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THE EFFECT OF HEAT TREATMENT OF WATERMELON SEED ON GERMINATION, HYPOCOTYL EMERGENCE AND ABSCISIC ACID CONTENT

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SUMMARY

Seeds of watermelon (*Citrullus lanatus*) with a moisture content of 7.2% were heat treated at 60 °C for 15 or 20 h, and their performances compared with that of untreated seeds. Radicle emergence of treated seeds was only slightly lower than that of the control when germinated at 25 °C. To ascertain the effect of the treatments on hypocotyl growth (emergence) *per se*, radicle emergence was synchronized in all treatments and emergence from deep plantings in sand determined. Small differences in emergence were observed when seeds were germinated at 25 °C, but seed treatment resulted in large reductions in emergence at 15 °C. This demonstrates that the hypocotyls developing from heat-treated, germinated seeds are less able to withstand low germination temperatures, which could be an important factor in emergence failure. Levels of free abscisic acid (ABA) were higher at 15 °C than at 25 °C, with hypocotyls of treated seeds containing more free ABA and its breakdown products than the control at the lower temperature.

INTRODUCTION

Seedling emergence is the product of two phases, namely, radicle emergence and subsequent pre-emergence shoot growth. In studies with apple embryos, Perino and Côme (1991) demonstrated that these phases are physiologically distinct and that they differ in their response to environmental factors such as temperature and oxygen.

Although it is known that inferior growth generally results from aged or low-vigour seeds, even when levels of germinability may be relatively high (Priestley, 1986; TeKrony and Egli, 1991), attempts are rarely made to ascertain whether poor emergence can be ascribed to germination (radicle emergence) failure, a breakdown in subsequent growth or both. Halmer and Bewley (1984) expressed the view that crop emergence losses are overwhelmingly due to a failure of seedlings to grow under the soil surface, rather than to germination failure, but they supplied no data or references to support their view.

In the present study the consequences of heat treatment on watermelon (*Citrullus lanatus*) seeds was investigated with respect to both germination and

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subsequent hypocotyl growth. Watermelon seeds with above 40% of oil are very likely to lose vigour and this may happen commonly in watermelon-growing parts of the world which can be characterized by hot and humid seed harvest periods (George, 1985). To determine the effect on hypocotyl growth *per se*, radicle emergence of the control and heat-treated seeds was synchronized before planting. Emergence was studied under favourable (25 °C) and cool (15 °C) conditions. The disparity in emergence between seed lots is usually much more evident under stressful conditions (Priestley, 1986).

Although the role of abscisic acid (ABA) in seed development, germination and dormancy has been well studied (Black, 1991), there is a lack of information on the role of this hormone in seed vigour and seedling emergence. Several reports indicated that ABA levels dropped in seeds subjected to dormancy-breaking treatment and that germination was inhibited by exogenous application (Walton, 1977). Determinations of ABA and its breakdown products were therefore made on hypocotyls of heat-treated and untreated watermelon seeds incubated at 25 and 15 °C.

MATERIALS AND METHODS

Watermelon (*C. lanatus* cv. Congo) seeds, harvested in May 1997, were purchased from MayFord Quality Seeds in July 1997. The seeds were kept in a sealed container in a cold room at 2 °C until heat treatments and subsequent experiments were commenced in March 1998.

Two heat treatments were applied. The seeds (moisture content 7.2%) were placed in sealed glass jars in a germination cabinet at 60 °C for 15 or 20 h. This temperature and the periods were determined according to preliminary experimental results. In preliminary experiments seeds were treated at 40, 50 and 60 °C for different periods ranging from 10 h to 10 d. The best results, reduction in rate but not in total germination were obtained at 60 °C with treatment lengths of 15 and 20 h. Germination (normal seedlings, well developed root and shoot) of the control (which was not subjected to heat treatment) and treated samples was determined at 25 °C in sand according to ISTA (1996), using four replicates of 50 seeds each per treatment.

