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Seed germination in a tomato male-sterile mutant is resistant to osmotic, salt and low-temperature stresses

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Abstract Seed germination in a male-sterile *7B-1* mutant in tomato is reletively more resistant to the inhibitory effects of a high osmoticum induced by mannitol and polyethylene glycol, to various salts, including NaCl, Na_2SO_4, KCl and K_2SO_4, and to low-temperature stress, compared to the wild-type (WT) seeds. The inhibitory effects of various stresses could be partly or completely overcome by fluridone (FLU), an inhibitor of abscisic acid (ABA) biosynthesis. However, lower concentration of fluridone was required for the *7B-1* mutant than for WT seeds, and the mutant seeds were more sensitive to the inhibitory effects of exogenous ABA. The data suggest that *7B-1* seed has a pre-existing level of elevated ABA which imparts resistance to the various stresses. The ability to regulate male sterility in the *7B-1* mutant by photoperiod, as previously reported by Sawhney (1997), and its resistance to abiotic stresses, as reported here, makes this a useful system for tomato breeding and in hybrid programs.

Keywords Abscisic acid · Abiotic stresses · Fluridone · Light · Male-sterile mutant · Tomato

Introduction

Male sterility in crop plants, spontaneous or induced, is a choice material for plant breeders for several reasons, including its use in backcrossing, interspecific hybridization, and in F_1 hybrid seed production. There are several male-sterile (*ms*) mutants known in nearly every crop, but their use in breeding programs has been limited for a variety of reasons (see reviews Kaul 1988; Rao et al. 1990). Some of the *ms* mutants are sensitive to abiotic stresses, especially temperature and drought, which can limit their use in breeding programs. The resistance of *ms* mutants to such factors, as well as to various salts, should be of added value for their use in practical applications, e.g., in the hybrid seed industry.

We recently isolated a single gene, recessive, male-sterile mutant, *7B-1*, in tomato (*Lycopersicon esculentum* Mill.) which is photoperiod-sensitive. In long days (LD, 16-h light/8-h dark) in summer field conditions, *7B-1* flowers are male-sterile and contain stamens that are shrunken and produce non-viable microspores (Sawhney 1997). In short days (SD, 8-h light/16-h dark), many of the flowers on *7B-1* plants are fertile and produce normal stamens and viable pollen. Thus, pure-line *7B-1* seed produced in short days can be used as the female parent for hybrid-seed production. Here we report that the *7B-1* mutant is also resistant to abiotic stresses including a high osmoticum, various salts and low temperatures, as shown by seed-germination tests. This makes the *7B-1* mutant an exceptionally attractive system for use in tomato-breeding programs.

Materials and methods

Plant material and growth conditions

The *7B-1* mutant was isolated as a photoperiod-sensitive male-sterile line in tomato (*L. esculentum* Mill., background, cv Rutgers) (Sawhney 1997). For all experiments, *7B-1* and wild-type (WT) seeds were obtained from plants grown in soil (Sunshine #1, Sun Gro Horticulture, USA) under SD (8-h light/16-h dark) in a growth chamber at a temperature regime of 25°C light/23°C dark.

Germination tests

Seeds were sterilized by soaking in 50% (v/v) Javex-5 solution (Colgate-Palmolive Canada, Inc., Toronto, Canada; 3% sodium

