Title

Novel cytonuclear combinations modify Arabidopsis seed physiology and vigor

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Running title

Seed traits under cytonuclear control

Issue section

Growth and development

Title Novel cytonuclear combinations modify Arabidopsis seed physiology and vigor

Highlight

Natural variation in Arabidopsis organelles and cytonuclear interactions influence seed dormancy, longevity and germination performance. Enhanced seed vigor was obtained through the creation of novel cytonuclear combinations.

Abstract

The influence of intraspecific variation in cytoplasmic genomes and cytonuclear interactions on key seed traits that can impact adaptation and agriculture has not been thoroughly explored, so far. Here, dormancy, germination performance and longevity of seeds have been assessed in Arabidopsis plants with novel cytonuclear combinations that disrupt coadaptation between natural variants of nuclear and cytoplasmic genomes. Although all three traits were affected by cytonuclear reshuffling, the sensitivity of seed traits to cytoplasmic change was dependent on the nuclear background. Both deleterious and, more surprisingly, favorable effects of novel cytonuclear combinations (in comparison with the nuclear parent) were observed, suggesting suboptimal genetic combinations exist in natural populations for these traits. Significant changes on dormancy and germination performance due to specific cytonuclear interacting combinations mainly occurred in opposite directions, in accordance with the previously proposed 'dormancy continuum'. Consistently, reduced sensitivity to exogenous ABA and faster endogenous ABA decay during germination were observed in a novel cytonuclear combination that also exhibited enhanced longevity and better germination performance, compared to its natural nuclear parent. Cytoplasmic genomes, therefore, represent an additional resource of natural variation for breeding seed vigor traits.

Keywords

cytolines, cytonuclear co-adaptation, cytonuclear interaction, dormancy, seed longevity, germination, seed vigor.

Abbreviations

CDT, controlled deterioration treatment; Gmax, maximum germination percentage; PH, post-harvest; RH, relative humidity

Introduction

The evolutionary success of flowering plants is largely due to the invention of seeds, which protect and scatter the next generation and provide it with resources upon germination. Germination is a complex process determined by the intrinsic properties of seeds and influenced by biotic and abiotic environment, aging-related damage, which can occur during seed storage, and dormancy, which prevents germination under favorable conditions. In nature, dormancy allows the seed to await for the favorable season for seedling success (Finch-Savage and Footitt, 2017). Once dormancy is released, the capacity of seeds to germinate in a wide range of environmental conditions contributes to adaptation, and germination speed has a fitness impact in competitive situations (Dubois and Cheptou, 2012). Germination is also a trait of primary interest for agriculture, where seed vigor, defined as the properties that ensure a fast and synchronized germination in field conditions (Finch-Savage and Bassel, 2016), is crucial for the quality of seed lots. In contrast to its adaptive role in natural conditions, dormancy is not a desirable trait in crops, as it leads to low and unsynchronized seedling emergence rates in agricultural production (Shu *et al.*, 2015). In the laboratory, seed vigor is assessed through the speed and uniformity of germination in favorable conditions, germination performance under stress conditions, and the ability to survive storage (Finch-Savage *et al.*, 2010; Rajjou *et al.*, 2012). Seed longevity, *i.e.* ability to survive and maintain germination performance despite physiological damage occurring during storage, is an important component of seed vigor (Rajjou and Debeaujon, 2008; Sano *et al.*, 2016).

Arabidopsis thaliana (hereafter Arabidopsis) has been widely used to decipher seed biology traits through mutant studies and genome-wide analyses (North *et al.*, 2010). Arabidopsis natural accessions display genetic variation for seed dormancy, longevity and germination vigor (Alonso-Blanco *et al.*, 2003; Clerkx *et al.*, 2004; Bentsink *et al.*, 2010; Vallejo *et al.*, 2010; Yuan *et al.*, 2016*a*). For example, the release of seed dormancy during post-harvest (PH) dry storage occurred at variable speeds in 112 Arabidopsis accessions, with the time of storage required to reach 50% of germination ranging from 3.5 to 264 days (Debieu *et al.*, 2013). In addition, Arabidopsis natural variation in seed dormancy and longevity has been shown relevant for adaptation in ecological studies (Donohue *et al.*, 2005*a*; Huang *et al.*, 2010; Kronholm *et al.*, 2012; Debieu *et al.*, 2013; Postma *et al.*, 2015). A number of quantitative trait loci (QTL) for dormancy, longevity, germination speed, and germination

33 tolerance to stresses have been reported (van Der Schaar *et al.*, 1997; Alonso-Blanco *et al.*,
34 2003; Clerkx *et al.*, 2004; Galpaz and Reymond, 2010; Bentsink *et al.*, 2010; Huang *et al.*,
35 2010; Joosen *et al.*, 2012; Nguyen *et al.*, 2012; Yuan *et al.*, 2016*b*). Further major advances in
36 our understanding of the genetic bases of natural variation in seed dormancy and
37 germination are being provided from the functional study of the genes underlying these loci
38 (Bentsink *et al.*, 2006; Shu *et al.*, 2015).
39 Comparatively, little is known about the role of cytoplasmic variation in the establishment of
40 seed traits important for adaptation or agriculture, despite the crucial role of mitochondria
41 and chloroplasts in seed quality and germination performance. Upon imbibition,
42 mitochondrial respiration resumes almost immediately to fuel the very active metabolism
43 which is required during imbibition and germination (Paszkiewicz *et al.*, 2017). Plastids are
44 the site of the early steps of ABA and GA syntheses. These two major hormones govern the
45 balance between dormancy and germination through their antagonist effects: ABA promotes
46 the induction and maintenance of seed dormancy, whereas GAs promote germination by
47 stimulating tegument and endosperm rupture and embryo cellular elongation (Finch-Savage
48 and Leubner-Metzger, 2006; Shu *et al.*, 2016). Both mitochondria and chloroplasts have
49 endosymbiotic origins and their co-evolution with the nucleus of the host cells has shaped
50 nuclear and organellar genomes, while organizing the compartmentalized metabolism of the
51 plant cell (Dyall *et al.*, 2004; Kutschera and Niklas, 2005). The tuning of mitochondrial and
52 chloroplast functions is under a complex and still elusive network of genetic and metabolic
53 regulations (Rurek, 2016; De Souza *et al.*, 2017). This is mainly ensured and controlled by
54 nuclear-encoded factors, some of which interact with organelle genes or their products.
55 Numerous genes encoding organellar proteins are expressed during germination and their
56 inactivation often affects the process (Yang *et al.*, 2011; Demarsy *et al.*, 2011; Savage *et al.*,
57 2013; Sew *et al.*, 2016). In addition, transcriptional regulation of specific mitochondrial
58 protein-encoding genes during seed germination has been largely documented (Howell *et*
59 *al.*, 2008; Narsai *et al.*, 2011; Law *et al.*, 2012). Also, plastid gene expression is required for
60 proper seed development in Arabidopsis (Bryant *et al.*, 2011) and maize (Sosso *et al.*, 2012).
61 Based on the severe consequences for seed development and germination arising from
62 anomalies in the genetic and physiological activities of plastids and mitochondria, it could be
63 assumed that natural cytoplasmic variation affecting these traits is strongly limited, if not
64 suppressed, by natural selection. However, a few studies have reported results suggesting

65 an impact of natural variation in cytoplasmic genomes on seed physiological traits. A seminal
66 study on reciprocal Arabidopsis F1s and backcross generations showed that cytoplasm
67 variation impacted germination efficiency (Corey *et al.*, 1976). A recent report on seed
68 germination performance of reciprocal hybrids between *Arabidopsis lyrata* populations
69 suggested that a disruption of cytonuclear coadaptation contributed to the lower
70 germination of F1 and F2 hybrid generations (Hämälä *et al.*, 2017). Furthermore, in a study
71 using Arabidopsis cytolines, each combining the nuclear genome of a natural variant with
72 the cytoplasmic genomes of a different variant, we previously demonstrated the impact of
73 cytoplasmic natural variation and cytonuclear interactions on adaptive traits in the field,
74 including germination (Roux *et al.*, 2016). Here, using the same cytolines, we have
75 investigated further the effect of natural genetic variation within organelles on specific
76 physiological seed traits relevant to adaptation and agriculture, namely dormancy,
77 germination performance and longevity. We found that genetic variation in organelle
78 genomes could impact these traits, depending on the nuclear background. Furthermore, we
79 uncovered novel cytonuclear combinations that displayed higher performance than their
80 natural nuclear parent, suggesting that cytoplasmic variation could potentially be used in
81 breeding of seed traits. The improved germination performance and seed longevity of a
82 novel cytonuclear combination was associated with more efficient endogenous ABA
83 degradation and lower exogenous ABA sensitivity.
84
85

Materials and Methods

Plant material

Cytolines are genotypes combining the nuclear genome of one parent with the organelle genomes of another conspecific parent. Hereafter, a cytoline possessing the cytoplasm of accession "A" and the nucleus of accession "B" is designated [A]B. We used series of cytolines derived from eight natural accessions of Arabidopsis, namely: Blh-1, Bur-0, Ct-1, Cvi-0, Ita-0, Jea, Oy-0, and Sha, which were selected for their wide genetic diversity (Mckhann *et al.*, 2004; Moison *et al.*, 2010). Cytolines were obtained by recurrent paternal backcrosses of hybrids from the di-allele cross (Roux *et al.*, 2016). Seed stocks of the 64 genotypes (56 cytolines and 8 parental accessions) are available at the Versailles Arabidopsis Stock Center (http://publiclines.versailles.inra.fr/cytoline/index).

