The influence of ABA INSENSITIVE 3 on the onset of desiccation tolerance in *Arabidopsis thaliana* seeds

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Abstract

Due to global warming and a consequent decrease in precipitation, researching the mechanisms involved in seed desiccation tolerance becomes of increasing importance for future conservation of desiccation sensitive species. Abscisic acid insensitive 3 (ABI3) has been suggested to be involved in the evolution of desiccation tolerance. The seeds of severe ABI3 mutants are desiccation intolerant after the maturation phase however, ABI3 mutants show some desiccation tolerance during the maturation phase. This makes ABI3 an interesting model to study the acquisition of desiccation tolerance in seeds. In this research a detailed assessment of *abi3-6* and Columbia wild type (WT) desiccation survival during the maturation phase is done. Seeds develop the ability to survive fast drying during the late maturation phase, before the onset of accelerated maturation drying. The way of drying has an influence on the acquisition of DT. If the seeds are dried inside the silique the seeds are able to continue to develop during drying and acquire desiccation tolerance in the early maturation phase. Fast dried abi3-6 seeds show a desiccation tolerant window consisting of two peaks. Indicating that the process of acquiring desiccation tolerance is a combination of several processes spread out over the maturation phase as opposed to one singular, ABI3 dependent, process.

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Introduction

Desiccation tolerance (DT) is the ability to survive in a desiccated state with a water content below 0.1g H_2O g^{-1}DW. Most seeds are desiccation tolerant (termed "orthodox");seeds that do not survive desiccation (termed "recalcitrant") are desiccation-sensitive (Bewley, Bradford, Hilhorst, & Nonogaki, 2013).

The majority of species with desiccation-sensitive seeds occur in evergreen rain forests where 46.6 percent of the species have recalcitrant seeds (Bewley et al., 2013; Tweddle, Dickie, Baskin, & Baskin, 2003). Economically important species such as mango, citrus, and rubber have recalcitrant seeds (Chin, 1980; Drew et al., 2007).

Climate model simulations predict several degrees of global temperature increase by 2100. Consequently, a decrease in precipitation in most tropical and mid latitude regions is predicted (Sherwood & Fu, 1997).Recalcitrant species are adapted to wet or flooded environments. The climate change poses an imminent threat to recalcitrant species as their seeds wouldn't survive prolonged drought (Bewley et al., 2013). Orthodox seeds can be dried to low water contents and frozen for prolonged storage. Conventional seed storage, is impossible for recalcitrant species, impeding the conservation of resources aimed at maintaining genetically healthy crop populations (Chin, 1980; Drew et al., 2007). Better insights into DT could provide alternate methods for the conservation of recalcitrant seeds.

DT does not only occur in seeds; species of bacteria, tardigrades, nematodes, and even cysts of crustaceans tolerate desiccation (Bewley et al., 2013; Crowe, 2014; Wharton, 2015). Animals and plants appear to be remarkably similar in their mechanisms concerning DT (Olivier Leprince & Buitink, 2015).

Desiccation causes damage to the cell's membranes, proteins, DNA, and RNA (Olivier Leprince & Buitink, 2015). The water content reduction in a cell causes membrane collapse, protein denaturation and reactive oxygen species (ROS) accumulation. ROS are highly reactive and "attack" nucleic acids, polysaccharides, proteins and membrane lipids. In orthodox seeds there are systems preventing ROS formation such as the shutdown of the photosynthetic pathway, and systems preventing ROS accumulation such as the exploitation of antioxidants (I. Kranner, Beckett, Wornik, Zorn, & Pfeifhofer, 2002; Ilse Kranner & Birtić, 2005).

In cells of orthodox seeds sucrose accumulates in the cytosol during the maturation phase when DT develops (Bewley et al., 2013). This disaccharide forms a glass that acts as a replacement solvent for water, preventing the denaturation of proteins and membrane collapse. However, glass formation (vitrification) is not enough to protect cells from desiccation damage (Crowe, Carpenter, & Crowe, 1998; Jaap et al., 1993). In orthodox seeds heat shock proteins (HSP), late embryogenesis abundant (LEA) proteins, and oleosins are suggested to protect proteins and membranes from denaturation and membrane collapse (Clegg, 2001; Frandsen, Mundy, & Tzen, 2001; Olivier Leprince & Buitink, 2015).