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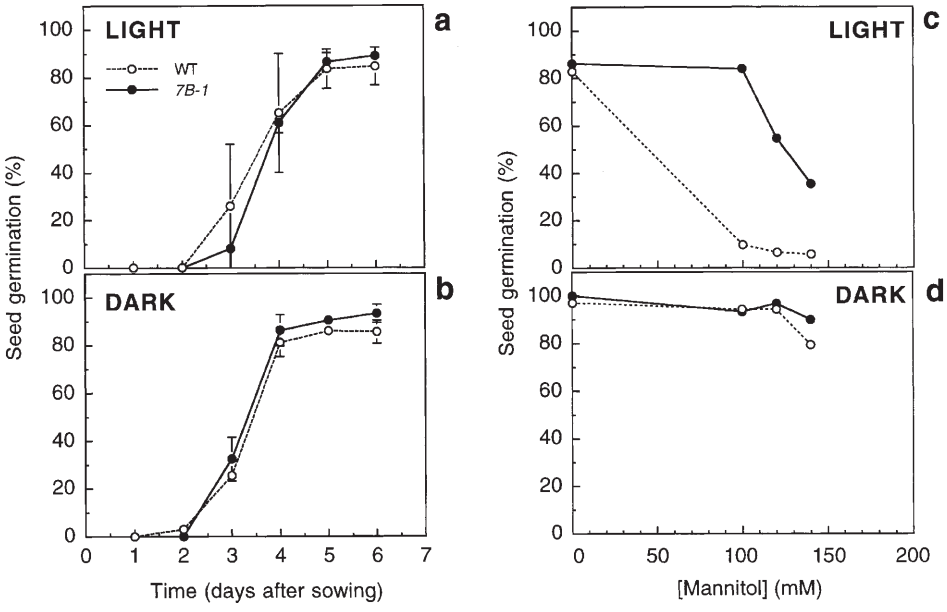

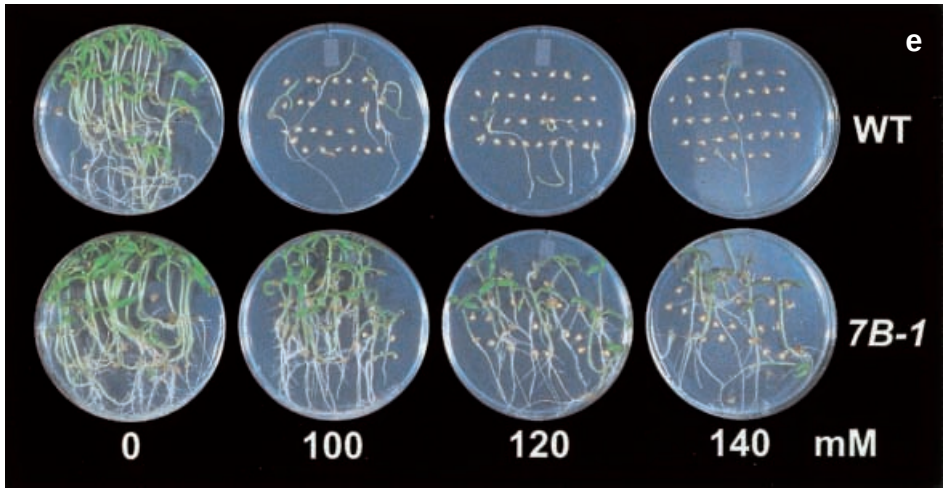

Fig. 1 Kinetics of seed germination in the WT and the *7B-1* mutant at normal temperature (25/23°C, light/dark) in the light (**a**) or in the dark (**b**), and the effect of mannitol (100–140 mM) on seed germination in both genotypes in the light (**c**, **e**) and in the dark (**d**). In **c** and **e**, seed germination was recorded after 12 days and in **d** after 7 days. In **a** and **b**, values represent means ±SE of four independent experiments. In **c** and **d**, the data from one representative experiment are shown. Similar results were obtained in 2–6 independent experiments

