Cold Moist Stratification and Acid Scarification Do Not Significantly Enhance Hemp Seed Germination

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Keywords. *Cannabis sativa*, recalcitrant seed, sulfuric acid

Abstract. **Hemp (*Cannabis sativa*) seed germination can be variable and unreliable. Exposure of seeds at 7 days postharvest for 10 minutes in sulfuric acid increased germination percent for 'Tsunami' but not for 'Cherry Wine' × 'Tsunami' or 'Tangerine' × 'Tsunami'. Seeds stored dry in plastic bags at room temperature (20 °C) for 60 days lost germination ability, but seeds undergoing cold (4 °C) moist stratification had maintained germination ability at >75% germination. Cold moist stratification for 30 to 60 days or exposure for 10 minutes to sulfuric acid may increase germination for recalcitrant hemp seed like 'Tsunami'.**

Seed germination ability of cannabis (*Cannabis sativa*) can be variable and influenced by genetics, mother plant health, growing environment during seed development, seed handling during drying, processing and storage, and seed age (Geneve et al. 2022; Langa et al. 2024). Both physical and physiological dormancy has been reported for cannabis. Low and inconsistent germination causes problems for propagators, breeders, and growers, including production delays, reduced yield, and wasted resources. We attempted to enhance germination rates and seedling vigor using chemical scarification with sulfuric acid (H_2SO_4) or cold (4 °C) moist stratification at different exposure durations.

Materials and methods

Feminized (all female) seed was produced by crossing female plants grown from seed of the cultivars Cherry Wine, Tsunami, and Tangerine, with plants of Tsunami that were masculinized with silver thiosulfate according to Lubell and Brand (2018). 'Cherry Wine' and 'Tsunami' were obtained as seed from Cheyenne Mountain Seed Company (Colorado Springs, CO, USA) in 2019 and 2021, respectively. 'Tangerine' was obtained as seed from Atlas Seed (Sebastopol, CA, USA) in 2021. Crosses were conducted simultaneously in a growth chamber with set points of 21 °C and 11 h photoperiod. There were three mother plants each of 'Cherry Wine' and 'Tangerine' and two mother plants of 'Tsunami'. Seed developed for ~42 d. Infructescences were harvested on 8 Jan 2025 and dried in metal perforated trays at room temperature. Seeds were cleaned within 7 d of harvest and pooled by maternal genotype. Only brown-colored, regularly formed seeds were used for experiments.

On 15 Jan 2025, seeds of each genotype, 'Cherry Wine' × 'Tsunami' (CWT) 'Tangerine' × 'Tsunami' (TT), and 'Tsunami' × 'Tsunami' (Tsunami or TSU), were soaked in H_2SO_4 (Sigma-Aldrich, St. Louis, MO, USA) for 0, 10, 30, or 60 min. There were 100 seeds per genotype per duration. The volume of H_2SO_4 per 100 seed was ~20 mL. After exposure to H_2SO_4, seeds were rinsed with deionized water, then soaked in ~20 mL of water for 24 h. On 16 Jan, seeds were randomly distributed to plastic 100 × 15-mm petri dishes lined on the bottom with #1 filter paper (Whatman, Marlborough, MA, USA) of 90-mm diameter. The experimental unit was a petri dish with 20 seeds. Dishes were held at room temperature (20 °C) and arranged by cultivar as a completely random design with five replications. Filter paper was initially moistened with 1.5 mL of deionized water. An additional 1.5 mL of water was added to filter paper on 17 Jan. More water at 1 mL was added on 18 and 19 Jan. Germination percent and length of germinated radicles were recorded on 19 Jan 2025, which was 3 d after moving seeds to petri dishes. A seed was considered germinated when the emerging radical elongated to a length of >3 mm.

On 15 Jan 2025, seeds of CWT, TT, and TSU, were placed in plastic resealable sandwich bags at 100 seeds per bag and two bags per genotype. Two tablespoons of fine sand was added to each bag. Sand was moistened with water until slightly damp. Bags were sealed and placed in a refrigerator at 4 °C. After 30 d, one bag per genotype was removed from the refrigerator, and the remaining bags were held at 4 °C for 30 additional days (60 d total). Seeds were cleaned of sand by rinsing with water, soaked in water for 24 h, and then randomly distributed to plastic petri dishes lined with filter paper as described for the H_2SO_4 experiment. Another set of seeds per genotype were stored dry at room temperature (20 °C) to serve as controls for the 30 and 60 d cold, moist stratified seed. After 30 and 60 d of dry storage at 20 °C, 100 seeds per genotype were soaked in water for 24 h and germinated in petri dishes as described. Experimental unit and design, number of replications, and data collection were as described for scarification.

