INFLUENCE OF TEMPERATURE LEVEL AND DURATION OF HEAT EXPOSURE DURING SEED MATURATION ON SEED GERMINATION AND SEEDLING GROWTH OF BARLEY

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A single brief exposure to high temperature stress during seed maturation stimulated increased seedling emergence in the freshly harvested barley seed when proper time-temperature relationship was - used. Within a range of 49°C. to 60°C. germinability was increased when the plants were directly stressed at 49°C. for 2 hours at 22 days after awn emergence or more safely by placing the plants at 38°C. raising the temperature during next 1½ hours to 54°C. holding at that temperature for 1 to 2 hours before subsequent cooling to greenhouse temperature and returning them to the pre-stress conditions. Stressing the seed abruptly at 54°C. for 2 hours reduced viability and the 60°C. stress killed the seed on account of intensified thermal injury. Seedlings raised from the seed stressed for 1 to 2 hours at 54°C. after 90 minutes heating from 38°C. to 54°C. were normal. Longer stress resulted in stunted seedlings with narrower leaves.

INTRODUCTION

Prevailing temperatures during maturation of seed while on parent plant are recognized to be associated with germinability and later growth. Increased germinability after moderate but warm maturation temperatures have been reported by several workers (Dotzenko, 1967; Evenari, 1965; Harrington and Thompson, 1952; Laude, 1967; Stearns, 1960; Von Abrams and Hand, 1956). The influence of a brief but more severe exposure during seed maturation has not been much investigated. Laude (1967) suggested the existence during seed maturation of a sensitivity gradient to high temperature stress in Atlas barley and reported an increased germinability at harvest in some instances. In his experiments barley stressed at 49°C. showed relatively higher emergence than that stressed at 54°C. but an exposure time of 5 hours seems to have depressed the expression of maximum stimulatory effect on seed germinability. Khan (1967) observed that germination of freshly harvested seed of Atlas barley is depressed following heat stress at 7 to 10 days after awn emergence, but is enhanced by the same stress applied some three weeks after awn emergence. The depression in these instances was attributable to reduced viability associated with thermal injury.

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The objective of the present investigation was to determine the responsiveness of barley during maturation to heat stress, and to consider the importance of the responses in terms of germination and seedling growth.

MATERIALS AND METHODS

A mildew-resistant type of Atlas barley, designated U.C.D. 2010, was grown in the greenhouse in 6-inch pots, filled with a mixture of two-thirds loam soil and one-third sand. Four plants per pot, watered as necessary to prevent moisture stress, were grown to maturity. Greenhouse temperatures were maintained near 21°C. day, and 15°C. night, except during the summer months when day temperatures reached 26°C. or slightly above. In winter to insure heading, the day lengths were extended to 17 hours by incandescent lighting which permitted an intensity of approximately 100 ft. C. at uppermost leaf height.

The age of the caryopsis during maturation was stated in terms of days after awn emergence of that spike carrying the caryopsis. A tag bearing the date of awn emergence was placed on each jointing culm. Plants were subjected to heat stress some three weeks after awn emergence. The stresses were applied in a chamber controlled as to air-temperature, humidity and light. Since the heating capacity of the stress chamber was such that the duration of time required to reach different temperature regimes varied considerably, the plants were moved from the greenhouse directly into the stress chamber when the air-temperature of the chamber had reached 49°C, 54°C or 60°C. (The latter was expected to be a near lethal temperature). They were maintained at each temperature for 2 hours then cooled for 30 minutes before returning to the pre-stress conditions.

For the heat stress at different durations of exposure, the plants were introduced into the stress chamber when the air-temperature in it had reached 38°C. During the next 90 minutes the temperature was gradually raised to 54°C. and held at that temperature for 1, 2 or 3 hours before the plants were removed from the chamber and returned to the greenhouse after subsequent cooling.

Seed was harvested when all green coloration had left the spike. Immediately upon harvest replicated 50-seed samples were planted at one half inch depth in moist sand and germinated in a growth chamber at constant 20°C. with 8 hours of low intensity illumination daily. Germinability was based on seedling emergence counts recorded for 21 days.