hypochlorite) for 25 min, and then rinsed extensively with sterile distilled water; 30 to 60 *7B-1* or WT seeds were germinated per Petri dish. The basal medium (BM) contained Murashige and Skoog (1962) salts, 1% (w/v) sucrose, 1 mM Mes, and 0.7% (w/v) agar (pH adjusted to 6.1 by KOH before autoclaving) with or without different concentrations of mannitol, polyethylene glycol (PEG 4000) (Fisher Scientific, Fair Lawn, N.J.), NaCl, KCl, Na_2SO_4, K_2SO_4, abscisic acid (ABA) (Sigma, St. Louis, Mo.) or fluridone {FLU, 1-methyl-3-phenyl-5-[(3-trifluoromethyl)phenyl]-4(4H)pyridone} (Eli Lilly, Indianapolis, Ind.). All the salts, mannitol, ABA and fluridone were added to the medium by sterile filtration (a 0.22-µm Millex-GS filter unit, Millipore Co., Bedford, Mass.) after autoclaving. Petri dishes with seeds were placed in an incubator at an optimal temperature regime of 25°C/23°C or in low temperatures at 16°C/13°C (light/dark), either under a 16-h light/8-h dark photoperiod or in continuous darkness. Illumination

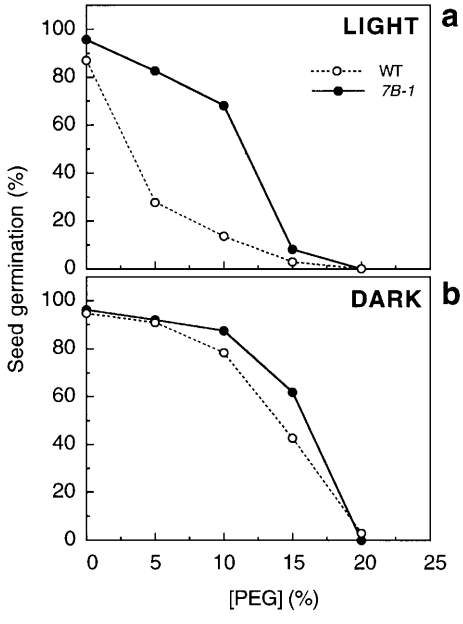

Fig. 2 Effect of PEG (5–20%) on WT and *7B-1* mutant seed germination in the light (**a**) and in the dark (**b**) at 25/23°C (light/dark). Seed germination was recorded after 7 days. The data from one representative experiment are shown. Similar results were obtained in two independent experiments ▶

