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The re-establishment of desiccation tolerance in germinated tomato (*Solanum lycopersicum*) seeds

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Research Paper

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Abstract

Desiccation tolerance (DT) of seeds, one of the plant's environmental adaptation mechanisms, allows them to survive as seeds in a quiescent state under extremely water-deficient conditions during the plant's life cycle, followed by seed germination and seedling establishment under favourable water conditions. The seed-DT is lost after radicle emergence; however, there is a developmental period called the 'DT window' during which the germinating seeds can re-induce DT following a cue from their ambient low water potential (i.e. mild osmotic stress). The DT re-inducibility within the DT window has been used as a model biosystem for understanding molecular mechanisms that activate/supress DT in a number of plant species. However, the characteristics of the DT window for species particularly important to the agroindustry are still largely fragmented. Here, physiological analyses were performed, aiming to elucidate the properties of the DT window in tomato, a model species for Solanaceae, holding a key strategic position for the seed industry and commercial use around the world. We revealed that (i) the DT window of tomato seeds is closed when the developing radicle reaches about 4 mm after germination, (ii) the most effective ambient water potential to re-induce DT into seeds is about −1.5 MPa and (iii) there is organ specificity of DT re-induction with hypocotyls, showing a longer DT window than cotyledons and roots in post-germination seeds.

Introduction

Desiccation tolerance (DT) can be defined as the ability of an organism to tolerate dehydration to about 10% residual water content, which is approximately equivalent to 50% relative humidity (RH) at 20°C, reducing the water potential to −100 MPa (Alpert, 2006). DT is an ancient trait in plants and is almost universal in land plants, but it is mostly confined to reproductive structures such as spores, pollen and seeds, with only a few species having DT in vegetative parts of plants, such as resurrection plants (Oliver et al., 2020). The majority of angiosperm species produce seeds termed 'orthodox seeds' that have DT and long-term dry storage (Roberts, 1973). The orthodox seeds acquire DT during seed development at the early maturation phase (Ooms et al., 1993; Ellis and Hong, 1994), and the timing of DT acquisition of seeds is not affected by environmental stresses compared to other seed traits such as seed longevity, suggesting that it is a highly robust and vital trait for angiosperm species (Righetti et al., 2015). Seeds lose their DT after radicle emergence; consequently, the established seedlings are sensitive to desiccation. For example, in *Medicago truncatula*, a model Fabaceae, more than 80% of the germinated seeds with radicles of 1 mm were still able to tolerate desiccation, whereas only 20% of the seeds were desiccation tolerant when their radicles were developed to 2 mm, and once the growing radicles reached 3 mm, the DT was almost completely lost (Buitink et al., 2003). In a model plant, *Arabidopsis thaliana*, the seed-DT began to be lost at the developmental stage of testa rupture, and the most seeds became desiccation-sensitive when their radicles were protruded (Maia et al., 2011). This suggests that the timing of suppression of DT in orthodox seeds is strictly controlled in a developmental programme during/post-germination processes.

A landmark discovery was made in the 1990s; when post-germination seeds (i.e. potentially desiccation-sensitive) were incubated with polyethylene glycol (PEG) prior to desiccation, the PEG-incubated seeds were tolerant to desiccation compared to non-PEG-incubated ones (Bruggink and van der Toorn, 1995). This DT bioassay with post-germination seed with or without PEG incubation has been used as a model system to elucidate the molecular mechanisms of seed-DT in many studies and resulted in uncovering a developmental time period (called 'DT window') during which seed-DT can be re-induced by applying mild osmotic stresses (i.e. using PEG) or the plant hormone abscisic acid (ABA), resulting in the prolongation of seed-DT to some extent (Dekkers et al., 2015). Successful conditions to re-establish the seed-DT have been reviewed in detail for 12 species (Peng et al., 2022), including those of herbaceous plants (e.g. *A. thaliana*, *Cucumis sativus*, *Impatiens walleriana*, *M. truncatula*,

Pisum sativum, *Vigna unguiculata* and *Xerophyta viscosa*) and woody plants (e.g. *Cedrela fissilis*, *Caragana korshinskii*, *Peltophorum dubium*, *Sesbania virgata* and *Tabebuia impetiginosa*). The fact that seed-DT re-induction is possible in a broad range of species implies that this DT window is an essential timeframe for seeds to optimize seedling establishment in changing environments (e.g. seedling establishment must be developmentally programmed to take place only where sufficient water is available through the wet–dry cycle of the soil, in combination with seed dormancy mechanisms) (Dekkers et al., 2015). The DT window generally spans up to a certain time point post-germination, where the degree of inducible DT is greatly impacted by the growth stages. For example, Arabidopsis seed-DT can be re-established by PEG and ABA, and both treatments on post-germination seeds with radicles up to 0.3 mm re-induced DT in more than 80% of the seeds, while on more developed seeds showing root hairs, the DT was re-induced in less than 40% of seeds (Maia et al., 2011, 2014). Similarly, PEG treatments of Medicago seeds re-induced DT in more than 80% of seeds with up to 2.7 mm long radicles, but in less than 10% of seeds when the growing radicles reached 4 mm (Buitink et al., 2003). In addition, different parts of seeds showed varying degrees of DT, in which roots appear to be the most desiccation-sensitive organ. After the DT assay in Medicago without PEG treatment, germinating seeds often demonstrated cotyledon growth, albeit resuming less radicle growth (Faria et al., 2005). This tendency is more prominent in the PEG-treated DT assay, where root growth re-initiation was only observed at the early post-germination phase, whereas a high proportion of healthy cotyledons were developed from all the tested post-germination stages (Sano et al., 2022), consistent with previous findings that a greater proportion of seeds treated by PEG succeed in re-inducing DT to cotyledons than to roots for a longer time frame of post-germination in Arabidopsis (Maia et al., 2011). Moreover, levels of osmotic potential, temperature and treatment time are known to be factors that influence the degree of re-establishment of seed-DT, and the optimal conditions vary among the reported plant species (Peng et al., 2022).

