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Research Paper

Seed development and viviparous germination in one accession of a tomato *rin* mutant

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In an experimental field, seed vivipary occurred in one accession of tomato *rin* mutant fruit at approximately 45–50 days after pollination (DAP). In this study, the possible contributory factors to this viviparous germination were investigated. Firstly, developing seeds were freshly excised from the fruit tissue every 5 days from 25–60 DAP. Germination occurred when isolated seeds were incubated on water, but was inhibited when they remained *ex situ* in fruit mucilage gel. The effect of abscisic acid (ABA) and osmoticum, separate and together, on germination of developing seeds was investigated. Additionally, ABA content in the seed and mucilage gel, as well as fruit osmolality were measured. The results showed that ABA concentrations in seeds were low during early development and increased later, peaking at about 50 DAP. ABA concentrations in *rin* accession were similar to those of the control cultivar and thus are not directly associated with the occurrence of vivipary. Developing seeds of *rin* accession are more sensitive than control seeds to all inhibitory compounds. However, osmolality in *rin* fruit at later developmental stages becomes less negative that is required to permit germination of developing seeds. Hence, hypo-osmolality in *rin* fruit may be an important factor in permitting limited viviparous germination.

Key Words: seed development, germination, *rin* accession, vivipary, ABA, osmolality.

Introduction

For many species, seeds cannot germinate precociously during development, but gain this capacity if they are removed from the maternal tissue or if developing embryos are dissected from other seed tissues, such as castor bean (Kermode and Bewley 1988). Also seed germination can be elicited by premature drying treatment, e.g. immature embryos of rape (Crouch and Sussex 1981) and rice (Stinissen *et al.* 1984) can germinate upon isolation from seeds and placed into culture, and maize kernels acquire the ability to germinate as early as 35 DAP (Oishi and Bewley 1990). Some treatments that cause a decrease in seed ABA content also promote precocious germination of immature soybean (Ackerson 1984) and maize embryos (Oishi and Bewley 1990, 1992).

For many orthodox seeds (embryos), their failure to germinate during development is related to the seed environment and/or maternal control (Bewley *et al.* 2013). Sometimes, however, a proportion of seeds germinate when still attached to the mother plant or are located *in situ* within a fruit, such as in maize (Eyster 1931), chayote (Aung *et al.* 1990), over-ripe tomato *sitiens* mutant (Groot and Karssen 1992), bell pepper (Marrush *et al.* 1998), Chinese cabbage (Ren and Bewley 1998, 1999) and rice (Miyoshi *et al.* 2000). The mechanism of precocious germination varies between species. For viviparous germination in over-ripe fruit of *sitiens*, an ABA-deficient tomato mutant, the minimum osmotic value of the fruit that permits mutant seeds germination is lower by 0.5 MPa than that which permits germination of wild-type seed, indicating that mutant seeds exhibit less sensitivity to osmotic inhibition (Groot and Karssen 1992). In contrast, deficiencies in endogenous ABA content result in seed germination in the silique of *Arabidopsis thaliana* (Karssen *et al.* 1983). In some maize viviparous mutants, correlations occur between lower ABA content, or embryo insensitivity to ABA, and vivipary (Robichaud *et al.* 1980). For rice *riv* mutants (*riv1-1*, *riv1-2*, *riv2*), seed insensitivity to ABA or a shorter period of sensitivity to it causes precocious germination in a humid environment (Miyoshi *et al.* 2000).

Precocious germination of some immature embryos (e.g. *Brassica napus*, *Phaseolus vulgaris* L, wheat, soybean, *Zea mays*, alfalfa) can be inhibited if ABA or osmoticum (mannitol or sucrose), or both are added in a culture medium

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(Crouch and Sussex 1981, Eisenberg and Mascarenhas 1985, Long *et al.* 1981, Neill *et al.* 1987, Triplett and Quatrano 1982, Xu *et al.* 1990). In fleshy-fruit-producing species such as muskmelon (Welbaum and Bradford 1988) and tomato (Berry and Bewley 1992), seeds are embedded in a highly moist environment and their inability to germinate during development is associated with low water potentials in, or inhibitory action by, the enclosing fruit tissues. But one accession of a tomato *rin* mutant (*rin* accession) exhibited some precocious germination; hence we have documented the characteristics of this *rin* seed during development, and the possible factors involved in its precocious germination were investigated, including potential roles of ABA in the seed and in fruit mucilage, as well as osmolality of the fruit tissue.

Materials and Methods

Seeds and plant material

Seeds of tomato (*Solanum lycopersicum*) cv. 'Dongnong706' (non-viviparous, as control) and the *rin* accession (viviparous) were stored and propagated at the Tomato Institute, Northeast Agricultural University, (Harbin, China). This homozygous *rin* accession (No: 12568) was a derived tomato accession harboring the *rin* mutation.

Tomato plants were grown in a controlled greenhouse with day- and night-temperatures of 25–28°C and 18–20°C respectively. To minimize variation in fruit development, flowers were hand-pollinated and tagged on the pollination date. Tomato fruits and seeds were harvested at 25–60 DAP. Because some non-conformity in seed development occurs within fruits, those located at the proximal end were used to avoid this.

