type (Fig. 7). ABI3, ABI5, RGL2 are emerging as important mediators of salinity and osmotic stress and controllers of ABA-GA homeostasis in imbibed seeds. ABA synthesised in the endosperm and released to the embryo activates the abundance and activity of the ABI3 and ABI5 transcription factors, and triggers an auto-feedback loop that maintains RGL2 mRNA levels high and represses cell wall modifying enzymes (Giraudat et al., 1992; Finkelstein and Lynch, 2000; Lee et al., 2002; Lopez-Molina et al., 2001 & 2002; Piskurewicz et al., 2008; Piskurewicz et al., 2009; Lee et al. 2010; Kang et al. 2015). Our data indicate that the ERf-mediated regulation of germination sensitivity to changing salinity levels interferes with that signalling loop.

A well-documented adaptive mechanism seeds have evolved to withstand unfavourable conditions such as high temperatures, cold, osmotic or salinity stress, and maintain embryo viability is secondary dormancy (Bewley, 1997) - a reversible, transient quiescent state induced and released in adaptation to fluctuating environmental conditions (Koornneef et al., 1982; Giraudat et al., 1992; Léon-Kloosterziel et al., 1996; Finch-Savage and Leubner-Metzger, 2006; Lefebvre et al., 2006; Weitbrecht et al., 2011; Ibarra et al., 2016). ABI3, ABI5 and RGL2 are prominent players in the regulation of secondary dormancy and increased sensitivity to ABA, upregulation of ABI3, ABI5 and RGL2 have been reported during early growth arrest in newly germinated Arabidopsis seedlings under water stress and salinity (Lopez-Molina et al., 2001; 2002). Here we find that loss of the ERf sensitises seed germination to salinity and frequently arrests it, and that this arrest is reversible, with germination readily resuming upon stress release and progressing to completion as fast as in seeds never exposed to stress (Fig. 6). Moreover, arrested seeds show an upregulation of the DOG1 gene (Fig. 7), a major controller of coat- and endosperm-mediated dormancy as takes place in the Arabidopsis seed. DOG1 interacts with GA and ABA signalling, upstream of ABI5, and appears to be an agent of environmental adaptation of germination among Arabidopsis accessions (Graeber, 2014; Dekkers et al., 2013; Née et al., 2017; Nishimura et al. 2018). Taken as a whole, these observations suggest that the ERf interacts with the molecular controls of secondary dormancy to appropriately cue and pace germination. While promotion of fast germination under stress may be seen as desirable, it also exposes the newly germinated seedling to risks of death should adverse conditions persist or worsen as
the embryo becomes directly exposed to the external environment with all its reserves already burnt. In such circumstances, germination delay or arrest could then be a useful protective strategy to maximise chances of survival through temporarily safeguarding the embryo against such a fate. In that light, the environment-dependent function of the ERf on germination speed would perform a vital adaptive function. Interestingly, only under extremely severe stress (~200 mM NaCl) does the loss of ER and ERL1 and/or ERL2 cause germination arrest in absolutely all seeds within a cohort. Under milder stress, some seeds do germinate at the same time as WT, others with increasing delay, and others are arrested until stress release, a mixed response that may balance risks of death and loss of fitness or ability to complete the life cycle in time.

In conclusion, plants must be endowed with a “surveillance” system for the perception and transduction of external environmental cues to internal compartments, and their integration with developmental pathways. This study illuminates a key role of the ERf in that elusive integrative network in seeds, to control the most critical decision in the cycle of life, when to initiate a new plant. Given the evolutionary conservation of the ERECTA receptor-kinases across a broad range of plant species, and the emerging intense interest in mucilage as a model for cell wall studies and an important adaptive feature, our findings open new avenues for unravelling the mechanisms seeds have evolved to control germination and tune it to local conditions for maximising chances of survival.

Gene accession numbers
AtER (At2g26330), AtERL1 (At5g62230), AtERL2 (At5g07180), (At4g37490), AtPDF2 (At1g13320), bHLH (At4g38070), PPR (At5g55840)

Supplementary Data
Supplementary Figure S1. NaCl-dependent effects of reduced ERf signaling on the time course of seed germination.
Supplementary Figure S2. Loss of ER alone or in combination with ERL1 and ERL2 sensitises seed germination to salinity in a dose-dependent manner.
Supplementary Figure S3. ERf-dependent sensitivity of seed germination to NaCl in an independent set of erf single, double and triple knock-out mutants.

Supplementary Figure S4. ERf promoter activity in mature dry seeds and germinating seeds.

Supplementary Figure S5. Similar sensitivity to salinity stress of selected ABA and GA biosynthetic gene expression levels in WT and erl1/seg erl2 seeds.

Supplementary Figure S6. Mature erl1 erl2 embryos exhibit similar radicle size and patterning than WT seeds.

Supplementary Figure S7. Relative abundance of total fatty acid methyl-esters (FAMES) and relative proportions of individual species in embryos at full seed maturity.

Supplementary Figure S8. Characteristics of the seed mucilage.

Supplementary Table S1. List of genotyping and RT-qPCR primers.

Acknowledgments
We thank Josephine Ginty and Kefan Peng for assistance with seed permeability assays and quantitative RT-PCR, respectively; Richard Phillips for help with measurements of seed ion contents; Guillaume Tcherkez for discussions on metabolites multivariate analysis; Keiko Torii for seeds of proERf:GUS reporter lines; the Nottingham Arabidopsis Stock Centre and SALK-Institute for mutant seeds; the Australian National University for funding.

References
Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., et al. (2003). Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science, 301:5633, 653–657.

Alvarado, V., Bradford, K. J. (2002). A hydrothermal time model explains the cardinal temperatures for seed germination. Plant, Cell and Environment, 25, 1061-1069.

Arsovski, A.A., Popma, T.M., Haughn, G.W., Carpita, N.C., McCann, M.C., Western, T.L. (2009). AtBXL 1 Encodes a bifunctional bD-Xylosidase / a-L-Arabinofuranosidase
required for pectic arabinan modification in Arabidopsis mucilage secretory cells. Plant Physiology, 150(3), 1219-1234.

Arsovski, A. A., Haughn, G. W., Western, T. L. (2010). Seed coat mucilage cells of Arabidopsis thaliana as a model for plant cell wall research. Plant Signaling and Behavior, 5:7, 796–801.

Bailly, C. (2004). Active oxygen species and antioxidants in seed biology. Seed Science Research, 14:2, 93–107.

Baskin, J. M., Baskin, C. C. (2004). A classification system for seed dormancy. Seed Science Research, 14:1, 1–16.

Becraft, P. W. (2002). Receptor kinase signaling in plant development. Annual Review of Cell and Developmental Biology, 18:1, 163-192.