The ability of seeds to re-induce DT is not only important for ecological adaptation in plants but also beneficial for agricultural production. An example is the widely used commercial technique for a pre-sowing treatment called seed priming, which can improve seed germination/seedling performance. The treatment involves the imbibition of seeds under controlled conditions to trigger metabolic processes for germination and subsequent drying of the seeds prior to the loss of seed-DT, so that seeds re-enter into a quiescent state of being viable (Varierl et al., 2010; Dekkers et al., 2015; Paparella et al., 2015). A wide range of priming treatments have been developed to meet the optimal priming conditions for different plant species, cultivars and lots. Nevertheless, an over-stimulation of seeds by priming (e.g. prolonged imbibition time) sometimes impairs the DT of primed seeds, known as over-priming, thereby causing the deterioration of seed quality such as a shortened seed life span and the formation of abnormal seedlings (Tarquis and Bradford, 1992; Fabrissin et al., 2021; Pagano et al., 2022). Studies on the loss and re-establishment of seed-DT will provide basic useful information to the seed industry; however, the re-induction of DT and the characterization of DT windows, especially for species important to the agroindustry, are still largely incomplete. Tomato, a model species for Solanaceae, holds a key strategic position for the seed industry, being one of the world's most important vegetables in terms of cultivation area, production, commercial use and consumption (World Processing Tomato Council, 2021). Here, we performed physiological analyses of seed-DT re-induction on tomato (*Solanum lycopersicum* L. cv. Micro-Tom) post-germination by using PEG treatments, aiming to characterize its DT window timing and re-activation mode.

Materials and methods

Plant materials

For seed production, tomato plants (*S. lycopersicum* L. cv. Micro-Tom) were grown in a greenhouse with standard conditions (23°C/19°C (day/night), a minimum photoperiod of 16 h provided by supplementary lighting, watered with a nutrient solution). Seeds from mature fruits were collected, and locular tissues were removed by incubation in a pectolytic enzyme solution (Lafazym CL Laffort, France) for 1 h, followed by extensive washing with water to remove the remnants of fruit tissues. Thereafter, seeds were equilibrated and dried at 44% RH using a saturated solution of K_2CO_3 at 20°C for 3 days (d), then hermetically stored at 4°C prior to seed physiological analyses.

Assessment of DT

Seeds were imbibed with sterile deionized water on filter paper in a Petri dish at 20°C for 3 d in the dark, then germinated seed samples with 1, 2, 3, 4 and 5 mm radicles were desiccated at 20°C for 3 d at 44% RH using a saturated solution of K_2CO_3. For mature seeds, no additional dehydration treatment was performed as they had already been dried for at least 3 d using a saturated solution of K_2CO_3 prior to their storage. The desiccated samples (mature seeds and germinated seeds) were then rehydrated with sterile deionized water on filter paper in a Petri dish and incubated in a growth chamber at 20°C under a 16 h photoperiod with 50 µmol m^{-2} s^{-1} light intensity. As the desiccation-sensitive organs show a growth arrest after the rehydration, the DT of each organ was evaluated based on the healthy developmental level of each organ (cotyledons: open-green at least 5 mm, hypocotyl: green at least 5 mm and roots: developed at least 10 mm) at 14 d after the incubation. Each healthy organ from a pool of 15 seedlings in 4 replicates was scored as DT- or desiccation-sensitive based on their ability to reach the corresponding criteria described previously, which led to percentages as the survival rate after the DT assay. The survival rate after the DT assay for whole seedlings was scored only when all organs (root, hypocotyl and cotyledon) fulfilled the criteria.

Re-establishment of DT

Seeds were germinated in water as described above, and samples with 1, 2, 3, 4 and 5 mm radicles were transferred to a PEG-8000 (Sigma-Aldrich, USA) solution with an osmotic potential of −0.5, −1.0, −1.5, −2.0, −2.5 and −3.0 MPa on filter papers and incubated at 10°C, aiming to block further growth of radicles for 3 d in the dark. The incubated samples were washed with sterile deionized water to remove residual PEG, then desiccated and rehydrated to score DT as described above. Photographs of germinated seedlings were acquired by using a digital microscope, Makrolite (Vision Engineering, UK).

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Tetrazolium viability test

Seed organ viability tests using a tetrazolium (TZ) staining were carried out as described previously (Santos et al., 2007) with a modified concentration of 1% (w/v) 2,3,5-triphenyltetrazolium chloride solution (Sigma-Aldrich, USA). Untreated and PEG-treated post-germination seeds with 1–5 mm radicles were dehydrated as described above. Seeds were then dissected longitudinally through the midsection of the embryonic axis using a surgical blade and incubated with the staining solution at 40°C for 3 h in the dark. Stained samples were observed by using the digital microscope Makrolite in triplicates of 20 seeds per treatment, and red-stained organs were considered viable.

Data plots and statistical analysis

Bar graphs with jitter points were drawn using the package ggplot2 (Wickham, 2016) (version 3.4.2) in R (R Core Team 2022) (version 4.2.2). Tukey–Kramer tests were performed using R to determine significant differences in multiple comparisons of values for the assessment of DT.

