Plant Physiol. (1987) 83, 723–725
0032-0889/87/83/0723/03/$1.00/0

Communication

(2-Chloroethyl)phosphonic Acid Promotes Germination of Immature Spores of *Ceratopteris richardii* Brongn.[1]

Received for publication October 29, 1986

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ABSTRACT

Freshly collected spores of strain Hn-n of *Ceratopteris richardii* Brongn. require storage for several months before attaining maximum germination rate. Treatments using (2-chloroethyl)phosphonic acid increased germination rate in freshly collected spores and decreased germination rate in older spores.

Freshly collected or immature spores of *Ceratopteris richardii* strain Hn-n exhibit a slower rate of germination than older or mature spores. Maximum germination rate occurs after room temperature storage for several months. Manipulation of various environmental factors, such as temperature, air pressure, acid scarification, presoaking, and light quality in previous attempts failed to accelerate this maturation process (TR Warne, LG Hickok, unpublished data). In this paper, we document the successful artificial maturation of immature spores by treatments with (2-chloroethyl)phosphonic acid.

MATERIALS AND METHODS

Plant Material. Spores were collected from greenhouse grown sporophytes of the inbred, homozygous diploid ($2n = 78$) strain of *Ceratopteris richardii* Brongn., Hn-n, (7–9) 6 d (*i.e.* immature), and 383 d (*i.e.* mature) prior to testing.

(2-Chloroethyl)phosphonic Acid Treatment. Dry spores were treated for 0 or 4 to 12 d prior to germination tests. For treatment, 1 to 5 mg spores were placed in a shell vial and sealed into a 500 ml Mason jar along with a separate shell vial containing either 0.5 ml Ethrel (Amchem Products, 21.3% [2-chloroethyl]phosphonic acid) and 1.0 ml of 1.0 N KOH ([2-chloroethyl]phosphonic acid treatment) or KOH alone (control).

Germination Assay. Following treatment, spores were soaked in distilled H_2O for 16 h, then surface sterilized according to standard procedures (11). Axenic cultures were established at a standard density of about 20 gametophytes cm^{-2} (9) on basal inorganic nutrients (10), pH 5.4, and maintained at a constant thermo-photoperiod of 27 ± 2°C and 23 ± 5 $W \cdot m^{-2}$. Spore germination, as identified by an emergent rhizoid, was determined from microscopic observation (×25) of 100 spores per dish. Initial time reference for timed germination counts was the time spores were soaked prior to sterilization.

RESULTS

Prior to treatment, slower germination rate was confirmed in the immature spores (Fig. 1). Maximum germination in the mature spores occurred by 7 d following soaking, whereas germination in the immature spores was substantially delayed. Treatment with (2-chloroethyl)phosphonic acid altered germination in both immature and mature spores (Table I). Enhancement of germination in immature spores occurred by the 4 d treatment and reduced germination in mature spores occurred by the 8 d treatment. At the 6 d treatment, a maximal effect occurred in immature spores relative to a minimal reduction of germination of mature spores. An examination of spore germination over time in 14 d treatments (Fig. 2) revealed that both immature and mature spores treated with (2-chloroethyl)phosphonic acid exhibited germination curves intermediate to control mature and immature spores.

DISCUSSION

These results demonstrate that treatment with (2-chloroethyl)phosphonic acid enhances germination rates in immature spores and inhibits germination rate in mature spores of strain Hn-n of *C. richardii*. This suggests an age-dependent spore maturation process, in which (2-chloroethyl)phosphonic acid treatments apparently accelerate a spore aging or ripening process in both immature and mature spores. These responses are likely related to the action of ethylene, since aqueous preparations of (2-chloroethyl)phosphonic acid hydrolyze and release free ethylene above pH 4.0 (1). Given a complete release of ethylene from the (2-chloroethyl)phosphonic acid in Ethrel, and no loss, a maximum of 0.74 mmol (1.48 mM) of ethylene was released during a treatment.

In contrast to the promotion of spore maturation in *C. richardii*, ethylene has been shown to inhibit early development, *i.e.* spore germination, in gametophytes of *Onoclea sensibilis* (2–4). In *O. sensibilis* ethylene does not affect imbibition of water by spores but effects inhibition of spore germination prior to DNA synthesis, nuclear migration and cell division (4, 6). Inhibition is overcome by light and appears related to photosynthesis and not phytochrome (3, 5).

The maturation requirement evident in Hn-n is not apparent in other diploid strains of *Ceratopteris* examined. Synthesized hybrids between Hn-n and another strain that lacks a spore maturation requirement (176D), segregate for both 'rapid' and 'delayed' germination types in the F2 sporophyte generation (TR Warne, LG Hickok, unpublished data). These observations suggest a genetic basis for this character. Recent techniques for the analysis of natural and induced variability in *Ceratopteris* should allow for verification and elucidation of the genetic basis of this trait (9).

[1] Supported by National Science Foundation grant DCB-85-11237.

