



Enhancing Physiological Quality of Aged Pak Choi (*Brassica rapa* L.) Seeds Through Different Invigoration Methods

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Abstract

Seed deterioration during storage significantly reduces the viability and productivity of pak choi (*Brassica rapa* L.). This study aimed to evaluate the efficacy of various synthetic and bio-organic invigoration agents combined with different soaking durations to restore the physiological quality of aged seeds (initial germination of 31%). The experiment was arranged in a factorial split-plot design. The main plots consisted of seven invigorating solvents: water (S1), shallot extract 50% (S2), mung bean sprout extract 5% (S3), bamboo shoot extract (S4), KNO₃ 1% (S5), GA₃ 100 ppm (S6), and PEG 6000 10% (S7). The sub-plots were three soaking durations: 15 (V1), 30 (V2), and 60 minutes (V3). The results indicated that a 30-minute soaking duration (V2) provided the optimal physiological window for metabolic repair without compromising desiccation tolerance. 10% PEG 6000 and 50% shallot extract emerged as the superior activators, consistently yielding the highest germination rates (up to 95.11%) and vigor indices (97.33%). While 10% PEG 6000 optimized post-emergence biomass, 50% shallot extract acted as a potent biostimulant due to its endogenous phytohormones. Furthermore, Radicle Emergence (RE) testing at 22 hours was identified as the most accurate diagnostic tool for predicting final germination capacity, particularly for the 30-minute treatment ($R^2 = 0.9943$). Conversely, bamboo shoot extract (S4) exhibited a strong inhibitory effect, likely due to allelopathic phenolic compounds, and is not recommended for invigoration. In conclusion, hydropriming or shallot extract treatment for 30 minutes offers a practical and effective strategy for rehabilitating aged pak choi seeds.

INTRODUCTION

Pak choi (*Brassica rapa* L.) is one of the most significant vegetable crops in Indonesia, highly favored for its nutritional value and short cultivation cycle. As market demand continues to rise, the provision of high-quality seeds becomes a critical factor for successful production (Indriyani et al., 2025). However, farmers often face constraints regarding seed availability, leading them to use seeds that have been stored for extended periods. These "aged seeds"

typically exhibit decreased quality, which results in low germination rates and uneven plant growth in the field (Meitankeisangbam et al., 2024). This loss of vigor presents a major economic threat, as suboptimal stand establishment results in the wasteful application of land, labor, and fertilizer resources (Reed et al., 2022).

The decline in seed performance during storage is primarily driven by seed deterioration, an inevitable physiological process characterized by a gradual loss of viability and vigor. In aging Brassicaceae, this deterioration is fueled by the excessive accumulation



of reactive oxygen species (ROS), which induces lipid peroxidation and macromolecular damage that compromises the plasma membrane's vital sensory functions (Dong et al., 2025). This decline is further exacerbated by the ultrastructural breakdown of mitochondria and the fragmentation of the endoplasmic reticulum; these changes collectively deplete energy reserves and disrupt protein synthesis, rendering the seeds incapable of meeting the metabolic demands of germination (Xing et al., 2025). The metabolism of deteriorated seeds is "restarted" and repaired under controlled conditions before actual growth begins. This treatment effectively rehabilitates seed performance and aligns the germination timing across the entire lot (Pradana et al., 2025).

Invigoration techniques through priming methods can be categorized into chemical and organic approaches, where chemical treatments frequently utilize Potassium Nitrate (KNO_3), Gibberellic Acid (GA_3), and Polyethylene Glycol (PEG) to optimize seed performance. KNO_3 acts as a versatile halonutrient that improves seed germination, seedling vigor, and total yield while simultaneously protecting against various abiotic stressors (MacDonald & Mohan, 2025). Similarly, GA_3 -based applications enhance early development and vigor by regulating essential physiological functions, including root architecture and stomatal conductance (Mahamud et al. 2025). Lei et al. (2021) mention that PEG provides a reliable osmotic framework designed to buffer plants against suboptimal environmental conditions.

Alternatively, organic seed invigoration serves as a sustainable approach (Umadi et al., 2025) and potent alternative by utilizing natural extracts, such as shallots, bamboo shoots, and bean sprouts. The application of shallot (*Allium ascalonicum* L.) extracts utilizes an endogenous blend of gibberellins, auxins, and cytokinins to trigger root morphogenesis and rehabilitate the physiology of aged seed (Siahaan et al., 2025). Concurrently, mung bean sprout (*Vigna radiata* L.) extract acts as an enzymatic and nutritional catalyst, streamlining germination by supplying metabolized solutes and enhancing the breakdown of internal reserves through exogenous amylolytic and proteolytic activity (Nugraha et al., 2021). Furthermore, the bioactive components in bamboo shoots supply critical phosphorus and phytohormones necessary for bypassing dormancy and fueling the ATP-driven metabolic surges required for cellular expansion (Rahmawati, 2022).