Results

Loss of DT in tomato after seed germination

In order to assess the process of loss of seed-DT in tomato, mature seeds and post-germination seeds with radicles ranging from 1 to 5 mm were subjected to DT assays (dehydration and rehydration) without applying a mild osmotic stress (i.e. non-PEG treatment) (Fig. 1A). At 14 d after rehydration, almost all mature seeds established normal seedlings, indicating that all tomato mature seeds withstand desiccation (Fig. 1B). In contrast, the seedling establishment was impaired following the DT assay in post-germination seeds, especially when the radicle growth reaches 4 mm. We observed a complete stop in radicle growth following this DT assay, suggesting that the tomato DT was gradually lost after germination and completely lost by the time the radicle length reached 4 mm. This observation was confirmed by a statistical analysis, where 100% of mature seeds showed DT (i.e. represented as seed survival after this DT assay), and this percentage of seeds was significantly reduced to 40% at germinated seeds with 1 mm radicles, followed by a continuous decrease to 0% of seed survival following this DT assay when the radicles of germinating seeds reached 4 mm (Fig. 1C).

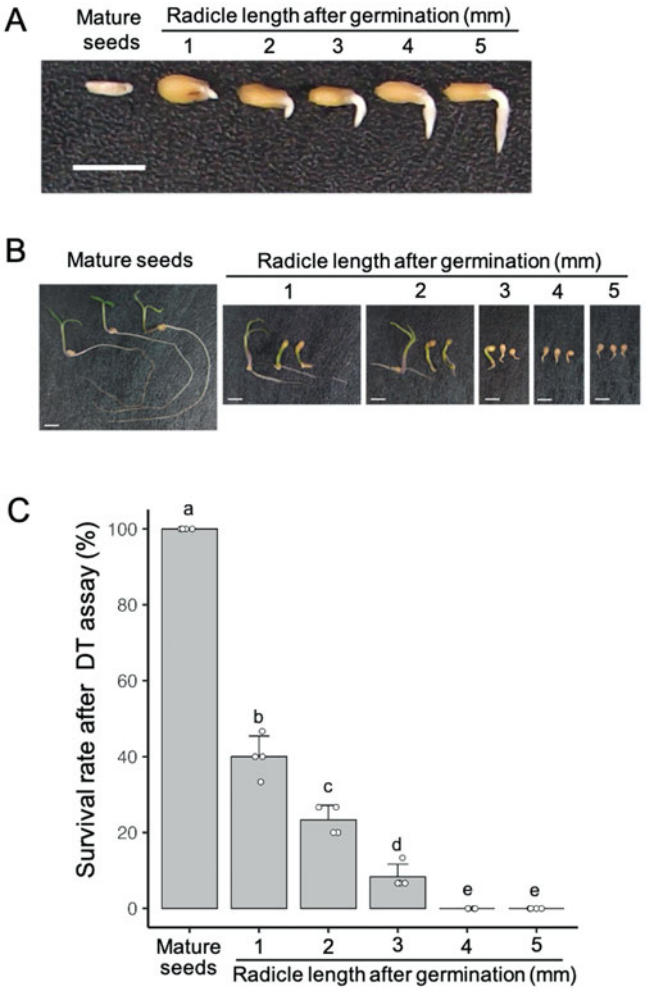

Figure 1. Loss of DT during post-germination development in tomato. (A) Germination and radicle elongation observations of tomato seeds. White bar scales represent 5 mm. (B) Two-week-old seedlings established after rehydration. The DT assay (dehydration and rehydration) was performed on mature seeds and post-germination seeds with different radicle lengths. White bar scales represent 5 mm. (C) Bar graphs with jitter points showing seed viability (%) (i.e. reflecting the ability of DT and % of healthy seedlings observed in 2-week-old seedlings after the rehydration). Values are means, error bars are SD ($n = 4$), and different letters indicate significant differences ($P < 0.05$, Tukey–Kramer tests).

PEG treatments induce DT in germinated tomato seeds

We next evaluated whether PEG treatments could be effective for re-establishing seed-DT in tomato, in which different concentrations of PEG-8000 (ambient water potential from −0.5 to −3.0 MPa) were tested on each post-germination seed with a radicle from 1 to 5 mm. The re-inducibility of DT varied greatly among the treatments (Fig. 2), and a relationship for DT re-induction between the ambient water potential with PEG and the radicle length of germinated seeds is summarized in Table 1. The most prominent DT re-induction was observed when using the external osmotic potential of −1.5 MPa on 1 mm radicle seeds, which resulted in 93% of seed survival. At all the other tested osmotic potentials (i.e. PEG concentrations), the re-inducibility of DT tended to decrease along with the radicle growth of germinated seeds. Although a slightly higher percentage of samples treated with −0.5 and −1.0 MPa osmotic potential also showed a re-induced DT compared to control samples at each radicle growth stage, apparent DT re-establishment was mainly observed in the samples treated with PEG, corresponding to −1.5 MPa external osmotic pressure. These results suggest that DT can be re-induced by a PEG treatment in post-germination tomato seeds, but that its re-induction occurs most effectively in 1 mm radicle seeds at the early post-germination growth stage and is preferentially treated with a specific range of PEG concentrations with an optimum corresponding to −1.5 MPa external osmotic pressure.