The genes that regulate protection against desiccation are collectively called the 'desiccome'. Expression of many of the genes that are included in the desiccome are regulated through abscisic acid (ABA) (Terrasson et al., 2013). ABA is a stress related signal molecule in all living kingdoms except archaea (Bruzzone et al., 2007; Hartung, 2010; Hauser, Waadt, & Schroeder, 2011). During seed development the ABA content peaks in the maturation phase when DT is acquired (Kanno et al., 2010).

During the maturation phase of *Arabidopsis thaliana* seeds LEAs, HSPs, and oleosins are highly expressed. Many LEA, HSP, and oleosin genes are controlled by a family of transcription factors(TFs) consisting of ABA INSENSITIVE3 (ABI3), FUSCA3 (FUS3), LEAFY COTELYDON2 and 1 (LEC2 and LEC1). All four TFs are involved in the ABA-GA antagonistic interaction responsible for dormancy in seeds and have an overlap in target genes (Holdsworth, Bentsink, & Soppe, 2008; Monke et al., 2012; To et al., 2006).

In the moss *Physcomitrella patens* ABA and ABI3are essential for the acquisition of DT. The transcription factorsFUS3 and LEC2 are not present in the genome of *P. patens* (Khandelwal et al., 2010), indicating that ABA and ABI3 could have been involved in early evolution of DT in the first land plants.

The seeds of Arabidopsis *ABI3* mutants are green, wrinkled, and desiccation sensitive or non-dormant, depending on the severity of the allele. These phenotypes are established during the maturation phase of seeds when the ABI3 content peaks (Delmas et al., 2013; Koornneef, Hanhart, Hilhorst, & Karssen, 1989; O. Leprince, van Aelst, Pritchard, & Murphy, 1998; Monke et al., 2012).

Wild type (WT) seeds lose their green colour during the late maturation phase. The loss of green colour could be rescued in *abi3-6,* through the overexpression of STAYGREEN 1/2 (SRG1/2). Nonetheless, DT was not rescued by over-expressing SRG1/2, indicating that the green colour and DT are not directly linked (Delmas et al., 2013). The green colour indicates an inability to shut down the photosynthetic system and prevent ROS formation. However, ROS prevention is suggested to be linked to longevity (Olivier Leprince, Pellizzaro, Berriri, & Buitink, 2016).

The *abi3, fus3,* and *lec2*mutants show a defect in

the production of seed reserves and DT (Angeles-Núñez & Tiessen, 2011; Focks & Benning, 1998; Keith, Kraml, Dengler, & McCourt, 1994; Zeng & Kermode, 2004).The storage of lipids in oil bodies and proteins in vacuoles provides resistance against cellular collapse upon drying in the late maturation phase, averting a wrinkled phenotype (O. Leprince et al., 1998). Many genes encoding for seed storage proteins are members of the ABI3 regulon (Monke et al., 2012; Zeng & Kermode, 2004). Energy storage could be part of DT through sugar accumulation leading to vitrification and oil body formation preventing damage due to cell shrinkage (Crowe et al., 1998; Ilse Kranner & Birtić, 2005). However, energy storage is not the sole factor in DT acquisition; ectopic continuous expression of ABI3, FUS3, or LEC2 in Arabidopsis restored seed reserve synthesis without restoring DT (Ooms, Wilmer, & Karssen, 1994; Roscoe, Guilleminot, Bessoule, Berger, & Devic, 2015).

Though ABI3 mutants show more defects next to the acquisition of DT, the *abi3-6* Arabidopsis mutant presents a very interesting model to study DT. The severe *abi3-6* mutant shows no dormancy and desiccation sensitivity at the very end of seed development, yet if the seeds are dried during the maturation phase they can survive desiccation. Assessing DT across the development of *abi3-6* in more detail, will allow future research to focus on DT transition points by transcriptome analysis which may provide knowledge to understand desiccation sensitivity in recalcitrant species.