According to Hasanović et al. (2025), the success of priming treatments depends on the precise duration of soaking to facilitate essential physiological repairs and hormonal realignment during Phase II of germination. Accurate timing is essential to avoid the dual risks of under-priming, which limits vigor, and over-priming, leading to irreversible developmental changes. Tanwal et al. (2023) added that exceeding this temporal threshold compromises the seed's ability to survive subsequent drying due to the loss of desiccation tolerance and the onset of oxidative stress.

Despite the documented benefits of various stimulants, the specific synergy between priming agents and soaking durations for optimizing pak choi performance remains insufficiently explored. Therefore, this study evaluated the efficacy of synthetic and bio-organic treatments to identify the optimal integration of invigorants and hydration periods for enhancing the physiological quality of aged seeds.

MATERIALS AND METHODS

Experimental Date and Place

This research was conducted from November to December 2025 at the Agronomy Laboratory, Agrotechnology Study Program, Faculty of Agriculture, Universitas Singaperbangsa Karawang. The study utilized aged pak choi (*Brassica rapa* L. cv. Nauli F1) seeds with an initial germination rate of 31%. The seeds were harvested in 2019 and stored at -4°C until 2025.

Experimental design

The treatments were arranged in a factorial split-plot design. The first factor, assigned to be the main plot, was invigoration solvent consisting of seven levels: water (S1), shallot extract 50% (S2), mung bean sprout extract 5% (S3), bamboo shoot extract (S4), KNO_3 1% (S5), GA_3 100 ppm (S6), and PEG 6000 10% (S7). The second factor, designated as the sub-plot, was soaking duration consisting of three levels: 15 minutes (V1), 30 minutes (V2) and 60 minutes (V3). This setup resulted in 21 treatment combinations, which were replicated three times to produce a total of 63 experimental units. With 25 seeds allocated per unit, a total of 1,575 seeds were required for entire experiment.

Seed Preparation

The germination test was carried out using the top of paper test method (ISTA, 2024). Seeds were

germinated in 17 x 5 x 10 cm boxes, using 25 seeds per box across three replicates. The bottom of each container was lined with one paper towel and three sheets of blotting paper. Germination was carried out at room temperature of $\pm 26^{\circ}\text{C}$ and relative humidity of 70-85%.

Experimental Procedure

Observations were made on radicle emergence (%), germination capacity (%), abnormal seedlings percentage (%), growth rate (normal seedling/etmal), maximum potential growth (%), vigor index (%), shoot length (cm) and radicle length (cm). Based on ISTA 2024, RE (radicle emergence) was calculated in by periodically counting radicle emergence after 24 hours and 48 hours. Observation was finished when the radicle approached 2 mm. Based on ISTA 2024, the first observation of the germination of pak choi was on the 5th day, and the final observation was on the 7th day. Abnormal seedling percentage was obtained by counting seeds that did not germinate normally on the 7th day. Vigor index was observed when the percentage of normal seedling was first calculated on the 5th day. Observation of the length of shoot and radicle was made on five samples of the seedling.

Statistical Analysis

Data analysis was performed using MINITAB 22 Statistical Software with an ANOVA test at $\alpha=5\%$ for testing the comparison of treatment means. If the results showed a significant effect, further testing was conducted using Tukey Test/Honestly Significant Differences (HSD) at $\alpha=5\%$ to determine the difference in means between treatments. A linear regression analysis using Google Spreadsheet was used to relate

radicle emergence test and normal seedling germination and vigor index.

RESULTS AND DISCUSSION

Radicle Emergence (RE) as a Vigor Diagnostic

Based on the 15-minute soaking duration (Figure 1), water (S1), PEG (10%) (S7), and shallot extract 50% (S2) effectively surpassed the 80% radicle emergence threshold. Water and PEG 10% reached this target the fastest (18 - 20 hours), followed by shallot extract 50% in 22 hours. Other treatments, including KNO_3 , GA_3 and various extracts, failed to reach 80% within 56 hours. According to Jovičić (2022), seed priming triggers a synchronized set of biological processes by allowing enough water uptake to initiate pre-germinative metabolism, while simultaneously restricting hydration to prevent full radicle protrusion.

At the 18th hour, 1% KNO_3 was the fastest treatment to exceed the 80% germination threshold (Figure 2), supporting Pandey's (2017) finding that chemical priming outperforms hydropriming in enhancing germination and vigor. Other treatments, including water, 10% PEG, 50% shallot extract, 100 ppm GA_3 , and 5% mung bean sprout extract, followed suit by stabilizing above the threshold within 20–24 hours. Notably, water and 50% shallot extract achieved nearly 100% consistency, with the S2V2 (shallot extract 30 minutes) combination reaching the highest vigor index (97.33%). In contrast, bamboo shoot extract showed inhibitory effects, peaking at only 30% and failing to meet the 80% target. 60-minute immersion treatment, 10% PEG (S7) was the fastest to exceed the 80% threshold (20th hour), followed closely by 100 ppm GA_3 and Water (Figure