Effect of PEG concentrations on DT re-induction

To statistically evaluate the effects of the PEG, we focused on seeds with 1 mm radicles, which corresponded to the most responsive post-germination stage to PEG treatments (Fig. 3). Significant DT re-induction for whole seedlings was confirmed on −1.5 MPa PEG-treated seeds compared to control (non-PEG-treated) ones (Fig. 3A). However, the number of seeds with re-induced DT

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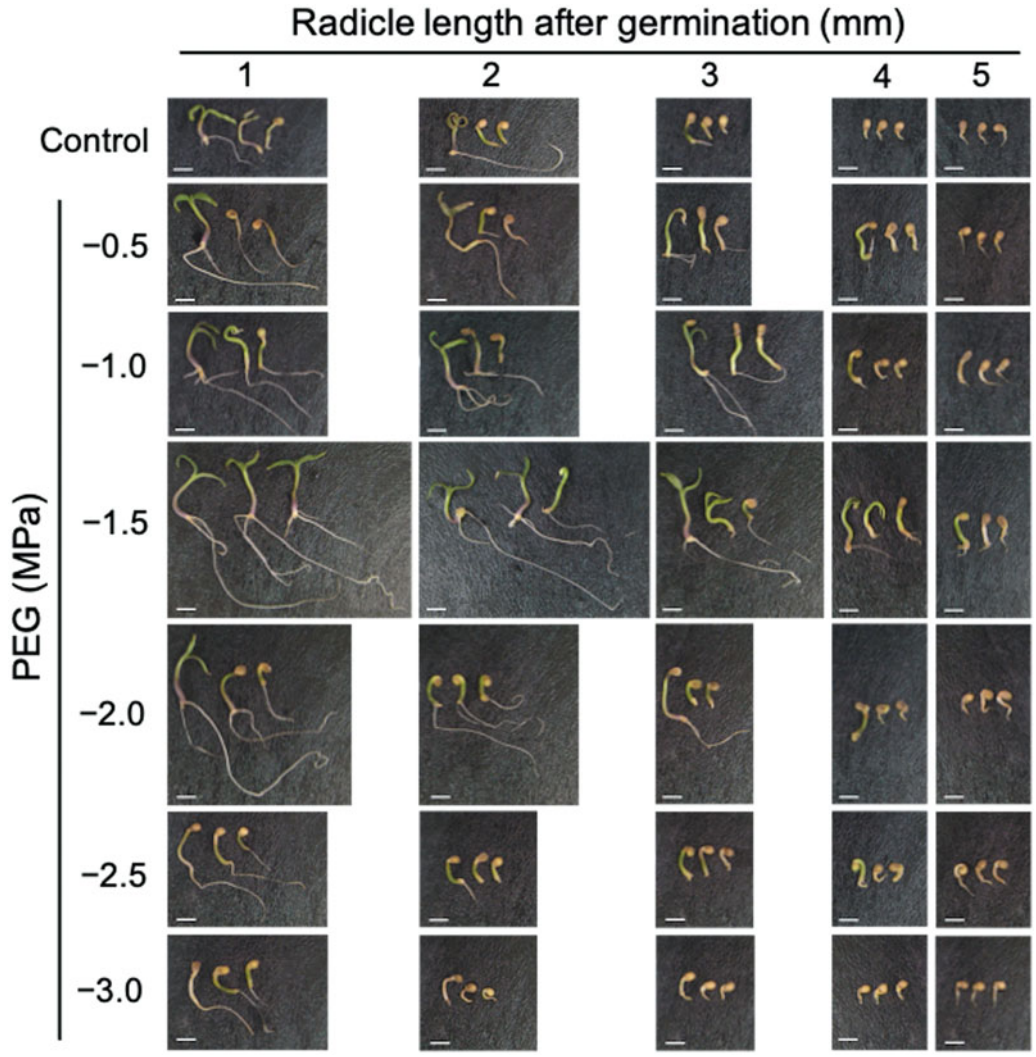

Figure 2. Effect of ambient water potential and growth of germinated seeds on DT re-inducibility. Two-week-old seedlings established after rehydration. For DT re-induction, post-germination seeds with different radicle lengths were treated with different PEG concentrations corresponding to different water potentials and then subjected to the DT assay (dehydration and rehydration) to evaluate seed survival. Controls are post-germination seeds that have been subjected to DT assays without PEG treatment. White bar scales represent 5 mm.

Table 1. Survival rate after the DT assay (%) of seedlings

		Radicle length after germination (mm)				
		1	2	3	4	5
Control		40 ± 5.4^{cd}	23.3 ± 3.8^{efgh}	8.3 ± 3.3^{hij}	0 ± 0^{j}	0 ± 0^{j}
PEG	−0.5 MPa	43.3 ± 3.8^{c}	26.7 ± 5.4^{defg}	11.7 ± 6.4^{ghij}	5 ± 3.3^{ij}	3.3 ± 3.8^{ij}
	−1.0 MPa	46.7 ± 5.4^{c}	31.7 ± 10^{cdef}	18.3 ± 6.4^{fghi}	13.3 ± 0^{ghij}	5 ± 6.4^{ij}
	−1.5 MPa	93.3 ± 5.4^{a}	66.7 ± 5.4^{b}	43.3 ± 6.7^{c}	23.3 ± 3.8^{efgh}	8.3 ± 3.3^{hij}
	−2.0 MPa	38.3 ± 6.4^{cde}	15 ± 10^{ghij}	15 ± 6.4^{ghij}	3.3 ± 3.8^{ij}	0 ± 0^{j}
	−2.5 MPa	15 ± 14.8^{ghij}	5 ± 6.4^{ij}	5 ± 3.3^{ij}	6.7 ± 5.4^{ij}	1.7 ± 3.3^{j}
	−3.0 MPa	3.3 ± 3.8^{ij}	0 ± 0^{j}	0 ± 0^{j}	0 ± 0^{j}	0 ± 0^{j}

Values are means ± SD ($n = 4$), and different letters indicate significant differences ($P < 0.05$, Tukey–Kramer tests).

was remarkably decreased for the seeds treated with −2.5 and −3.0 MPa PEG, indicating that a PEG treatment with water potential below −2.5 MPa has a negative effect on the DT re-establishment. Aiming to assess the difference in DT between seed organs in tomato, DT for healthy roots, hypocotyls and cotyledons were further investigated by using the 1 mm radicle seeds.

