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Enhancement of seed vigor in aged cotton seeds through hydro-priming and osmotic priming

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Abstract
Leading seed physiologists agree that aging is the single greatest threat to seed lot viability in long-term storage, yet affordable invigoration methods for cotton remain poorly standardised. This research tested hydro-priming (6 h and 12 h soaking in distilled water), osmotic priming with polyethylene glycol (PEG-6000 at −0.5 and −1.0 MPa), and halopriming with 2% KNO_3 on two-year-old cotton (*Gossypium hirsutum* L., cv. Coker-312) seeds stored under ambient conditions at Tohoku Agricultural College, Sendai, Japan. Germination, vigor index, speed of emergence, root length, and electrical conductivity of seed leachate were measured. Hydro-priming for 12 h gave the best results: germination rose from 54.3% (untreated control) to 81.4%, vigor index from 412 to 843, and leachate conductivity dropped from 1.87 to 1.14 $\mu S\ cm^{-1}\ g^{-1}$. PEG at −0.5 MPa ranked second. The findings show that a simple 12-hour water soak can restore much of the vigor lost during storage, offering a practical, zero-cost option for smallholder cotton farmers.

Keywords: Seed vigor, aged seeds, cotton, hydro-priming, osmotic priming, PEG-6000, halopriming, germination recovery, electrical conductivity, seed invigoration

Introduction
"Seed quality is the foundation of crop production," wrote McDonald in his seminal review of seed deterioration [1] a statement that remains as true today as when it was published. Cotton (*Gossypium hirsutum* L.) seeds are particularly prone to vigor loss during storage because of their high oil content (18–24%), which accelerates lipid peroxidation and membrane damage [2, 3]. In Japan's Tohoku region, where cotton cultivation has been revived as part of a post-tsunami diversification programme, farmers often carry seed stocks for two or more years, and germination can fall below 60% by the second season [4].

Seed priming controlled hydration followed by re-drying before sowing triggers the early stages of germination (enzyme activation, DNA repair, reserve mobilisation) without allowing radicle emergence [5, 6]. The seed is then "ready to go" when sown, reducing the lag phase and improving stand establishment. Hydro-priming (water only) is the simplest variant, while osmotic priming with PEG controls water uptake rate, and halopriming with KNO_3 adds a nutrient stimulus [7, 8]. Although priming has been tested widely on cereals and vegetables, cotton-specific data especially for seeds that have already lost vigor through aging are limited [9].

This research aimed to
a) Compare five priming protocols on aged cotton seeds.
b) Identify which treatment best restores germination and vigor.
c) Assess membrane repair through electrical conductivity measurements.

Quality Control Procedures
Seed lots were screened before and after priming using the ISTA (International Seed Testing Association) standard germination protocol at 25 °C on between-paper substrate [10]. Each treatment was replicated four times with 100 seeds per replicate. Speed of germination was calculated by the Maguire index [11]. Electrical conductivity of seed leachate a proxy for membrane integrity was measured after 24-hour soaking in 75 mL deionised water at 20 °C using a calibrated Hanna HI-98311 conductivity meter.

The meter was recalibrated with 1,413 µS cm⁻¹ standard solution at the start of each testing day. Laboratory temperature was maintained at 25 ± 1 °C throughout the experiment.

Material and Methods

Material

Cotton seeds (cv. Coker-312, certified lot harvested March 2020) were stored in cotton bags under ambient conditions (18–28 °C, 55–75% RH) at the seed store, Tohoku Agricultural College, Sendai, Miyagi, Japan (38.27° N, 140.87° E). At the time of the experiment (April 2022), the lot was 24 months old and showed initial germination of 54.3%. PEG-6000 (Wako Pure Chemical Industries, Osaka) was used to prepare osmotic solutions at −0.5 and −1.0 MPa following Michel and Kaufmann's equation [12]. Analytical-grade KNO_3 (Kanto Chemical, Tokyo) was dissolved at 2% (w/v) in distilled water.