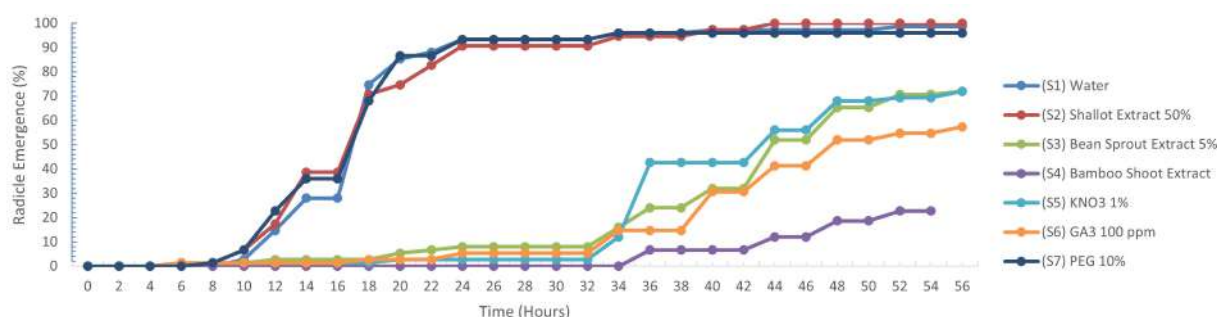


Figure 1. Radicle Emergence test in seeds treated with 15-minute soaking duration

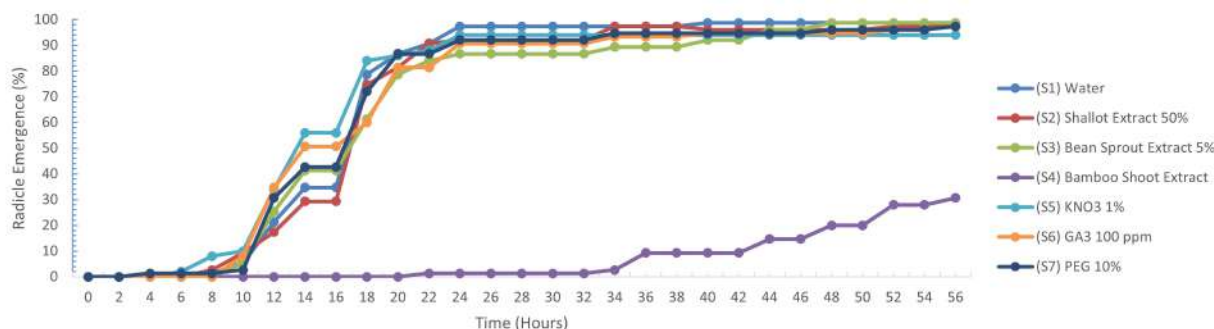


Figure 2. Radicle Emergence test in seeds treated with 30-minute soaking duration

3). By the 24th hour, most treatments (S2, S5, S3) also surpassed this mark, suggesting that a 60-minute duration optimizes synchronous metabolic activation. While water eventually reached 100% stability, bamboo shoot extract (S4) remained inhibited, peaking at only 15%. This suppression aligns with Moura et al. (2018), who noted the allelopathic potential of bamboo extracts and their negative impact on seedling establishment.

The 30-minute soaking duration (V2) demonstrated the most optimal performance compared to the 15 and 60-minute durations in accelerating synchronous radicle emergence. At the 30-minute mark, nearly all growth activators surpassed the 80% threshold, significantly faster than at 15 minutes, with 1% KNO₃ (S5) being the fastest to reach the target (2 mm) by the 18th hour, which is the fastest treatment to determine RE. Demir (2023) highlights the RE test as an efficient vigor assay for evaluating the success of invigoration treatments. Unlike traditional germination tests that are time-consuming, RE test offers a rapid assessment by quantifying the percentage of seeds that achieve a radicle length of at least 2 mm within a brief timeframe. Although the 60-minute duration

(V3) showed effectiveness, the 30-minute treatment (V2) offered the optimal balance between accelerated metabolic activity and vigor stability. This was particularly evident in the 50% shallot extract (S2V2) treatment, which yielded the highest vigor index of 97.33% (Table 2). The superior performance of this concentration is attributed to its rich auxin profile; as reported by Tarigan et al. (2022), shallot extract contains 2,4-D (2.82 ppm), NAA (0.77 ppm), and IAA (0.75 ppm), which collectively stimulate cell division and radicle elongation.

In terms of solvent efficiency, 10% PEG (S7), water (S1), and 50% shallot extract (S2) consistently maintained high vigor levels (exceeding 80-90%) regardless of the soaking duration. In contrast, bamboo shoot extract (S4) acted as a natural inhibitor across all time intervals, failing to achieve the 80% vigor threshold and resulting in a high incidence of fresh, ungerminated seeds.