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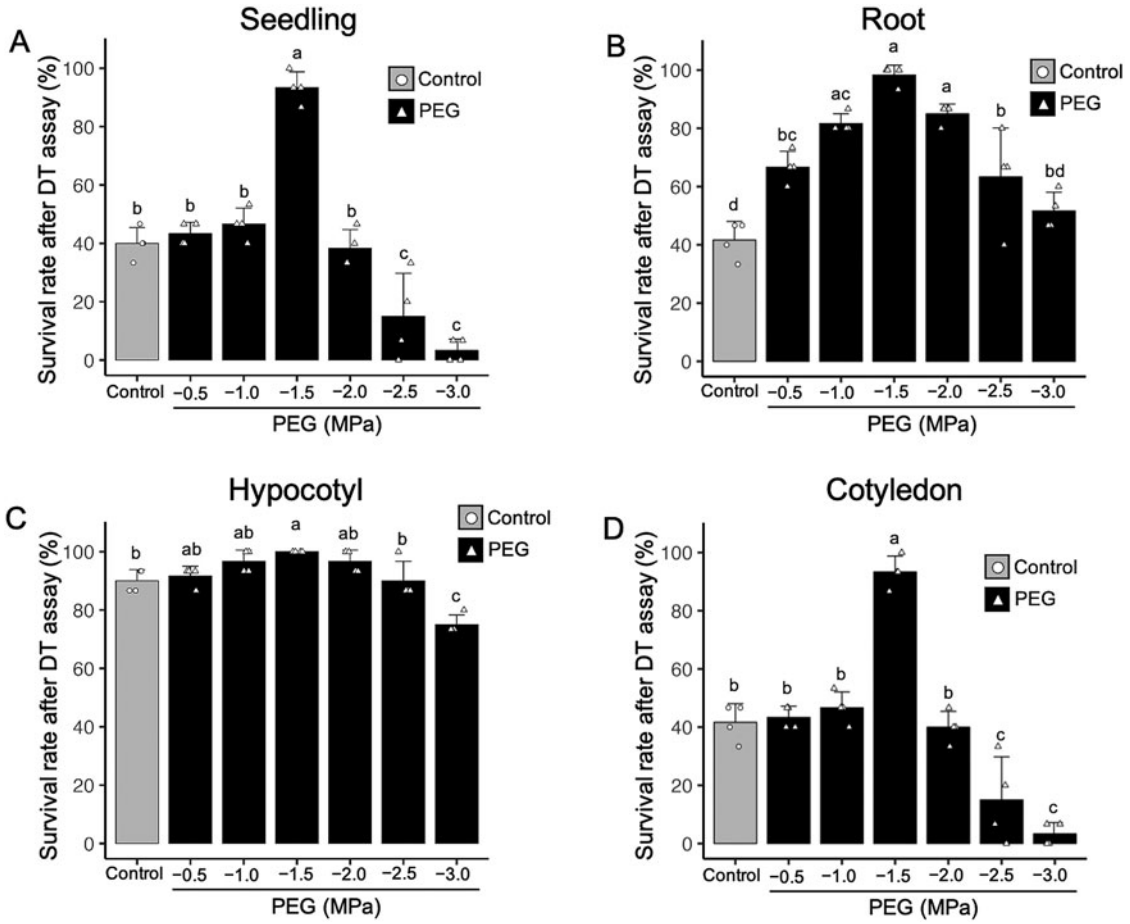

Figure 3. Effect of ambient water potential on DT re-inducibility for early stages of post-germination seeds. Bar graphs with jitter points show the DT (%) of post-germination seeds with 1 mm radicles. For DT re-induction, 1 mm radicle seeds were treated by different PEG concentrations corresponding to different water potentials and then subjected to the DT assay (dehydration and rehydration) to evaluate the seed survival (i.e. ability to re-induce DT). Controls are post-germination seeds that have been subjected to DT assays without PEG treatment. Healthy-established seedlings (A), roots (B), hypocotyls (C) and cotyledons (D) were scored 2 weeks after the rehydration. Values are means, error bars are SD ($n = 4$) and different letters indicate significant differences ($P < 0.05$, Tukey–Kramer tests).

Similar to the levels of whole seedlings, about 40% of seeds developed healthy roots in the control using the DT assay without PEG, but the most successful DT re-induction was achieved with again −1.5 MPa PEG treatment on the roots. Nevertheless, unlike whole seedlings, the positive effect for root-DT re-induction was observed in all other tested concentrations of PEG (Fig. 3B). This suggests that germinated roots in tomato are highly reactive to ambient water potential. As for hypocotyls, their DT re-induction was well established even without PEG treatment (Fig. 1B) and demonstrated more than 80% of the seed being able to tolerate desiccation in all tested conditions except for −3.0 MPa PEG treatment (Fig. 3C), indicating that hypocotyls may have a higher capacity to re-induce DT than other organs of tomato seeds. For cotyledons, the pattern of DT re-inducibility (Fig. 3D) was almost identical to that of the whole seedling (Fig. 3A). Thus, re-activation of DT in cotyledons may be one of the limiting factors for germinated tomato seeds to re-establish DT in whole seeds.