Materials & Methods

Plant growth conditions:

Arabidopsis thaliana plants, accession Columbia (Col-0, N60000) and *abi3-6* mutant plants, were grown on Rockwool plugs (MM40/40; Grodan), at 22° C, under 16H day 8H night conditions, watered with Hyponex nutrient solution (1 g l-1, http://www.hyponex.co.jp/en) three times a week (growth conditions A) or twice a day (growth conditions B).

Desiccation survival assessment (DSA):

Columbia wild type and *abi3-6*mutant plants were grown under the above mentioned conditions. Staging of the developing seeds was done by tagging individual flowers on the day of anthesis. Immature seeds were collected by opening unripe siliques using a needle and forceps.

The siliques were divided into three treatments: directly germinated (DIR), dried in (IN), and dried out (OUT). The DIR siliques were collected in 50 or 100 µM GA 'for DSA1 and DSA2 respectively' to prevent desiccation. The DIR seeds were excised from the siliques within 50 min after harvest for the germination assessment. The IN siliques were dried in micro tubes for 7 days. The IN seeds were then excised from the silique and dried for three more days on a petri dish with 3H filter paper before the germination assessment. The OUT seeds were excised from the siliques within 30 min after harvest and dried for 3 days on a petri dish with 3H filter paper before the germination assessment.

The drying for the treatments IN and OUT was done at 22°C, in a cabinet with air flow and 30% RH in the dark. The germination assessment was done placing seeds in petri dishes on 3H filter paper, humidified with 1.5mL of 50 or 100µM gibberellin solution 'for DSA1 and DSA2 respectively', at22°C, in cabinets with constant light. The percentage of germination (survival) was scored after five or seven days 'for DSA1 and DSA2 respectively'.

DSA1 was done on plants under growth conditions A. The time points were assessed at different days in a random order.
Each treatment contained six biological replicates from different plants. Each replicate was represented by one silique, on average 28 seeds.

DSA2 was done on plants under growth conditions B. The dried IN treatment was not done in DSA2. The percentage of germination (survival) was scored after seven days. The time points were assessed at different days in a chronological order. From 20DAF onwards the seeds showing seedling establishment were removed and the remaining seeds and underdeveloped seedlings were stratified for 3 days at 4°C before being placed back at 22°C to germinate for 7 days.
Each treatment contains one biological replicate represented by 2 siliques (approximately 60 seeds) from different plants.

Water content determination.

Columbia wild type and *abi3-6* mutant siliques, grown under conditions B, were harvested in micro tubes containing water soaked filter paper. Approximately 100 seeds were excised from siliques, weighed within 10 minutes after excision and after 24H of drying at 105°C. The seeds were weighed using the Perkin Elmer AD-4 auto balance.
Each treatment contains one biological replicate

represented by 4 siliques (approx. 100 seeds) from different plants.

Results

Desiccation survival assessment 1.