Invigoration Treatment

ANOVA results (Table 2) revealed that the physiological quality of aged pak choi seeds was significantly influenced by the main effects of

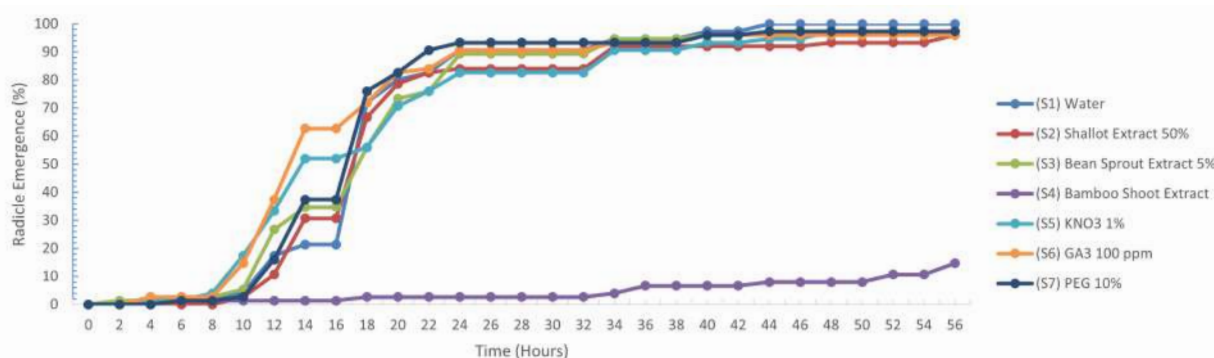


Figure 3. Radicle Emergence test in seeds treated with 60-minute soaking duration

Table 1. Effects of invigoration treatments on the physiological quality of pak choi seeds

Source	Germination Rate (%)	Abnormal Seedling Rate (%)	Fresh Seed (%)	Vigor Index (%)	Growth Rate (%NS/Etmal)	Maximum Potential Growth (%)	Radicle Length (cm)	Shoot Length (cm)	RE 18 Hours	RE 20 Hours	RE 22 Hours
S1	92.70 a	6.22 bc	0.00 b	92.40 a	24.00 a	99.1 a	41.90 b	48.00 b	75.10 a	84.00 a	87.10 a
S2	95.10 a	3.55 c	0.40 b	94.20 a	24.40 a	98.7 ab	42.00 b	49.40 b	70.70 a	78.20 a	85.30 a
S3	82.70 a	11.60 ab	0.40 b	80.40 b	19.40 b	94.2 a	31.90 c	50.50 b	40.00 b	52.40 b	55.60 b
S4	35.10 c	11.10 ab	48.00 a	23.55 c	4.60 c	46.2 c	27.30 c	29.70 c	0.89 c	0.89 c	1.33 c
S5	77.30 b	11.10 ab	1.80 b	73.80 b	18.27 b	88.4 b	41.30 b	54.10 ab	45.30 b	51.60 b	54.70 b
S6	79.60 b	15.10 a	0.90 b	73.30 b	18.10 b	94.2 ab	42.00 b	53.50 b	44.90 b	55.60 b	56.00 b
S7	94.70 a	4.44 b	0.00 b	90.70 a	24.10 a	99.1 a	56.20 a	61.10 a	72.00 b	85.30 a	88.00 a
V1	68.60 b	16.40 a	8.00 a	63.40 b	14.90 b	85.0 b	38.10 b	44.00 b	57.30 a	36.80 b	74.39 a
V2	85.30 a	6.28 b	7.40 a	82.30 a	21.20 a	91.6 a	44.00 a	53.00 a	60.80 a	70.90 a	70.70 a
V3	85.00 a	4.38 b	6.70 a	80.80 a	20.90 a	89.3 ab	38.40 b	51.40 a	31.40 b	67.20 a	38.8 b

Remarks: S1=Water, S2=Shallot Extract 50%, S3=Mung Bean Sprout Extract 5%, S4=Bamboo Shoot Extract, S5=KNO₃ 1%, S6=GA₃ 100 ppm, S7=PEG 6000 10%, V1=15 minutes, V2=30 minutes, V3=60 minutes.

Means followed by the same letters in the same column are not significantly different according to HSD.

invigoration solvent (S), soaking duration (V), and their interaction (S*V). The solvent effect was highly significant ($p < 0.01$) for primary vigor indicators, including germination rate, radicle/shoot length, and radicle emergence, while the S*V interaction highlights that priming efficacy is intrinsically linked to the duration of the exposure. This suggests that the hydration window primarily governs the kinetics of metabolic repair and rehydration rather than physical dormancy (Taer, 2024). While parameters like maximum growth potential demonstrated high experimental precision (CV = 22.19%), other variables, such as abnormal seedling and fresh seed rates, showed extreme variability (CV > 110%). Malek et al. (2022) state that this inconsistency likely reflects the non-uniform nature of seed deterioration during the aging process and the sporadic distribution of these categories across replicates.