Effect of post-germination growth on DT re-induction

In order to statistically assess DT re-inducibility by PEG treatments with respect to post-germination seed growth, we compared control (non-PEG-treated) samples with −1.5 MPa PEG-treated ones, as it was the most effective condition to re-induce the DT (Fig. 4). DT re-establishment for whole seedlings was observed at all the post-germination stages from 1 to 4 mm radicle seeds but not in 5 mm compared to control ones (Fig. 4A), indicating that the 4 mm radicle is the oldest developmental stage for the DT window of tomato seeds. The DT for healthy roots, hypocotyls and cotyledons was also investigated with the −1.5 MPa PEG-treated samples and revealed that the patterns of DT re-inducibility of roots (Fig. 4B) and cotyledons (Fig. 4D) were almost identical to those of the whole seedling (Fig. 4A), and their DT was not re-inducible in 5-mm radicle seeds. As for hypocotyls, the number of seeds that were able to re-induce DT was higher than those for other organs, and the PEG treatment showed efficiency at 5 mm radicle seeds with a survival rate of 28% following the DT assay, which is significantly higher than in control (Fig. 4C), implying that hypocotyls may have a longer DT window than other organs in tomato seeds.

Desiccation-tolerant/-sensitive organs in seeds

To biochemically assess the desiccation-tolerant/-sensitive organs in tomato seeds, the viability test using TZ staining was carried out (Fig. 5A). In the PEG-treated seeds with 1 mm radicle, all

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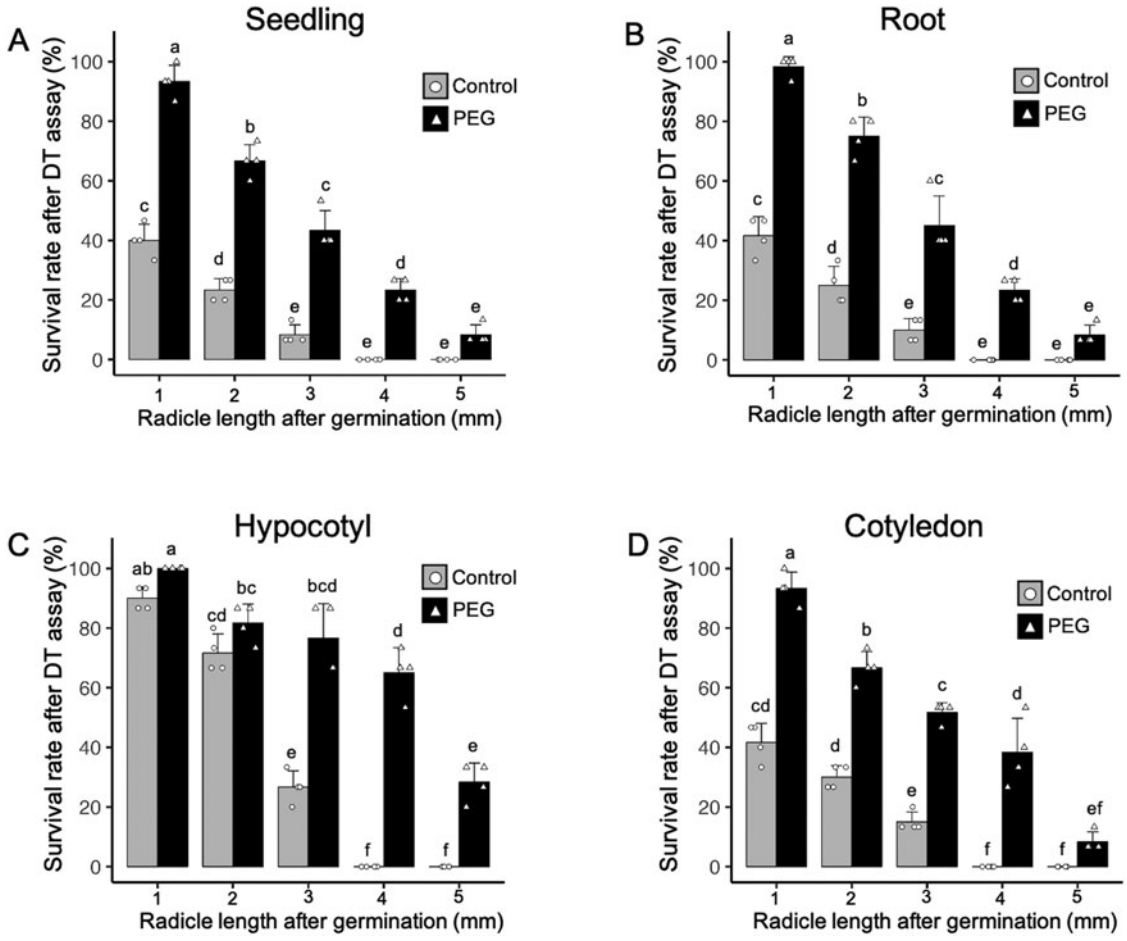

Figure 4. Effect of the growth stage of germinated seeds on DT re-inducibility by PEG. Bar graphs with jitter points showing seed survival (%) reflect the ability to re-induce DT in post-germination seeds with different radicle lengths. For DT re-induction, post-germination seeds were treated with −1.5 MPa PEG and then subjected to the DT assay (dehydration and rehydration) to evaluate the seed survival. Controls are post-germination seeds that have been subjected to DT assays without PEG treatment. Healthy-established seedlings (A), roots (B), hypocotyls (C) and cotyledons (D) were scored 2 weeks after the rehydration. Values are means, error bars are SD ($n = 4$) and different letters indicate significant differences ($P < 0.05$, Tukey–Kramer tests).