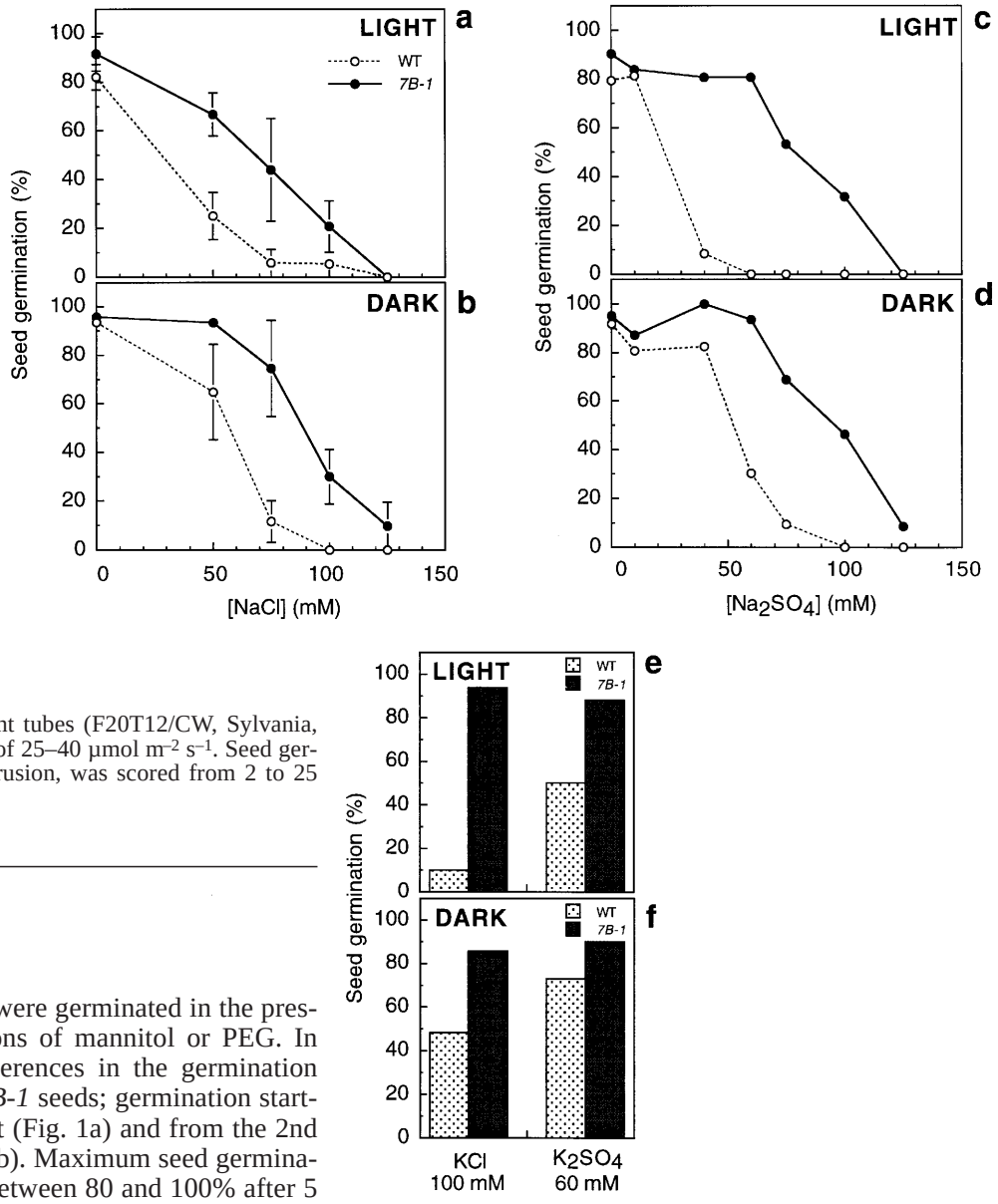

Fig. 3a–f Effects of salt stress on WT and *7B-1* mutant seed germination. Seeds were sown on BM supplemented with or without NaCl (50–125 mM) (**a**, **b**), Na_2SO_4 (10–125 mM) (**c**, **d**), KCl (100 mM) (**e**) and K_2SO_4 (60 mM) (**f**), and incubated at normal temperatures, 25/23°C (light/dark), in the light or in the dark. Seed germination was recorded after 13 days. In **a** and **b**, values represent mean seed germination ±SE of four independent experiments. Data from one representative experiment are shown in **c**, **d**, **e** and **f**, while similar results were obtained in 2–3 independent experiments

was provided by white fluorescent tubes (F20T12/CW, Sylvania, USA) with a photon flux density of 25–40 µmol m^{-2} s^{-1}. Seed germination, defined as radicle protrusion, was scored from 2 to 25 days after sowing.

Results

High osmoticum stress

Wild-type and mutant seeds were germinated in the presence of various concentrations of mannitol or PEG. In controls, there were no differences in the germination rates between the WT and *7B-1* seeds; germination started on the 3rd day in the light (Fig. 1a) and from the 2nd to 3rd day in the dark (Fig. 1b). Maximum seed germination in both genotypes was between 80 and 100% after 5 to 6 days in the light or in the dark (Fig. 1a, b). Mannitol (100–140 mM) inhibited WT seed germination in the light by 80 to 95%, compared to the control (Fig. 1c, e). In contrast, *7B-1* seeds were highly resistant to the osmotic stress of mannitol; germination was not affected by 100 mM of mannitol and was reduced to approximately 50% and 40% at 120 and 140 mM, respectively (Fig. 1c, e). In the dark, there was no difference in WT and mutant seed-germination with various mannitol concentrations (Fig. 1d). PEG also drastically affected WT seed germination in the light (Fig. 2a). With 5% PEG, WT seed germination was only approximately 20%, whereas in *7B-1* seeds it was about 80%. Further, 10% PEG yielded approximately 10% germination of WT seeds, but 70% in mutant seeds (Fig. 2a). With 15% and 20% PEG, germination was nearly completely inhibited in both genotypes. In the dark, *7B-1* and WT seed-germination showed a similar response to various concentrations of PEG (Fig. 2b).