Beeckman, T., De Rycke, R., Viane, R., Inzé, D. (2000). Histological study of seed coat development in Arabidopsis thaliana. Journal of Plant Research, 113:1110, 139–148.

Bemis, S. M., Lee, J. S., Shpak, E. D., Torii, K. U. (2013). Regulation of floral patterning and organ identity by Arabidopsis ERECTA-family receptor kinase genes. Journal of Experimental Botany, 64:17, 5323–5333.

Bewley, J. D. (1997). Seed germination and dormancy. The Plant Cell, 9(7), 1055–1066.

Bewley, J. D., Bradford, K., Hilhorst, H. (2013). Seeds: physiology of development, germination and dormancy - J.D. Bewley, K. Bradford, H. Hilhorst, H. Nonogaki eds. Springer. Chapter 4.

Boyer, J.S. (1982). Plant productivity and environment. Science, 218, 443-448.

Braam, J., Davis, R.W. (1990). Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell, 60:3, 357-364.

Bradford, K.J. (1990). A water relations analysis of seed germination rates. Plant Physiology, 94:2, 840-849.

Bundy, M. G. R., Thompson, O. A., Sieger, M. T., Shpak, E. D. (2012). Patterns of cell division, cell differentiation and cell elongation in epidermis and cortex of Arabidopsis pedicels in the wild type and in erecta. PLoS ONE, 7:9, e46262.

Clerkx, E. J. M., El-Lithy, M. E., Vierling, E., Ruys, G. J., Vries, H. B.-D., Steven P.C. Groot, et al. (2004). Analysis of natural allelic variation of arabidopsis seed germination and seed longevity traits between the accessions Landsberg erecta and Shakdara, using a new recombinant inbred line population. Plant Physiology, 135, 432-443.

Day, R.C., Herridge, R.P., Ambrose, B.A., Macknight, R.C. 2008. Transcriptome analysis of proliferating Arabidopsis endosperm reveals biological implications for the control of
syncytial division, cytokinin signalling, and gene expression regulation. Plant Physiology, 148, 1964–1984.

de Lorenzo, L., Merchan, F., Laporte, P., Thompson, R., Clarke, J., Sousa, C., Crespi, M. (2009). A novel plant leucine-rich repeat receptor kinase regulates the response of Medicago truncatula roots to salt stress. The Plant Cell, 21:2, 668–680.

Debeaujon, I., Koornneef, M. (2000). Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiology, 122, 415-424.

Debeaujon, I., Koornneef, M. (2000). Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiology, 122, 415-424.

Decreux, A., Messiaen, J. (2005). Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. Plant and Cell Physiology, 46(2), 268–278.

Dekkers, B. J. W., Pearce, S., van Bolderen-Veldkamp, R. P., Marshall, A., Widera, P., Gilbert, J., et al. (2013). Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. Plant Physiology, 163, 205-215.

Dekkers B.J., He H., Hanson J., Willems L.A.; Jamar D.C.; Cueff G.; Rajjou L.; Hilhorst H.W., Bentsink L. (2016). The Arabidopsis DELAY OF GERMINATION 1 gene affects ABSCISIC ACID INSENSITIVE 5 (ABI5) expression and genetically interacts with ABI3 during Arabidopsis seed development. Plant Journal, 85:4, 451-465.

DeRose-Wilson, L., Gaut, B. S. (2011). Mapping salinity tolerance during Arabidopsis thaliana germination and seedling growth. PLoS ONE, 6:8, e22832.

Dilkes, B.P., Spielman, M., Weizbauer, R., Watson, B., Burkart-Waco, D., Scott, R.J., Comai, L. 2008. The maternally expressed WRKY transcription factor TTG2 controls lethality in interploidy crosses of Arabidopsis. PLoS Biology, 6, 2708-2720.

Donohue, K., de Casas, R. R., Burghardt, L., Kovach, K., Willis, C.G. (2010). Germination, postgermination adaptation, and species ecological ranges. Review of Ecology, 41:1, 293–319.

Etchells, J. P., Provost, C. M., Mishra, L., Turner, S. R. (2013). WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. Development, 140:10, 2224–2234.

Ferjani, A., Horiguchi, G., Yano, S., Tsukaya, H. (2007). Analysis of leaf development in fugu mutants of arabidopsis reveals three compensation modes that modulate cell expansion in determinate organs. Plant Physiology, 144, 988-999.

Finch-Savage, W. E., Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. The New Phytologist, 171:3, 501–523.

Finkelstein, R. R., Lynch, T. J. (2000). The arabidopsis abscisic acid response gene ABI5
encodes a basic leucine zipper transcription factor. The Plant Cell, 12(4), 599–609.

Finkelstein, R., Reeves, W., Ariizumi, T., Steber, C. (2008). Molecular aspects of seed dormancy. Annual Review of Plant Biology, 59, 387–415.

Fry, S. C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annual Review of Plant Physiology. 37, 165-186.

Galpaz, N., Reymond, M. (2010). Natural variation in Arabidopsis thaliana revealed a genetic network controlling germination under salt stress. PLoS ONE, 5:12, e15198.

Garcia, D., Saingery, Chambrier, P., Mayer, U., Jürgens, G., Berger, F. (2003). Arabidopsis haiku mutants reveal new controls of seed size by the endosperm. Plant Physiology, 131:1661–1670.

Garcia, D., Gerald, J.N.F., Berger, F. (2005). Maternal control of integument cell elongation and zygotic control of endosperm growth are coordinated to determine seed size in Arabidopsis. Plant Cell, 17, 52–60.

Ghanem, M. E., Han, R. M., Classen, B. (2010). Mucilage and polysaccharides in the halophyte plant species Kosteletzkya virginica: Localization and composition in relation to salt stress. Journal of Plant Physiology, 167, 382-392.

Giraudat, J., Hauge, B. M., Valon, C., Smalle, J., Parcy, F., Goodman, H. M. (1992). Isolation of the Arabidopsis ABI3 gene by giositional gloning. The Plant Cell, 4:10, 1251-1261.

Godiard, L., Sauviac, L., Torii, K. U., Grenon, O., Mangin, B., Grimsley, N. H., Marco, Y. (2003). ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. Plant Journal, 36:3, 353–365.

Graeber, K., Linkies, A., Steinbrecher, T., Mummenhoff, K., Tarkowská, D., Turečková, V., et al. (2014). DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. Proceedings of the National Academy of Sciences of the United States of America, E3575-3580.

Griffiths, J. S., Tsai, A. Y.-L., Xue, H., Voiniciuc, C., Sola, K., Seifert, G. J., et al. (2014). SALT-OVERLY SENSITIVE5 mediates Arabidopsis seed coat mucilage adherence and organization through pectins. Plant Physiology, 165:3, 991–1004.