organs such as cotyledon, hypocotyl and root were coloured red by TZ, indicating that these organs were still alive after dehydration. However, in the control (non-PEG-treated) seeds with 1 mm radicles, the TZ viability was observed in all hypocotyl (100%), while approximately 50% of the 1 mm radicle seeds displaying TZ-stained roots and cotyledons (Fig. 5B). This result corroborates our previous observations that (i) many hypocotyls retain DT but most roots and cotyledons are more sensitive to desiccation; in other words, beginning to lose roots- and cotyledons-DT at the early stage of post-germination in the control (non-PEG condition) while (ii) PEG treatment with −1.5 MPa efficiently re-induced DT to roots and cotyledons of 1 mm radicle seeds (Figs 3 and 4). Furthermore, red staining representing the tissues viability was less observed in the control seeds with 5-mm radicles. This result is consistent with the results of germination tests (Figs 1C and 4), suggesting a complete loss of DT in all organs at 5 mm radicle seeds without PEG treatment. When the 5-mm radicle seeds were treated by PEG, we detected partial staining, especially from the hypocotyls in 43% of seeds (Fig. 5B), re-emphasizing that the tomato hypocotyl has a longer re-inducibility of DT (i.e. DT window) than other organs when treated with −1.5 MPa PEG. In contrast, the percentages of viable roots and cotyledons were low in PEG-treated 5 mm radicle seeds (i.e. about 15–20%) and close to zero in the control (non-PEG-treated) 5 mm radicle seeds, again highlighting that the DT window of tomato seeds is closing at this developmental stage.

Discussion

Optimum water potential for DT re-induction in tomato seeds

In this study, we showed that PEG treatment with −1.5 MPa induced significant DT in post-germination seeds in tomato; however, no positive effect was observed at other water potentials (Figs 2 and 3A). This result highlighted that only a specific ambient water potential can trigger DT re-induction at the post-germination stages of tomato seeds.

Dry seeds usually have water potentials between −350 and −50 MPa. When seeds are soaked in water (pure water corresponds to 0 MPa), the large difference between the dry seed tissue water potential and the ambient water potential results in rapid water influx into seeds, activating seed cell metabolism for germination (Bradford, 1995). Meanwhile, seeds cannot germinate in soil without sufficient water. The minimum ambient water potential required for seed germination, known as base water potential, varies among plant species. Many horticultural plant

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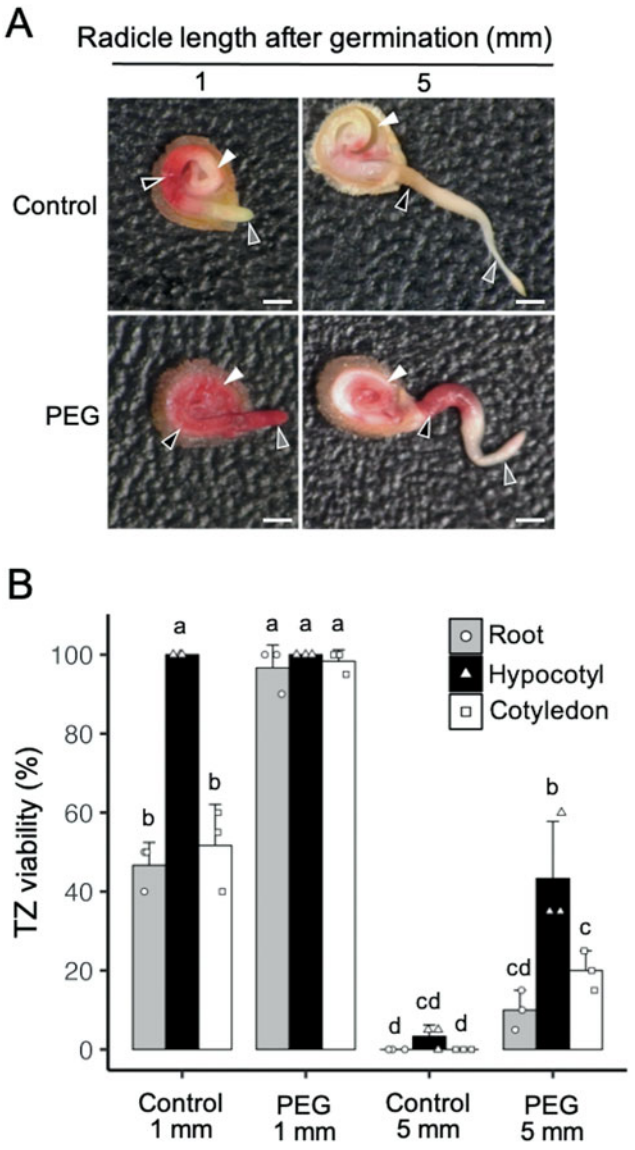

Figure 5. TZ viability test on germinated seeds after dehydration. (A) TZ viability test on post-germination seeds after dehydration. Germinated seeds with 1 and 5 mm radicles were not PEG-treated (control) or treated with −1.5 MPa PEG and then dehydrated, followed by staining with 1% (w/v) 2,3,5-triphenyltetrazolium chloride solution. White, black and grey arrowheads indicate the cotyledon, hypocotyl and root of seedlings, respectively. White bar scales represent 1 mm. (B) Bar graphs with jitter points showing the percentage (%) of seedlings displaying a red staining (i.e. also called TZ viability, %) of the germinated seeds not PEG-treated (control) or −1.5 MPa PEG-treated, following dehydration. Values are means, error bars are SD ($n = 3$), and different letters indicate significant differences ($P < 0.05$, Tukey–Kramer tests).

species have been shown to have relatively higher base water potentials (0 to −1.0 MPa) compared to crop and tree species (−0.6 to less than −2.0 MPa) (Dürr et al., 2015). In tomato seeds, PEG treatment corresponding to −1.0 MPa has been reported to significantly inhibit germination (Liptay and Schopfer, 1983; Florido et al., 2018), suggesting that the base water potential of tomato is around −1.0 MPa. However, few data are available on the minimum ambient water potential for growth of germinated seeds, especially immediately after radicle emergence. In order to achieve sufficient DT re-induction using PEG treatment, the water potential of PEG should be slightly lower than the base water potential to prevent radicle elongation, as the DT re-inducibility decreased concomitantly with radicle growth (Fig. 4A).