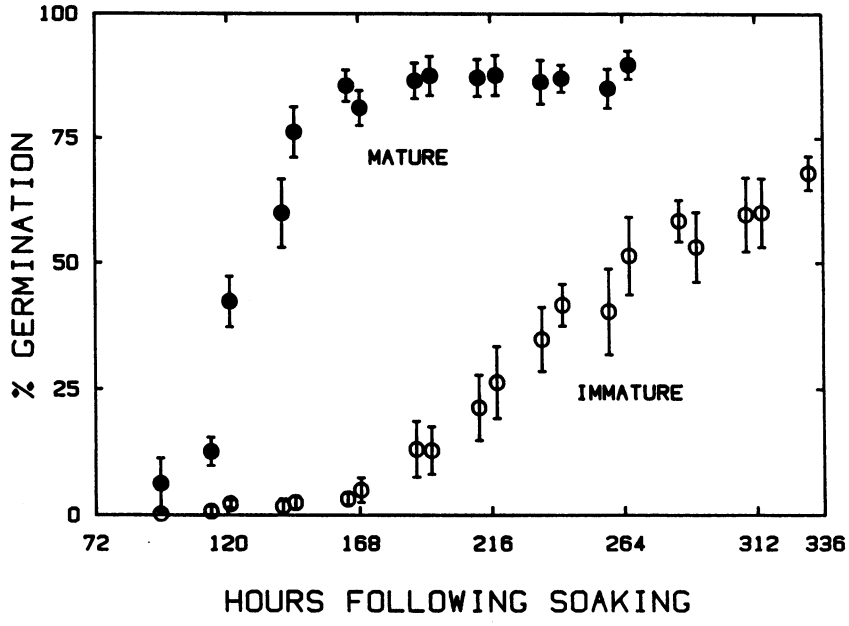

FIG. 1. Mean ± SD (n = 4) percent germination in untreated immature and mature spores of Hn-n.

Table I. *Germination at 6 Days following Soaking of Immature and Mature Spores Exposed to (2-Chloroethyl)phosphonic Acid Treatment for 0 to 14 Days*

Numbers in parentheses indicate germination as a percentage of untreated mature spores.

Treatment Time	Germination			
	Immature		Mature	
	Untreated	Treated	Untreated	Treated
d		% ± SD		
0	2.5 ± 1.3 (3.3)		75.9 ± 5.2	
4	3.8 ± 2.2 (4.6)	42.0 ± 7.4 (51.4)	81.8 ± 3.6	76.3 ± 6.8 (93.3)
6	2.5 ± 0.7 (3.5)	61.5 ± 6.5 (86.3)	71.3 ± 6.5	67.0 ± 7.1 (94.0)
8	3.8 ± 2.5 (7.4)	45.8 ± 5.3 (90.1)	50.8 ± 6.1	42.0 ± 12.6 (82.8)
10	3.3 ± 1.3 (4.1)	73.5 ± 3.0 (93.3)	78.8 ± 3.6	65.0 ± 4.1 (82.5)
12	1.5 ± 2.1 (2.0)	71.8 ± 5.7 (93.8)	76.5 ± 4.0	46.8 ± 12.0 (61.1)
14	18.8 ± 9.7 (24.5)	46.8 ± 5.1 (61.1)	76.5 ± 6.4	53.5 ± 7.5 (69.9)

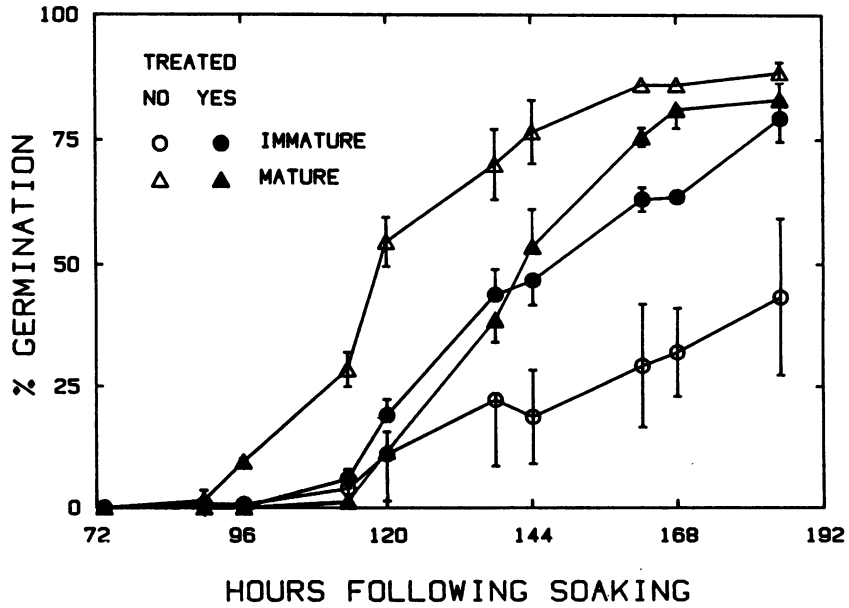

FIG. 2. Mean ± SD (n = 2) percent germination in immature and mature spores of Hn-n treated with or without (2-chloroethyl)phosphonic acid for 14 d prior to a germination test.

Acknowledgments—We thank Hugh Britten and Jennifer Panter for technical assistance and Dr. Otto J. Schwarz for helpful comments and discussion.

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