Salt stress

The sensitivity of the *7B-1* mutant and WT seeds to various concentrations of NaCl, KCl, Na_2SO_4 and K_2SO_4 was tested. WT seed germination was strongly inhibited by NaCl. For example, with 50 mM of NaCl, WT seed germination in the light was approximately 30%, whereas that of mutant seed was 70% (Fig. 3a). Similarly, while 75-mM of NaCl nearly completely inhibited the germination of WT seeds, in mutant seeds it was approximately 40%. *7B-1* seeds were also less sensitive than WT seeds to NaCl in the dark (Fig. 3b), as well as to Na_2SO_4 in both the light (Fig. 3c) and the dark (Fig. 3d). However, the inhibition of WT seed germination was stronger in the light than in the dark. Additionally, mutant seeds germinated better than the WT in KCl and K_2SO_4. This effect was more pronounced in the light (Fig. 3e) than in the dark (Fig. 3f).

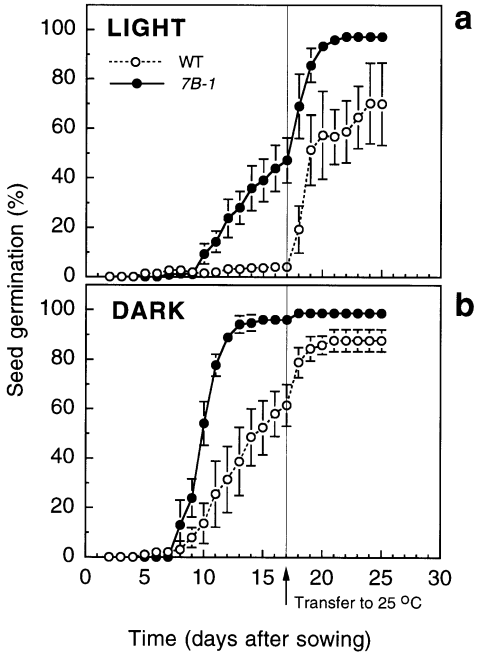

Fig. 4 Effect of low temperature, 16/13°C (light/dark), on WT and *7B-1* mutant seed-germination in the light (**a**) and in the dark (**b**). Seed germination was recorded each day for 25 days. The *arrow* indicates the day (17) when Petri dishes with the seeds were transferred from low temperature to normal temperature, 25/23°C (light/dark). Values represent mean seed germination ±SE of four independent experiments. For germination of control seeds at normal temperature in the light or in the dark see Figs. 1a and b, respectively

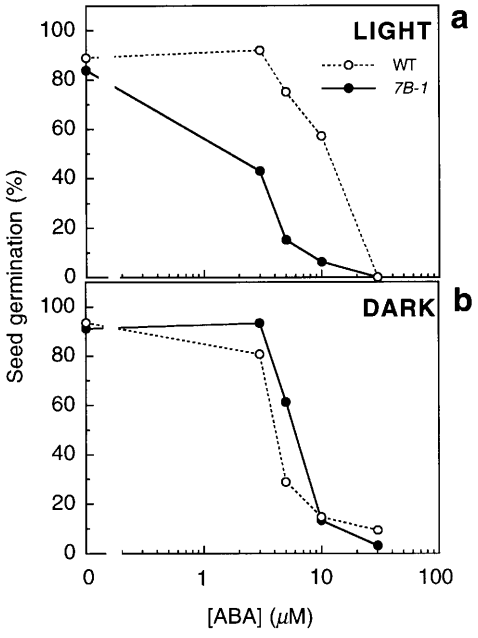

Fig. 5 Effect of ABA on germination in WT- and *7B-1* mutant-seeds in the light (**a**) and in the dark (**b**) at an optimal temperature, 25/23°C, light/dark. Seeds were sown on BM supplemented with or without ABA (5–30 μM). Seed germination was recorded after 7 days. The data from one representative experiment are shown. Similar results were obtained in four independent experiments