Germination of developing seeds imbibed in water

At 25, 30, 35, 40, 45, 50, 55, and 60 DAP, developing seeds were harvested from cv. Dongnong706 and *rin* accession fruit (precociously germinated seeds in the *rin* fruit were removed), and incubated in 1% (v/v) HCl for 1 h to remove the locular and mucilage gel surrounding the seeds. These seeds (100 seeds per each of 3 replicates) were then placed on 2 layers of filter-paper soaked in 6 mL sterile water in Petri dishes (9 cm diam × 1.5 cm height) for 7 d. During testing, the seeds were transferred to identical Petri dishes with fresh water every 2 d. Percent germination (radicle protrusion) was recorded at 12-h intervals.

Germination of developing seeds incubated in ABA or osmoticum or their combination

ABA (Sigma, MYM Biological Technology Company) solutions of 0.2 μM, 2 μM, 10 μM, 20 μM, 40 μM, 50 μM, 100 μM, 1 mM and 10 mM ware used. Polyethylene glycol 6000 (PEG6000, Kermel, Tianjin) solutions of 100 g/L (–0.166 MPa), 150 g/L (–0.325 MPa), 200 g/L (–0.534 MPa), 300 g/L (–1.1 MPa) and 400 g/L (–1.878 MPa) were prepared according to Michel and Kaufmann (1973). The combined PEG-ABA solutions were prepared according to Michel (1983): I: 100 g/L PEG + 0.2 μM ABA, II: 200 g/L PEG + 0.2 μM ABA, III: 100 g/L PEG + 2 μM ABA, IV: 200 g/L PEG + 2 μM ABA.

For the Dongnong706 and *rin* accession, (after precociously-germinated seeds had been removed from the latter), isolated seeds (30–55 DAP) were placed on 2 layers of filter paper soaked in 6 mL ABA or PEG solutions or 6 mL sterile water (control) in a Petri dish for 7 d. Isolated 45–55 DAP seeds were placed on 2 layers of filter paper soaked in 6 mL combined PEG-ABA solution or 6 mL sterile water (control). During the test period seeds were transferred to new Petri dishes containing fresh ABA, PEG or PEG-ABA solutions every 2 d. Percent germination was determined after 7 d. There were 3 replicates per treatment with 30 seeds for each replicate.

After 7 d non-germinated seeds from each treatment were rinsed and transferred to Petri dishes containing 6 mL sterile water and germination recorded again after 7 d.

ABA concentration in seeds and mucilage gel

ABA extraction and determination were according to Jun (1997) with some modifications. Seeds of the 2 tomato lines (*rin* accession and Dongnong706) at 30–55 DAP were removed from the fruit and the surrounding mucilage gel removed. Both seeds and mucilage gel (0.5 g each) were frozen in liquid nitrogen and powdered, then immersed in pre-chilled 80% (v/v) aqueous methanol for 12 h at 4°C. The extracts were adjusted to pH 8 using 0.2 mmol/L monosodium phosphate (NaH_2PO_4) and extracted 3 times in the mixture of petroleum ether and ethyl acetate (volume ratio 1:1). Then 0.2 g polyvinyl pyrrolidone was added to the aqueous phase and adjusted to pH 2.8 with 0.2 mol/L citric acid. This was extracted in ethyl acetate and the upper ester phase was collected and condensed in a rotary evaporator and dissolved in 5 mL mobile phase (a mixture of acetonitrile, methanol and 0.6% [v/v] acetic acid in a 5:50:45 ratio) and was filtered using a 0.45 μm membrane. The ABA in the extract was determined using high performance liquid chromatography (HPLC; DIONEX P680 HPLC, USA). The parameters for HPLC were: (1) The ratio of mobile phase of acetonitrile:methanol:0.6% (v/v) acetic acid was 5:50:45; (2) The flow rate was maintained at 0.8 mL/min; (3) Wavelength of detection was 254 nm; (4) C_{18}HICHROM316A-LOK (KU) column size was 150 mm × 4.9 mm; (5) Sampling volume was 10 μL; (6) Room temperature (RT).

Determination of osmolality in cv. Dongnong706 and rin accession fruit

At each of 30–55 DAP three developing fruits were surface sterilized. The juice from the fruit tissue was filtered and collected, according to Groot and Karssen (1992), and its osmolality was measured using an AUTO. F. P. Osmometer FM-8P (Shanghai Medical University Instruments, Shanghai, China).

Statistical analyses

Statistical analyses were performed using The Statistical Program from Social Sciences (SPSS) Version 20.0 according to Xiang and Han (2007). Data were analyzed using one-way ANOVA. The means and sample variances were equal in all experiments. Standard deviation (SD) were all provided in all figures. Data are the means of 3 replicates ± SD. Bars with different letters in the figures indicate significant differences at $p < 0.05$ according to Duncan's multiple range test.

Results

Viviparous germination in rin accession fruit

Viviparous germination occurred in *rin* accession seeds when they were still located in the fruit attached to the mother plant (**Fig. 1**). A few precociously-germinated seeds appeared at 45 DAP, with short emerged radicles (**Fig. 1A**). At 50 DAP the radicles were extended and more seeds precociously germinated, to approximately 10% of total (**Fig. 1B**). Thereafter, the percentage of precocious germination increased to about 15–20%, and young seedlings with visible cotyledons appeared in the 55 DAP fruit (**Fig. 1C**). When the fruits remained attached to the maternal plant for 4 weeks after reaching the 55 DAP stage, precociously germinated seeds, with extended roots exhibiting root hairs, accounted for less than half of total number in fruit. Meanwhile, the mucilaginous substance in the fruit became depleted and a cavity appeared, but only after germination was completed (**Fig. 1D**). However, some precocious germination did occur during development, even when seeds were embedded in a considerable amount of mucilage gel (**Fig. 1A–1C**). Because of this observed increasing precocious germination of *rin* seeds during fruit development, it was appropriate to investigate the developing tomato seed and fruit characteristics.