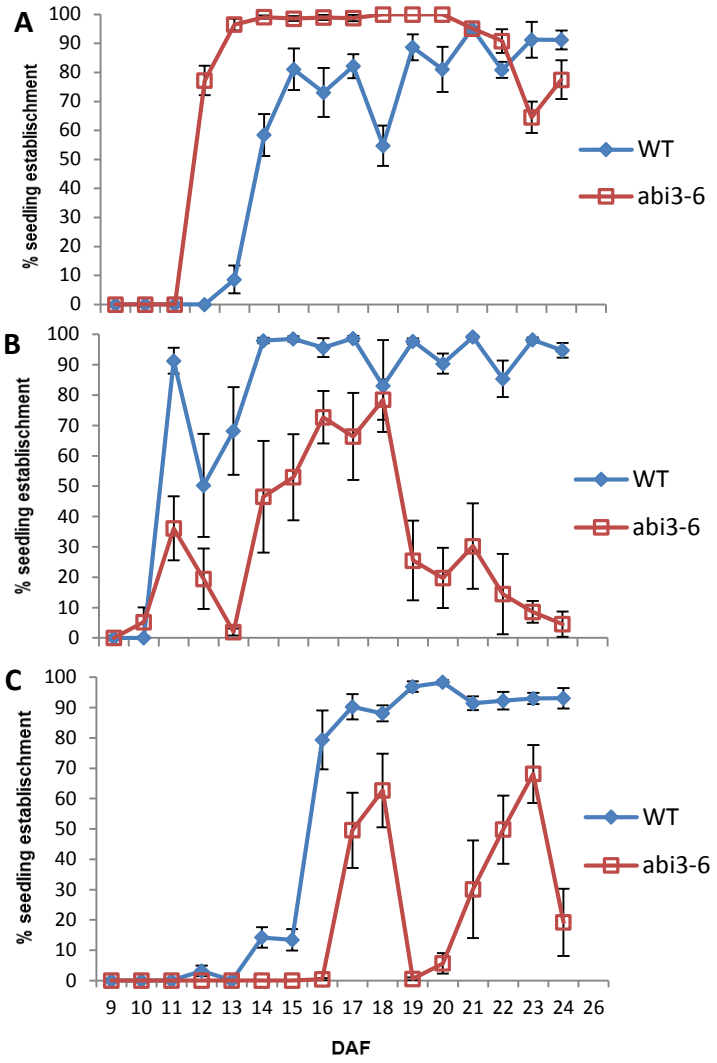

Fig1: percentage of average seedling establishment per silique after 5 days per Day After Flowering (DAF), with error bars showing the standard error (6 siliques) for Colombia WT (—◆—) and *abi3-6* (—□—).
A, seeds germinated directly after harvest. (DIR)
B, seeds first dried inside silique for 7 days then excised and dried outside the silique for 3 days. (IN)
C, seeds germinated after 3 days of drying outside of the silique(OUT).

In order to assess whether seeds survive desiccation, the ability of seeds to establish seedlings without drying was first ascertained as a reference.
when not dried (fig1A) *abi3-6* showed almost full seedling establishment from 12 DAF onwards, until 20 DAF. At 10 DAF, 20% of the *abi3-6* seeds showed some protrusion however, no full seedling establishment was observed. The WT seeds showed more than 50% seedling establishment 3 days after the *abi3-6* seeds. The directly germinated WT seeds did not attain full seedling establishment on average. The non-dried *abi3-6* seeds showed a higher germination percentage and reached full seedling establishment on average.
Seeds dried inside the silique (fig 1B) show earlier seedling establishment compared to the directly geminated and dried OUT seeds. Both in *abi3-6* and WT some seeds show protrusions at 9 DAF however, no full seedlings were established. The average seedling establishment of *abi3-6* stayed lower compared to the DIR germinated seeds. abi3-6 and WT showed a decrease in germination at 13 and 12 DAF respectively. The period of desiccation survival for seeds first dried inside the silique was longer compared to that of seeds dried outside of the silique. On average more seedlings were establishment, for the dried IN seeds compared to the dried OUT seeds, 32% and 19% percent respectively.
WT seeds dried outside of the silique (fig1 C) started germinating later in development compared to the other treatments. The WT seedling establishment fluctuation seemed less in the dried out seeds compared to WT directly germinated and WT dried inside the silique. The dried out *abi3-6* seeds started to germinate at 17 DAF, and peaked with an average of 62 percent showing seedling establishment at 18 DAF where all siliques showed some seedling establishment. At 19 and 20 DAF the average seedling establishment stayed below 10%, and no siliques showed more than 20% seedling establishment. From 21 till 23DAF the average seedling establishment rises again till 68% and at 23 DAF there were no siliques showing no seedling establishment. At 24DAF the average seedling establishment drops below 20%.

Desiccation survival assessment 2.