Hamann, T. (2012). Plant cell wall integrity maintenance as an essential component of biotic stress response mechanisms. Frontiers in Plant Science, 3, 77.

Hamilton, E. S., Schlegel, A. M., Haswell, E. S. (2015). United in diversity: mechanosensitive ion channels in plants. Annual Review of Plant Biology, 66, 113–137.
Hanke, D. E., Northcote, D. H. (1975). Molecular visualization of pectin and DNA by ruthenium red. Biopolymers, 14:1, 1–17.

Harpaz-Saad, S., McFarlane, H. E., Xu, S., Divi, U. K., Forward, B., Western, T. L., Kieber, J. J. (2011). Cellulose synthesis via the FEI2 RLK/SOS5 pathway and cellulose synthase 5 is required for the structure of seed coat mucilage in Arabidopsis. The Plant Journal, 68:6, 941–953.

Haswell, E. S., Verslues, P. E. (2015). The ongoing search for the molecular basis of plant osmosensing. The Journal of General Physiology, 145:5, 389–394.

Haughn, G. W., Western, T. L. (2012). Arabidopsis seed coat mucilage is a specialized cell wall that can be used as a model for genetic analysis of plant cell wall structure and function. Frontiers in Plant Science, 3(64).

Holdsworth, M. J., Bentsink, L., Soppe, W. J. J. (2008). Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytologist, 179:1, 33–54.

Hou, X. W., Tong, H. Y., Selby, J., DeWitt, J., Peng, X. X., He, Z. H. (2005). Involvement of a cell wall-associated kinase, WAKL4, in Arabidopsis mineral responses. Plant Physiology, 139:4, 1704–1716.

Ibarra, S.E., Tognacca, R.S., Dave, A., Graham, I.A., Sanchez, R. A., Botto, J. F. Molecular mechanisms underlying the entrance in secondary dormancy of Arabidopsis seeds, Plant Cell Environment, 39, 213-221

Ingram, G., Gutierrez-Marcos, J. (2015). Peptide signalling during angiosperm seed development. Journal of Experimental Botany, 66:17, 5151–5159.

James, G.O., Hocart, C.H., Hillier, W., Chen, H., Kordbacheh, F., Price, G. D., Djordjevic, M. A. (2011). Fatty acide profiling of Chlamydomonas reinhardttii under nitrogen deprivation. Bioresource Technology, 102:3, 3343-3351.

Jordá, L., Sopeña-Torres, S., Escudero, V., Nuñez-Corcuera, B., Delgado-Cerezo, M., Torii, K. U., Molina, A. (2016). ERECTA and BAK1 receptor like kinases interact to regulate immune responses in Arabidopsis. Frontiers in Plant Science, 7:15140, 897.

Jiang, W.B., Huang, H.Y., Hu, Y.W., Zhu, S.W., Wang, Z.Y., Lin, W.H. (2013). Brassinosteroid regulates seed size and shape in Arabidopsis. Plant Physiology, 162:4, 1955-1977.

Kang, J., Yim, S., Choi, H., Kim, A., Lee, K. P., Lopez-Molina, L., et al. (2015). Abscisic acid transporters cooperate to control seed germination. Nature Communications, 6, 8113.
Kazachkova, Y., Khan, A., Acuna, T., López-Diaz, I, Carrera, E., Khozin-Goldberg, I., Fait, A., Barak, S. (2016). Salt induces features of a dormancy-like state in seeds of Eutrema (Thellungiella) salsugineum, a halophytic relative of arabidopsis. Frontiers in Plant Science, 7, 1071

Kim, J. B., Carpita, N. C. (1992). Changes in esterification of the uronic-acid groups of cell-wall polysaccharides during elongation of maize coleoptiles. Plant Physiology, 98(2), 646–653.

Kim, S.G., Lee, A.K., Yoon, H.K., Park, C.M. (2008). A membrane-bound NAC transcription factor NTL8 regulates gibberellic acid-mediated salt signaling in Arabidopsis seed germination. The Plant Journal, 55:1, 77–88.

Koornneef, M., Van der Veen, J. H. I (1980). Induction and analysis of gibberellin-sensitive mutants in Arabidopsis thaliana (L.) Heynh. Theoretical Applied Genetics 586:1:257–263.

Koornneef, M., Joma, M. L., Brinkhorst-van der Swan, D. L., Karssen, C. M. (1982). The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.) heynh. Theoretical and Applied Genetics, 61:4, 385–393.

Kucera, B., Cohn, M. A., Leubner-Metzger, G. (2005). Plant hormone interactions during seed dormancy release and germination. Seed Science Research, 15:4, 281–307.

Lease, K. A., Lau, N. Y., Schuster, R. A., Torii, K. U., Walker, J. C. (2001). Receptor serine/threonine protein kinases in signalling: analysis of the ERECTA receptor-like kinase of Arabidopsis thaliana. The New Phytologist, 151:1, 133–143.

Lee, K. P., Piskurewicz, U., Turečková, V., Strnad, M., Lopez-Molina, L. (2010). A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. Proceedings of the National Academy of Sciences, 107:(44, 19108–19113.

Lee, S., Cheng, H., King, K. E., Wang, W., He, Y., Hussain, A., et al. (2002). Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes and Development, 16:5, 646–658.

Lefebvre, V., North, H., Frey, A., Sotta, B., Seo, M., Okamoto, M., et al. (2006). Functional analysis of Arabidopsis NCED6 and NCED9 genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. Plant Journal, 45:3, 309–319.

Léon-Kloosterziel, K. M., van de Bunt, G. A., Zeevaart, J., Koornneef, M. (1996). Arabidopsis mutants with a reduced seed dormancy. Plant Physiology, 110:1, 233–240.
Linkies, A., Müller, K., Morris, K., Turečková, V., Wenk, M., Cadman, C. S. C., et al. (2009). Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using Lepidium sativum and Arabidopsis thaliana. The Plant Cell, 21:12, 3803–3822.

Lionen, J., Schwender, J. (2009). Analysis of metabolic flux phenotypes for two arabidopsis mutants with severe impairment in seed storage lipid synthesis. Plant Physiology 151, 1617-1634.

Liu, P.-P., Koizuka, N., Homrichhausen, T. M., Hewitt, J. R., Martin, R. C., Nonogaki, H. (2005). Large-scale screening of Arabidopsis enhancer-trap lines for seed germination-associated genes. Plant Journal, 41:6, 936–944.

Liu, X., Hu, P., Huang, M., Tang, Y., Li, Y., Li, L., Hou1, X. (2016). The NF-YC–RGL2 module integrates GA and ABA signalling to regulate seed germination in Arabidopsis. Nature Communications, 7, 12768.