Low temperature stress

At optimal temperatures (25/23°C, light/dark), WT- and *7B-1* mutant-seeds showed no differences in either germination rate or in % germination (Figs. 1a, b). Low temperature (16/13°C, light/dark) almost completely inhibited the germination of WT seeds in the light, and the maximum germination was approximately 5% after 17 days (Fig. 4a). In the dark, inhibition of WT seed germination by low temperature was less than that in the light, but was still only approximately 60% after 17 days of sowing (Fig. 4b). In contrast, seed germination in *7B-1* was more tolerant to low temperatures than WT seeds. At 17 days after sowing, there was 50% germination in mutant seeds compared to no germination in the WT (Fig. 4a). In the dark, *7B-1* seed germination was accelerated compared to the light and, in 12 days, maximum (>90%) germination was observed compared to 30% in the WT. When WT and *7B-1* seeds were transferred from the low temperature to the optimal temperature, the germination was fully restored in the light as well as in the dark (Figs. 4a, b).

ABA and stress tolerance

One possible mechanism by which plants resist abiotic stress is by the accumulation of the plant hormone abscisic acid (ABA), since endogenous ABA levels are known to increase in stressed plant tissues (Goldbach and Michael 1976; Daie and Campbell 1981; Mäntylä et al. 1995). The possibility that the tolerance to abiotic stresses in the *7B-1* mutant could be related to altered ABA physiology was examinated. We tested the responses of WT and *7B-1* seeds to exogenous ABA, and to FLU, an inhibitor of ABA biosynthesis (Gamble and Mullet 1986; Saab et al. 1990; Xu and Bewley 1995), under osmotic, salt, and low-temperature stresses.

In the light, WT seed germination in the presence of ABA (3–30 μM) was slightly inhibited at 5 μM, 50% at 10 μM, and was totally inhibited at 30 μM (Fig. 5). In the mutant, seed germination was apparently more sensitive to exogenous ABA than in the WT. For example, 50% inhibition of germination was caused by 2 μM of ABA in the mutant compared to 10 μM of ABA in the WT (Fig. 5a). In the dark, however, WT and *7B-1* seeds showed a similar response to ABA (Fig. 5b).

Fluridone enhanced the rate of germination in both WT and *7B-1* seeds in the light (Fig. 6a) and in the dark (Fig. 6b). FLU also overcame the inhibitory effect of mannitol on seed-germination in the light (Fig. 6c, compare to Fig. 1c). Inhibition of germination by NaCl at 100 mM was partially overcome by 10 μM, and fully by 100 μM, of FLU, respectively, in both WT as well as in *7B-1* seeds (Fig. 6d). Similar results were obtained for seed germination in the dark and for other salts (data not