In order to confirm the results of DSA1 in relation to the water content determination, DSA2 was done with plants grown under the same growth conditions as the water content determination. The points 23 and 25 DAF where not measured in DSA 2.
The WT seeds germinated on 100µm GA started to establish full seedlings at 12 DAF. The percentage increases till a peak at 14 DAF where 72% of the seeds fully germinate and 82% showed some kind of protrusion (data not shown). From 14 till 19 DAF the amount of germination declines. After 19 DAF the amount of germination increased again till all seeds fully germinated at 26 DAF. The WT seeds germinated in water started germinating after 20DAF, and reached 100% germination at 26 DAF. At 20 DAF flowering all stratified seeds germinated on water established seedlings while only 53 percent of the GA stratified seeds established seedlings (fig 2A)

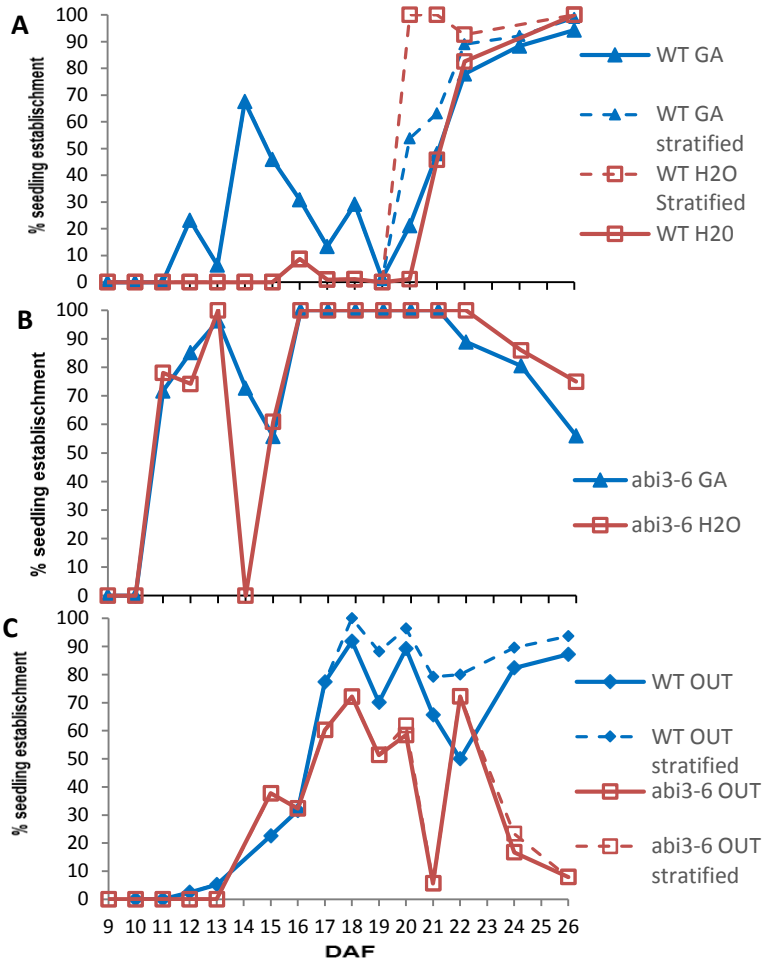

Fig2: percentage of seeds germinating per Day After Flowering (DAF)
A, Columbia WT seeds germinated directly after harvest in GA (—◆—) and H_2O (—□—) The dotted line represent the non-germinated seeds that germinated after extra stratification. This was only done from 20 DAF onwards
B, *abi3-6*seeds germinated directly after harvest in GA (—◆—) and H_2O (—□—) extra stratification made no difference in the values from 20 DAF onwards
C, seeds germinated after 3 days of drying outside of the silique(OUT).The two strains are: Colombia WT (—◆—) and *abi3-6* (—□—) The dotted line represent the non-germinated seeds that germinated after extra stratification. This was only done from 18 DAF onwards.

The directly germinated *abi3-6* showed germination and distorted seedling establishment at 9 DAF (data not shown). Full seedling establishment started at 11 days. At 14 DAF seedling establishments dropped for the GA and water treatments. The seeds germinated in water showed less than 1% germination and no seedlings were established at 14 DAF. The seeds germinated in GA reached their low point at 15 DAF where 19% of the seeds showed some protrusion or distorted seedling establishment and 55% of the seeds showed seedling establishment. At 16 DAF both treatments showed 100% seedling establishment till 21 DAF after which both showed a drop in seedling establishment. The final drop in seedling establishment was steeper in the GA treatment. There were no differences in seedling establishment after stratification from 20 DAF onwards (fig2 B).