Unless specified, the seed stocks used were obtained in a growth chamber and previously used in the study of (Roux *et al.*, 2016). They are conserved in controlled conditions (4°C, 15-30% relative humidity, RH) in the Versailles Arabidopsis Stock Center. These seed lots had been stored for at least 18 months when used for the experiments described here.

Two additional seed productions were realized for dormancy studies in a growth chamber under the same conditions as in Roux *et al.* (2016) (light 16 h at 21°C, dark 8 h at 18°C), using two plants per genotype, each on either of the two available shelves. An additional seed production of the Sha and [Blh-1]Sha lines was realized in the greenhouse, seeds from four individuals were pooled for each genotype.

Seed dormancy assays

For the 64 genotypes, the depth of dormancy was measured on freshly harvested seeds then at 3, 6 and 9 months of after-ripening (designated 0PH, 3PH, 6PH and 9PH). Seed imbibition was conducted in Petri dishes (Ø 55 mm) on filter papers with three sheets of absorbent paper (Roundfilter paper circles, Ø 45 mm; Schleicher & Schuell, Dassel, Germany) covered by a black membrane filter (ME 25/31, Ø 45 mm; Schleicher & Schuell, Dassel, Germany) wetted with 1.3 mL of ultrapure water. Seeds were then incubated in a controlled culture room under continuous light (Philips TRM HOW/33 RS tubes, 70 $\mu mol.m^{-2}.s^{-1}$) at 15°C or 25°C. Germination (protrusion of the radicle after rupture of the testa and endosperm) was assessed daily during twelve days by visual examination.

117 Results were analyzed independently for each PH time and considering both germination
118 temperatures in order to better control the variability of germination success, except for
119 0PH whose only results at 15°C were considered because none of the genotypes germinated
120 at 25°C upon harvest. The [Sha]Cvi-0 and [Ct-1]Jea genotypes were excluded from the
121 analyzed dataset due to missing data for at least one biological replicate.
122
123 *Germination performance tests in challenging conditions*
124 The complete set of 64 genotypes was tested for germination performance on water and
125 NaCl, after stratification. Seeds were placed on a blue blotter papers (Anchor Paper
126 Company, SGB1924B) positioned on the germination medium (Phytoblend, 0.5% in water)
127 with or without NaCl, in square Petri dishes (120 x 120 x 17 mm). Because seed germination
128 tolerance to NaCl is variable from one accession to another (Galpaz and Reymond, 2010),
129 each nuclear series was tested on the NaCl concentration (Table S1) that resulted in
130 approximately 50% germination of the parental accession in a preliminary experiment. Each
131 dish contained four to eight genotypes and two or three replicates were realized.
132 Stratification treatment was applied at 4°C in the dark for four days. Then, dishes were
133 transferred into a phytotron system providing continuous light from three sides at 25°C
134 (Sanyo MLR-351H, Versatile environmental test chamber). Germination time was counted
135 from the transfer into the phytotron. For the following of germination kinetics, images were
136 taken at 5 to 6 time points between 24 and 72 hours of germination with a digital camera
137 (Nikon Coolpix-P510, Nikon, France). Germination was visually assessed using ImageJ
138 (http://rsbweb.nih.gov/ij/). The maximum germination percentage (Gmax) was considered
139 reached when no additional germination was observed after another 24 hours.
140 Following the same procedure, the Sha nuclear series was tested on mannitol (200 mM) or
141 KCl (100 mM). ABA (50 µM) sensitivity tests were identically realized on Sha, [Blh-1]Sha and
142 [Ita-0]Sha, except that germination was monitored visually daily during four days.
143
144 *Controlled Deterioration Treatment (CDT)*
145 The CDT was performed in order to mimic natural seed aging (Tesnier and Strookman-
146 Donkers, 2002; Rajjou *et al.*, 2008). These experiments were performed on the Sha and Ct-1
147 nuclear series, on seeds from the Versailles Arabidopsis Stock Center.

148 Briefly, dry mature seeds were first equilibrated at 75% relative humidity (20°C) during 3
149 days. After this step, day 0 controls were immediately dried back in a desiccator with
150 Silicagel for three days. Treatment was done by storing the seeds (at 75% RH, using sealed
151 boxes with saturated NaCl solution) for 10, 20, 30 and 40 days at 35°C. After the treatment,
152 seeds were dried back on Silicagel as above. Germination percentage was measured after
153 seed imbibition on water under continuous light at 23°C.
154
155 *Measurement of Na and K ion contents in Arabidopsis seeds*
156 A germination experiment was set as described above for germination performance, with or
157 without NaCl (100 mM), except that seeds were collected either immediately after
158 stratification or after 20 hours of germination. Three replicates were realized. Seeds were
159 rinsed three times with 50 mL of sterile water and dried at 100°C overnight. The dry weight
160 of each sample was measured and samples were digested in 2 mL of 70% HNO_3 for a total of
161 3h with temperature ramping from 80 to 120°C. Potassium and sodium contents were
162 determined with a Varian AA240FS atomic absorption spectrometer (Agilent Technologies,
163 Santa Clara, Ca, USA) and concentrations calculated by comparison with Na^+ and K^+
164 standards.
165
166 *Measurement of ABA content in Arabidopsis seeds*
167 ABA content was measured on dry seeds and on stratified seeds after 6 h of germination,
168 using the same experiment setting as described above for germination performance, with or
169 without NaCl (100 mM). Twenty mg of seeds were ground in a mortar with liquid nitrogen
170 and lyophilized with a freeze-drier. For each sample, 10 mg of freeze-dried powder were
171 extracted with 0.8 mL of acetone/water/acetic acid (80/19/1 v:v:v). ABA stable labelled
172 isotope used as internal standard was prepared as described in Le Roux *et al.* (2014). Two
173 nanograms of each standard were added to the sample. The extract was vigorously shaken
174 for 1 min, sonicated for 1 min at 25 Hz, shaken for 10 min at 4°C in a Thermomixer
175 (Eppendorf®), and then centrifuged (8,000 *g*, 4°C, 10 min). The supernatants were collected,
176 and the pellets were re-extracted twice with 0.4 mL of the same extraction solution, then
177 vigorously shaken (1 min) and sonicated (1 min; 25 Hz). After the centrifugations, the three
178 supernatants were pooled and dried (final volume of 1.6 mL). Each dry extract was dissolved
179 in 140 µL acetone, filtered, and analyzed using a Waters Acquity ultra performance liquid

180 chromatograph coupled to a Waters Xevo triple quadrupole mass spectrometer TQS (UPLC-
181 ESI-MS/MS). The compounds were separated on a reverse-phase column (Uptisphere C18
182 UP3HDO, 100*2.1 mm*3µm particle size; Interchim, France) using a flow rate of 0.4 $mL.min^{-1}$
183 and a binary gradient: (A) acetic acid 0.1 % in water (v/v) and (B) acetonitrile with 0.1 %
184 acetic acid. The following binary gradient was used (t, % A): (0 min, 98 %), (3 min, 70 %), (7.5
185 min, 50 %), (8.5 min, 5 %), (9.6 min, 0%), (13.2 min, 98 %), (15.7 min, 98 %). Mass
186 spectrometry was conducted in electrospray and Multiple Reaction Monitoring scanning
187 mode (MRM mode), in negative ion mode (see Le Roux *et al.* 2014 for monitored
188 transitions). Relevant instrumental parameters were set as follows: capillary 1.5 kV (negative
189 mode), source block and desolvation gas temperatures 130°C and 500°C, respectively.
190 Nitrogen was used to assist the cone and desolvation (150 $L.h^{-1}$ and 800 $L.h^{-1}$, respectively),
191 argon was used as the collision gas at a flow of 0.18 $mL.min^{-1}$.
192
193 *Statistical analyses*
194 All analyses were performed with the R software. For all experiments, the selected models
195 and the contrast tests are detailed in Methods S1.
196 For all experiments measuring seed germination, germination success was analyzed using a
197 generalized linear model (McCullagh and Nelder, 1989) assuming a binomial distribution
198 with the dispersion parameter fixed at unity and a logit link function.
199 For each experiment, the model was selected as follows: (i) factors included in the model
200 were defined according to the experimental design; (ii) starting from the most complete
201 model, nested models were sequentially fitted and the best one according to BIC was
202 selected. Once the model selected, the contrasts relevant to the addressed question were
203 tested, and p-values were adjusted using the Bonferroni procedure to control the family-
204 wise error rate (FWER). A contrast was declared significant if its adjusted p-value was lower
205 than 0.05.
206 We performed a contrast test procedure to detect significant interacting cytoplasm x
207 nucleus combinations: each test concerned a pair of cytoplasms (C1 and C2) and a pair of
208 nuclei (Na and Nb), designed in the text as 'cytonuclear interacting combination'; a
209 significant p-value indicated that the effect of changing cytoplasm C1 to C2 in the Na nuclear
210 background differed from the effect of the same change in the Nb background, H0
211 {([C1]Na-[C2]Na) - ([C1]Nb-[C2]Nb) = 0}. The analysis of data from the complete set of 64

