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Hormonal Control of Pepper Seed Germination

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Abstract. **Germination rate of pepper (*Capsicum annuum* L.) seed was reduced and germination became less uniform as temperature was lowered from 25° to 15°C. There was no evidence of a leachable or extractable germination inhibitor being activated or formed during the exposure to low temperature. Auxin and kinetin applications did not alter germination rates, but gibberellins (GA_3 and GA_{4+7}) increased germination rates. GA_{4+7} was slightly more effective than GA_3 in stimulating a germination rate increase. AMO 1618 effectively reduced germination rates.**

The control of germination at suboptimal temperature may be governed by inhibitors or promoters. Abdul-Baki and Stoner (1) found a growth promoter in leachate from low-temperature-germinating tomato seed PI 341984. Leachates from seeds of 'Red Rock' tomato, which germinated poorly at low temperatures, inhibited or slowed germination of PI 341984 seeds at low temperatures.

The leachate from PI 341984 promoted germination of 'Red Rock' tomato seeds at 10°C. The promotive and inhibitory components of the leachates were not isolated or identified, but they were found to be highly specific and restricted in nature to tomato seeds. Maluf and Tigchelaar (5) reported that germination of noncold-germinating lines of tomato was improved by adding activated carbon to the germination media. They suggested that the inhibition or reduced germination at 10° in noncold-germinating lines was due, in part, to a low-temperature-triggered activation or formation of an inhibitor.

We investigated possible hormonal control of pepper seed germination at optimal and suboptimal temperatures. Studies were conducted to determine if low temperatures stimulated the formation or activation of an endogenous germination inhibitor, and to find whether germination rate could be increased by the addition of growth promoters.

Germination procedure. 'Early Calwonder' pepper seeds were germinated at 15° and 25°C on moistened Whatman No. 1 and No. 3 filter papers in 5.5- or 9-cm Petri dishes. All treatments were replicated 4 times with 25 seeds per replicate. Germination data were taken at 24-hr intervals. Seeds with visible radicles were counted as germinated. The mean number of days to germination (MDG) was calculated by the formula adapted from an emergence index used by Gerson and Honma (2).

Inhibitor activity after low temperature imbibition. Air-dry seed (5g) were leached in 40 ml of cold distilled water on a reciprocating shaker for 0, 2, 4, or 8 days at 4° ± 0.5°C. The water was changed midway during each leaching period and saved. Moist (fully imbibed) and dry seeds were also subjected to the 4° temperature as controls. At the end of each leaching period, seeds were removed for germination tests.

Leachates from each of the time periods were combined, frozen at −20°C, and were concentrated by freeze-drying. Residue was taken up in 10 ml of distilled water and then filtered through a double thickness of Miracloth. Seeds were germinated in 1 ml of sample, representing leachate from 0.5 g of seed. The osmotic potential of the sample was determined using a vapor pressure osmometer. In order to determine if osmotic potential of the leachate might affect germination rate, comparison treatments were prepared using PEG-6000 or a 0.2 M Na_2HPO_4 buffer. Osmotic potential of the leachates averaged −2 bars.

Seeds leached for 0, 2, 4, or 8 days, representing about 4.5 g of air-dry seed, were homogenized in distilled water for 5 min. The homogenate was filtered and washed through a double thickness of Miracloth, centrifuged for 10 min at 3600× *g*, and freeze-dried. The residue was taken up in 9 ml of distilled water and 1 ml was used for germination tests.

Pepper seed germination under optimal conditions was uniform with most seeds exhibiting radicle emergence after 4 days of imbibition. However, at 15°C, germination became less uniform with radical emergence occurring in some seeds after 7, 8, and 9 days of imbibition. Gerson and Honma (2) screened 80 genotypes of *Capsicum annuum* for emergence rates at low temperatures and observed that average emergence indices increased from 17.8 at 18° to 26.5 at 16° and further increased to 41.5 at 13°.

Table 1. The effect of leachate from 0.5 g of seed on the germination of pepper seed at 25° and 15°C.

	Mean days to germination	
Treatment	25°C	15°C
Untreated control	4.6 b[z]	8.5 d
2-day leachate	5.3 a	10.8 b
4-day leachate	5.4 a	10.1 bc
8-day leachate	5.6 a	10.6 b
−2 bar PEG-6000	4.4 b	9.7 c
−2 bar Na_2HPO_4	5.4 a	11.6 a

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

Leaching seeds in cold distilled water had no effect on the MDG or total germination at 15° or 25°C compared with unleached seed (data not presented). At 25°, seeds germinated slower in the leachate extract and a comparable Na_2HPO_4 buffer than in the untreated control (Table 1). When germinated at 15°, untreated seeds germinated more rapidly than all other treatments. Total germination was similar to the untreated controls in all treatments (data not presented).

Filtrates from ground seeds, whether or not they had been previously leached, reduced the rate of germination when compared with the untreated control at 15° or 25°C (Table 2). When compared to germination in osmotic solutions, germination rates at 15° were similar to the leached seed treatments. Total germination was unaffected by germinating the seeds in a ground seed extract (data not presented). Thus, there did not appear to be any evidence that a leachable factor is responsible for low-temperature inhibition of pepper seed germination as was reported for tomato seed (1, 5). A −2 bar Na_2HPO_4 buffer solution reduced the speed of germination similar to the leachates.