Tomato seed germinability (cv. Dongnong706 and rin accession) at different developmental stages

The cumulative germination time course for freshly isolated seeds of cv. Dongnong706 and *rin* accession imbibed on water are plotted in **Fig. 2**. For both cultivars, a few seeds became able to germinate by 30–35 DAP, and a gradual and earlier increase in germination percentage was observed with greater seed maturity, exactly, germinated well from 40 DAP onwards. These results indicated that isolating the seeds from their surrounding tissues elicited germination. However, the Dongnong706 seeds were unable to germinate throughout development when they were still attached to the placenta in the fruit; for *rin* accession, despite the ability of some developing seeds to germinate precociously, the majority of seeds at 45–55 DAP were prevented from germinating in the fruit while the mucilage was still present (**Fig. 1A–1C**). Also, their germination was completely or largely inhibited when isolated 45–55 DAP seeds of both cultivars were replaced *ex situ* on mucilage gel extracted from fruits at the same time of development, but recovered well again after transferring these seeds to water

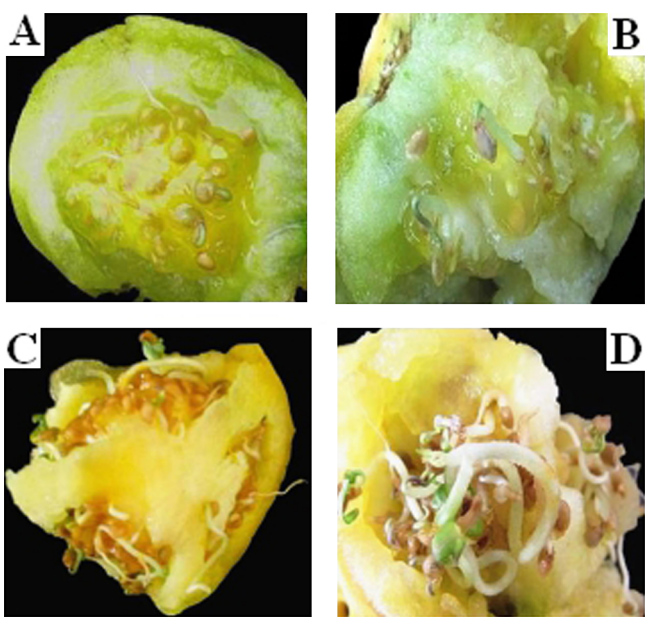

Fig. 1. Viviparous seed germination in *rin* accession fruit. Viviparous germination percent in *rin* accession fruit at different developmental times: (A) 45 DAP 6.94 ± 4.24%, (B) 50 DAP 9.87 ± 2.36%, (C) 55 DAP 16.62 ± 2.51%, (D) 4 weeks after 55 DAP 45.2 ± 7.23%. Data are the mean ± SD of precociously germinated seeds in 5 fruits for each time of development.

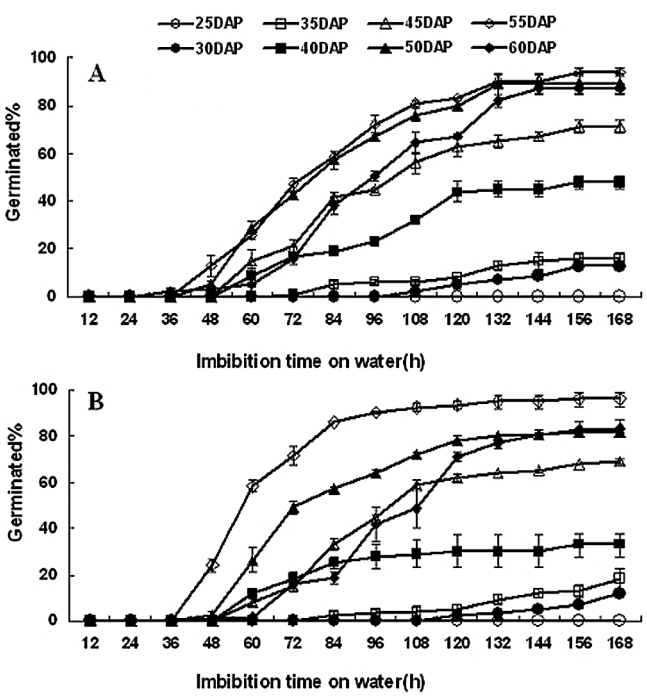

Fig. 2. Germination of tomato seeds isolated during development (different days after pollination, DAP) and incubated on sterile water for 7 d. (A) Dongnong706 as control, (B) *rin* accession.

(data not shown). Thus the developing seeds placed in mucilage gel are prevented from germinating, but can recover this ability on subsequent transfer to water.