212 genotypes (8 parents and 56 cytolines) thus allows for the testing of 784 cytonuclear
213 interacting combinations (28 pairs of nuclei x 28 pairs of cytoplasms).
214 ABA content and Na^+/K^+ ion ratio were analyzed using linear models. The selection of the
215 model and tests for effects were conducted as described above.
216

217 **Results**

218 *Effects of cytoplasmic variations and cytonuclear interactions on seed dormancy*

219 Following on from the observation of differences in germination rate in field-grown
220 Arabidopsis cytolines (Roux *et al.*, 2016), we sought to evaluate whether cytoplasmic
221 variation and cytonuclear interactions specifically impacted seed primary dormancy and the
222 kinetics of its release during after ripening storage. Germination of seeds of the complete set
223 of 64 genotypes (8 natural accessions and 56 cytolines) was tested at harvest and three, six
224 and nine months post-harvest (0PH, 3PH, 6PH or 9PH), without any stratification treatment,
225 in two temperature conditions, 15°C and 25°C (Fig. 1 and S1). As expected from the wide
226 variation for dormancy among the panel of parental accessions, the nuclear background had
227 a strong effect on germination. Notably, no or low germination was obtained with seeds of
228 the Ita-0 nuclear series in any conditions. For this reason, these genotypes were excluded
229 from the subsequent analyses. Most seed lots germinated better at 15°C (Fig. 1) than at 25°C
230 (Fig. S1), as previously reported for Col-0 freshly harvested seeds (Leymarie *et al.*, 2012),
231 except those of the Bur-0 nuclear series, which reached almost 100% germination at 25°C at
232 harvest, but displayed lower germination success at 15°C.

233 We tested whether germination of cytolines was modified compared to their natural nuclear
234 parent. Table 1 shows that changing organelle genomes had effects on dormancy, but not to
235 the same extent nor in the same direction in all nuclear backgrounds. When effects were
236 observed, foreign cytoplasms tended to enhance dormancy in Ct-1, Jea, Oy-0 and Sha
237 nuclear backgrounds (lower germination in cytolines compared to nuclear natural parent),
238 whereas they rather lowered dormancy in Blh-1, Bur-0, and Cvi-0 backgrounds (higher
239 germination in cytolines). Interestingly, the impact of a given cytoplasm differed according
240 to the nucleus it was associated with, suggesting that dormancy was influenced by
241 cytonuclear interactions. We thus tested for significant cytonuclear interacting combinations
242 impacting dormancy, *i.e.* for differences between two given nuclei in the effect of a given
243 change of cytoplasm, for all possible pairs of cytoplasms and all possible pairs of nuclei (see
244 Material and Methods and Methods S1 for details). As some genotypes were excluded from
245 the analysis due to missing data or total absence of germination, 441 combinations were
246 tested for 0PH data, and 505 for data at other PH times. After Bonferroni corrections for
247 multiple tests, we found 177 significant cytonuclear interacting combinations at 0PH, and
248 232, 186, and 204 at 3PH, 6PH and 9PH, respectively (Table S2). This indicated that the depth

249 of seed primary dormancy is modulated by genetic variation in interacting nuclear- and
250 organelle-encoded partners.
251 As seen in Table 1, observed effects of foreign cytoplasms on dormancy often persisted in
252 successive PH times, which was somewhat expected since an effect on the depth of
253 dormancy at harvest would still be detectable after some after-ripening, according to the
254 kinetics of dormancy release. Similarly, we observed persisting effects of cytonuclear
255 interacting combinations over several PH times (Table S2): among the 391 cytonuclear
256 interacting combinations that influenced germination at any PH time, 237 (60%) were
257 significant at two PH times or more and 47 (12%) at all four PH times and, in the vast
258 majority (between 86 % to 100 %) of the cases, they modified germination in the same
259 direction (Table 2), in accordance with a persistence of the observed effects during after-
260 ripening.
261 Interestingly, the rate of dormancy release during after-ripening also seemed to be modified
262 by foreign cytoplasms in some cases: as seen in Table 1, no difference compared to the
263 natural nuclear parent at 0PH could be followed by either better (*e.g.* [Jea]Cvi-0) or lower
264 (*e.g.* [Sha]Blh-1) germination percentage in later PH times. In addition, a modification in the
265 kinetics of dormancy release was indicated by a change in the contrast sign between PH
266 times in two cases: [Oy-0]Sha germinated less than Sha at 0PH but better at following PH
267 times; oppositely, [Bur-0]Cvi-0 germinated better than Cvi-0 at 0PH, but less at 9PH.
268 Similarly, we interpreted opposite signs of contrast for the effects of a cytonuclear
269 interacting combination significant at to PH times (Table 2) as indication that it altered the
270 kinetics of dormancy release.
271
272 *Effects of cytoplasmic variations and cytonuclear interactions on germination performance*
273 In order to assess whether a disruption of cytonuclear co-adaptation also affected the
274 germination performance of seeds after dormancy release, we measured the germination of
275 the complete set of genotypes under permissive and challenging conditions, specifically in
276 the presence of NaCl, at least 18 months after harvest and after stratification. We used salt
277 stress to enhance differences in germination performance that could be difficult to assess
278 after the stratification treatment. Because seed germination tolerance to NaCl is variable
279 from one accession to another (Galpaz and Reymond, 2010), each nuclear series was tested
280 on the NaCl concentration (Table S1) that resulted in 50% germination of the parental

281 accession in a preliminary experiment. Time plots are shown in Fig. S2. Despite the
282 stratification treatment, 100% of germination was not reached on water in Ct-1 and Cvi-0
283 nuclear series, although germination had reached the plateau (Gmax). Nevertheless, we
284 considered the Gmax as an indicator of germination performance and tested, in each
285 nuclear series, the germination performance of cytolines compared to the natural accession,
286 averaged on the two conditions (Fig. 2, see Methods S1 for details). The results clearly
287 showed that the sensitivity of germination to a cytoplasm change was very variable among
288 the nuclear backgrounds, Blh-1 being very sensitive whereas Ct-1 and Oy-0 were not. In
289 general, when affecting germination, foreign cytoplasms had a negative effect. However,
290 three cytolines ([Ct-1]Ita-0, [Ct-1]Jea and [Blh-1]Sha) did germinate better than their
291 respective nuclear parent. After Bonferroni correction for multiple tests, 202 out of 784
292 cytonuclear interacting combinations (26%, Table S2) significantly impacted germination
293 success in this experiment. Thus, as dormancy, germination performance was influenced by
294 interactions between organelle- and nuclear-encoded factors that vary among the panel of
295 parental accessions.
296 Among the 505 cytonuclear interacting combinations that were tested both for germination
297 performance and for dormancy, 92 were significant for both traits. This represented 80% of
298 the interacting combinations impacting germination performance (92/115), but only 23% of
299 those impacting dormancy for at least one PH (92/392). In addition, the cytonuclear
300 interacting combinations that influenced both dormancy and germination performance
301 impacted germination success more often in the same direction in both experiments than
302 would be randomly expected (Table 3, Chi^2 test pval <0.001), indicating a negative
303 correlation between dormancy and germination performance.
304
305 *Contribution of individual accessions to cytonuclear interactions that impact germination*
306 The above analyses, the impact on germination traits of a shuffling of genetic compartments
307 depended on the origin of the genomes. To better assess this variation, we analyzed the
308 contribution of individual accessions to cytonuclear interacting combinations affecting
309 dormancy or germination performance. Fig. 3 indicates, for each accession, the number of
310 significant cytonuclear interacting combinations that involved its cytoplasm or its nucleus
311 and that impacted dormancy depth, conservatively estimated as effects maintained through
312 all PH times (Fig. 3a) or germination performance after storage (Fig. 3b). Among the 47

313 cytonuclear interacting combinations that impacted dormancy depth, 28 involved the Ita-0
314 cytoplasm and 33 involved the Jea nucleus. Among the 202 significant interacting
315 combinations that affected germination performance after storage, 100 involved the Blh-1
316 cytoplasm and 76 the Sha nucleus. When considering the contribution of accessions to
317 cytonuclear interacting combinations that affect both dormancy and germination
318 performance, the Blh-1 and Ita-0 cytoplasms and the Sha nucleus clearly emerged (Fig.S3).
319
320 *Effects of cytoplasm variation on seed longevity in Sha and Ct-1 nuclear backgrounds*
321 Along with dormancy and germination performance, longevity is an important component of
322 seed vigor. Thus, we addressed whether this trait was also under the influence of
323 cytoplasmic variation. We selected the Sha and Ct-1 series because these two nuclear
324 backgrounds represented two extreme cases in their contribution to cytonuclear effects in
325 the previous experiments (Fig. 3 and S3). The longevity of seeds from these two cytoline
326 series was estimated by measuring their viability (germination percentage in standard
327 conditions) after 0, 10, 20, 30 or 40 days of controlled deterioration treatment (CDT) (Fig. 4).
328 We used the same seed lots as in the assays for germination performance after storage. In
329 this experiment, all genotypes of the Ct-1 series reached 100% germination. We concluded
330 that the partial seed imbibition followed by desiccation included in the CDT treatment, even
331 for the control, acted as a priming treatment releasing the residual dormancy observed in
332 the experiment on germination performance described above. The kinetics of viability loss
333 were different in the two nuclear backgrounds, revealing an expected nuclear effect on seed
334 longevity (Fig. 4). Interestingly, four cytolines appeared less sensitive to CDT than their
335 natural nuclear parent, whereas none was more sensitive (Table 4, see Methods S1 for
336 details). In addition, in both nuclear backgrounds, the selected statistical models included an
337 interaction between the cytoplasm and the length of CDT treatment, which suggested that
338 the loss of germination ability between two treatment durations was under the influence of
339 the cytoplasmic genomes. We could not test directly for significant cytonuclear interacting
340 combinations, because the two series were measured in different experiments and the
341 results analyzed independently. Nevertheless, in Blh-1/Sha and Ita-0/Sha cytoplasm pairs,
342 individual cytoplasms had opposite effects in the two nuclear backgrounds (Table S3),
343 indicating an influence of cytonuclear epistasis on germination after CDT. Strikingly, the Sha