The dried out WT seeds slowly started establishing seedlings from 12 DAF till 16 DAF after which there was a steep increase in seedling establishment and the seedling establishment percentage fluctuates between 70% and a 100 %. The dried out *abi3-6* starts establishing seedlings after 13DAF. The seeds from 14 DAF were moved after 1 day of drying because of a scheduling error and this point is deleted from the data. *Abi3-6* dried out seeds peaked at 18 DAF where 72% of the seeds established seedlings after desiccation. At 21 DAF only 5% establishes seedlings. At 22 DAF there is a peak of 70% seedling establishment after which there is decline in seedling establishment till 8% at 26DAF. Seeds were stratified after counting from 18DAF onwards. The WT seeds established more seedlings for all stratified time points while there were only minor changes in the *abi3-6* seedling establishment (fig2 C).

Water content

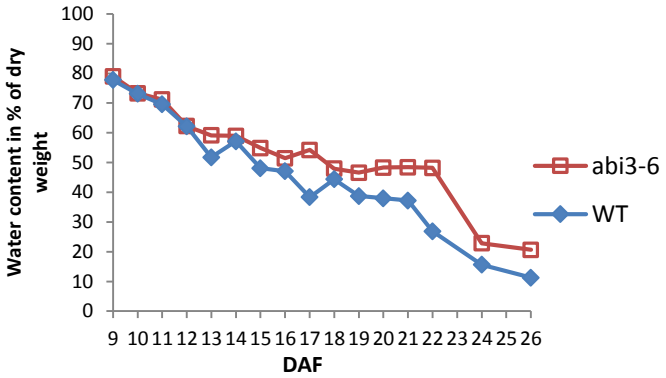

Fig 3 water content in % of DW per Day After Flowering (DAF) for Colombia *WT* (—◆—) and *abi3-6* (—□—) plants grown under the same conditions as DSA2.

The water content determination (fig3) was done for plants grown under the same circumstances as DSA2. The points 23 DAF and 25DAF were not measured. WT and *abi3-6* started at a water content around 80% (78% and 79% respectively). The WT seeds showed a steady linear decline in WC (a=-3.7, R^2=0.96), However, the graph seems to easily divide into three separate sections; from 9-13 DAF with a steeper drop in water content compared to 14 till 20 DAF, and from 21 till 26 DAF when the WC seems to rapidly decline again ending at 11% I.e. 0.11g H_2O g DW^{-1}. The *abi3-6* decline in WC was not significantly linear (R^2=0.89). For the first 4 points the WC decline of *abi3-6* seems similar to the decline in WT, from 13 DAF onward the *abi3-6* water concentration decline stagnated at 50% till 22 DAF after which there was a steep drop in WC till 24 DAF. The water content was not measured at 23 DAF impeding a more precise determination

of the onset of the decline in water content. At 26 DAF the WC of abi3-6 is 20% I.e. 0.2 g H_2O g DW^{-1}.

Discussion

Dormancy

The evolution of desiccation tolerance was most likely accompanied by the evolution of dormancy; both are hypothesized to be evolved as a solution for survival in fluctuating environments during the transition from aquatic to terrestrial life (Costa et al., 2016). ABI3 is involved in the acquisition of dormancy and DT (Costa et al., 2016; Holdsworth et al., 2008).

In fig 2A it is shown that there is dormancy in the first part of the WT maturation phase. Seeds show more germination under GA treatment compared to the water control. Unfortunately, GA alone was not always enough to break dormancy in the WT seeds. There are additional hormone independent pathways creating dormancy (Holdsworth et al., 2008; Murphey et al., 2015). In fig 2A after 20 DAF it is seen that additional stratification was needed to break dormancy in all seeds. During stratification of wild type seeds ABI3 levels decrease in the absence of ABA inhibiting ABI3 dependent dormancy (Lopez-Molina, Mongrand, McLachlin, Chait, & Chua, 2002). ABA and dormancy levels differ between seeds within siliques (Kanno et al., 2010; Mitchell, Johnston, & Bassel, 2016). This could explain why the WT seeds did not reach a 100% average seedling establishment when germinated on GA contrary to *abi3-6* seeds (fig1,2 A,C; fig2 B); The difference being the result of issues concerning dormancy.