In consideration of factors involved in the suppression of precocious germination, information is available on the inhibitory role of ABA on immature embryos in vitro, e.g. in wheat, soybean, maize, alfalfa (Obendorf and Wettlaufer 1984, Rivin and Grudt 1991, Triplett and Quatrano 1982, Xu and Bewley 1991), and likewise maize vivipary can be induced by treating kernels with fluridone or other pyridinones, which decrease their ABA content (Fong *et al.* 1983, Oishi and Bewley 1990). In fleshy-fruit species, such as tomato (Berry and Bewley 1992) and muskmelon (Welbaum *et al.* 1990), it is suggested that it is the osmotic potential of the fruit tissue that plays an important role in prevention of precocious germination. Finkelstein and Crouch (1986) showed that high osmoticum, rather than ABA, can mimic the environment of the developing rape seed growing in a non-mucilaginous silique. Hence, we determined, in the following experiments if ABA, osmoticum or a combination of these most simulated the inhibitory effect of the mucilage gel in preventing developing tomato seed germination.

Effect of ABA, osmoticum, or both on germination of cv. Dongnong706 and rin accession at different developmental stages

Seeds isolated from their fruits at various stages of development were incubated in ABA to test for their germinability (**Fig. 3**). **Fig. 3A** shows that the control seeds germination was less affected at 10 µM and lower concentrations, but was effectively inhibited at 20 µM and above, with 100 µM being completely inhibitory at all stages of development. Recovery of germination was tested by subsequent transfer of the non-germinated seeds from high concentrations of ABA (20 µM and above) to water for 7 d (**Fig. 4**). Poor germination of 30 and 35 DAP seeds was probably due to their immaturity because seeds placed directly on water of these times germinated poorly also (**Fig. 4A**, **4B**). Mature seeds (even those incubated in high ABA for 7 d) exhibited normal germination when transferred to water (**Fig. 4C–4F**), indicating that there was no irreversible influence of the inhibitor on germination ability, even after incubation in 100 µM ABA.

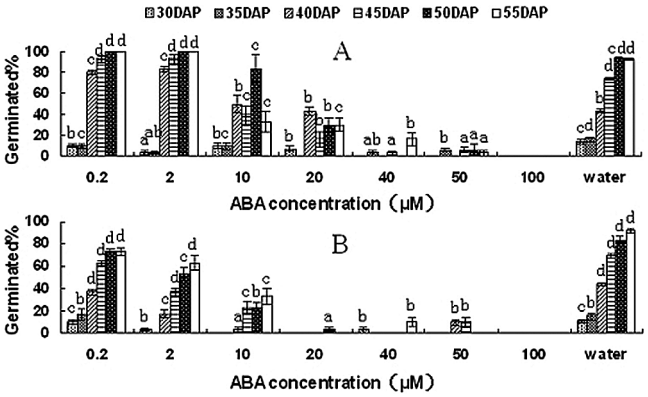

Fig. 3. Germination of isolated 30–55 DAP seeds incubated on ABA for 7 d. (A) Dongnong706 as control, (B) *rin* accession.

Throughout development, *rin* seed germination was more inhibited by 0.2 and 2 µM ABA than were the developing control seeds, and particularly at 10 µM and higher concentrations (**Fig. 3B**). Upon transferring non-germinated *rin* seeds from 20 µM ABA and above to water, recovery of germination was low at 30–40 DAP (**Fig. 4A–4C**), and becoming greater in the more mature seeds (**Fig. 4D–4F**). But in comparison with the results for the control cultivar, the

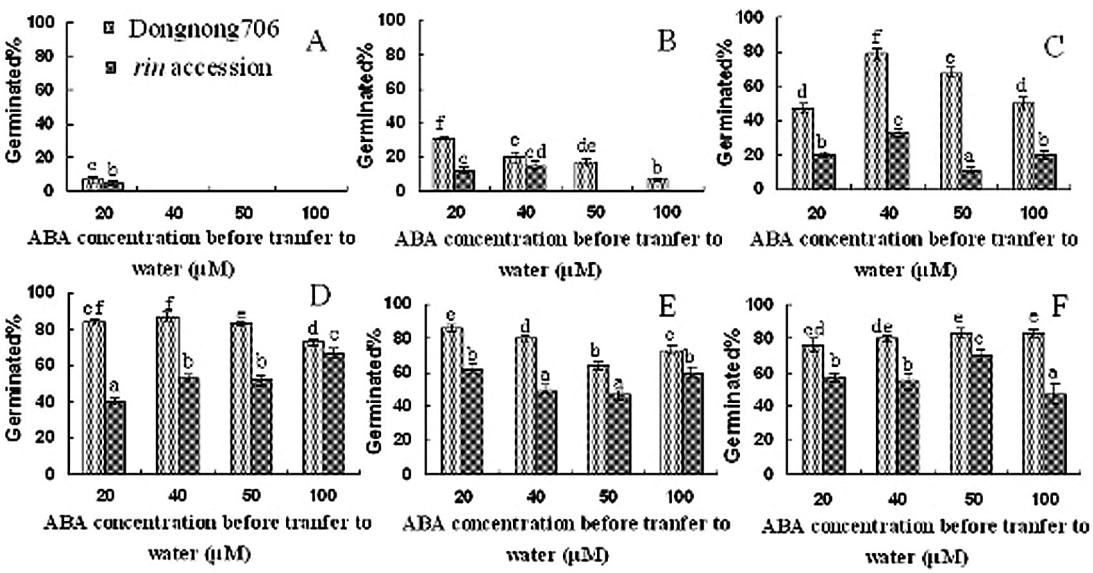

Fig. 4. Germination of seeds (Dongnong706 and *rin* accession) at different times of development that were first incubated for 7 d on 20–100 µm ABA before transfer to sterile water for another 7 d. (A) 30 DAP, (B) 35 DAP, (C) 40 DAP, (D) 45 DAP, (E) 50 DAP, (F) 55 DAP.

rin seeds exhibited less recovery at all times of development when germination occurred (**Fig. 4B–4F**). Also seeds of both cultivars initially incubated in 1 and 10 mmol ABA did not recover their ability to germinate on subsequent transfer to water, presumably because a high concentration of the hormone remained within the seed (data not shown).