344 cytoplasm performed better than the Blh-1 and the Ita-0 cytoplasms in the Ct-1 nuclear
345 background but worse when associated with its natural nuclear partner.
346
347 *Physiological clues for germination performance of the [Blh-1]Sha line*
348 A closer look at the 202 cytonuclear interacting combinations that influence germination
349 performance revealed that the [Blh-1]Sha combination was involved in 46 (23%, Table S2) of
350 them, indicating that this cytonuclear combination had a major effect on germination. In
351 addition, this genetic combination also had a strong impact on CDT tolerance. We thus
352 decided to get further insight into the behavior of [Blh-1]Sha seeds.
353 In order to test whether the peculiar behavior of the [Blh-1]Sha seeds was due to the
354 conditions of seed production, we tested germination performance of [Blh-1]Sha and Sha
355 seeds from two productions in different environments, growth chamber and greenhouse.
356 We observed higher germination performance of the cytoline compared to the natural
357 accession for both seed productions (Fig. S4). An environmental cause of the higher
358 germination performance of [Blh-1]Sha was therefore very unlikely. However, we also
359 observed that this positive effect did not persist in seed samples that had been stored
360 several months at room temperature in the laboratory.
361 We then tested whether the germination performance of [Blh-1]Sha was possibly
362 maintained in the presence of mannitol (200 mM) and KCl (100 mM), by analyzing the
363 germination efficiency of the Sha cytoline series (Fig. S5). The concentrations applied were
364 chosen to compare results to those of the NaCl treatment (100 mM) in the germination
365 performance experiment. The germination rate of the [Blh-1]Sha cytoline was consistently
366 higher than that of the other genotypes under both osmotic and ionic stresses, although
367 these stresses had a milder effect than the NaCl treatment on all genotypes, as previously
368 reported (Munns and Tester, 2008). The germination curve of [Blh-1]Sha on KCl grouped
369 with those of the other lines in the absence of KCl.
370 It was conceivable that the better germination performance of [Blh-1]Sha seeds would be
371 amplified by an increased tolerance to salt in germination under NaCl stress. Since Na^+/K^+
372 ratio is a key indicator of salt tolerance (Shabala and Cuin, 2008), potassium and sodium
373 contents were analyzed in seeds of [Blh-1]Sha, [Ita-0]Sha and Sha. The [Ita-0]Sha was used as
374 a control cytoline with germination performance similar to Sha (Fig. 2). Very little sodium
375 accumulated in seeds in the absence of NaCl. When germinated on NaCl, both [Blh-1]Sha

376 and [Ita-0]Sha seeds showed a lower Na^+/K^+ ratio than Sha seeds (Fig. 5). As the two
377 cytolines had different germination responses on NaCl compared to Sha, we considered it
378 unlikely that the Na^+/K^+ balance is the primary cause of the increased germination success of
379 the [Blh-1]Sha cytoline on NaCl. These results reinforced our hypothesis that the intrinsic
380 germination capacity of [Blh-1]Sha seeds was enhanced compared to its natural parent.
381 We then focused on ABA content and sensitivity to further explore physiological parameters
382 that could contribute to the enhanced germination performance of [Blh-1]Sha seeds. We
383 measured the endogenous ABA content of seeds from [Blh-1]Sha, [Ita-0]Sha, and Sha lines.
384 ABA contents were measured in both dry and stratified seeds placed under germination
385 conditions for six hours, in the presence or absence of NaCl (Fig. 6a). The experiment was
386 repeated after 10 further months of storage in controlled conditions (Versailles Stock
387 Centre), yielding similar results. The combined analysis of data from both experiments (see
388 Methods S1 for details) indicated that dry seeds of [Blh-1]Sha contained more ABA than
389 those of [Ita-0]Sha and Sha (p-values < 0.001), whereas they contained less ABA after six
390 hours of germination, either on water or NaCl (p-values < 0.01). The sensitivity of
391 germinating seeds to exogenous ABA (50 µM) was tested on the same seed stocks after the
392 second experiment (Fig. 6b). The [Blh-1]Sha seeds were less sensitive to ABA treatment than
393 Sha seeds. Taken together, the results suggest that both endogenous ABA metabolism and
394 ABA sensitivity are modified in [Blh-1]Sha seeds compared to Sha seeds.

395 **Discussion**

396 In this study, we examined seed physiological traits important for adaptation and of
397 relevance to agriculture, in relation to cytoplasmic genetic variants and to the coadaptation
398 between genomic compartments. Cytolines are original and valuable genetic resources to
399 enlighten cytoplasmic and cytonuclear effects (Roux *et al.*, 2016). The analysis of a cytoline
400 series from a given nuclear parent allows assessment of the consequences of a disruption of
401 cytonuclear coadaptation in the considered nuclear background. The di-allele cross design
402 used to produce the cytolines is suited to the detection of cytonuclear genetic interactions.
403 When examining seed traits, this approach does not distinguish effects acting at the level of
404 the mother plant from those acting at the level of the zygote(s). Nevertheless, as both the
405 mother plant and progeny have the same genotype, it does not preclude the detection of
406 cytoplasmic or cytonuclear effects, keeping in mind that they could act in the mother plant
407 and/or in the progeny.

408 Dormancy was assessed by monitoring germination of freshly harvested seeds, or seeds
409 after three, six or nine months of after-ripening, without stratification. Disruption of
410 cytonuclear coadaptation had variable effects on dormancy depending on the nuclear
411 background, both in the number of cytolines with modified dormancy and in the direction of
412 the effect. When dormancy differed in a cytoline compared to its parent, it tended to be
413 deeper when the parent was weakly dormant (*e.g.* Jea, Sha) and weaker in the deeply
414 dormant Cvi-0 background. This suggests that coadapted variants of nuclear and organellar
415 genes contributed to adaptive dormancy. However, this was not always verified: in the
416 weakly dormant Bur-0, novel cytonuclear combinations enhanced germination. The Bur-0
417 nuclear series was remarkably different from the others regarding germination at the two
418 tested temperature conditions: in contrast to the other nuclear series, the seeds of the Bur-0
419 nuclear series had no dormancy phenotype at 25°C (Fig. S1) but displayed dormancy at 15°C
420 (Fig.1). A peculiar response to sowing temperature of Bur-0 seeds was also reported in a
421 study examining a set of 73 accessions where they displayed almost 100% germination at
422 26°C and around 50% germination at 18°C (Schmuths *et al.*, 2006). Genetic variation for the
423 rate of dormancy loss after-ripening was previously reported for Arabidopsis (Barua *et al.*,
424 2012). Here, by revealing that novel genetic combinations modify the kinetics of dormancy
425 release, we provide evidence that this rate can be modulated by cytonuclear genetic
426 interactions.

427 Germination performance was estimated through the germination percentage of seeds in
428 normal and challenging conditions. The effects of foreign cytoplasms tended to lower
429 germination performance, which is expected if optimal germinative success is assumed for
430 natural coadapted genotypes. However, some novel cytonuclear combinations had better
431 germination performance than their nuclear parent, suggesting that natural accessions do
432 not always have optimal germination performance, at least in laboratory conditions. A good
433 germination efficiency under challenging stress conditions was reported as a component of
434 seed vigor and related to germination speed (Finch-Savage and Bassel, 2016; Yuan *et al.*,
435 2016*a*). Although the limited number of time points precluded the precise fitting of
436 germination curves and reliable comparison of germination speeds, examination of the
437 germination time plots (Fig. S2) strongly suggested that cytolines with higher germination
438 performance also germinated quicker than their natural parent, whereas those performing
439 worse germinated slower. In addition, the germination percentage was influenced by
440 cytonuclear interactions independently of the presence of salt (no third-order level of
441 interaction), which was consistent with the idea that the detected cytonuclear effects affect
442 germination performance *per se* rather than salt tolerance. This was further verified in
443 [Blh-1]Sha and [Ita]Sha where a marker of salt tolerance (lower Na+/K+ ratio) was not linked
444 to germination performance, whereas the specific effect of the Blh-1 cytoplasm on ABA
445 decay rate was observed both on water and NaCl. We therefore conclude that our analysis
446 identified effects of cytoplasm variation and cytonuclear interactions on a component of
447 seed vigor. As longevity is also associated to seed vigor, we assessed germination after CDT
448 in Ct-1 and Sha nuclear series, which presented contrasted sensitivities to cytoplasmic
449 change for germination performance and dormancy. Strikingly, disruption of cytonuclear co-
450 adaptation had no or positive effect on seed longevity, at least for the genetic variation
451 tested. This was unexpected since lower seed longevity is generally indicative of low seed
452 vigor and an unfit genotype. It is interesting to note that [Sha]Ct-1 and [Blh-1]Sha displayed
453 both higher longevity and germination performance compared to their natural parents. It is
454 conceivable that both phenotypes revealed the same underlying beneficial effect on seed
455 vigor of the new cytonuclear combinations. However, the [Ita-0]Sha cytoline, which had a
456 higher seed longevity than Sha, did not germinated better. A negative correlation between
457 dormancy and seed longevity determined by co-located QTLs was observed in recombinant
458 inbred-line populations of Arabidopsis (Nguyen *et al.*, 2012). This negative correlation was