Heydecker (4) reported similar results with pepper seed extracts. The delay in germination rate imposed by the extracts could not be fully explained by the osmotic potential of the extract which was duplicated with PEG-6000. He believed that one of the components of the extract imposed a delay in ger-

Table 2. The effect of an aqueous solution from ground leached seed, 0.5 g/treatment, on pepper seed germinated at 25° and 15°C.

	Mean days to germination	
Treatment	25°C	15°C
Untreated control	4.8 b[z]	9.3 c
0-day leachate	7.2 a	10.9 abc
2-day leachate	6.6 a	12.1 a
4-day leachate	7.5 a	11.2 ab
8-day leachate	7.2 b	11.2 ab
−2 bar PEG-6000	4.4 b	9.7 c
−3 bar PEG-6000	4.5 b	12.3 a
−2 bar Na_2HPO_4	5.4 b	11.6 a

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

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Table 3. The effect of GA_{4+7} on germination of pepper seeds at 25°C and 15°.

GA_{4+7}[z] concn (ppm)	25°C		15°C	
	Mean days to germination	Total germination (%)	Mean days to germination	Total germination (%)
0	4.7 a[y]	79 a	12.9 a	81 a
10	4.2 b	82 a	11.5 b	79 a
50	3.9 bc	78 a	10.7 c	79 a
100	3.8 c	75 a	10.8 c	86 a
500	3.7 c	80 a	10.7 c	82 a
1000	3.7 c	82 a	10.9 c	85 a

[z]Gibberellic acid was in 0.05 M Na_2HPO_4 buffer.
[y]Mean separation within columns by Duncan's multiple range test, 5% level.

mination. However, in our study, germination rate was not reduced further by increasing leaching time. Thus, there does not appear to be a leachable inhibitor formed or activated during the exposure to low temperature, but merely a salt-osmotic effect on germination rate due to the addition of the leachate itself.

Growth-regulator effects. Either GA_3, GA_{4+7}, or indoleacetic acid (IAA) were added at 0 to 1000 ppm or kinetin was added at 0 to 100 ppm to moisten the filter papers for germination tests. The solutions were adjusted to pH 6.5.

IAA and kinetin treatments had no effect on increasing the germination rate or altering the total germination of pepper seeds at 15° or 25°C (data not presented). Applications of GA_3 (data not presented) and GA_{4+7} (Table 3) at 15° or 25° generally enhanced germination rates when compared to untreated seeds. GA_{4+7} appeared to be more effective than GA_3 in increasing germination rate.

GA_{4+7} at 0, 100, and 1000 ppm was combined with 2′isopropyl-4′-(trimethylammonium chloride)-5′methyl-phenyl piperidine carboxylate (AMO-1618), an inhibitor of GA synthesis, at 0, 100, and 1000 ppm and 1.5 ml of each combination was used as a germination medium for pepper seeds at 25°C. Filter paper was not used in this experiment.

Application of AMO-1618 at 100 or 1000 ppm in the absence of GA_{4+7} reduced the rate of germination when compared to the untreated control (Table 4). Application of GA_{4+7} overcame the inhibition of AMO-1618 on germination rate. There was no effect of varying the concentration of GA on this enhancement. AMO-1618 did not effect the total germination (data not presented).

GA_{4+7} was more effective than GA_3 in stimulating low-temperature germination in cucumber (6) and species of Labiatae (8). Sosa-Coronel and Motes (7) reported that GA_3 stimulated several different pepper genotypes to germinate faster with more uniform radicle lengths in aerated water columns at 30°C. Additionally, GA_3 was found to stimulate germination to the same extent after seeds were preimbibed for 24 hr in water. This implied to the authors that GA stimulated germination processes after seed imbibition and activation. They did not use GA_{4+7} in their work. We found a greater stimulation of germination rate by using GA_{4+7} compared to GA_3.

AMO-1618, an inhibitor of GA synthesis, delayed germination of pepper seeds. This delay in germination was overcome with GA. Harvey and Oaks (3) found that AMO-1618 at 2000 μM concentrations inhibited protease and amylase synthesis in excised corn endosperms. Additions of GA_3 did not overcome this inhibition. It appeared from the present study that low levels of GA may be synthesized prior to radicle protrusion in pepper seeds. Gibberellic acid synthesis may not be a prerequisite of germination but, in effect, could be necessary to allow radicle protrusion to proceed at a rapid pace. Added GA stimulated germination at 15° and 25°C by possibly triggering or enhancing some germination process, such as the formation or activation of enzymes as seen in monocots or by increasing embryo growth, allowing germination to proceed at a more rapid rate.

Table 4. The effect of AMO-1618 on the germination rate of pepper seeds at 25°C.

GA_{4+7} concn (ppm)	Mean days to germination		
	AMO-1618		
	0 ppm	100 ppm	1000 ppm
0	5.6 b[z]	6.7 a	6.7 a
10	4.4 cd	4.5 cd	4.8 c
100	3.8 d	4.2 cd	4.2 cd
1000	4.0 d	4.0 d	4.2 cd

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

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