Data were subjected to multiple comparisons by Fisher's least significant difference test ($P < 0.05$) using statistical software (SAS version 9.4; SAS Institute, Cary, NC, USA).

Results and discussion

Exposure to H_2SO_4 for 10 min decreased germination by ~50% for CWT and TT, but increased germination by 66% for TSU (Table 1; Fig. 1). As exposure duration to H_2SO_4 increased from 10 to 60 min, germination percent and radicle length generally decreased for all three genotypes. It is possible that TSU had a thicker seedcoat than the other two genotypes and was more resistant to acid penetration and damage to the embryo. Seed size of TSU (31 mm^3) is 88% greater than CWT (16 mm^3).

For all genotypes, there was no difference in germination percent or

Received for publication 25 Apr 2025. Accepted for publication 27 May 2025.

Published online 11 Jul 2025.

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https://doi.org/10.21273/HORTTECH05682-25

Table 1. Germination percent and radicle length for *Cannabis sativa* genotypes 'Cherry Wine' × 'Tsunami' (CWT), 'Tangerine' × 'Tsunami' (TT), and 'Tsunami' (TSU), after seeds, at 7 d postharvest, were scarified with sulfuric acid (H_2SO_4) for 0, 10, 30, or 60 min or received cold (4 °C), moist stratification or held dry at room temperature (20 °C) for 30 or 60 d.

		CWT		TT		TSU	
Exposure duration	Exposure conditions	Germination (%)	Radicle length (mm)	Germination (%)	Radicle length (mm)	Germination (%)	Radicle length (mm)
Scarification							
0 min		60 a[i]	14.6 a	81 a	26.1 a	38 bc	23.1 a
10 min	H_2SO_4	30 b	5.5 b	43 b	5.4 b	63 a	14.9 b
30 min	H_2SO_4	25 bc	4.3 bc	11 c	3.5 b	53 ab	13.7 b
60 min	H_2SO_4	14 c	3.2 c	18 c	3.4 b	28 c	6.9 c
Stratification							
30 d	4 °C, moist sand	87 a	14.2 b	82 ab	21.5 b	72 ab	20.1 ab
30 d	20 °C, dry	75 ab	13.4 b	84 a	21.0 b	54 b	16.5 b
60 d	4 °C, moist sand	79 a	22.6 a	84 a	45.1 a	76 a	23.1 a
60 d	20 °C, dry	64 b	22.8 a	72 b	28.1 b	27 c	23.0 a

[i] Letters within column, within scarification or stratification, depict Fisher's least significant difference groupings at $P < 0.05$ (n = 5).

Fig. 1. Germinated seeds of *Cannabis sativa* 'Tsunami' after no exposure to sulfuric acid (A) or 10-min exposure to sulfuric acid (B).

radicle length between cold moist stratified seed and nonstratified seed after 30 d (Table 1). After 60 d of dry storage in plastic bags at room temperature, germination ability of TT and TSU declined, but seed given 60 d of cold moist stratification maintained a high germination percent of >75%. Ripening for 60 d generally enhanced radicle growth upon germination. Exposure of seeds to cold temperatures of 4 °C for 3 d and 10 °C for 5 d did not enhance germination for 14 varieties of industrial hemp and the cultivars Merlot and Berry Blossom, respectively (Elias et al. 2020; Islam et al. 2022).

Reported temperatures for storing hemp seed vary widely and range from −20 to 21 °C (Small and Brookes 2012). Small and Brookes (2012) recommended storage at 5 °C to maintain germination ability for 6 years. We regularly store hemp seed dry in resealable plastic bags in a refrigerator at 4 °C and have not observed declines in germination ability for seed stored >6 years.

In conclusion, a 10-min exposure to H_2SO_4 may enhance germination percent for recently harvested recalcitrant seed of ≥30 mm[3]. When time permits, providing seeds a 30- to 60-d cold, moist stratification may enhance germination success. Cold storage at 4 °C as opposed to room temperature may preserve the germination ability of hemp seed overtime.

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