The biochemical efficacy of seed priming agents

serves as a fundamental determinant of post-germination and physiological vigor, with water (S1) exhibiting functional parity to shallot extract 50% (S2) and PEG 6000 (S7) through divergent metabolic trajectories. Shallot extract 50% (S2) yielded peak germinative performance (95.11% germination; 94.22% vigor index), an effect attributed to a synergistic matrix of phytohormones (IAA, GA, and cytokinins) (Paiman, 2023), sulfur-based metabolites, and antioxidants that function as biostimulants to catalyze cellular proliferation and mitigate rehydration-induced oxidative stress (Rhaman, 2022). In contrast, the osmoticum PEG 6000 (S7) proved superior in optimizing post-emergence biomass, achieving maximal radicle (56.16 cm) and shoot (61.09 cm) elongation by modulating water potential to avert imbibitional (Wang et al., 2023). These outcomes parallel the metabolic activation triggered by hydro-priming, which stimulates alpha-amylase activity

Table 2. ANOVA results of the effects of invigoration treatment

Source	Germination Rate (%)	Abnormal Seedling Rate (%)	Vigor Index (%)	Growth Rate (%NS/Etmal)	Fresh Seed (%)	Maximum Growth Potential (%)	Radicle Length (cm)	Shoot Length (cm)	RE 18 Hours	RE 20 Hours	RE 22 Hours
S	**	**	**	**	**	**	**	**	**	**	**
V	**	**	**	**	ns	**	**	**	**	**	**
S*V	**	**	**	**	ns	**	**	**	**	**	**
CV	29,75 %	110,98%	35,89%	42%	234,35%	22,19%	28,21%	25,56%	64,67%	61,78%	61,06%

Remarks: S = Solvent, V = Soaking duration, S*V = Interaction, **=Very Significant, *= Significant, ns = No Significant, CV = Coefficient of Variation, RE = Radicle Emergence.

and mitochondrial respiration to facilitate reserve mobilization and intrinsic DNA repair (Cañizares et al., 2024; Xing et al., 2025). Conversely, bamboo extracts exhibited strong allelopathic activity, likely mediated by the release of phytotoxic secondary metabolites such as phenols, tannins, and flavonoids; these compounds significantly suppressed not only seed germination (Moura et al., 2018) but also the subsequent elongation of the radicle and plumule (Rawat et al., 2017).

Seed invigoration efficiency is highly dependent on exposure kinetics, with a 30-minute soaking duration (V2) identified as the physiological optimum. While a 15-minute interval (V1) resulted in a 16.38% abnormality rate due to insufficient metabolic reactivation, a 60-minute duration (V3) offered no benefit and might impair radicle elongation through hypoxia or solute leaching. The goal is to sustain Phase II metabolism for DNA repair without transitioning into Phase III, where desiccation tolerance is lost (Pagano et al., 2023). Such precise timing is vital for "reactivating" metabolic pathways in seeds deteriorated by long-term storage (Nithyadevi et al., 2022). Priming solvent composition significantly influences embryo protrusion speed, with water (S1), shallot extract (S2), and PEG 6000 (S7) showing the strongest stimulatory effects during the 18–22-hour window. These agents accelerate the germination sequence by optimizing enzymatic mobilization and metabolic readiness (Pagano et al., 2023). In contrast, bamboo shoot extract (S4) serves as a metabolic inhibitor, leading to minimal emergence and developmental arrest, consistent with the inhibitory effects noted by Moura et al. (2018). The integrated data suggests that the efficacy of the invigoration treatment is highly dependent on the synergy between the solvent type and soaking duration. While water (S1), shallot extract (S2), and PEG 6000 (S7) show high compatibility with the 30-minute soaking duration (V2) to enhance germination and vigor, the poor performance of S4 regardless of time suggests biochemical interference with the germination process. For practical application, the use of water (S1) for 30 minutes (V2) is recommended to achieve maximum seedling uniformity and growth potential.

Synergistic Interaction of Solvent and Duration on Seed Physiology

The interaction results in Table 3 reveal that the combined application of specific solvents and

longer soaking duration provides the most robust recovery for aged seeds. This section focuses on the physiological synergies and the dynamics of the RE test, which is a key vigor indicator for Brassicaceae (Shinohara et al., 2021). The interaction between solvent type and soaking duration serves as a critical determinant for seed physiological enhancement, with the combinations of S1V2 (water, 30 min), S2V2 (Shallot Extract, 30 min), and S7V1 (PEG 6000, 15 min), representing the observed physiological optima. While S2V2 attained the highest vigor index of 97.33%, this superior performance likely stems from the specific kinetic requirements of shallot extract; the diffusion of its complex organic molecules, such as auxins and phenolics, into the seed embryo is a relatively slower process compared to the rapid imbibition of pure water (Taer, 2024).

Conversely, all combinations involving bamboo shoot extract (S4) consistently performed the lowest, characterized by severe germination inhibition (30.67%–37.33%) and a high percentage of fresh, ungerminated seeds, reaching up to 50.66%, regardless of the applied soaking duration. These results confirm that bamboo shoot extract (S4) likely contains natural inhibitors; this suppression is attributed to allelochemicals, such as phenolic acids, which disrupt cell division and essential physiological metabolism during the initial stages of growth (Rawat et al., 2017). Conversely, the combination of GA₃ (S6) with 30-minute (V2) and 60-minute (V3) soaking duration restored germination to over 90%, proving that the hormonal signal provided by GA₃ is the most effective means of overcoming the physiological stagnation of aged Brassica seeds (MacDonald & Mohan, 2025).