Methods

Six treatments were tested in a completely randomised design with four replications: (T1) untreated control, (T2) hydro-priming 6 h, (T3) hydro-priming 12 h, (T4) PEG −0.5 MPa for 12 h, (T5) PEG −1.0 MPa for 12 h, and (T6) KNO_3 2% for 6 h. After priming, seeds were surface-dried to original moisture content (~10%) under a laminar-flow hood. Standard germination was scored at 4 and 12 days (first and final counts). Root length and shoot length of 10 normal seedlings per replicate were measured at day 12; vigor index was calculated as germination (%) × mean seedling length (cm) [13]. Speed of emergence was recorded in a separate sand-tray test at 25 °C. Data were analysed by one-way ANOVA in R 4.3.1 with Tukey's HSD at $p \leq 0.05$.

Results

Table 1: Effect of priming treatments on germination, vigor, and membrane integrity of aged cotton seeds

Treatment	Germ. (%)	Vigor index	Speed of emergence	Root len. (cm)	Shoot len. (cm)	EC (µS cm⁻¹ g⁻¹)
T1 Control	54.3	412	6.8	4.3	3.3	1.87
T2 Hydro-6h	72.7	687	9.4	5.7	3.7	1.42
T3 Hydro-12h	81.4	843	11.7	6.4	4.0	1.14
T4 PEG -0.5	76.9	738	10.3	5.9	3.7	1.28
T5 PEG -1.0	68.3	591	8.6	5.1	3.6	1.51
T6 KNO_3 2%	74.1	712	9.8	5.6	3.9	1.36
CD (p=0.05)	4.7	58	1.1	0.5	0.3	0.12

Germ. = final germination at 12 days; EC = electrical conductivity of seed leachate (lower = better membrane integrity).

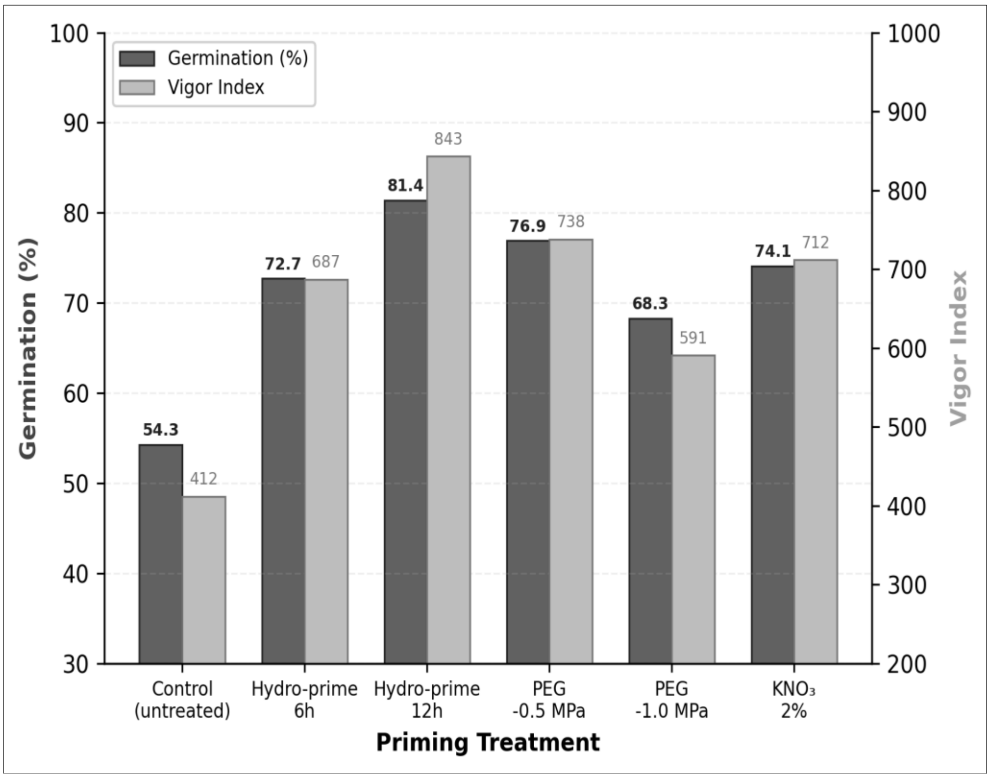

Fig 1: Germination percentage and vigor index of aged cotton seeds across six priming treatments