Llorente, F., Alonso-Blanco, C., Sánchez-Rodríguez, C., Jordá, L., Molina, A. (2005). ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus Plectosphaerella cucumerina. The Plant Journal, 43:2, 165–180.

Lopez-Molina, L., Mongrand, S., Chua, N. H. (2001). A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 98:8, 4782–4787.

Lopez-Molina, L., Mongrand, S., McLachlin, D. T., Chait, B. T., Chua, N.H. (2002). ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. Plant Journal, 32:3, 317–328.

Luo, M., Dennis, E.S., Berger, F., Peacock, W.J., Chaudhury, A. 2005. MINISEED3 (MINI3), a WRKY family gene and HA IKU2 (IKU2), a leucine-rich repeat (LRR) kinase gene are regulators of seed size in Arabidopsis. Proceedings of the National Academy of Science USA 102, 17531–17536.

Macquet, A., Ralet, M.-C., Kronenberger, J., Marion-Poll, A., North, H. M. (2007). In situ, chemical and macromolecular study of the composition of Arabidopsis thaliana seed coat mucilage. Plant Cell Physiology, 48:7, 984-999.

Masle, J., Gilmore, S. R., Farquhar, G. D. (2005). The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. Nature, 436:7052, 866–870.

Molina, I., Ohlrogge, J. B., Pollard, M. (2008). Deposition and localization of lipid polyester
in developing seeds of Brassica napus and Arabidopsis thaliana. Plant Journal, 53: 3, 437–449.

Monshausen, G. B., Haswell, E. S. (2013). A force of nature: molecular mechanisms of mechanoperception in plants. Journal of Experimental Botany, 64:15, 4663–4680.

Müller, K., Linkies, A., Vreeburg, R. A. M., Fry, S. C., Krieger-Liszkay, A., Leubner-Metzger, G. (2009). In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiology, 150:4, 1855–1865.

Müller, K., Tintelnot, S., Leubner-Metzger, G. (2006). Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of Lepidium sativum (cress) and endosperm rupture of cress and Arabidopsis thaliana. Plant and Cell Physiology, 47:7, 864–877.

Munns, R., Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59:1, 651–681.

Née, G., Kramer, K., Nakabayashi, K., Yuan, B., Xiang, Y., Miatton, E., Finkemeier, I., Soppe, W.J.J. (2017). DELAY OF GERMINATION1 requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. Nature Communications, 8, 72.

Nishimura, N., Tsuchiya, W., Moresco, J.J., Hayashi, Y., Satoh, K., Kaiwa, N., Irisa, T., Kinoshita, T., Schroeder, J.I., Tates, J.R., Hirayama, T., Yamazaki, T. (2018). Control of seed dormancy and germination by DOG1-AHG1 PP2C phosphatase complex via binding to heme. Nature Communications, 9, 2132.

Nonogaki, H. (2014). Seed germination - emerging mechanisms and new hypotheses. Frontiers in Plant Science, 5, 233.

North, H. M., Berger, A., Saez-Aguayo, S., Ralet, M.-C. (2014). Understanding polysaccharide production and properties using seed coat mutants: future perspectives for the exploitation of natural variants. Annals of Botany, 114:6, 1251–1263.

Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., Tran, L. S. P. (2013). Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. Journal of Experimental Botany, 64:(2, 445–458.

Penfield, S., Meissner, R. C., Shoue, D. A., Carpita, N. C., Bevan, M. W. (2001). MYB61 is required for mucilage deposition and extrusion in the Arabidopsis seed coat. The Plant Cell, 13, 2777-2791.

Penfield, S.P., Rylott E.L., Gilday, A.D., Graham S., Larson, T.R., Graham, I.A. (2004). Reserve mobilisation in the Arabidopsis endosperm fuels hypocotyl elongation in the dark, is independent of abscissic acid, and requires phosphoenolpyruvate carboxykinase1.
The Plant Cell, 16, 2705-2718.

Penfield, S., Li, Y., Gaday, A.D., Graham, S., Graham, I.A. (2006) Arabidopsis ABA INSENSITIVE 4 regulates lipid mobilisation in the embryo and reveals repression of seed germination by the endosperm. The plant Cell, 18, 1887-1899.

Peng, L. C., Hocart, C. H., Redmond, J. W., Williamson, R. E. (2000). Fractionation of carbohydrates in Arabidopsis root cell walls shows that three radial swelling loci are specifically involved in cellulose production. Planta, 211:3, 406–414.

Pettolino, F. A., Walsh, C., Fincher, G. B., Bacic, A. (2012). Determining the polysaccharide composition of plant cell walls. Nature Protocols, 7:9, 1590–1607.

Pillitteri, L. J., Bemis, S. M., Shpak, E. D., Torii, K. U. (2007). Haploinsufficiency after successive loss of signaling reveals a role for ERECTA-family genes in Arabidopsis ovule development. Development, 134:17, 3099–3109.

Piskurewicz, U., Jikumaru, Y., Kinoshita, N., Nambara, E., Kamiya, Y., Lopez-Molina, L. (2008). The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. The Plant Cell, 20:10, 2729–2745.

Piskurewicz, U., Turečková, V., Lacombe, E., Lopez-Molina, L. (2009). Far-red light inhibits germination through DELLA-dependent stimulation of ABA synthesis and ABI3 activity. The EMBO Journal, 28:15, 2259–2271.

Quesada, V., García-Martínez, S., Piqueras, P., Ponce, M. R., Micol, J. L. (2002). Genetic architecture of NaCl tolerance in Arabidopsis. Plant Physiology, 130:2, 951–963.

Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., Job, D. (2012). Seed germination and vigor. Annual Review of Plant Biology, 63, 507–533.

Ralet, M.-C., Crépeau, M.-J., Vigouroux, J., Tran, J., Berger, A., Sallé, C., et al. (2016). Xylans provide the structural driving force for mucilage adhesion to the Arabidopsis seed coat. Plant Physiology, 171, 165-178.

Rautengarten, C., Usadel, B., Neumetzler, L. (2008). A subtilisin-like serine protease essential for mucilage release from Arabidopsis seed coats. The Plant Journal, 54, 466-480.

Ren, Z., Zheng, Z., Chinnusamy, V., Zhu, J., Cui, X., Iida, K., Zhu, J. K. (2010). RAS1, a quantitative trait locus for salt tolerance and ABA sensitivity in Arabidopsis. Proceedings of the National Academy of Sciences, 107:12, 5669–5674.
Rédei, J.P. (1992). A note on Columbia wild type and Landsberg erecta. In Methods in Arabidopsis Research. Koncz, C., Chua, N. H. and Schell, J. eds. World Scientific Publishing Co Inc.