Seed viability was determined by means of tetrazolium test (Moore, 1962) and excised embryo technique. For the latter, embryos, from the caryopses of freshly harvested stressed and non-stressed seeds were separated from the endosperm with a sharp scalpel after 4 to 6 hours of soaking in water. A small amount of endosperm adhered to one end of these separated embryos. This end was placed in contact with moist filter paper in 60×15 mm. petri dishes. The embryos were incubated at constant 20°C. with 8 hours of light daily, and those which produced both radicle and plumule were considered germinated.

RESULTS AND DISCUSSION

Greenhouse grown barley matured seed some 30 days after awn emergence. Heat stresses of 49°C., 54°C. or 60°C. for two hours were applied on 22nd day after awn emergence. The germinability of this seed at harvest is presented in Table 1. Different temperature regimes were highly significant. Controlled seed, not stressed, revealed a degree of fresh seed dormancy. The seed stressed at 49°C. resulted in highest emergence. 54°C. and 60°C. gave lower emergences attributable to reduced viability associated with thermal injury. The viability of the seed was appraised before the emergence test by means of tetrazolium test (Moore, 1962) and excised embryo technique. Seeds which failed to germinate were recovered from the sand after 21 days, rinsed and tested with tetrazolium. (Table 2).

TABLE 1.—*Effect of different air temperatures applied for 2 hours on 22nd day after awn emergence on seed germinability at harvest.*

Temperature °C	Average number of seedlings[1] emerged at 7 days	14 days	21 days
Control ..	24.0 b[2]	29.3 b	30.0 b
49 ..	43.0 a	46.6 a	47.3 a
54 ..	18.0 b	20.6 b	20.6 c
60 ..	1.0 c	1.0 c	1.0 d

1. Average of three 50-seed samples.

2. Duncan's Multiple Range at 1% probability. Any two means within each emergence period which do not share a letter in common differ significantly.

TABLE 2.—*Estimate of seed viability after heat stress but before the emergence test and on recovered seed after the test.*

Temperature° C	Before emergence test				Recovered seed	
	Excised embryo test		Tetrazolium test		Tetrazolium test	
	No. tested	No. germinated	No. tested	No. respiring[1]	No. tested	No. respiring
Control	15	15	25	25	25	25
49 ..	15	15	25	25	..	..
54 ..	15	6	25	11	25	0
60 ..	15	0	25	0	25	0

1. Respiration processes within living tissues of seed release hydrogen, which combines with the colourless tetrazolium solution and produces red stain in living, healthy tissue, *e.g.*, embryo and scutellum.

The control seeds and those stressed at 49°C. were viable and respiring, but the seed stressed at 54°C. showed less than 50 per cent viability. After the emergence test all the recovered control seeds were still respiring. Failure to germinate was probably due to this seed still being in a state of fresh seed dormancy. No seed stressed at 49°C. was recovered as practicably all of it had germinated. None of the seed stressed at 54°C. revealed respiration indicating that all the viable seeds had germinated and only non-viable seeds remained ungerminated in the sand. The 60°C. stress had killed the seed.

Since there was not appreciable difference in germinability of the control seed and the seed stressed at 54°C., the fresh seed dormancy in the former appeared to be equal to the heat injury in the latter. It was felt desirable to investigate next the effects of different durations of exposure at 54°C. and to evaluate the extent in difference of response when the plants were not abruptly shocked at this temperature as in the previous case. The germinability at harvest of the seed stressed on the 22nd day after awn emergence for different durations at 54°C. after 90 minutes' gradual heating from 38°C. to 54°C. is given in Table 3. Exposures of 1 and 2 hours at 54°C. after 90 minutes of gradual heating from 38°C. to 54°C. gave significantly greater emergence than the control up to 14 days. Thereafter, their difference with the control became non-significant by the end of 21 days due to disappearance of dormancy in the control seed by that time. Three hours at 54°C. revealed no superiority either over the control or 1 to 2 hours stress at 54°C. Excised embryo and tetrazolium tests performed on the freshly harvested seed (Table 4) indicated that the seed stressed for 3 hours at 54°C resulted in considerably lower viability probably due to thermal injury to the seed.