To determine viability (total radicle emergence) and mean time to germination (radicle emergence) seeds of the control and two heat treatments were incubated in quartz germination sand with their micropyles protruding above the surface. The containers were sandwich boxes (24 × 22 × 6 cm) and incubation proceeded at 25 °C. Four replicates of 50 seeds each were used. The seeds were regarded as germinated when the radicle had attained a length of 1–2 mm. Germination counts were made at 5-h intervals until no further germination occurred.

Mean time to germination was calculated according to the formula of Ellis and Roberts (1980): $\Sigma n.D/\Sigma n$, where n = number of seeds germinated on day D, and D = number of days of incubation.

To determine the effect of heat treatments on emergence without the confound-

ing effect of differences in germination rate, radicle emergence in all treatments was synchronized before planting the germinated seeds in sand. This was accomplished by incubating large numbers of seeds of each treatment in sand at 25 °C and distributing commencement of germination according to each treatment's predetermined germination rate. At the commencement of the emergence trial, germinated seeds (radicle protrusion of 1–2 mm) were selected from each treatment and planted.

The emergence experiment was conducted in quartz germination sand in sandwich boxes (described above). To increase the severity of the emergence potential test, germinated seeds were planted at a depth of 3.5 cm. Incubation temperatures of 25 (optimum) and 15 °C (cool) were used, with four replicates of 25 seeds each per treatment. Preliminary experiments showed that 13 °C was too low and 18 °C was too high to create a stressful environment for watermelon seeds. Therefore 15 °C was selected as the cool stress temperature. Emergence counts (that is, when seedlings appeared above the surface) were made daily for 20 d. Total emergence was determined as well as mean time to emergence, using the formula of Ellis and Roberts (1980) with emergence counts substituted for germination counts.

Analyses of variance were conducted on the data of the germination and emergence experiments and least significant differences calculated according to Tukey (Steel and Torrie, 1960)

For ABA determinations, germinated seeds were incubated as described for the emergence trial using seeds from only the control and 20-h heat treatment. Samples were removed for analysis after emergence had begun, that is, after incubation for 4 d at 25 °C and after incubation for 13 d at 15 °C. Analyses were performed on hypocotyls after removal of the roots and cotyledons. Free abscisic acid (ABA) and its breakdown products, phaseic acid (PA), diphaseic acid (DPA) and epi-DPA were extracted and determined according to Cowan and Railton (1987). Determination was by means of high pressure liquid chromatography (HPLC) on a reverse-phase column (Ultrasphere ODS, 4.6 mm × 15 cm) eluted with a gradient of 0–100% ethanol in 0.5% acetic acid at a flow rate of 1 cm^3 min^{-1}. The eluent was monitored at a wavelength of 254 nm. Samples were injected three times. Extraction and determination were performed on two replicates of each treatment.

RESULTS AND DISCUSSION

Heat treatment of seeds for 15 h at 60 °C decreased the percentage of normal seedlings (well developed root and shoot) in the standard germination test from 93.5% to 86.5%, while treatment for 20 h at the same temperature caused a further reduction to 78.0% (Table 1). In contrast, heat treatment had a small and non-significant effect on viability (radicle emergence, 2 mm radicle formation) (Table 1). Mean time to radicle emergence was increased by both heat

Table 1. Germination of control and heat-treated watermelon seeds at 25 °C.

Heat treatment	Radicle emergence (%)	Mean time to radicle emergence (d)	Germination (% normal seedlings)
Untreated control	95.0 (2.0)†	2.54 (0.36)	93.5 (4.4)
60 °C for 15 h	93.0 (2.5)	2.82 (0.11)	86.5 (6.8)
60 °C for 20 h	91.3 (5.37)	3.16 (0.29)	78.0 (5.4)

†Figures in parentheses are s.e.m.

treatments, although the difference was statistically significant only in the case of the 20-h treatment (Table 1).