Saez-Aguayo, S., Ralet, M.-C., Berger, A., Botran, L., Ropart, D., Marion-Poll, A., North, H. M. (2013). PECTIN METHYLESTERASE INHIBITOR6 promotes Arabidopsis mucilage release by limiting methylesterification of homogalacturonan in seed coat epidermal cells. The Plant Cell, 25:1, 308–323.

Sánchez-Rodríguez, C., Estévez, J. M., Llorente, F., Hernández-Blanco, C., Jordá, L., Pagán, I., et al. (2009). The ERECTA receptor-like kinase regulates cell wall–mediated resistance to pathogens in Arabidopsis thaliana. Molecular Plant-Microbe Interactions: MPMI, 22:8, 953–963.

Seo, M., Hanada, A., Kuwahara, A., Endo, A., Okamoto, M., Yamauchi, Y., et al. (2006). Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. The Plant Journal, 48:3, 354–366.

Sessions, A., Weigel, D., Yanofsky, M. F. (1999). The Arabidopsis thaliana MERISTEM LAYER 1 promoter specifies epidermal expression in meristems and young primordia. The Plant Journal, 20:2, 259–263.

Shen, H., Zhong, X., Zhao, F., Wang, Y., Yan, B., Li, Q., Chen, G., Mao, B., Wang, J., Li, Y., Xiao, G., He, Y., Xiao, H., Li, J., He, Z. (2015). Overexpression of receptor-kinase ERECTA improves thermotolerance in rice and tomato. Nature Biotechnology, 33, 996-1003.

Shiu, S.-H., Karlowski, W. M., Pan, R., Tzeng, Y.-H., Mayer, K. F. X., Li, W.-H. (2004). Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. The Plant Cell, 16:5, 1220–1234.

Shpak, E. D., Berthiaume, C. T., Hill, E. J., Torii, K. U. (2004). Synergistic interaction of three ERECTA-family receptor-like kinases controls Arabidopsis organ growth and flower development by promoting cell proliferation. Development, 131:7, 1491–1501.

Shpak, E. D., McAbee, J.M., Pillitteri, L.J., Torii, K. U. (2005). Stomatal patterning and differentiation by synergistic interactions of receptor kinases. Science, 309:5732, 290-293.

Sivaguru, M., Ezaki, B., He, Z. H., Tong, H. Y., Osawa, H., Baluska, F., et al. (2003). Aluminum-induced gene expression and protein localization of a cell wall-associated receptor kinase in Arabidopsis. Plant Physiology, 132:4, 2256–2266. Stamm, P.,
Ravindran, P., Mohanty, B., Tan, E. L., Yu, H., Kumar, P. P. (2012). Insights into the molecular mechanism of RGL2-mediated inhibition of seed germination in Arabidopsis thaliana. BMC Plant Biology, 12.

Steber, C. M., McCourt, P. (2001). A role for brassinosteroids in germination in Arabidopsis. Plant Physiology, 125:2, 763–769.

Torii, K. U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R. F., Komeda, Y. (1996). The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. The Plant Cell, 8:4, 735–746.

Uchida, N., Lee, J. S., Horst, R. J., Lai, H.-H., Kajita, R., Kakimoto, T., et al. (2012). Regulation of inflorescence architecture by intertissue layer ligand-receptor communication between endodermis and phloem. Proceedings of the National Academy of Sciences of the United States of America, 109:16, 6337–6342.

Uchida, N., Shimada, M., Tasaka, M. (2013). ERECTA-family receptor kinases regulate stem cell homeostasis via buffering its cytokinin responsiveness in the shoot apical meristem. Plant and Cell Physiology, 54:3, 343-351.

Wagner, T. A., Kohorn, B. D. (2001). Wall-associated kinases are expressed throughout plant development and are required for cell expansion. The Plant Cell, 13:2, 303–318.

Wang, Z., Wang, J., Bao, Y., Wu, Y., Zhang, H. (2010). Quantitative trait loci controlling rice seed germination under salt stress. Euphytica, 178:3, 297–307.

Wang, A., Garcia, D., Zhang, H., Feng, K., Chaudhury, A., Berger, F., Peacock, W.J., Dennis, E.S., Luo, M. (2010). The VQ motif protein IKU1 regulates endosperm growth and seed size in Arabidopsis. Plant Journal 63: 670–679.

Weitbrecht, K., Muller, K., Leubner-Metzger, G. (2011). First off the mark: early seed germination. Journal of Experimental Botany, 62:10, 3289–3309.

Western, T. L., Skinner, D. J., Haughn, G. W. (2000). Differentiation of mucilage secretory cells of the Arabidopsis seed coat. Plant Physiology, 122:2, 345–355.

Western, T. (2012). The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. Seed Science Research, 22, 1-25.

Wharton, M. J. (1955). The use of tetrazolium test for determining the viability of seeds of the genus Brassica. Proceedings of the International Seed Test Association. 20, 81-88.

Willats, W., Knox, J. P., Mikkelsen, J. D. (2006). Pectin: new insights into an old polymer are starting to gel. Trends in Food Science and Technology, 17, 97-104.

Xing, H.T., Guo, P., Xia, X.L., Yin, W.L. (2011). PdERECTA, a leucine-rich repeat receptor-like kinase of poplar, confers enhanced water use efficiency in Arabidopsis. Planta, 234,
Figure Legends

Figure 1. The three ERECTA family members synergistically control the timing and pace of seed germination under salinity.

A, B, T_{50} values (h post-stratification) for testa rupture (A) and endosperm rupture (B). C, Time interval (h) between the two steps. Experiment repeated 5 times with different seed
batches. As the triple mutant is sterile, the segregating progeny of *er erl1+/- erl2* plants was used to investigate germination of triple mutant seeds, and is referred to in text and figures as *er erl1/seg erl2*. A-C, Values are means and s.e.m. (n = 4 plates, 30 seeds per genotype per plate). Different letters above bars denote significant differences by two-way ANOVA and Tukey HSD pair-wise tests (*P* < 0.001).

**Figure 2. Germination-specific function of the ERf on sensitivity to salinity.**

A-D, Time-course of endosperm rupture for *er, er erl1, er erl2* or *er erl1/seg erl2* seeds over a 10 d incubation period on 0 (A, C) or 150 mM NaCl agar media (B, D) following imbibition and stratification either directly on media (A, B) or in water prior to plating (C, D). E, Seedling relative expansion rates (d⁻¹) on 0 or 150 mM NaCl media. Seeds were first germinated on NaCl-free media and then transferred to fresh 0 mM or 150 mM NaCl plates for monitoring their expansion over the next 72 h, measurements of whole seedling projected area on images captured using *ImageJ*. Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests (*P* < 0.001), n = 7.