459 not verified here: we observed deeper dormancy at 0PH and higher tolerance to CDT in the
460 [Ita-0]Sha cytoline than in Sha. This suggests that the cytonuclear interaction effects on seed
461 longevity and dormancy in this cytoline are not driven by the loci reported in Nguyen's work
462 or, not exclusively, that this cytoplasm change has different impacts on the Sha allelic effects
463 on seed dormancy and longevity at these loci. It is also conceivable that the apparently
464 contradiction between our result and that from Nguyen *et al.* (2012) come from differences
465 in the conditions used to estimate seed longevity, *i.e.* survival after several years of dry
466 storage versus CDT for 10-40 days.
467 Some accessions contributed more than others to the cytonuclear interacting combinations
468 affecting germination, and individual accessions contributed differently to interactions that
469 affect dormancy depth and germination performance after storage. For example, the Jea
470 nucleus had the highest contribution to significant cytonuclear interacting combinations for
471 dormancy. This suggests that, when changing the organellar partners of cytonuclear
472 epistasis involved in dormancy tuning, Jea alleles lead to different consequences on the
473 phenotype than alleles from other parents.
474 We found that the majority of cytonuclear interacting combinations that modulated
475 germination performance also altered dormancy, with a negative correlation between
476 dormancy and germination performance in most cases (Table 3). This is consistent with the
477 idea that dormancy and germination speed are parts of a continuum that control
478 germination (Finch-Savage and Leubner-Metzger, 2006). We exclude that residual dormancy
479 of Ct-1 and Cvi-0 series induced a bias toward this conclusion because the contributions of
480 these nuclei to significant cytonuclear interacting combinations shared by germination
481 performance and dormancy experiments are among the fewest observed (Fig. S3). The
482 continuity of the biological process underlying dormancy and seed vigor was recently
483 brought back to light by a report that linked genetic variation in ABA sensitivity with seed
484 germination performance in *Brassica oleracea* (Awan *et al.*, 2018). ABA content was also
485 reported to be related to germination speed in *B. oleracea* (Morris *et al.*, 2016). In this
486 context, our results on the [Blh-1]Sha cytoline seem particularly relevant. At the moment, a
487 formal causal link cannot be made between the enhanced germination vigor of the cytoline
488 on one hand, and faster ABA decay or reduced ABA sensitivity on the other hand.
489 Nevertheless, these observations provide an interesting path for further research. In parallel
490 to deciphering the physiological consequences of novel genetic combinations that modulate

491 seed performance, genetic studies should also lead to the identification of gene variants that
492 underlie the effects reported here.
493 One remarkable and unexpected result of the present study is the enhanced seed behavior
494 of some cytolines compared to natural genotypes that were shaped by coevolution.
495 Interestingly, the cytoplasm of the drought adapted Kas-1 accession was reported to have a
496 strong and negative effect on water use efficiency in a QTL analysis (Mckay *et al.*, 2008), also
497 suggesting that the natural genotype was not optimal for this trait. It is conceivable that
498 trade-offs between adaptive characters have led to the maintenance, in natural variants of
499 Arabidopsis, of suboptimal genomic combinations for adaptive traits. Indeed, trade-offs
500 between traits under selection, including germination, have been previously shown to
501 underlie natural genetic variation in Arabidopsis (Donohue *et al.*, 2005*b*; Todesco *et al.*,
502 2010; Debieu *et al.*, 2013). In light of these results, we suggest that more attention is given
503 to cytoplasmic variation and its effect on seed vigor in the genetic resources of cultivated
504 species.
505

506 **Supplementary data**

507 Methods S1. Statistical models and contrast tests.

508 Fig. S1. Dormancy release during after ripening in cytoline series, germination at 25°C.

509 Fig. S2. Germination time curves of cytoline series on water and NaCl.

510 Fig. S3. Contribution of individual accessions to cytonuclear interacting combinations

511 impacting dormancy and germination performance.

512 Fig. S4. Germination performance of [Blh-1]Sha and Sha seeds from two environments.

513 Fig. S5. Germination time curves of the Sha cytoline series on mannitol and KCl.

514 Table S1. NaCl concentrations used in the germination performance experiment

515 Table S2. Results of tests for significant cytonuclear interacting combinations in dormancy

516 and germination performance experiments.

517 Table S3. Results of tests for significant effect of cytoplasms on germination after CDT.

518

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Table 1. Impact of foreign cytoplasms on germination after various post-harvest times in the different nuclear backgrounds.

Nuclear background[a]	cytoplasm[b]	0 PH		3 PH		6 PH		9 PH	
		Pval[c]	direction of effect[d]	Pval	direction of effect	Pval	direction of effect	Pval	direction of effect
Blh1	Bur-0	***	+	***	+	***	+	ns	
	Ct-1	ns		ns		ns		*	-
	Cvi-0	ns		ns		ns		ns	
	Ita-0	*	+	**	+	ns		ns	
	Jea	ns		ns		**	+	ns	
	Oy-0	ns		ns		ns		ns	
	Sha	ns		ns		***	-	***	-
Bur-0	Blh-1	ns		ns		ns		ns	
	Ct-1	**	+	***	+	ns		ns	
	Cvi-0	***	+	*	+	***	+	ns	
	Ita-0	**	+	***	+	ns		ns	
	Jea	*	+	***	+	***	+	***	+
	Oy-0	ns		ns		ns		ns	
	Sha	ns		***	+	ns		*	+
Ct-1	Blh-1	ns		ns		ns		ns	
	Bur-0	ns		ns		ns		ns	
	Cvi-0	*	-	ns		**	-	***	-
	Ita-0	ns		ns		ns		ns	
	Jea	ns		ns		ns		ns	
	Oy-0	ns		ns		ns		ns	
	Sha	ns		***	+	ns		ns	
Cvi-0	Blh-1	NA		ns		ns		**	-
	Bur-0	***	+	ns		ns		**	-
	Ct-1	***	+	***	+	***	+	ns	
	Ita-0	*	+	ns		***	+	ns	
	Jea	NA		ns		ns		***	+
	Oy-0	NA		ns		ns		ns	
Jea	Blh-1	***	-	**	-	***	-	***	-
	Bur-0	***	-	***	-	ns		ns	
	Cvi-0	ns		*	-	***	-	***	-
	Ita-0	***	-	***	-	***	-	***	-
	Oy-0	ns		ns		ns		ns	
	Sha	ns		ns		ns		ns	
Oy-0	Blh-1	ns		ns		ns		ns	
	Bur-0	**	-	ns		ns		ns	
	Ct-1	***	-	ns		ns		ns	
	Cvi-0	***	-	ns		ns		ns	
	Ita-0	***	+	ns		ns		ns	
	Jea	*	-	ns		ns		ns	
	Sha	ns		ns		ns		ns	
Sha	Blh-1	ns		ns		ns		*	+
	Bur-0	***	-	ns		***	-	**	-
	Ct-1	**	-	ns		ns		ns	
	Cvi-0	**	-	ns		ns		ns	
	Ita-0	***	-	ns		ns		ns	
	Jea	ns		ns		ns		ns	
	Oy-0	***	-	***	+	***	+	*	+

[a] The results for the Ita-0 nuclear background were not analyzed due to extremely low germination in all conditions (see Fig. 1 and Fig. S1).

[b] For each nuclear background, missing foreign cytoplasms correspond to genotypes excluded before analysis due to missing data or total absence of germination (NAs at 0PH).

[c] For each PH time, a difference in germination success between the genotype and its nuclear parent was tested (see Methods S1 for details). For 3PH, 6PH and 9PH experiments, the cytoplasm effects must be interpreted as averaged on germination temperatures. Pvalue : ***<0.001<**<0.01<*<0.05; ns, non significant.

[d] the direction of the effect is indicated by the sign of the contrast result. (+) indicates that the tested genotype had a higher germination than its natural nuclear donor. (-) indicates the opposite result.

Table 2. Comparison of directions of the effects of significant cytonuclear interacting combinations shared by two PH times.

comparison	number of shared cytonuclear interactions	number of identical direction of effects[a]	number of opposite directions of effects[b]
0PH vs 3PH	103	95	8
0PH vs 6PH	82	71	11
0PH vs 9PH	77	69	8
3PH vs 6PH	124	123	1
3PH vs 9PH	110	103	7
6PH vs 9PH	130	130	0

[a] Identical direction of effects corresponds to identical signs of contrast results in Table S2. It indicates that the considered cytonuclear interacting combination affected germination success in the same direction at the two PH times.

[b] Opposite directions of effects corresponds to opposite signs of the contrast results in Table S2. It indicates that the considered cytonuclear interacting combination affected germination success in opposite directions at the two PH times.