The molecular processes involved in the DT re-induction have been well studied at the transcriptomic level, which resulted in identification of DT-associated gene networks that overall facilitate a programmed reversion from a metabolically active state of germinating seeds to a quiescent state of dry seeds (Maia et al., 2011; Terrasson et al., 2013; Costa et al., 2015; Peng et al., 2017). Nevertheless, PEG treatments with water potentials below −2.5 MPa in tomato had a negative effect on DT re-establishment (Fig. 3A), indicating that the re-induction of DT is not simply caused by low water potential mimicking the dry seed condition. Similarly, a specific optimal water potential with PEG in a range of −1.2 to −2.5 MPa has been demonstrated to be crucial for the re-establishment of seed-DT in other plant species such as maize (Huang and Song, 2013), Medicago (Buitink et al., 2003), *T. impetiginosa* (Vieira et al., 2010), *C. korshinskii* (Peng et al., 2017), Arabidopsis (Maia et al., 2011) and *S. virgata* (Costa et al., 2016), although these treatments were performed under different temperatures between 4 and 25°C. The optimal water potential is also critical in agricultural seed priming treatments; for example, PEG-primed seed germination was enhanced at −1.25 MPa but impaired at −1.5 MPa in tomato (Govinden-Soulange and Levantard, 2008). Further physiological analysis using various DT re-induction conditions (e.g. different temperature conditions combined with various PEG concentrations) would be important to identify more optimal conditions for seed-DT induction in horticulture and crop species.

Organ specificity of seed-DT in tomato

Organ-/tissue-specific differences in DT during the post-germination process have been reported in several plant species, including Medicago (Faria et al., 2005; Sano et al., 2022), Arabidopsis (Maia et al., 2011), mung bean (Tian et al., 2019), *C. korshinskii* (Peng et al., 2017) and pea (Wang et al., 2021). The common characteristic is that the ability to re-acquire DT is lost at younger stages in roots of post-germination compared to cotyledon and hypocotyl, and/or the lost root-DT is hardly re-established by PEG treatment compared to cotyledons and hypocotyl. However, our results unexpectedly demonstrated that the survival rate reflecting the loss of DT in the post-germination process of tomato was the highest in hypocotyls but similarly lower in cotyledons and roots in control (non-PEG-treated) samples (Figs 4 and 5B). In addition, the patterns of DT re-inducibility by PEG for roots (Fig. 4B) and cotyledons (Fig. 4D) were almost identical throughout the studied post-germination stages and stopped in 5-mm radicle seeds, while hypocotyl-DT was still able to be re-induced in 5-mm radicle seeds (Fig. 4C). These results suggest that the DT windows of cotyledon and root are ending at the same time during post-germination, and this DT window is shorter than in tomato seed hypocotyl. The acquisition of DT during seed formation is often referred to as a requirement for seeds to have a longer life span and storability (Sano et al., 2016; Ballesteros et al., 2020). Interestingly, the viability of cotyledons in tomato seeds has been shown to be highly associated with seed longevity and storability based on TZ viability tests (Guadalupe et al., 2022). As there were overlaps in the genetic networks activated in the DT re-induction process after germination and in DT acquisition during seed maturation (Terrasson et al., 2013; Costa et al., 2015), the

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molecular mechanisms underlying the difficulty of DT re-induction in tomato cotyledons after germination may somehow be linked to the deterioration process of the cotyledons during seed storage following seed formation.

Again, in terms of root-DT after germination, our results show a low survival rate after the DT assay, consistent with previous plant species reports. It should be noted that the root marked the highest sensitivity of external osmotic pressure for DT re-induction among tested seed organs, as it was significantly induced at −0.5 MPa PEG treatment (Fig. 3B), indicating that the mild osmotic external environment may be enough to re-induce root-DT in tomato. By focusing on root development, Dekkers et al. (2015) hypothesized on the role of a DT window to cope with the water deficit in Arabidopsis, suggesting an ecological switch in post-germination growth. Indeed, within the DT window, germinating/germinated seeds are able to stop growing and return to a quiescent desiccated state, but growth can resume when water is available. When temporarily outside the DT window, germinated seeds start to grow root hairs that anchor the seed to the soil and allow it to take up water more efficiently from its environment, although the seedlings become irreversibly desiccation-sensitive. The competence of tomato roots to re-induce DT in response to mild osmotic stimuli may contribute to a dehydration-sensitive response in the soil under natural conditions at the early stage after germination. Moreover, the hypothesis by Dekkers et al. (2015) may also explain the longer DT window in cotyledon and hypocotyl, which are more prone to dehydration during the early stage of seedling establishment than the root tissues that are more protected in the soil. However, this last speculation is challenged by our results on tomato. Indeed, even if most of the post-germination DT re-induction was more efficient in cotyledons in other published species, in tomato, cotyledons appeared to have the same sensitivity as roots. However, further studies from ecological and morphological perspectives are needed to make sense of the physiological roles of the varied DTs in different seed organs, while the organs showing different DTs will be key research materials to elucidate the molecular mechanisms by which plant DT is switched on and off in specific organs.

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