**Fig 3. ERf transcripts are present in mature dry seeds and ERf de novo transcription is activated early during germination.**

*ERf* gene expression in WT dry seeds (“Dry”) and germinating seeds at: the end of imbibition and stratification (stage I); 20 h later (stage II, testa rupture); and 52 h later (stage III-G, endosperm rupture completed on control media). Non-germinated seeds at that time on 150 mM NaCl (label III-NG) were sampled and analysed separately. Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests (*P* < 0.001), n = 4 seed pools per genotype and treatment, of 300 seeds each. The experiment was repeated 3 times.

**Figure 4. The ERf regulates seed germination sensitivity to salinity mostly via interactions with its ionic effects, but is also involved in the control of germination under osmotic stress.**
A, Percentage of seeds with endosperm rupture for WT and the NaCl hyper-sensitive mutants er erl1, er erl2, and er erl1/seg erl2, on day 1, 2, 3 and 7 post-stratification as a function of media osmotic pressure ($\pi_e$) varied through supplementation of PEG_8000 at concentrations ranging from 0 to 171g L$^{-1}$. n=100-200 seeds per replicate. B, Germination response over an extended range of PEG concentrations, in an independent experiment with a different seed batch. Data points depict the percentage of seeds exhibiting endosperm rupture 6d post-stratification. n= 100-200 seeds per replicate. C, Kinetics of seed germination under 0.99 MPa $\pi_e$ induced by NaCl. Same seed batch as in (B). The arrow points to germination scores on d6 when, under iso-osmotic conditions induced by PEG, at least 90% seeds had germinated (see panel B). n = 3 plates, 30 seeds per plate and per genotype. Experiments replicated 3 times.

Figure 5. Seed germination in the salinity hypersensitive er, er erl1, er erl2 and er erl1/seg erl2 mutants is more sensitive to external NaCl than KCl concentrations under iso-osmotic conditions.

Time-course of germination under iso-osmotic conditions (media osmotic pressure, $\pi_e$) induced by supplementation of either NaCl or KCl. Percentages of seeds exhibiting endosperm rupture 4 d post-stratification (means and s.e.m.; n = 3 plates, 30 seeds per plate and per genotype; experiments replicated twice).

Figure 6. Seed germination readily resumes upon salinity stress removal.

Time-course of seed germination on 150 mM NaCl media (0-490 h), and after transfer to NaCl-free media (arrow on x-axis). The experiment was repeated 3 times. Values are means and s.e.m. (n = 4 plates, 30 seeds per genotype per plate). Different letters above data points denote significant genetic differences at each time point by one-way ANOVA and Tukey HSD pair-wise tests ($P < 0.05$). “NS” at the final time point indicates that genetic differences were non-statistically significant by one-way ANOVA.

Figure 7. The ERf interacts with the sensitivity of seed germination to exogenous ABA and with the expression of major ABA and GA signalling genes.
A, Germination response to exogenous ABA application. Data points represent $T_{50}$ values and s.e.m. for testa rupture (A) and endosperm rupture (B) ($n = 3$ plates, with 30 seeds of each genotype). The experiment was repeated 3 times. C, $ABI3$, $ABI5$, $RGL2$ and $DOG1$ gene expression in dry seeds (“Dry”) and seeds sampled at: the end of imbibition and stratification (stage I); 20 h later (stage II, testa rupture); and 52 h later (stage III-G, endosperm rupture). Non-germinated seeds on 150 mM NaCl media (label III-NG) were analysed separately. A-C, Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P < 0.05$).

Figure 8. The ERf function in seed germination sensitivity to salinity is maternally controlled and shows partial overlap with an ERf function in the determination of seed size.

A, Seed projected area (mm$^2$); means and s.e.m. ($n \geq 400$ seeds per genotype, from 11 siliques). Letters indicate significant differences by one-way ANOVA and Tukey HSD pair-wise tests ($P < 0.001$). B, Relative expansion rate (mm$^2$ mm$^{-2}$ d$^{-1}$) of mature embryos excised from enclosing tissues, over a 72 h incubation period on 0 or 150 mM NaCl media, ($n = 7$). Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P < 0.001$). C, Time-course of germination for WT and $er erl1$ seeds, and F1 seeds generated from their reciprocal crosses. Similar results were obtained from crosses between WT and $er erl2$ flowers (data not shown). D, Size of F1 seeds from reciprocal crosses between WT and $er erl1$+-/+ $erl2$ flowers ($n=86$ to 143 seeds per cross). Different letters indicate significant differences by one-way ANOVA and Tukey HSD pair-wise tests ($P < 0.001$). C-D, Crosses were made between flowers at similar positions on the main inflorescence; seeds were harvested at the same time, 3 weeks after crossing.

Figure 9. The ERf is involved in the control of seed coat permeability mucilage composition and salinity-dependent role in the regulation of germination speed.

A, Seed coat permeability to tetrazolium red ($n = 4$ seed pools; some s.e.m. are hidden by symbols). * denotes statistical significance ($P < 0.001$) by two-way ANOVA and Scheffe post-hoc test. B, Seed sodium content 24 h post-stratification on 0 mM or 150 mM NaCl media, ($n=3$ seed pools). Letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P = 0.42$ and 0.39 for genotype effect under control and salt
treatment, respectively). C, Correlation between mass of water soluble mucilage per seed and seed size. Means and s.e.m. (n = 4 seed pools per genotype, 40 mg seeds per pool, average seed weight and area determined on sub- aliquots; experiment replicated 3 times). Regression lines: 0 mM NaCl, y=36.6x-0.20, r² =0.84; 150 mM NaCl, y=36.3x+0.002, r² =0.81. Similar results were obtained with size expressed as area. D, GalUA/Gal and Rhm/Xyl ratios. Letters besides points indicate statistical significance of differences in GalUA/Gal (P<0.05) by one-way ANOVA and Tukey post-hoc tests, compared to all unlabelled data points. P=0.08 for differences in Rhm/Xyl between er erl1/seg erl2 and WT. E, Testa rupture (TeR) and endosperm rupture (EnR) T₅₀ values for intact seeds and “demucilaged” seeds. Mean values per genotype (n = 3 plates; 30 seeds per genotype per plate). Labelled points denote genotypes where removal of the outer water soluble mucilage significantly advanced germination on 150 mM NaCl media. The 1:1 line represents the bisextrix, where mucilage removal is neutral. F, TCH3 gene expression in WT and er erl1/seg erl2 dry and imbibed seeds during the three germination phases, n=4 seed pools per genotype and NaCl condition, of 300 seeds each.