ABI3 mutants do not show dormancy and stay in a developmental state that more closely resembles a developing seedling (Nambara, Keith, McCourt, & Naito, 1995; Nambara et al., 2002). *Abi3-6* seems to have some dormancy at 14 DAF in DSA2 (fig2 B) where more seeds germinated in GA compared to water. In DSA1 there was not one silique showing less than 95% germination between 13 and 20 DAF. A similar experiment harvested from the same plants did not show this dip in germination (data not shown). For *abi3-6* the extra stratification from 20 DAF onwards made no difference in seedling establishment for directly germinated seeds, and only small differences for dried out seeds (fig2 B,C); this indicates that there is no dormancy in *abi3-6* 'conform literature' and the dip at 14 DAF was a human error.

Speed of drying and DT

DT is defined as survival upon rehydration after drying below 0.1g H_2O g DW^{-1} (Alpert, 2005). However the way of drying has an influence on desiccation survival, more seeds survived the slower drying IN compared to the faster drying OUT (fig1 B,C). Ooms, van der Veen, and Karsen (1994) described the effect of different drying methods on the desiccation sensitive *aba1-1/abi3-1* mutant. In their case the germination percentage only reached above 50% when the seeds where dried slow or inside the silique. This indicates that the rate of water loss is essential for DT induction in immature seeds(Ooms et al., 1994).

The "slow" dried IN seeds survived slow drying before the non-dried seeds are able to establish seedlings (fig1 A,B),indicating that there is some seed development during drying inside the silique. At the point of the highest *abi3-6* drying IN survival the WC was around 50% (fig3). Still allowing metabolic processes and the development of protective mechanisms to take place (Walters, 2015).

In our experiments both WT and *abi3-6* only survive fast drying when the seeds are more mature. This was also observed by Ooms et al. (1994), and is probably due to the further development of the seed and the subsequent synthesis of protective mechanisms (Bewley et al., 2013; Ooms, van der Veen, et al., 1994).

Abi3-6 seemed "reluctant" to desiccate; retaining a higher water content compared to WT seeds from 13 DAF onwards. After 22 DAF when the seeds start drying at a faster pace (fig 3) *abi3-6* desiccation survival drops in all graphs. At 23 DAF the percentage of seeds surviving an additional 3 days of fast drying in DSA1 equalled the percentage of seeds surviving direct germination (fig1 A,B). Indicating that desiccation protective mechanisms are in place at 23 DAF. On average less than 10% of the slow dried seeds survived at 23 DAF. Recalcitrant seeds are known to survive to lower water contents when dried rapidly compared to when they are dried slowly; this could be due to the longer time spent drying causing ROS to accumulate to lethal levels (Pammenter & Berjak, 2014).

At most of the time points for the dried *abi3-6* seeds there is a lot of variation in seedling establishment within and between the siliques, as can be observed from the average percentage and error bars of 6 siliques respectively. One reason for this variation could be asynchronous seed development within and between siliques. One evolutional advantage of this variation is called batch hedging where the mean population fitness is reduced 'the average seedling establishment',

while the chances of the next generation to survive under sup-optimal conditions 'desiccation' are increased (Mitchell et al., 2016).

Desiccation tolerance in the recalcitrant abi3-6

Desiccation tolerance after "fast" drying develops during the late maturation phase (fig1,2 C), before the increase in maturation drying, at 21 and 22 DAF for WT and *abi3-6* respectively (fig3).

After the maturation drying WT seeds showed a water content of 0.1g H_2O g DW^{-1}, and full seedling establishment at 26 DAF (fig3, fig2 A). After 3 more days of additional drying, ensuring desiccation (Nambara et al., 1995), more than 90% of the seeds established seedlings (fig2 C); confirming WT seeds as orthodox (Walters, 2015).
After the maturation drying, at 26 DAF, the *abi3-6*water content was around 0.2g H_2O g DW^{-1} (fig3). 20% WC is the threshold for onset of instant seed mortality in recalcitrant seeds. However, more than75% of the *abi3-6* seeds survived direct germination at 26DAF (fig2,1 A); this places *abi3-6* on the border between recalcitrant and intermediate seeds (Walters, 2015).After desiccation less than 10% of the *abi3-6* survived at 26DAF.