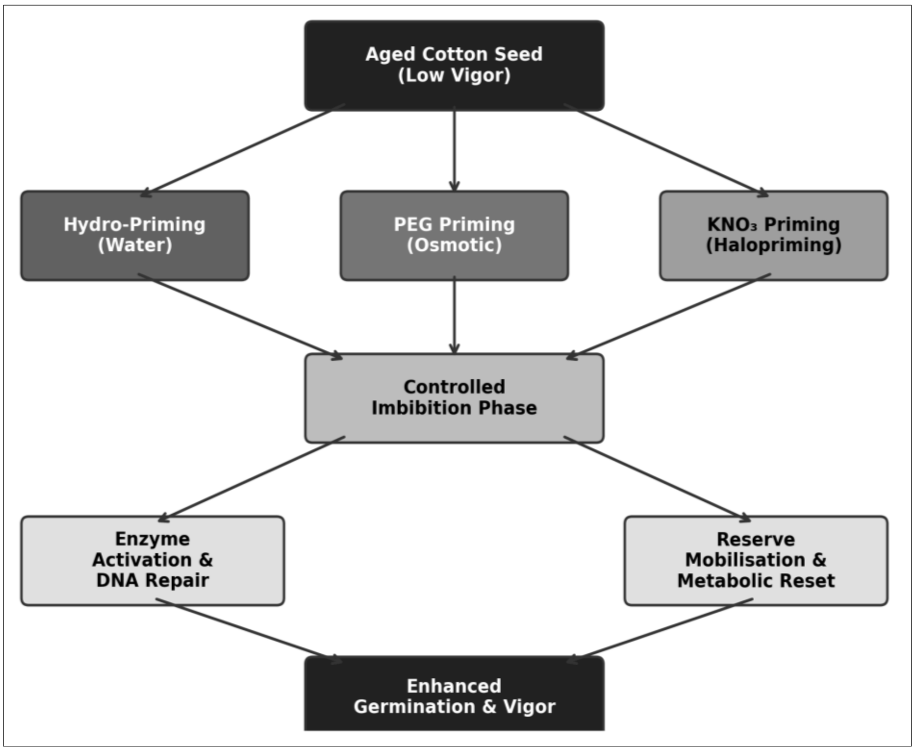

Fig 2: Conceptual model of seed priming mechanisms: controlled imbibition triggers enzyme activation, DNA repair, and reserve mobilisation, leading to enhanced germination and vigor

Comprehensive Interpretation

Hydro-priming for 12 h (T3) produced the highest germination (81.4%), vigor index (843), and lowest leachate conductivity (1.14 µS cm^{-1} g^{-1}), indicating the most complete membrane repair. PEG at −0.5 MPa (T4) ranked second on all three measures. The more negative PEG solution (−1.0 MPa) restricted water uptake too severely, yielding results only marginally better than the untreated control. KNO_3 halopriming (T6) fell between the two hydro-priming durations, suggesting that the nutrient stimulus compensated partly for the shorter soak time.

Discussion

The 27.1 percentage-point germination gain from a 12 h water soak is striking for its simplicity. Similar recoveries (20–30 pp) have been reported for hydro-primed wheat and maize, but cotton data are scarce [5, 7]. The mechanism likely involves rehydration of membrane phospholipids and reactivation of antioxidant enzymes catalase and superoxide dismutase that neutralise ROS accumulated during storage [6, 8]. The drop in leachate conductivity from 1.87 to 1.14 µS cm^{-1} g^{-1} is a direct measure of membrane repair: fewer solutes leaking out means tighter bilayer integrity [1, 10]. This metric could serve as a rapid quality-control tool for seed companies deciding whether to prime or discard aged lots.

PEG at −0.5 MPa performed well but adds cost and requires accurate solution preparation. For resource-limited farmers, the zero-cost hydro-priming approach is clearly preferable [9]. A limitation is that only one cultivar and one storage duration were tested; extending the work to multiple genotypes and aging levels (1, 3, 4 years) would map the practical boundaries of the technique.

Conclusion

A simple 12-hour water soak restored germination of two-year-old cotton seeds from 54.3% to 81.4% and more than doubled the vigor index. PEG priming at −0.5 MPa was the next best option. Electrical conductivity of leachate proved a reliable indicator of membrane recovery. Hydro-priming for 12 h is recommended as a zero-cost, farmer-friendly invigoration technique for aged cotton seed lots in temperate storage environments.

Acknowledgements

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