Supplementary Figure S1. NaCl-dependent effects of reduced ERf signaling on the timing and pace of seed germination.

A-D, Percentages of seeds exhibiting testa rupture (A, C) and endosperm rupture (B, D) on 0 mM NaCl (A, B) and 150 mM NaCl media (C, D) as a function of time (h post-stratification). Data points are means and s.e.m. (n = 4 plates per condition, 30 seeds per genotype in each plate). The experiment was repeated 5 times.

Supplementary Figure S2. Loss of ER alone or in combination with ERL1 and ERL2 sensitises seed germination to salinity in a dose-dependent manner.

A, Percentages of seeds exhibiting endosperm rupture 4 d post-stratification on 0, 100, 150 or 200 mM NaCl (means and s.e.m.; n = 3 plates per condition, 30 seeds per genotype per plate). The experiment was repeated 3 times. B, Percentages of germinated seeds over a 10 d incubation period on 200 mM NaCl media for WT and the four NaCl-hypersensitive erf mutants. Different letters indicate significant differences by one-way ANOVA and Tukey HSD pair-wise tests (P≦0.001), n = 4 plates).
Supplementary Figure S3. Erf-dependent sensitivity of seed germination to NaCl in an independent set of erf single, double and triple knock-out mutants.

A, ERL1 and ERL2 expression is abolished in the erl1-5 (SALK_019567), and erl2-2 (SALK_015275C) mutants. B, T$_{50}$ values for testa and endosperm rupture in WT and erf mutants carrying the er2, erl1-5, or erl2-2 alleles. C. Time-interval between testa and endosperm rupture. A-C. Means and s.e.m. are shown (n = 4 plates, 30 seeds per genotype in each plate; experiment replicated 3 times). Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P<0.001$).

Supplementary Figure S4. Erf promoter activity in mature dry seeds and germinating seeds. A-C, GUS staining patterns for embryos dissected from mature dry seeds, just before sowing (A), and then from germinating seeds incubated on 0 mM NaCl media (B) or 150 mM NaCl media (C), at the end of stratification (0 h time point) and daily thereafter until all seeds had germinated.

Supplementary Figure S5. Similar sensitivity to salinity stress of selected ABA and GA biosynthetic gene expression levels in WT and er erl1/seg erl2 seeds.

ABA2, NCED9, GA3OX1 and GA3OX2 relative gene expression in dry seeds (“Dry”), after imbibition and stratification (I), and at the end of the next two germination phases (stages II, III; see Methods; III-NG non-germinated seeds yet on 150 mM NaCl media). n=4 pools of seeds per genotype and media, of 300 seeds each). Different letters within each graph indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P<0.01$).

Supplementary Figure S6. Mature er erl1 erl2 embryos exhibit similar radicle size and patterning than WT seeds.

A, Representative photographs of WT and er erl1 erl2 mature embryos excised from dry seeds dissected out of siliques of the same age and similar positions on the main inflorescence. Embryos were cleared and imaged by differential interference microscopy. B-
E, Morphometric analysis: cotyledon and radicle lengths (B); number of hypocotyl cells (C); cotyledon size (D); and number of cotyledon epidermal cells (E). * denotes significant differences by 2-tailed paired t-tests ($P = 0.0011; n=5$).

Supplementary Figure S7. Relative abundance of total fatty acid methyl-esters (FAMES) and relative proportions of individual species in embryos at full seed maturity. A, Amount of FAMES in mature embryos. Data points show amounts for 4 independent pools of 50 embryos for each genotype. Genotypes sharing a letter above the box are not statistically different by one-way ANOVA and Tukey pair-wise tests ($P \leq 0.05$). B, Percentages of medium-chain fatty acids (C16, C18; tall bars, dark shade colors) and mono-unsaturated fatty acids (shorter bars, paler shade colors); the complements to 100% represent long-chain fatty acids (C20,C22) and polyunsaturated fatty acids, respectively. C, Proportions of individual fatty acids relative to the total amount of FAMES. Measurements were done by GC-MS on 4 pools of 50 mature embryos excised from dry seeds.

Supplementary Figure S8. Characteristics of the seed mucilage. A, B, Ruthenium red staining of WT and erf seed mucilage after 2 h imbibition in 0 mM NaCl (top row) or 150 mM NaCl solution (bottom row), with gentle shaking (A) or in presence of 10 mM Tris-HCl without shaking (B). Ruthenium red stains acidic pectins. C, Proportions (%) of monosaccharides in seed water soluble mucilage (n = 4 pools of seeds, 40 mg seeds per pool). Genetic differences for individual sugars were not statistically different by one-way ANOVA and Tukey HSD pair-wise tests at $P<0.05$, but $P=0.055$ to 0.08 for differences in xylose content between WT and er1, erl1, er erl1, er erl2 and er erl1/seg erl2 mucilage. Rhm, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; GalUA, Glucoronic acid measured in the water soluble mucilage, which typically represent 60-70% of the total Arabidopsis seed mucilage (Ralet et al. 2016).

Supplementary Table S1. List of genotyping and RT-qPCR primers
Figure 1. The three ERECTA family members synergistically control the timing and pace of seed germination under salinity.

A, B, $T_{50}$ values (h post-stratification) for testa rupture (A) and endosperm rupture (B). C, Time interval (h) between the two steps. Experiment repeated 5 times with different seed batches. As the triple mutant is sterile, the segregating progeny of $er$ $erl1+/-$ $erl2$ plants was used to investigate germination of triple mutant seeds, and is referred to in text and figures as $er$ $erl1/seg$ $erl2$. A-C, Values are means and s.e.m. ($n = 4$ plates, 30 seeds per genotype per plate). Different letters above bars denote significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P < 0.001$).
Figure 2. Germination-specific function of the ERf on sensitivity to salinity.

A-D, Time-course of endosperm rupture for er, er erl1, er erl2 or er erl1/seg erl2 seeds over a 10 d incubation period on 0 (A, C) or 150 mM NaCl agar media (B, D) following imbibition and stratification either directly on media (A, B) or in water prior to plating (C, D). E, Seedling relative expansion rates (d⁻¹) on 0 or 150 mM NaCl media. Seeds were first germinated on NaCl-free media and then transferred to fresh 0 mM or 150 mM NaCl plates for monitoring their expansion over the next 72 h, measurements of whole seedling projected area on images captured using ImageJ. Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P < 0.001$), $n = 7$. 
Fig 3. \textit{ERf} transcripts are present in mature dry seeds and \textit{ERf de novo} transcription is activated early during germination.