Various concentrations of osmoticum have distinctively different effects on germination of isolated seeds during their development, as demonstrated in **Fig. 5**. Except for the two earliest times, the control seeds isolated during development germinated well in the presence of 100 g/L osmoticum. Seeds at 40–55 DAP germinated less on 150 g/L osmoticum and on 200 g/L osmoticum only a little at 50–55 DAP, and higher concentrations were completely inhibitory. Thus, the more negative the osmotic potential, the more effectively are seeds prevented from precocious germination. When the non-germinated seeds from the various osmotica were transferred to water there was poor recovery of germination of 30–35 DAP seeds, likely due to their immaturity (**Fig. 6A**, **6B**), but more mature seeds (40–55 DAP) germinated well upon transfer to water (**Fig. 6C–6F**), even after incubation at the highest concentrations.

Less germination of developing *rin* seeds occurred with increasing concentrations of osmoticum compared to that of the control seeds (**Fig. 5B**). There was some germination on 100 g/L osmoticum but it was more strongly inhibited on 150 g/L PEG and almost none at 200 g/L or higher. After transfer of the mutant seeds to water after 7 d, their germination recovery was less than that of control seeds, particularly at the earlier developmental times (**Fig. 6A–6C**).

Fig. 3 and **Fig. 5** show the changing sensitivity of isolated seeds to ABA and osmoticum, but singly. In several species, ABA has a similar effect to osmoticum in preventing precocious germination, e.g. maize, alfalfa, soybean, rape (Finkelstein *et al.* 1985, Neill *et al.* 1987, Obendorf and Wettlaufer 1984, Xu *et al.* 1990). Hence, it was of interest to determine if there is a synergistic inhibitory effect when the two are combined. In order to make it clearer and easier to compare the results, some ABA and osmoticum data are repeated in **Fig. 7** together with those of the combined effects. Using 45–55 DAP developing seeds of the control cultivar, it is apparent that greater inhibition of germination was achieved by addition of 0.2 μM ABA to 100 and 200 g/L osmoticum than with ABA alone, and with increasing ABA concentrations the inhibitory effect was greater. The decrease in germination percentage caused by the combination was greater than the sum of the individual effects (**Fig. 7A**). For example, at 50 DAP no inhibition occurred on 0.2 μM ABA alone and germination on 100 g/L osmoticum alone was inhibited by 7%. Inhibition by their combination, however, was 62%. After the non-germinated seeds that were in contact with the combination of inhibitory compounds were

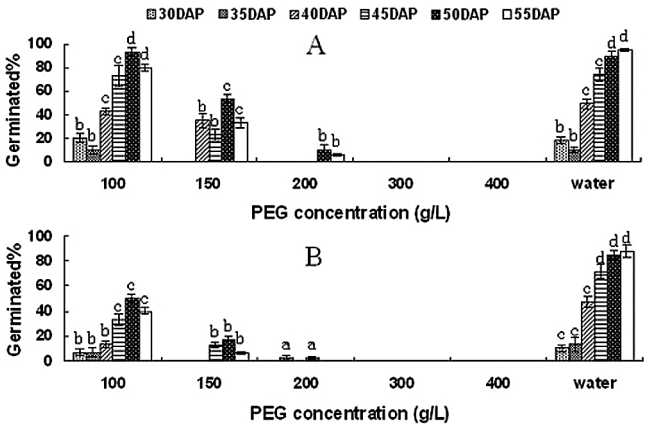

Fig. 5. Germination of isolated 30–55 DAP seeds incubated on the osmoticum (PEG6000) at various concentrations for 7 d. (A) Dongnong706 as control, (B) *rin* accession.