Table 3. Comparison of directions of effects of cytonuclear interacting combinations significant both in the dormancy and germination performance experiments.

comparison[a]	number of shared significant cytonuclear interacting combinations	number of identical direction of effects[b]	number of opposite directions of effects[c]
Gmax vs 0PH	49	36	13
Gmax vs 3PH	59	45	14
Gmax vs 6PH	37	20	17
Gmax vs 9PH	43	30	13

[a] Gmax stands for germination success in the experiment testing germination performance; xPH stands for germination success after x months PH in the dormancy experiment.

[b] Identical direction of the effects correspond to identical signs of the contrasts results in Table S2. Based on the writing of the contrasts, it indicates that the considered cytonuclear interacting combination affected germination success in the same direction in both experiments, thus dormancy and germination performance in opposite directions.

[c] On the same basis, opposite directions of the effects correspond to opposite signs of the contrasts results in Table S2. It indicates that the considered cytonuclear interacting combination affected germination success in opposite directions in both experiments, thus dormancy and germination performance in the same direction.

Table 4. Impact of foreign cytoplasms on germination after CDT in the Sha and Ct-1 nuclear backgrounds

cytoplasm[a]	Ct-1 nuclear background		Sha nuclear background	
	Pval[b]	direction of effect[c]	Pval	direction of effect
Blh-1	ns		***	+
Bur-0	*	+	ns	
Ct-1			ns	
Cvi-0	ns		ns	
Ita-0	ns		*	+
Jea	ns		ns	
Oy-0	ns		ns	
Sha	***	+		

[a] Each foreign cytoplasm was tested for effect compared to the parental cytoplasm (see Methods S1 for details). The cytoplasm effects must be interpreted as averaged on {10, 20 ,30} days of CDT.

[b] Pvalue : ***<0.001<**<0.01<*<0.05; ns, non significant.

[c] (+) indicates that the tested genotype had a higher germination after CDT than its natural nuclear donor. (-) indicates that the tested genotype had a lower germination after CDT than its natural nuclear donor.

Figure legends

Fig. 1 Dormancy release during after ripening in cytoline series.

Each panel shows boxplots of the maximum germination percentage at 15°C for one cytonuclear combination 0, 3, 6 and 9 months after harvest (mph). Rows present genotypes sharing their nucleus and columns those sharing their cytoplasm. Natural accessions are framed in red. The [Sha]Cvi-0 cytoline could not be tested due to the small amount of seeds obtained by hand pollination on this male-sterile genotype.

Fig. 2 Germination performance of cytoline series.

Each panel shows boxplots of the maximum germination percentage (Gmax) of a nuclear series of cytolines, highlighting the natural parental accession in a red dotted frame. White, germination on water; blue, germination on NaCl. Significant differences of germination performance, averaged on the presence of salt, of cytolines compared to their nuclear natural parent are indicated with red stars. Pvalue: ***<0.001<**<0.01<*<0.05 (see Methods S1 for details).

Fig. 3 Contribution of individual accessions to cytonuclear interacting combinations impacting germination traits.

Cytonuclear interacting combinations are plotted according to the contribution of individual accessions. For each accession, the number of combinations involving its cytoplasm (orange) or its nucleus (blue) that significantly impacted dormancy depth at the four PH times (panel a) or germination performance after storage (panel b) are indicated upon the total number of tested cytonuclear interacting combinations (grey).

Fig. 4 Kinetics of loss of germination efficiency during artificial aging.

Controlled deterioration treatment (35°C, 75% relative humidity) was applied for different durations to seeds from cytolines of the Ct-1 (a) and Sha (b) nuclear series. The means of maximum germination percentage from three replicates were plotted. Vertical bars indicate standard deviations from the mean.

The colors of the graphs indicate the cytoplasms of the genotypes: black, Blh-1; blue, Bur-0; orange, Ct-1; green, Cvi-0; purple, Ita-0; brown, Jea; cyan, Oy-0; red, Sha.

Fig. 5 Ratios of Na+ and K+ ion contents in seeds from lines with different cytoplasms associated with the Sha nucleus

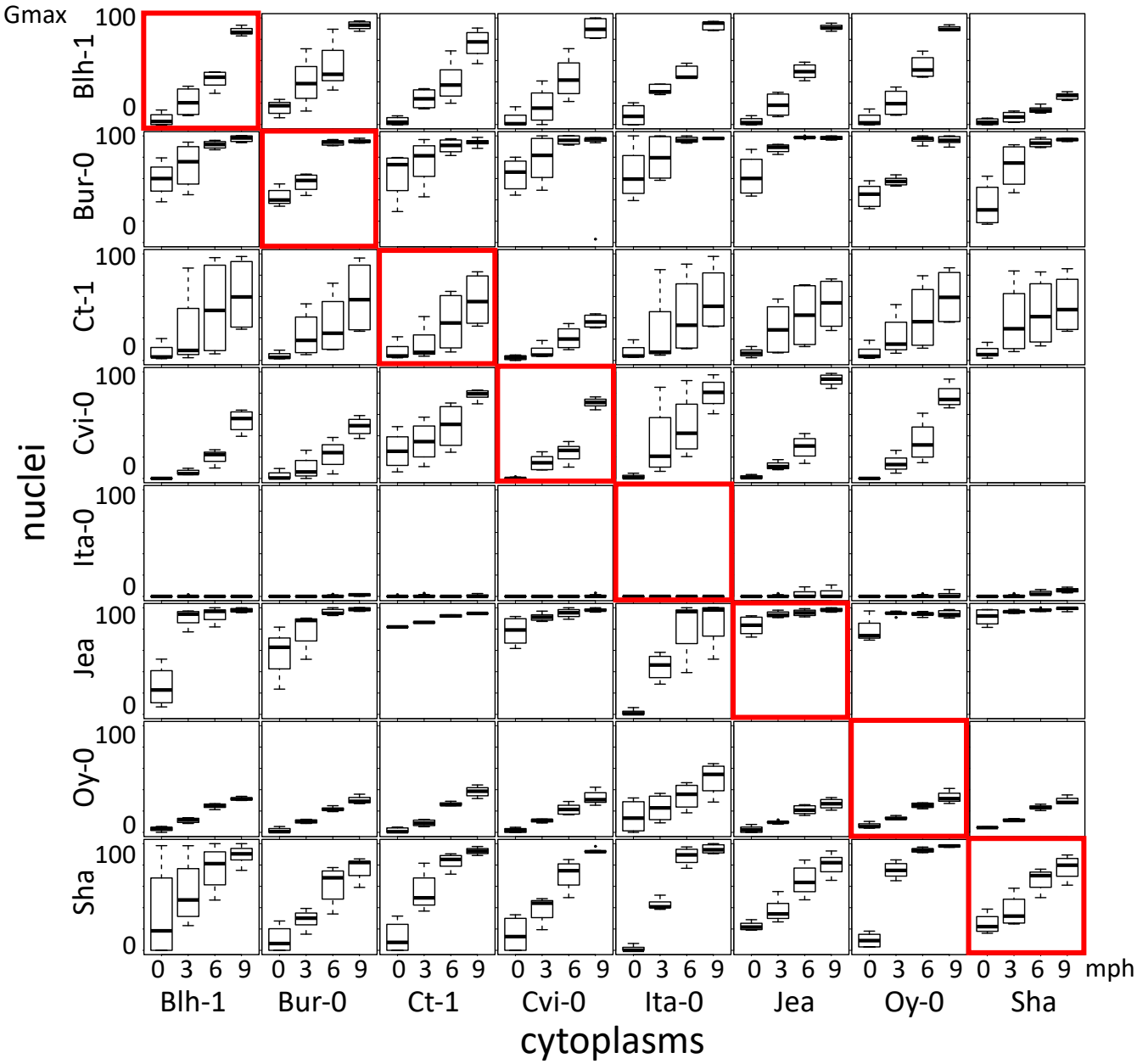
Ratios of Na+ and K+ ion contents just after stratification (white boxplots) or after 20h of germination (blue boxplots) measured in seeds on 100 mM NaCl. The Na/K ratios were different between the three genotypes in pairwise contrast tests. On the figure, only p-values for comparisons of the cytolines to Sha are shown, ***<0.001<**<0.01.

Fig. 6 ABA content and sensitivity in seeds from lines of the Sha nucleus series.

a. ABA content of seeds from [Blh-1]Sha, [Ita-0]Sha and Sha genotypes, at the dry stage (left panel), or after stratification followed by 6 hours of germination on water (middle panel) or on salt (right panel). Results of comparisons between the cytolines and Sha in the plotted experiment are shown, **<0.01<*<0.05; ns, not significant.

b. Germination of stratified seeds from [Blh-1]Sha, [Ita-0]Sha and Sha on water (white boxplots) or on 50 µM ABA (blue boxplots). Germination rate was monitored after 4 days. Results of the test for a different germination response to treatment of each cytoline compared to the parent (significant cytoplasm x treatment effect) are indicated; * pval <0.05; ns, not significant (see Methods S1 for details).