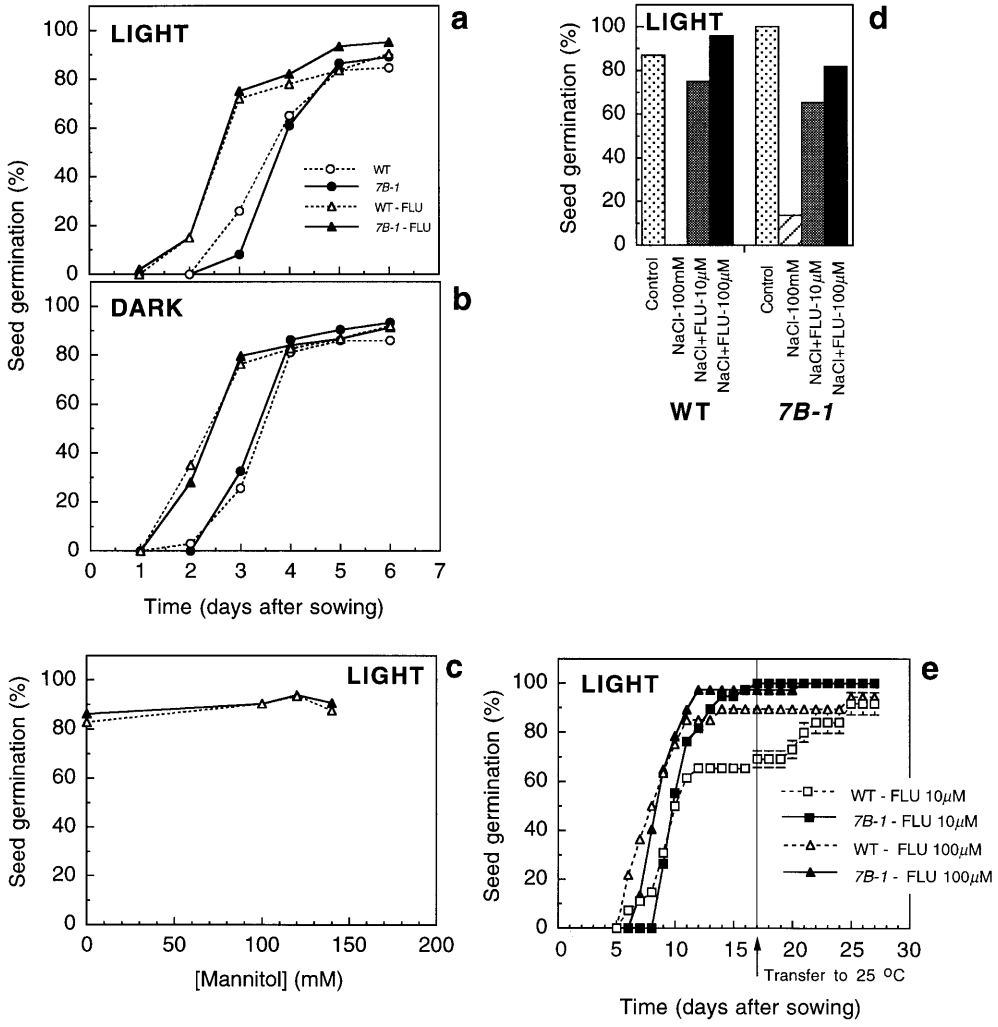

Fig. 6a–e Effect of FLU on germination in WT- and *7B-1* mutant-seeds in normal temperature conditions (25/23°C, light/dark) or under various abiotic stresses. The kinetics of WT- and *7B-1* mutant-seed germination in the presence of FLU (10 µM) in the light (**a**) and in the dark (**b**), and the effect of FLU (10 µM) + 100–140 mM mannitol on seed germination (**c**). Seed germination was recorded each day for 6 days (**a**, **b**) or after 7 days (**c**). The data from one representative experiment are shown, while similar results were obtained in two to three independent experiments. **d** Seed germination in WT and the *7B-1* mutant in the absence or presence of NaCl (100 mM) and/or fluridone (10 or 100 µM) in the light at normal temperature (25/23°C, light/dark). Seed germination was recorded and evaluated 13 days after seed sowing. The data of one representative experiment are shown. Similar results were obtained in three independent experiments. **e** Seed germination in WT- and *7B-1* mutant seeds in the light at low temperature (16/13°C, light/dark), in the presence of 10 µM or 100 µM of FLU. Seed germination was recorded for 27 days. The *arrow* indicates the day (17) when Petri dishes with the seeds were transferred to normal temperature (25/23°C, light/dark). Values represent mean seed germination ±SE of four independent experiments. For germination of control seeds in the light at optimal temperature see Fig. 1a

shown). Finally, FLU at concentrations of 10 µM partially, and at 100 µM completely, suppressed the inhibitory effect of low temperature on WT seed germination in the light (Fig. 6e). In contrast, 10 µM of FLU was sufficient to allow maximum germination of *7B-1* seeds at low temperature (Fig. 6e). In the dark, 10 µM of FLU fully restored low-temperature-induced inhibition of seed germination in both WT and *7B-1* seeds (data not shown).