Regarding the dynamics of RE, the speed of germination was most effectively accelerated by S1V2, S2V2, and S7V2, all of which exceeded 85% emergence within 22 hours. The performance of water, 30 min (S1V2) is particularly noteworthy, as it reached 90.66% emergence by the 22-hour mark. While PEG 6000 (S7) demonstrated remarkable temporal stability by maintaining high emergence rates across all soaking durations, other treatments like GA₃ at 15 minutes (S6V1) and bamboo shoot extract (S4) exhibited significant delays or total failure in radicle breakthrough during the first 20 hours. This confirms that while specific solvents can initiate metabolic repair, hydropriming with water for 30 minutes (S1V2) is equally sufficient to trigger the transition to active germination.

Table 3. Effects of interaction between solvent and soaking duration on the physiological quality of pak choi seeds

Source	Germination Rate (%)	Abnormal Seedling Rate (%)	Fresh Seed (%)	Vigor Index (%)	Growth Rate (%NS/Etm al)	Maximum Potential Growth (%)	Radicle Length (cm)	Shoot Length (cm)	RE 18 Hours	RE 20 Hours	RE 22 Hours
S1V1	90.70 a	8.00 cde	0.00 b	90.70 a	23.20 a	98.70 a	49.33 abcd	54.10 abc	74.66 a	85.30 a	88.00 a
S1V2	94.70 a	4.00 de	0.00 b	93.30 a	24.90 a	98.70 a	46.30 abcde	49.50 abcd	78.66 a	86.70 a	90.70 a
S1V3	93.30 a	6.67 de	0.00 b	93.30 a	23.80 a	100.00 a	30.10 fgh	40.30 cdef	72.00 a	80.00 a	82.70 a
S2V1	93.30 a	6.67 de	0.00 b	93.30 a	24.50 a	100.00 a	39.60 defgh	46.60 bcdef	70.66 a	74.70 a	82.70 a
S2V2	94.70 a	1.33 e	0.00 b	97.30 a	24.60 a	98.70 a	46.70 abcde	49.50 abcd	74.66 a	81.30 a	90.70 a
S2V3	94.70 a	2.67 de	1.30 b	92.00 a	24.10 a	97.30 a	41.50 bcdefg	52.10 abcd	66.66 a	2.66 b	82.70 a
S3V1	64.00 b	22.7 abc	0.00 b	60.00 b	10.80 a	86.70 ab	30.00 fgh	33.00 ef	2.66 b	5.33 b	6.66 b
S3V2	90.70 a	8.00 cde	0.00 b	88.00 a	22.70 a	98.70 a	24.00 h	61.70 ab	61.33 a	78.70 a	84.00 a
S3V3	93.30 a	4.00 de	0.00 b	93.30 a	26.60 a	97.30 a	28.50 gh	56.90 ab	56.00 a	73.30 a	76.00 a
S4V1	37.30 cd	10.70 cde	50.70 a	26.70 cd	4.97 cd	48.00 d	34.00 defgh	32.80 ef	0.00 b	0.00 b	0.00 b
S4V2	37.30 cd	17.30 bcd	45.30 a	26.70 cd	5.14 cd	54.70 cd	24.00 h	28.70 f	0.00 b	0.00 b	1.30 b
S4V3	30.70 d	5.33 de	48.00 a	17.30 d	3.71 d	36.00 d	24.00 h	28.20 f	2.66 b	2.66 b	2.66 b
S5V1	45.30 bsd	26.70 ab	4.00 b	42.70 bc	7.42 bcd	72.00 bc	29.30 gh	38.30 def	1.33 b	2.66 b	2.66 a
S5V2	90.70 a	4.00 de	1.33 b	88.00 a	24.00 a	94.70 a	53.30 ab	62.70 a	78.66 a	81.30 a	85.30 a
S6V3	93.30 a	4.00 de	0.00 b	90.70 a	23.40 a	98.70 a	41.30 bcdefgh	61.40 ab	56.00 a	79.70 a	76.00 a
S6V1	92.00 a	37.30 a	0.00 b	40.00 c	8.42 bc	92.00 ab	32.00 efgh	40.00 cdef	2.66 b	2.66 b	2.66 b
S6V2	92.00 a	4.00 de	0.00 b	90.70 a	22.30 a	96.00 a	48.30 abcde	59.50 ab	60.00 a	81.30 a	81.30 a
S6V3	54.70 bc	4.00 de	2.66 b	89.30 a	23.50 a	96.00 a	45.80 abcdefg	56.90 ab	72.00 a	82.70 a	84.00 a
S7V1	94.70 a	2.67 de	0.00 b	90.70 a	4.97 cd	48.00 d	59.20 a	63.50 a	68.00 a	86.70 a	86.70 a
S7V2	94.70 a	5.33 de	0.00 b	92.00 a	5.14 cd	54.70 cd	51.80 abc	60.10 ab	72.00 a	86.70 a	86.70 a
S7V3	94.70 a	5.33 de	0.00 b	89.30 a	3.71 d	35.00 d	57.50 a	59.70 ab	72.00 a	82.70 a	90.70 a

Remarks: S1=Water, S2=Shallot Extract 50%, S3=Mung Bean Sprout Extract 5%, S4=Bamboo Shoot Extract, S5=KNO₃ 1%, S6=GA₃ 100 ppm, S7=PEG 6000 10%, V1=15 minutes, V2=30 minutes, V3=60 minutes. RE = Radicle Emergence. Means followed by the same letters in the same column are not significantly different according to HSD.