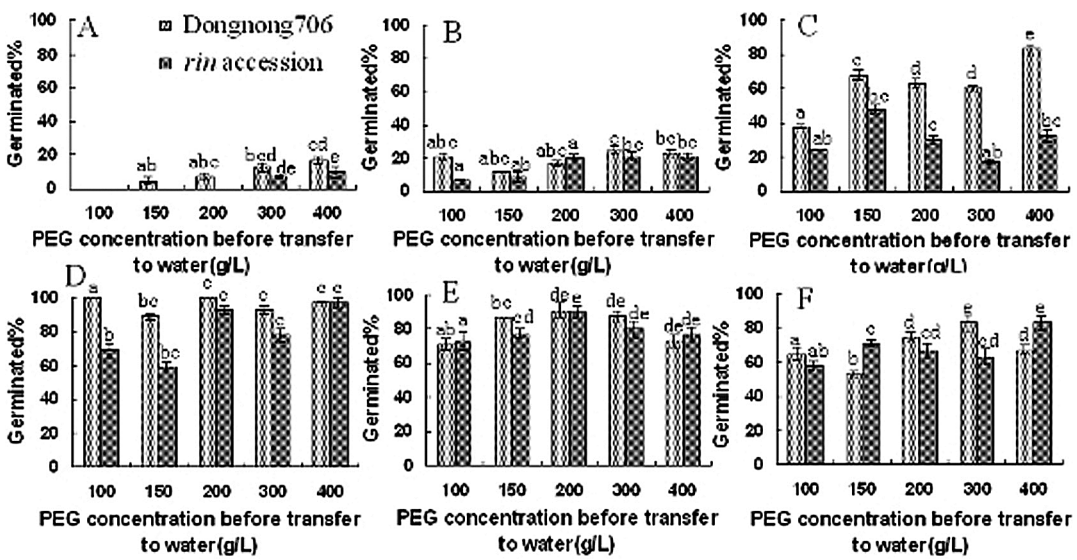

Fig. 6. Germination of seeds (Dongnong706 and *rin* accession) at different times of development first incubated for 7 d on various concentrations of osmoticum before transfer to sterile water for another 7 d. (A) 30 DAP, (B) 35 DAP, (C) 40 DAP, (D) 45 DAP, (E) 50 DAP, (F) 55 DAP.

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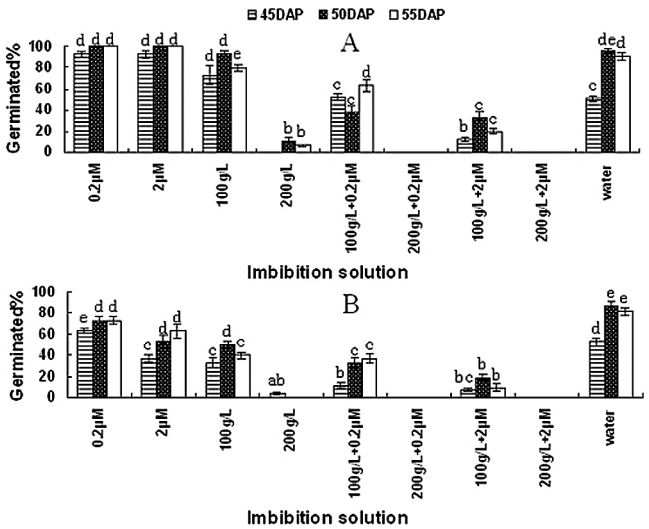

Fig. 7. Comparison of the inhibitory effect of ABA or osmoticum alone, or combinations of these, on germination of isolated 45, 50, and 55 DAP seeds. (A) Dongnong706 as control, (B) *rin* accession.

transferred to water, germination recovered again (**Fig. 8**), slightly better in 45 and 50 DAP seeds than in 55 DAP seeds (**Fig. 8A–8C**).

While 45–55 DAP *rin* seed germination was inhibited in the presence of the two germination inhibitors combined, although the effect was approximately additive, with the stronger influence coming from the osmoticum. Also *rin* seeds germination was more inhibited by the two combined than the control seeds (**Fig. 7**). Following transfer of the seeds to water after 7 d, germination was high and similar in both *rin* and control seeds at these three developmental times (**Fig. 8A–8C**), even though the former was more sensitive to the inhibitory treatments.

When comparing the results for isolated *rin* seeds on ABA, osmoticum, and both combined, with those obtained for isolated control seeds of Dongnong706, it is apparent that the former seeds are more sensitive to all inhibitory conditions (**Figs. 3**, **5**, **7**). For example, 20 μM ABA very effectively blocked germination of the *rin* seeds but was less effective on Dongnong706. Likewise at 100 and 200 g/L osmoticum, germination percentage was lower in the *rin* seeds, as it also was when these concentrations were combined with 0.2 and 2 μM ABA. Thus the differences between the cultivars in their responses to these inhibitory conditions, as determined by capability to complete germination, do not explain the precocious germination occurring in *rin* accession fruit; the *rin* seeds are not less sensitive to either ABA or their osmotic environment. Experiments were therefore conducted to determine if there are any differences in the *in vivo* concentrations of ABA in the seeds and fruit mucilage during development and of osmoticum in the fruit environment.

ABA concentration in seeds and fruit mucilage gel

Subsequently, ABA concentration was measured in seeds and fruit mucilage during development of the *rin* accession and control seeds. ABA content of both the *rin* accession and Dongnong706 seeds was low during early development and increased as they matured, peaking at about 50 DAP. After that time, ABA declined again in both cultivars (**Fig. 9A**). Until 50 DAP the *rin* seeds contained more ABA than the control, but were similar thereafter. However, no viviparous germination was observed during development of the Dongnong706 seeds. This suggests that variations in seed ABA concentration do not explain the differences in precocious germinability of these two cultivars.