Discussion

In field conditions, plants are often exposed to various abiotic stresses, such as drought, low and high temperatures, and high salinity (Siminovitch and Cloutier 1983; Lee and Chen 1993; Mäntylä et al. 1995; Ryu et al. 1995), conditions which can significantly reduce crop productivity (Epstein et al. 1980). Mutants with reduced sensitivity to abiotic stresses, and especially stress-tolerant male-sterile mutants, can be useful systems for crop producers and breeders. Thus, male-sterile mutants, in addition to their use as female parents in hybrid programs, could be less predisposed to various environmental stresses. To our knowledge, no male-sterile mutants

have been reported in tomato which show resistance to abiotic stresses, e.g., drought and high salinity.

Seed germination in the male-sterile *7B-1* mutant showed resistance to osmotic stress induced by mannitol or PEG, to the various salts tested, and to low temperatures. The effects of high salinity and low temperature are, in part, caused by impaired water absorption and transport; therefore, these stresses may, in part, be considered as different forms of osmotic stress (Ryu et al. 1995; Xiong et al. 1999). The tolerance of plant tissues to abiotic stresses, including drought, has been related to an increase in endogenous ABA in some systems (Quarrie 1980; Chen et al. 1983; Zeevaart and Creelman 1988; Chandler and Robertson 1994; Quarrie et al. 1994). Additionally, plant tissues, including tomato, exposed to stressful conditions show increased levels of endogenous ABA (Goldbach and Michael 1976; Daie and Campbell 1981; Mäntylä et al. 1995). Thus, WT and *7B-1* seeds exposed to various stresses, may well have an increase in endogenous ABA. This suggestion is supported by the observations that FLU, an inhibitor of ABA synthesis, partially or completely overcame the inhibitory effects of all of the stresses, i.e., high osmoticum, high salinity and low temperature. However, less FLU was needed for *7B-1*- than for WT-seed, particularly for the restoration of low-temperature stress. This reduced requirement may be due to the elevated levels of pre-existing ABA in *7B-1* seed. Thus, the *7B-1* seed may be regarded as pre-adapted to abiotic stresses. The greater sensitivity of *7B-1* seed, compared to the WT, to germinate in the presence of exogenous ABA may also be interpreted in terms of the high ABA content of the seed. Finally, the fact that ABA-deficient mutants in tomato and *Arabidopsis* are impaired in tolerance to various abiotic stresses (Koornneef et al. 1982; Bray 1988; Heino et al. 1990; Chen and Plant 1999) supports the suggestion that the *7B-1* mutant has elevated level of endogenous ABA.

Inhibition of WT seed germination under the abiotic stresses tested was much more pronounced in the light than in the dark. This indicates that light controls, or amplifies, the inhibitory effects of abiotic stresses on seed germination. Interestingly, *7B-1* seed germination was less sensitive to the light-induced amplification in all the abiotic stresses tested. Light is known to regulate ABA biosynthesis/metabolism in plants (Tillberg and Björkman 1993; Kraepiel et al. 1994; Baraldi et al. 1995; Weatherwax et al. 1996, 1998). Our observations on the inhibition of seed germination by mannitol, various salts and low temperature in the light support the notion that light controls plant responses to abiotic stresses via the regulation of ABA synthesis.

In conclusion, the *7B-1* mutant is potentially a useful system for tomato breeders, both for its use as a female parent in hybrid-seed production, and for its resistance to abiotic stresses.

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