After synchronization of germination, emergence of seedlings at 25 °C was slightly less in the case of treated seeds, but values did not differ significantly from that of the control (Table 2). Incubation at 15 °C reduced emergence in the control treatment from 100% to 92% but emergence of seedlings of the treated seeds was reduced to 43% and 48% for the 15- and 20-h treatments respectively. The results show that the statement that treated seeds (aged) are generally less able to withstand environmental stress (Priestley, 1986) also applies to shoots (hypocotyls) developing from such seeds, and supports similar conclusions drawn from studies on shoots of fresh and treated (aged) maize grains (Dreyer and van de Venter, 1992). It underlines the importance of assessing the effect of seed deterioration not only on seed viability, but also on subsequent pre-emergence growth, especially under stress conditions. Mean time to emergence in the heated seed treatments was increased above that of the control at both 25 and 15 °C incubation temperatures, but the differences were not statistically significant.

At 25 °C incubation temperature, hypocotyls of the control treatment contained slightly more ABA (approximately 0.1 ng g^{-1} fresh mass) than those of the heated seed treatment (Table 3). The latter contained more breakdown products of ABA, but the total amount of these compounds was relatively small in both cases. However, the moisture contents of the hypocotyls and the sand were not measured. Incubation at 15 °C increased the free ABA in control hypocotyls by a factor of 1.3, while that in the hypocotyls from treated seeds was more than doubled. The concentration of ABA breakdown products was also increased at the

Table 2. Emergence of seedlings of control and heat-treated watermelon seeds at 15 and 25 °C after radicle emergence had been synchronized in all treatments.

	Total emergence (%)		Mean time to emergence (d)	
Heat treatment	25 °C	15 °C	25 °C	15 °C
Untreated control	100 (0.0)†	92 (6.5)	3.33 (0.1)	11.88 (0.6)
60 °C for 15 h	94 (6.9)	43 (17.0)	3.91 (0.1)	12.18 (0.4)
60 °C for 20 h	96 (5.0)	48 (8.6)	4.07 (0.0)	13.40 (1.4)

†Figures in parentheses are s.e.m.

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Table 3. Concentration of abscisic (ABA) and total breakdown products (PA, DPA, epi-DPA) (ng g^{-1} fresh mass) in hypocotyls from untreated control and heat-treated watermelon seeds at 15 and 25 °C after radicle emergence had been synchronized in all treatments.

	25 °C		15 °C	
Heat treatment	Free ABA	Total ABA breakdown products	Free ABA	Total ABA breakdown products
Untreated control	0.464 (0.02)†	0.003 (0.0001)	0.619 (0.05)	0.191 (0.06)
60 °C for 20 h	0.373 (0.06)	0.036 (0.004)	0.944 (0.3)	0.254 (0.08)

†Figures in parentheses are s.e.m.

lower temperature with hypocotyls from the treated seeds showing the highest concentration of both free ABA and its breakdown products.

In all stresses studied, available data indicate that ABA levels increase as stress increases (Morgan, 1990). Stress tolerance appears to be associated with higher levels of ABA (Larqué-Saavedra and Wain, 1976; Morgan, 1990). Although levels of ABA in watermelon hypocotyls were increased by low temperature stress in the present study, higher levels of both ABA and breakdown products were found in hypocotyls of treated seeds than in those of the control. This is contrary to what was expected as the hypocotyls arising from treated seeds were shown to be less tolerant of the low temperature. However, moisture contents of hypocotyls were not measured. Comparison of treated and untreated seedling moisture contents would be useful regarding total ABA content. No other reports comparing ABA levels in seedlings from fresh and treated seeds could be found. This is, clearly, a field which requires much further investigation.

CONCLUSION

This work showed that poor emergence is not only due to radicle emergence failure but also to breakdown of subsequent seedling growth. This is accentuated under stressful temperature conditions. Regarding watermelon seed production, high temperatures (imitated by heat treatment) at harvest or during the storage period can cause low vigour and this in turn reduces percentage emergence. ABA content is well correlated with this low emergence potential.

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