A possible linkage between ABA content of the fruit mucilage gel and the ability of the seeds to germinate *in situ* was assessed also for the two cultivars (**Fig. 9B**). The ABA concentration in the *rin* fruit mucilage was calculated in terms of total μM present, and at 45–55 DAP was approximately equivalent to 20 μM ABA, a concentration adequate to prevent developing seed germination (**Fig. 3B**). However, some developing seeds still exhibited vivipary at this

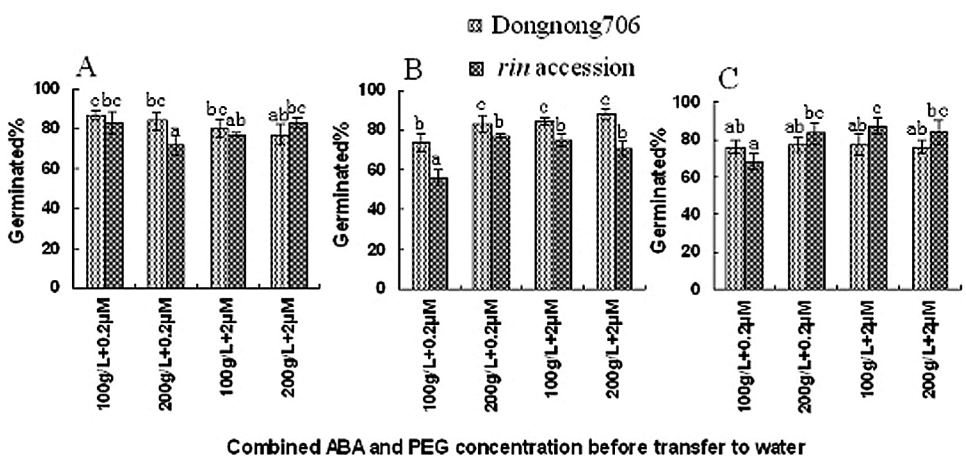

Fig. 8. Germination of seeds (Dongnong706 and *rin* accession) at different times of development after initial incubation for 7 d on combinations of ABA and osmoticum, and later transferred to sterile water for another 7 d. (A) 45 DAP, (B) 50 DAP, (C) 55 DAP.

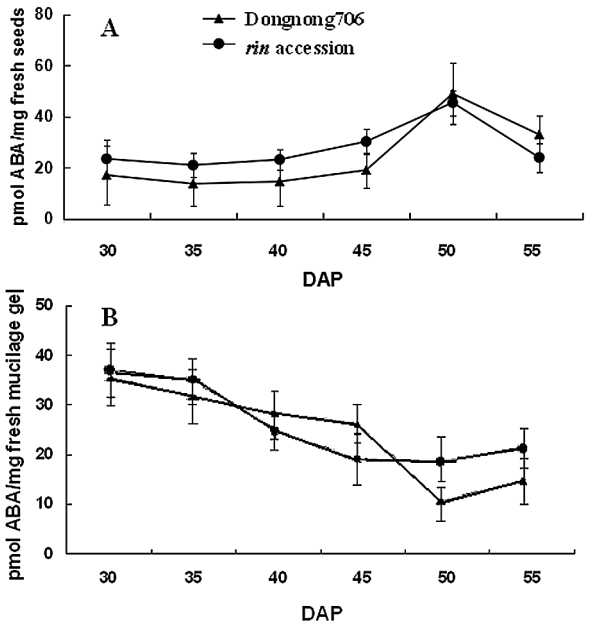

Fig. 9. ABA concentration in developing 30–55 DAP seeds (A) and in fruit mucilage (B) of 2 tomato cultivars (—▲—, Dongnong706; —●—, *rin* accession). The values are the mean of 3 replicates ± SD.

time (**Fig. 1A–1C**). In comparison, the ABA concentration in the control fruit mucilage at 50 and 55 DAP was about 12 and 15 μM, respectively, a concentration that would allow precocious germination of the developing seeds (**Fig. 3A**); but none occurred in the fruit. This is indicative that ABA alone in the mucilage gel does not restrict precocious germination *in situ*.

Osmolality in the rin accession and Dongnong706 fruit mucilage gel

Because mucilage gel from developing tomato fruits inhibits germination of both *rin* and control seeds (see section 3.2; Berry and Bewley (1992)), and because ABA is not the sole factor involved, the osmotic concentration of the *rin* accession and control fruits was therefore measured. Osmolality in the *rin* fruit mucilage gel initially increased by about 20% during fruit development, but declined again after 45 DAP (**Fig. 10**). The osmolality in the mucilage gel at 45, 50 and 55 DAP was 369, 357 and 342 mOsm/kg, respectively, indicating that osmotic potential in the *rin* fruit became less negative with time of development, when precocious germination increased. Especially at about 4 weeks after 55 DAP stage, when the mucilaginous substance in the fruit became depleted and no inhibitory effect exert on seeds, the precocious germination percent has reached 45% (**Fig. 1D**). Osmolality in the control fruit mucilage increased throughout development and according to Van't Hoff law (1882), the equation (π = iCRT, T is variable), the corresponding osmotic potential became more negative than that of a 200 g/L PEG solution, the threshold value that permitted germination of 50 and 55 DAP seeds (**Fig. 5A**).

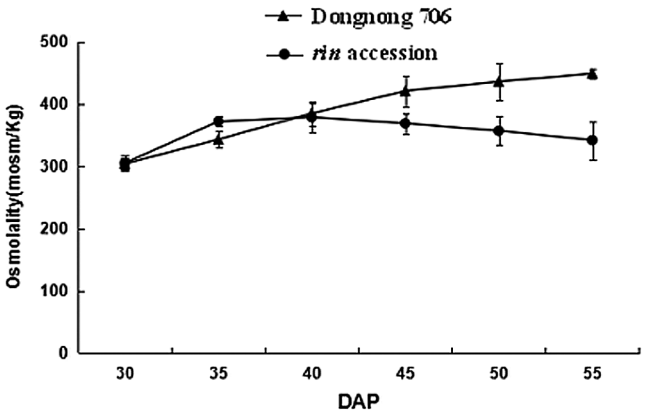

Fig. 10. Osmolality of developing 30–55 DAP fruit mucilage gel in the tomato (—▲—, Dongnong706; —●—, *rin* accession). The values are the mean of 3 replicates ± SD.