From a practical and economic perspective, S1V2 (water for 30 minutes) emerges as the most efficient recommendation for vigor enhancement. Since its results are statistically on par with shallot extract (S2) and PEG 6000 (S7) for most vigor parameters, the use of water offers a significantly more cost-effective, accessible, and practical alternative for large-scale applications. While PEG 6000 10% at 15 minutes (S7V1) remains the superior choice for maximizing structural elongation, such as radicle and shoot length, S1V2 provides a highly pragmatic balance of high germination, speed, and simplicity. Adopting the S1V2 protocol ensures effective invigoration without the added costs or preparation time associated with specialized extracts or chemical agents, while successfully avoiding the inhibitory effects observed in suboptimal treatments.

The RE counts at 18, 20, and 22 hours are perhaps

the most sensitive indicators of seed vigor in this study. The "Ageing/Repair Hypothesis" posits that high-vigor seeds have a shorter lag phase and thus emerge earlier than low-vigor or aged seeds (Powell, 2022). In Table 3, the treatments S1V1, S1V2, S2V1, S2V2, S5V2, S6V2, and S7V1-V3 all achieved RE percentages at 18 hours exceeding 60-70%. This rapid emergence suggests that these invigoration methods effectively perform the necessary metabolic repairs during the soaking phase (Shinohara et al., 2021).

Notably, the KNO₃ (S5) and GA₃ (S6) treatments at V2 (30 min) exhibited a substantial increase in RE values compared to V1. This suggests a "threshold effect" where 30 minutes of exposure to these ionic and hormonal triggers is necessary to catalyze the biochemical pathways for radicle protrusion (Taer, 2024). The RE test has been validated by ISTA for

Brassica napus and *Raphanus sativus*, and our results confirm its high sensitivity for *Brassica rapa*, as it clearly differentiates the speed of metabolic recovery between different invigoration regimens (Shinohara et al., 2021). High RE values at 18 hours are correlated with greater field emergence and seedling competitive ability, as they indicate that the seeds have completed their repair processes and are ready for rapid heterotrophic growth (Powell, 2022).

The highest germination rates (approx. 94.67%) were achieved by combinations such as S1V2 (water), S2V2 and S2V3 (shallot extract), and S7V1, S7V2, S7V3 (PEG 6000). The stability of PEG 6000 across all durations highlights its efficiency as an osmotic regulator that prevents the seeds from proceeding to phase III prematurely, regardless of soaking duration (MacDonald & Mohan, 2025). In contrast, the natural extract of shallot (S2) required at least 30 minutes to express its full potential, likely because the diffusion of its larger organic molecules (auxins and phenolics) into the seed embryo is a slower process compared to simple water uptake (Taer, 2024).

The interaction between solvent type and soaking duration significantly affected seed enhancement, with S1V2 (water, 30 min), S2V2 (shallot extract, 30 min), and S7V1 (PEG 6000, 15 min), representing the physiological optima. Although S2V2 achieved the highest absolute vigor index of 97.33%, the S1V2 treatment performed comparably, yielding a germination rate of 94.67% and a vigor index of 93.33%. The natural extract of shallot (S2) required at least 30 minutes to express its full potential, likely because the diffusion of its larger organic molecules (auxins and phenolics) into the seed embryo is a slower process compared to simple water uptake (Taer, 2024).

Correlation Values in between Radicle Emergence (RE) and Viability Parameters

Regression analysis shows a significant positive correlation ($R^2=0.88$) between 18-hour Radicle Emergence (RE) and final germination (Figure 4). The 30-minute soaking duration (V2) offered the highest reliability ($R^2 = 0.9223$; Figure 4b), indicating optimal metabolic synchronization. While the 60-minute duration (V3) had the steepest slope ($R^2 = 0.8897$; Figure 4c) and the 15-minute duration (V1) showed a strong fit ($R^2 = 0.8906$; Figure 4a), the 18-hour RE remains a valid rapid predictor of viability. These results align with Ozden (2020) and Nugraheni (2023), who link early emergence to high vigor, driven by DNA repair and cell cycle synchronization (Bewley et al., 2013).

Across all soaking durations, 20-hour RE showed a strong positive correlation ($R^2= 0.89$) with final germination (Figure 5). The coefficient of determination (R^2) is a number that shows how well your statistical model fits. The 30-minute interval (V2) demonstrated the highest predictive precision ($R^2 = 0.9854$; Figure 5b), outperforming the 60-minute ($R^2 = 0.9636$; Figure 5c) and 15-minute ($R^2 = 0.8966$; Figure 5a) treatments. These findings confirm that 20-hour RE is a highly reliable predictor of seed viability, particularly at the 30-minute optimum. This aligns with Kamanga (2024), who asserts that effective invigoration enhances quality by accelerating RE and increasing both germination rates and seedling normality.