Fig. 1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Blh-1
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Jea
Oy-0
Sha
nuclei
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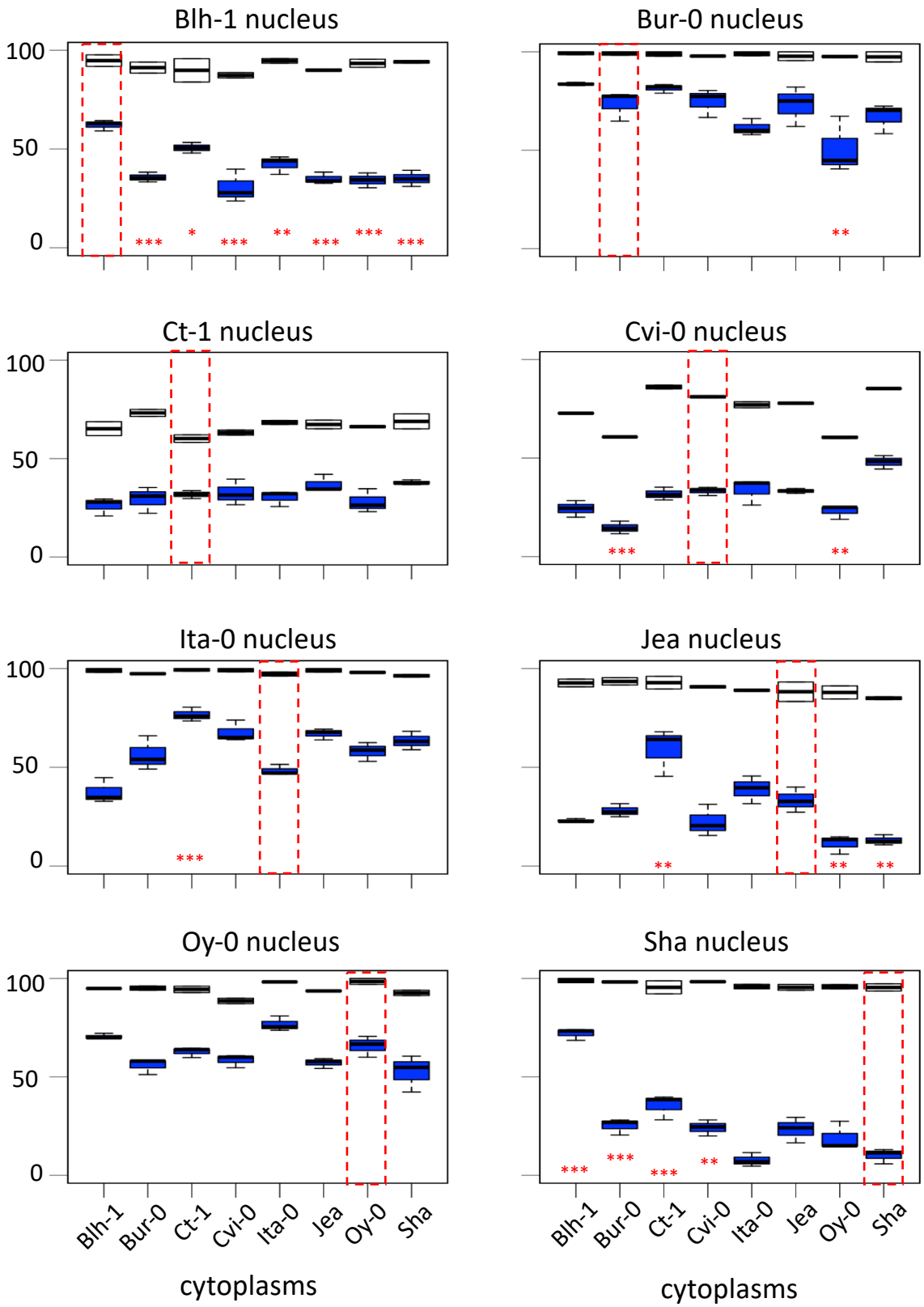
mph
cytoplasms

Fig. 2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Bur-0 nucleus
Ct-1 nucleus
Cvi-0 nucleus
Ita-0 nucleus
Jea nucleus
Oy-0 nucleus
Sha nucleus
100
50
0
Blh-1
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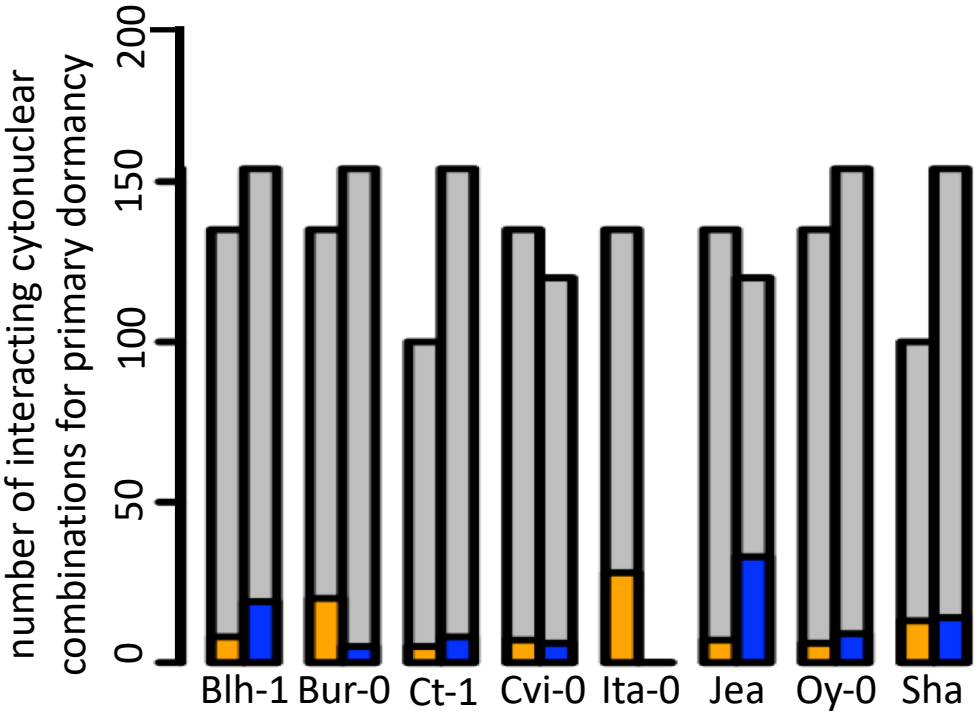
Jea
Oy-0
Sha
cytoplasms
cytoplasms

Fig. 3

a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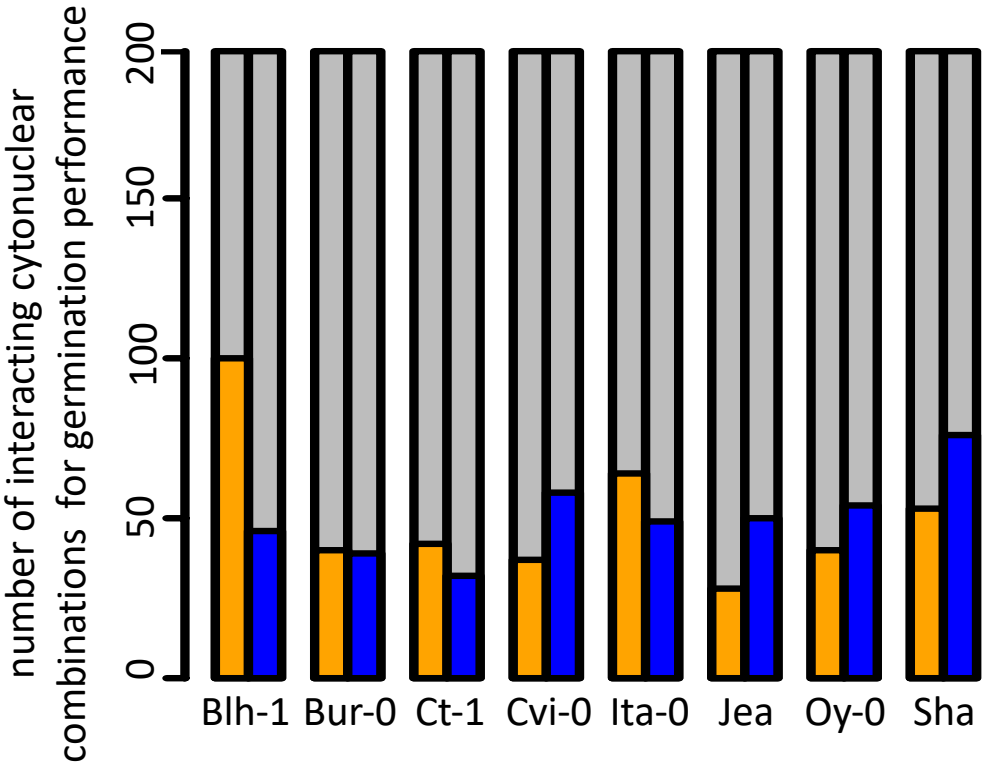
Oy-0
Sha