Therefore, a possible explanation for the few viviparously-germinating seeds in the *rin* accession fruits is decreasing osmolality in *rin* fruit at more mature stages, and some of the seeds are able to escape from the inhibitory effects of the osmotic environment.

Discussion

In summary, developing seeds of tomato can be elicited to germinate precociously after isolation from their surrounding fruit tissue and incubation on water (**Fig. 2**), and therefore maturation drying is not essential for developing seed germination; excision from the fruit is sufficient. As reported for muskmelon (Welbaum and Bradford 1988, Welbaum *et al.* 1990) and tomato (Berry and Bewley 1991, 1992), seed germinability occurs early during development but removal of the seeds from their surrounding fruit tissue is a prerequisite for precocious germination. Also the mucilage gel can exert an inhibitory influence on germination of the majority of developing tomato seeds (**Fig. 1A–1C**; see section 3.2). This conclusion is in accordance with that of Berry and Bewley (1992) who suggested that fruit tissues play an important role in suppressing precocious germination.

Both ABA and osmoticum can inhibit the germination of isolated tomato seeds, and a combination of the two is additive. This inhibition can be reversed by subsequent transfer of the seeds to water. And ABA decreased in its ability to prevent germination as the seeds increased in their maturity beyond 35 DAP (**Fig. 3**). This could be due to a decrease in sensitivity of the seeds to ABA as they mature, as in developing alfalfa embryos (Xu and Bewley 1991), for example. Tolerance of osmotic stress increases as the seeds mature in the fruit (**Fig. 5**), as shown for muskmelon seeds (Welbaum *et al.* 1990). Also the slight synergistic inhibitory action of the two combined on germination of developing seeds is consistent with, but less than that in muskmelon (Welbaum *et al.* 1990) or alfalfa (Xu and Bewley 1991).

Seeds of a *rin* accession exhibit a limited amount of

precocious germination in the developing fruit. But when comparing the results for isolated *rin* seeds on ABA, osmoticum, and both combined, with those obtained for isolated control seeds of Dongnong706, it can be concluded that the *rin* seeds exhibited more sensitive to all the inhibitory conditions in the prevention of germination. This is different from the observations made on some mutants of the tomato (*sitiens* mutant), corn, *Arabidopsis*, and wheat, which exhibit embryonic or seed insensitivity to osmoticum or ABA and undergo precocious germination (Groot and Karssen 1992, Koornneef *et al.* 1984, Robichaud *et al.* 1980, Walker-Simmons 1987). Also the precocious germination of *rin* seeds appears not to be due to differences in ABA content of either the seeds or of the surrounding fruit mucilage gel (**Fig. 9**), in contrast to other species in which reduced ABA content in embryo or seed tissues can elicit premature germination, such as in soybean embryos, fluridone-treated corn kernels, and *Arabidopsis* or rape seeds (Ackerson 1984, Finkelstein *et al.* 1985, Karssen *et al.* 1983, Oishi and Bewley 1992, Walker-Simmons 1987). However, the osmotic potential of mucilage surrounding the *rin* seeds is less negative during late development than in the control seeds, raising the possibility that a few seeds *in situ* are less inhibited by their environment and are thus able to germinate precociously. This mechanism is different from that of viviparous germination in the tomato *sitiens* mutant, which has a low ABA content in the fruit. The primary cause of vivipary for *sitiens* mutant is reduced sensitivity of the seed to osmotic stress, however, even though the osmotic potential in the mutant fruit is similar to that of the control wild type (Groot and Karssen 1992).

Taking the decreasing osmolality after 45 DAP into account, it is not difficult to argue whether there are some linkages between the lower osmotic potential and its non-ripening characteristic of *rin* fruit. Throughout the developmental process of fruits, neither burst of ethylene production nor elevated respiration rate was present due to the *rin* mutation (Giovannoni 2007, Kumar *et al.* 2012, Lincoln and Fischer 1988, Vrebalov *et al.* 2002). The changes in some features, including the color-turning, fruit softening, aroma production and texture alterations, were suppressed as well (Fujisawa *et al.* 2013, Vrebalov *et al.* 2002). Many metabolites and nutritional substances fail to accumulate, such as flavonoids, lycopene, carotenoid, soluble sugars, and organic acids (Giovannoni 2007, Vrebalov *et al.* 2002). This may be used to explain why its osmotic potential became less negative, compared to the control tomato fruits. Also, normal ripening of the fruit appears to involve the changes in the metabolic constituents in the fruitfull tissue (Klee and Giovannoni 2011). Tomato seeds are placed within fleshy fruits for their development and dispersal (Karlova *et al.* 2014). Therefore, unveiling the mechanism of the ripening-inhibition phenotype, its decreasing osmolality and seed vivipay, will provide clues into the improvement of this fruit-related breeding programme. Exactly how the fruit developmental model altered the metabolic components in fruits and how the seed was responsive to its environment signaling stimuli in *rin* fruit, remain to be elucidated.

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