When the seed was directly stressed some three weeks after awn emergence for 2 hours at 54°C. more than 50 per cent reduction in viability was observed. The abrupt increase in temperature seems to have intensified injury which could be effectively reduced by placing the plants at 38°C. raising the temperature to 54°C. during a 90 minutes' interval and maintaining at that temperature for 1 to 2 hours. The nature of the "Conditioning effect" of 1½ hours of raising temperature was not studied, but would appear to warrant investigation.

TABLE 3.—*Effect of durations of air temperature stress applied on the 22nd day after awn emergence on the germinability of seed at harvest.*

Time at 54°C after 90 minutes heating from 38°C to 54°C.	Average number of seedlings[1] emerged at		
	7 days	14 days	21 days
Control ..	21.0[2] c	33.0 b	44.6 a
1 hr. ..	43.0 a	49.0 a	49.0 a
2 hrs. ..	29.6 b	41.3 a	41.6 a
3 hrs. ..	19.3 c	27.3 b	27.3 b

1. Average of three 50-seed samples.
2. Duncan's Multiple Range at 1% probability. Any two means within each emergence period which do not share a letter in common differ significantly.

The effect of different durations of heat stress at 54°C. on the subsequent seedling growth was studied. The germination test was commenced 1 day after harvest on slanted substrates at constant 20°C. with a photoperiod of 8 hours. In this technique twenty-five seeds were planted in a row at the mid section of a moist horizontal blotting-paper. A single ply of thin dry tissue, such as Kleenex, was placed over the seeds. A fine mist of water was sprayed over the tissue. The clinging moist tissue held the seeds firmly in place so that the substrate could be slanted with the embryo ends of the seeds facing downward and other ends upwards. For support the blotting-paper was placed on a rigid piece of non-toxic plastic. A rack was used to hold the slanted substrate sheet with the basal portion in a water reservoir. The plumules grew straight

TABLE 4.—*Estimate of seed viability after heat stress but before the emergence test.*

Time at 54°C after 90 minutes' heating from 38°C to 54°C.	Before emergence test			
	Excised embryo test.		Tetrazolium test.	
	No. tested	No. germinated	No. tested	No. respiring[1]
Control ..	15	15	25	25
1 hr. ..	15	15	25	25
2 hrs. ..	15	14	25	24
3 hrs. ..	15	9	25	16

1. Respiring tissue within seed, *e.g.*, embryo and scutellum develop red stain with colorless tetrazolium solution.

TABLE 5.—*Root and shoot growth of the seedlings at* 10 *days after planting of the seed stressed approximately three weeks after own emergence.*

Hours at 54°C after 90 minutes' heating from 38°C to 54°C	Average seed weight (gms)	Average emergence[2] at 10 days	Average length[3]	
			Root (cm)	Shoot (cm)
Control ..	2.5 a	30.0	6.9	13.7
1 ..	2.3 b[4]	46.6	7.0	12.9
2 ..	2.2 b	40.0	7.2	13.0
3 ..	2.3 b	26.0	4.6	5.7

1. and 2. Average of three 50-seed lots.

3. Average of 25 seedlings.

4. Duncan's Multiple Range at 1% probability. Any two means within each treatment which do not share a letter in common differ significantly.

upward and the radicles downward along the slanted substrate. Ten days after planting, the shoot growth was measured from the hypocotyl to the tip of the first leaf and the root growth from the point of radicle emergence to the tip of the longest root. The results are presented in Table 5. Although the control seed was heavier than the stressed seed, there was no appreciable difference in the root and shoot length of the seedlings emerging from the control seed and the seed stressed for 1 to 2 hours at 54°C. (after the 90 minutes' heating from 38°C to 54°C). The longer exposure of 3 hours resulted in decreased viability, and reduced root and shoot growth. These seedlings were stunted in growth and had narrower leaves as compared to the normal seedlings. These observations suggest that temperature stress rather than the seed size exerted greater influence on seedling growth.

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