\textit{ERf} gene expression in WT dry seeds (“Dry”) and germinating seeds at: the end of imbibition and stratification (stage I); 20 h later (stage II, testa rupture); and 52 h later (stage III-G, endosperm rupture completed on control media). Non-germinated seeds at that time on 150 mM NaCl (label III-NG) were sampled and analysed separately. Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P < 0.001$), n=4 seed pools per genotype and treatment, of 300 seeds each. The experiment was repeated 3 times.
Fig. 4

(A) Seeds with endosperm rupture (%) over time with varying media osmotic pressure (MPa).

(B) Seeds with endosperm rupture (%) over PEG-8000 concentration (g L⁻¹) showing a threshold at π₀ = 0.99 MPa.

(C) Seeds with endosperm rupture (%) over time with π₀ = 0.99 MPa for NaCl treatment.
Figure 4. The ERf regulates seed germination sensitivity to salinity mostly via interactions with its ionic effects, but is also involved in the control of germination under osmotic stress.

A, Percentage of seeds with endosperm rupture for WT and the NaCl hyper-sensitive mutants er erl1, er erl2, and er erl1/seg erl2, on day 1, 2, 3 and 7 post-stratification as a function of media osmotic pressure ($\pi_e$) varied through supplementation of PEG_8000 at concentrations ranging from 0 to 171g L$^{-1}$. n=100-200 seeds per replicate. B, Germination response over an extended range of PEG concentrations, in an independent experiment with a different seed batch. Data points depict the percentage of seeds exhibiting endosperm rupture 6d post-stratification. n= 100-200 seeds per replicate. C, Kinetics of seed germination under 0.99 MPa $\pi_e$ induced by NaCl. Same seed batch as in (B). The arrow points to germination scores on d6 when, under iso-osmotic conditions induced by PEG, at least 90% seeds had germinated (see panel B). n = 3 plates, 30 seeds per plate and per genotype. Experiments replicated 3 times.
Figure 5. Seed germination in the salinity hypersensitive er, er erl1, er erl2 and er erl1/seg erl2 mutants is more sensitive to external NaCl than KCl concentrations under iso-osmotic conditions.

Time-course of germination under iso-osmotic conditions (media osmotic pressure, $\pi_e$) induced by supplementation of either NaCl or KCl. Percentages of seeds exhibiting endosperm rupture 4 d post-stratification (means and s.e.m.; n = 3 plates, 30 seeds per plate and per genotype; experiments replicated twice).
Figure 6. Seed germination readily resumes upon salinity stress removal.

Time-course of seed germination on 150 mM NaCl media (0-490 h), and after transfer to NaCl-free media (arrow on x-axis). The experiment was repeated 3 times. Values are means and s.e.m. (n = 4 plates, 30 seeds per genotype per plate). Different letters above data points denote significant genetic differences at each time point by one-way ANOVA and Tukey HSD pair-wise tests ($P < 0.05$). “NS” at the final time point indicates that genetic differences were non-statistically significant by one-way ANOVA.
Figure 7. The ERf interacts with the sensitivity of seed germination to exogenous ABA and with the expression of major ABA and GA signalling genes.

A, B, Germination response to exogenous ABA application. Data points represent $T_{50}$ values and s.e.m. for testa rupture (A) and endosperm rupture (B) ($n = 3$ plates, with 30 seeds of each genotype). The experiment was repeated 3 times. C, $ABI3$, $ABI5$, $RGL2$ and $DOG1$ gene expression in dry seeds (“Dry”) and seeds sampled at: the end of imbibition and stratification (stage I); 20 h later (stage II, testa rupture); and 52 h later (stage III-G, endosperm rupture). Non-germinated seeds on 150 mM NaCl media (label III-NG) were analysed separately. A-C, Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests ($P < 0.05$).
Figure 8. The ERf function in seed germination sensitivity to salinity is maternally controlled and shows partial overlap with an ERf function in the determination of seed size.

A, Seed projected area (mm²); means and s.e.m. (n≥ 400 seeds per genotype, from 11 siliques). Letters indicate significant differences by one-way ANOVA and Tukey HSD pair-wise tests (P < 0.001). B, Relative expansion rate (mm² mm⁻² d⁻¹) of mature embryos excised from enclosing tissues, over a 72 h incubation period on 0 or 150 mM NaCl media, (n = 7). Different letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests (P < 0.001). C, Time-course of germination for WT and er erl1 seeds, and F1 seeds generated from their reciprocal crosses. Similar results were obtained from crosses between WT and er erl2 flowers (data not shown). D, Size of F1 seeds from reciprocal crosses between WT and er erl1+/- erl2 flowers (n=86 to 143 seeds per cross). Different letters indicate significant differences by one-way ANOVA and Tukey HSD pair-wise tests (P < 0.001). C-D, Crosses were made between flowers at similar positions on the main inflorescence; seeds were harvested at the same time, 3 weeks after crossing.
Figure 9. The ERf is involved in the control of seed coat permeability mucilage composition and salinity-dependent role in the regulation of germination speed.

A, Seed coat permeability to tetrazolium red (n = 4 seed pools of 50 mg each; some s.e.m. are hidden by symbols). * denotes statistical significance (P < 0.001) by two-way ANOVA and Scheffe post-hoc test. B, Seed sodium content 24 h post-stratification on 0 mM or 150 mM NaCl media, (n=3 seed pools). Letters indicate significant differences by two-way ANOVA and Tukey HSD pair-wise tests (P = 0.42 and 0.39 for genotype effect under control and salt treatment, respectively). C, Correlation between mass of water soluble mucilage per seed and seed size. Means and s.e.m. (n = 4 seed pools per genotype, 40 mg seeds per pool, average seed weight and area determined on sub-aliquots; experiment replicated 3 times). Regression lines: 0 mM NaCl, y=36.6x-0.20, r² =0.84; 150 mM NaCl, y=36.3x+0.002, r² =0.81. Similar results were obtained with size expressed as area. D, GalUA/Gal and Rhm/Xyl ratios. Letters besides points indicate statistical significance of differences in GalUA/Gal (P<0.05) by one-way ANOVA and Tukey post-hoc tests, compared to all unlabelled data points. P=0.08 for differences in Rhm/Xyl between er erl1/seg erl2 and WT. E, Testa rupture (TeR) and endosperm rupture (EnR) T50 values for intact seeds and “demucilaged” seeds. Mean values per genotype (n = 3 plates; 30 seeds per genotype per plate). Labelled points denote genotypes where removal of the outer water soluble mucilage significantly advanced germination on 150 mM NaCl media. The 1:1 line represents the bisextrix, where mucilage removal is neutral. F, TCH3 gene expression in WT and er erl1/seg erl2 dry and imbibed seeds during the three germination phases, n=4 seed pools per genotype and NaCl condition, of 300 seeds each.