The dried out abi3-6showed two peaks of desiccation survival in DSA1. All siliques show seedling establishment at 18 and 23 DAF, while there are no siliques with more than 20% seedling establishment at 19 and 20 DAF. This indicates that both peaks in desiccation survival are occurring in all siliques. In DSA2 the window of DT is bigger and new maximum amounts of germination compared to DSA1 are observed at 15,16,19,20 DAF (fig1,2 C). This bigger DT window could be due to different external and technical factors such as the different watering method and the larger amount of time before the seeds where scored for germination, or due to intrinsic factors such as maternal variability (Mitchell et al., 2016). In DSA2 There is a drop in seedling establishment at 21 DAF creating two peaks of desiccation survival. An increase in replicates could cause both peaks in desiccation survival to overlap, creating one larger average desiccation survival peak with a higher standard deviation.

Both experiments showed no desiccation survival at the final harvest (data not shown). In both DSA DT is acquired during and lost during the maturation phase of the abi3-6 mutant. This "desiccation tolerant window" in abi3-6 suggests that the acquisition of DT consists of different mechanisms over time.
Similar researches, in a Landsberg erecta (Ler) background, used the *aba1-1/abi3-1* double mutant as a model for recalcitrance. The less severe *abi3-1* is DT after maturation, the ABA deficient *aba1-1/abi3-1* double mutant is not. Compared to this research less detailed DSAs using the *aba1-1/abi3-1* double mutant, showed only 1 peak of DT during the maturation phase (Koornneef et al., 1989; Ooms, van der Veen, et al., 1994; Ooms, Wilmer, et al., 1994). A compared to this research less detailed DSA of *abi3-5*, also in a Ler background, showed signs of a second DT peak (Jaap et al., 1993). This suggests that ABA may be involved in one of the ABI3 independent desiccation survival peaks.
ABA is synthesized in maternal and zygotic tissues at different stages during development, and is suggested to have distinct physiological functions depending on origin and stage of seed development. During the development of seeds there are two peaks in ABA concentration. The first peak, between 8 and 12 DAF, is before our acquisition of DT (fig1,2 C). The second peak, around 18 DAF, coincides with our first peak of DT for *abi3-6* (fig1,2 C). This indicates that ABA could have been involved in our first peak of desiccation survival in *abi3-6* (Gazzarrini, Tsuchiya, Lumba, Okamoto, & McCourt, 2004; Kanno et al., 2010; Okamoto et al., 2006).
possible candidates for an ABA independent desiccation peak are Dehydration Responsive Element Binding proteins (DREBs). DREBs have been annotated as an ABA independent road to DT (Gutierrez, Van Wuytswinkel, Castelain, & Bellini, 2007; Sakuma et al., 2006). Ooms et al. (94) suggested DREBs as the responsible genes for ABA independent desiccation tolerance in the *aba1-1/ abi3-1* double mutant (Ooms, van der Veen, et al., 1994). Later research found evidence of crosstalk between some DREB and ABA (Lee et al., 2010). ABA responsive elements are found in all DREB genes, however DREBs also have ABA independent binding motives which are known to respond to abiotic stresses (Sazegari, Niazi, & Ahmadi, 2015). perpetuating DREBs as a possible candidate gene causing an ABA independent desiccation peak in *abi3-6* seeds.

Conclusions

Desiccation tolerance is acquired during the late maturation of seeds before fast maturation drying. The desiccation sensitive seeds of *abi3-6* can survive desiccation at some points during the maturation phase, yet not after. Indicating that the process of DT acquisition is a combination of several processes, instead of one singular *abi3-6* dependent process. The speed of drying has a big influence on the ability to survive desiccation. *Abi3-6* seeds are able to develop and acquire desiccation tolerance during drying inside the silique increasing the overall survival when dried slow.

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