Regression analysis confirms a strong positive correlation between 22-hour RE and final germination. The 30-minute soaking duration (V2) showed the highest predictive accuracy ($R^2 = 0.9943$; Figure 6b), indicating that 22-hour RE can explain final germination variations with extreme precision. While the 60-minute ($R^2 = 0.967$; Figure 6c) and 15-minute soaking ($R^2 =$

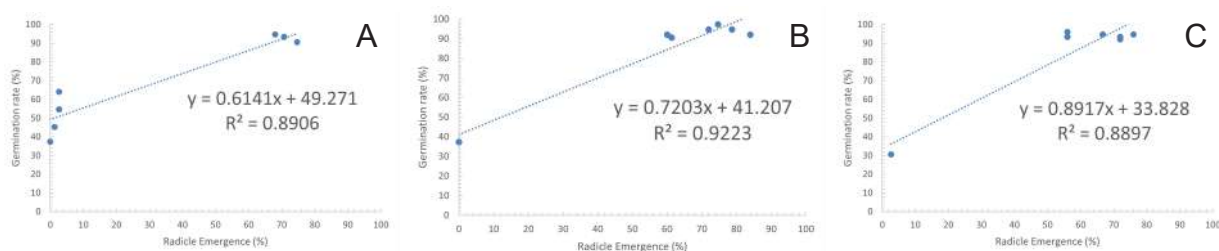


Figure 4. Regression between germination rate and radicle emergence 18 hours in seeds treated with 15-minute (A), 30-minute (B) and 60-minute (C) soaking duration

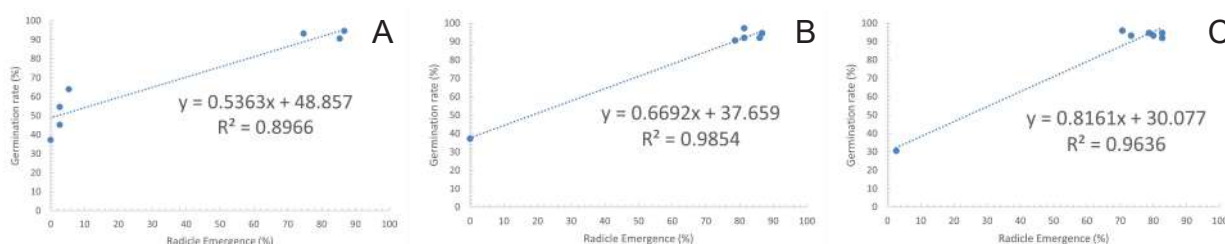


Figure 5. Regression between germination rate and radicle emergence 20 hours in seeds treated with 15-minute (A), 30-minute (B) and 60-minute (C) soaking duration

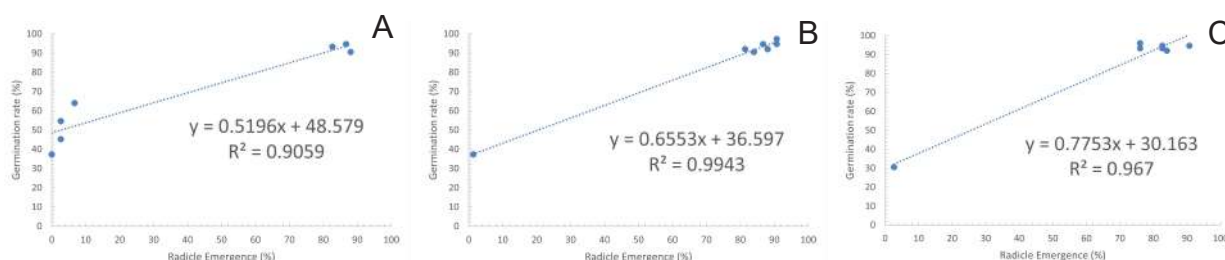


Figure 6. Regression between germination rate and radicle emergence 22 hours in seeds treated with 15-minute (A), 30-minute (B) and 60-minute (C) soaking duration

0.9059; Figure 6a) also showed strong relationships, the 30-minute interval remains the most stable indicator. These findings align with Demir (2023), who identifies RE as a critical diagnostic tool for quantifying seed vigor and the effectiveness of invigoration.

CONCLUSIONS

The 30-minute soaking duration is optimal, providing the best balance between metabolic acceleration and seedling quality. Water, 10% PEG 6000, and 50% shallot extract proved to be superior activators, consistently yielding the highest germination and vigor indices. Radicle Emergence (RE) testing at 22 hours proved to be the most accurate rapid indicator ($R^2 = 0.9943$). Water can be used as an alternative treatment option because it is cheaper than chemical treatment. Conversely, bamboo shoot extract is not recommended for seed invigoration as it significantly inhibits the germination process.

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