b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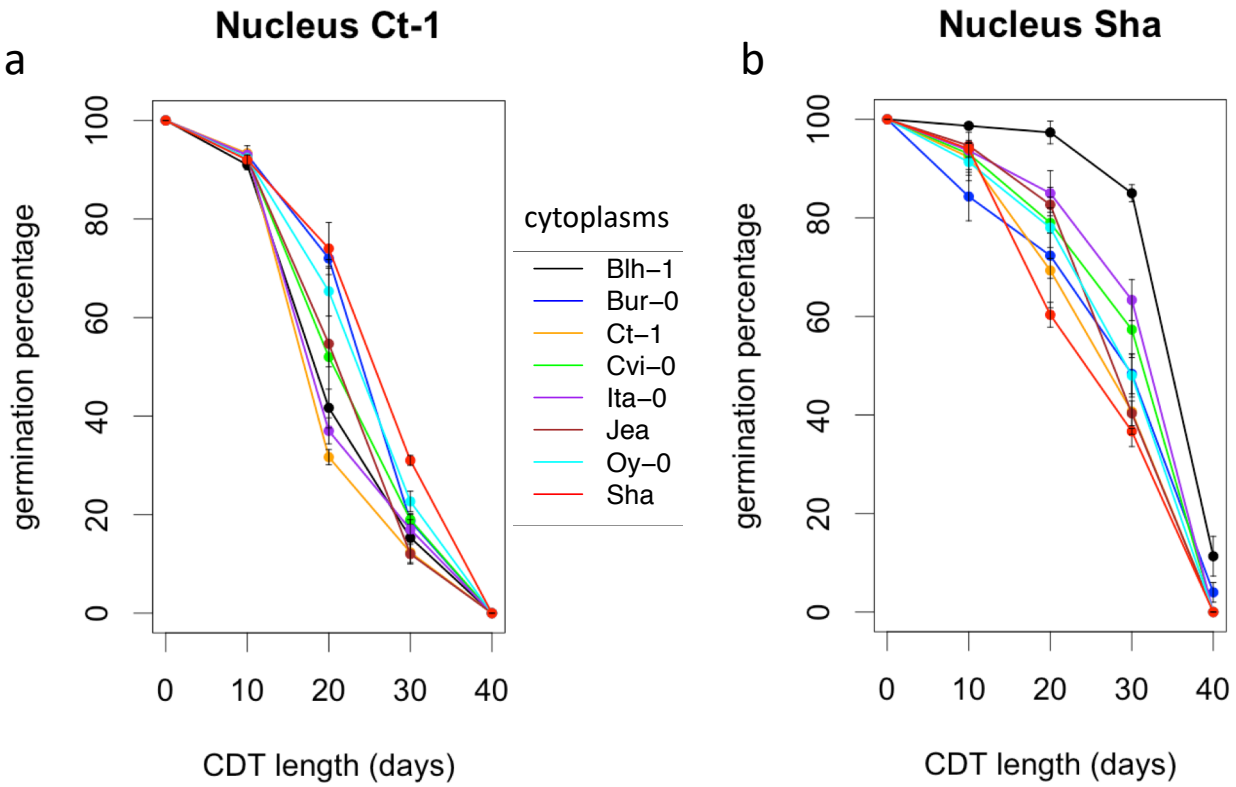
Sha

Fig. 4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Nucleus Ct-1
germination percentage
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40
60
80
100
0
10
20
30
40
CDT length (days)
cytoplasms
Blh−1
Bur−0
Ct−1
Cvi−0
Ita−0
Jea
Oy−0
Sha
b
Nucleus Sha
germination percentage
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20
40
60
80
100
0
10
20
30
40
CDT length (days)

Fig. 5

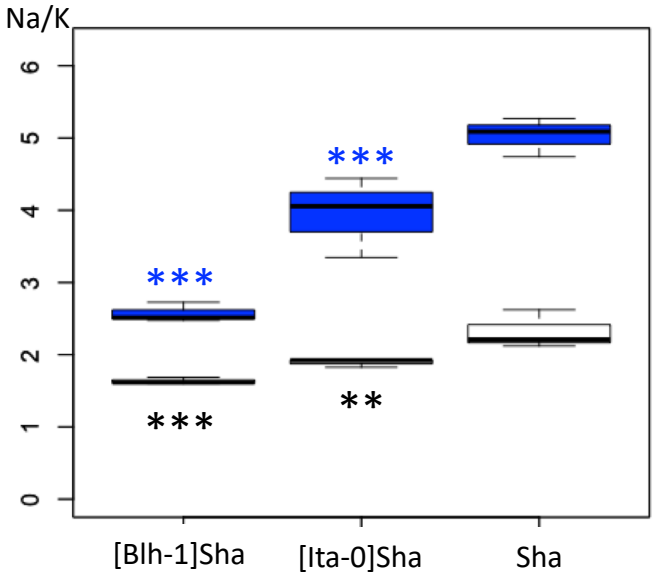
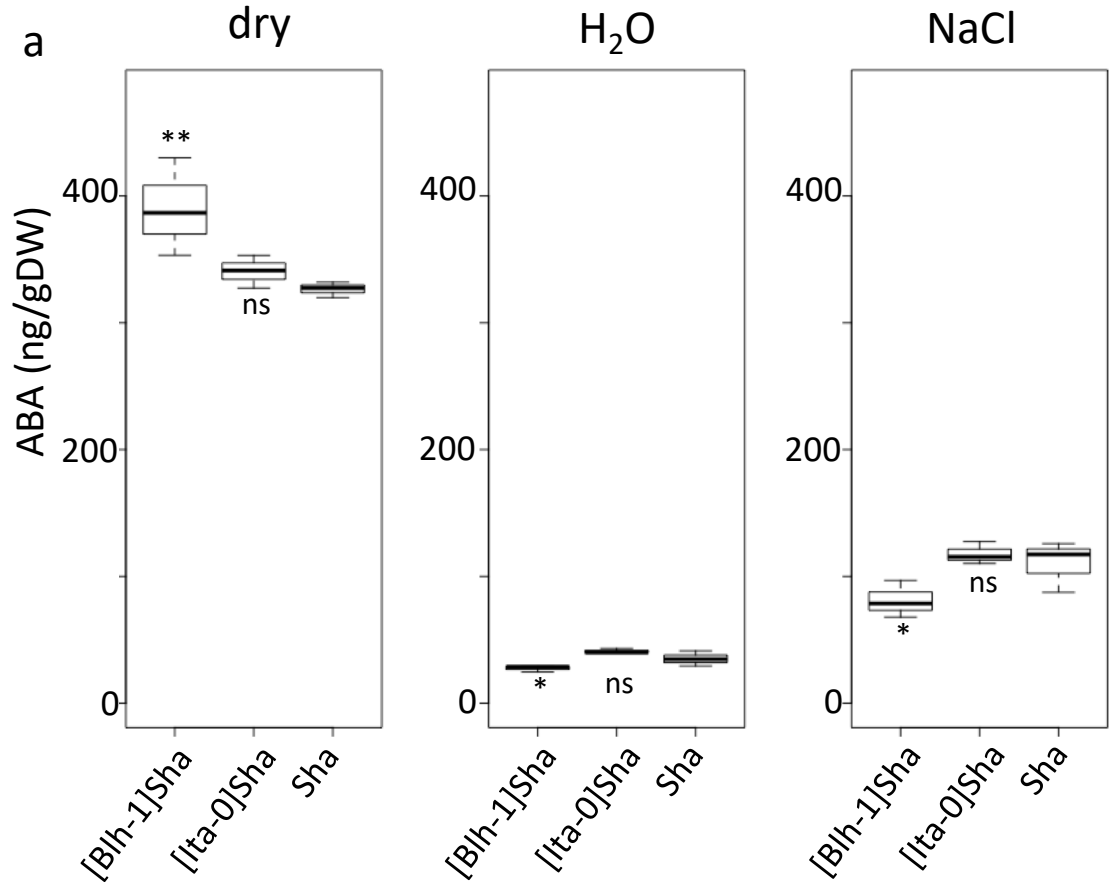


Fig. 6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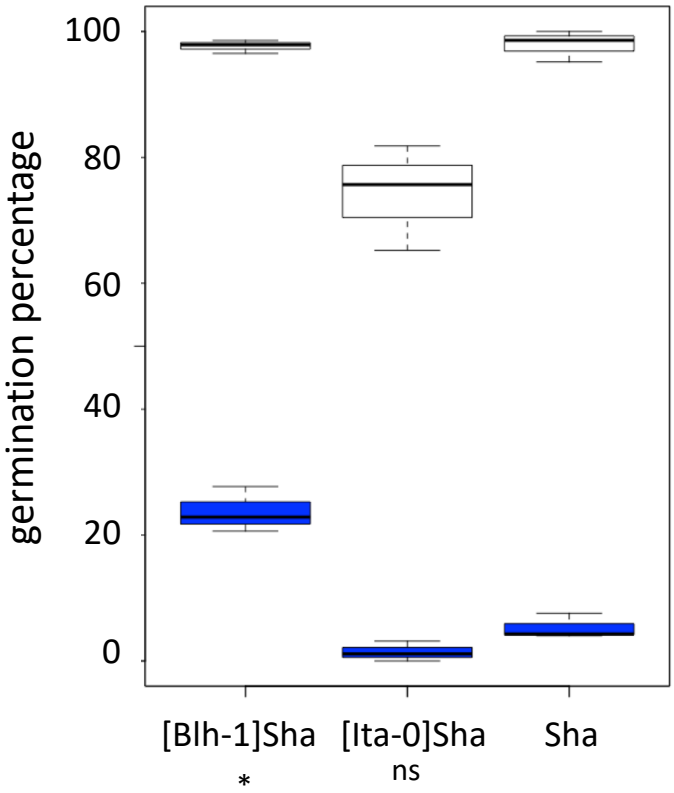
NaCl
ABA (ng/gDW)
400
200
0
**
ns
*
ns
*
ns
[Blh-1]Sha
[Ita-0]Sha
Sha


b
germination percentage
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80
60
40
20
0
[Blh-1]Sha
*
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ns
Sha