Agro Bali : Agricultural Journal
Vol. 9 No. 2: 332-342, July 2026

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

Effects of Dormancy-Breaking Agents and Vernalization Temperatures on Dormancy Release and Sprouting of Shallot Bulbs

Masrinda Oktavia, Elkawakib Syam'un♥, and Tigin Dariati

Department of Agronomy, Faculty of Agriculture, Universitas Hasanuddin, Makassar, Indonesia

♥Corresponding author email: elkawakibsyam@gmail.com

Article history: submitted: April 29, 2026; accepted: July 8, 2026; available online: August 11, 2026

Abstract. Post-harvest dormancy in shallot (*Allium cepa* L. var. aggregatum) seed bulbs delays sprouting and disrupts planting schedules. While gibberellic acid (GA_3), hydrogen peroxide (H_2O_2), and vernalization each break dormancy, their combined effects have not been evaluated. The study, conducted in October 2025 at the Hasanuddin University Teaching Farm in Indonesia, is the first to evaluate the factorial effects of GA_3 or H_2O_2 combined with vernalization on dormancy breaking. Lokana shallot bulbs underwent vernalization for 14 days at room temperature, 5°C, or 10°C, followed by treatment with either no agent, GA_3 (100 mg L^{-1}, 6 h), or H_2O_2 (20%, 2 h), using a 3 × 3 randomized complete block design (27 plots, 1.215 plants). Data analysed using ANOVA followed by HSD at the 5% level and Pearson's correlation. Significant interactions ($p < 0.05$) between dormancy-breaking agents and vernalization were observed for most germination rate and sprouting time parameters. The combination of H_2O_2 treatment with 5°C vernalization produced the fastest sprouting, achieving the highest sprouting rate index (SSI = 6.91) and rate coefficient (CVG = 12.78), the shortest mean sprouting time (MSpT = 7.86 days), and the lowest time to 50% sprouting (T50 = 7.06 days), outperforming both the control (SSI = 2.82; MSpT = 16.90 days; T50 = 16.86 days) and GA_3 treatments. Sprouting synchrony and uniformity remained unaffected. The combined application of H_2O_2 and short-duration cold vernalization represents a practical and cost-effective approach to reducing dormancy and expediting planting readiness in shallot seed production.

Keywords: dormancy breaking; hydrogen peroxide; shallot bulb; sprouting performance; vernalization

1. Introduction

Shallots (*Allium cepa* L. var. aggregatum) are a horticultural commodity of significant economic importance. Demand is increasing due to their functional properties as sources of antioxidants and bioactive compounds (Moldovan et al., 2022), as well as a documented rise at the national level (Indonesia Central Bureau of Statistics, 2024; Center for Agricultural Data and Information Systems, 2025). Sustainable production depends on the timely availability of high-quality bulb seeds. However, efficient seed supply is often limited by premature sprouting and post-harvest dormancy, both of which inhibit shoot and root development (Kiran et al., 2024; D'Angelo & Goldman, 2019).

Prolonged dormancy delays planting schedules and reduces the efficiency of the shallot seed supply chain. This dormancy is regulated by a complex hormonal network, including abscisic acid (ABA), gibberellins (GA), auxins, and brassinosteroids, with ABA serving as the primary inhibitor of germination (Puccio et al., 2022). Dormancy is generally maintained by a high ABA/GA ratio, and release occurs when this ratio decreases (Sano & Marion-Poll, 2021). However, this regulatory mechanism is organ-specific and does not always align with the classical antagonism model, as shown by transcriptome and phytohormone analyses of ethylene-treated onion bulbs (Alamar et al., 2020). In addition to hormonal regulation, reactive oxygen species such as hydrogen peroxide (H_2O_2) act as signaling molecules for dormancy release but can be harmful oxidants at high concentrations (Farooq et al., 2021; Li et al., 2022). Cold temperatures also contribute by lowering ABA levels and increasing endogenous GA concentrations in onion bulbs, thereby reducing the ABA/GA ratio and promoting germination (D'Angelo & Goldman, 2018).

Previous research has assessed these three dormancy-breaking strategies separately. For instance, D'Angelo &

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

Goldman (2019) demonstrated that soaking dormant onion bulbs in a 20% H_2O_2 solution for two to four hours accelerated and standardized root growth. Similarly, exogenous application of GA_3 has been shown to induce dormancy release in garlic bulbs by modifying gibberellin-related gene expression (Dong et al., 2022). In shallots, GA_3 at concentrations up to 100 mg L^{-1} has been reported to improve plant morphological traits (Marlin et al., 2021). For environmental interventions, D'Angelo & Goldman (2018) identified an optimal vernalization period of 12–14 weeks at 10°C for long-day onions. Marlin et al. (2021) found that six weeks of vernalization at 8°C significantly increased flowering in tropical shallots, although there was notable intervarietal sensitivity. Collectively, these findings suggest that the effectiveness of H_2O_2, GA_3, and vernalization depends on species and agroecological context, and each method has been studied as an independent treatment.

Although GA_3, H_2O_2, and vernalization have each been evaluated individually (D'Angelo & Goldman, 2018, 2019; Dong et al., 2022; Marlin et al., 2021), their combined effects have not been tested in a single factorial design in shallots. Marlin et al. (2021) examined GA_3 and vernalization together, but these treatments were not factorially combined, which prevents conclusions about their interaction effects. This research gap is important because $H_2O_2$and vernalization affect overlapping physiological pathways, particularly ABA metabolism and GA biosynthesis (Ishibashi et al., 2017). Evidence from model plant systems suggests that reactive oxygen species such as H_2O_2 may interact synergistically with GA and ABA pathways during dormancy release (Grainge et al., 2022). Currently, information on the interactive effects of GA_3 and H_2O_2 with vernalization temperature on dormancy breaking in shallot bulbs is limited. Therefore, this study hypothesizes that GA_3 and H_2O_2 independently promote dormancy release, that lower vernalization temperatures significantly reduce the endogenous ABA/GA ratio, and that a significant interaction exists between dormancy-breaking agents and vernalization temperature, resulting in higher germination rates than either factor alone.

To address these gaps and hypotheses, this study evaluates the effectiveness of GA_3 and H_2O_2, combined with different vernalization temperatures, in accelerating dormancy release and improving shallot bulb germination. The study also investigates the interactions among these factors using a single factorial design. The main scientific contribution is a novel combined approach that advances the mechanistic understanding of dormancy regulation in Allium species. Practically, the results are expected to assist breeders and farmers in reducing bulb dormancy, expediting planting schedules, and enhancing the efficiency of quality shallot seed production, thereby supporting the sustainability of the national seed supply.

2. Materials and Methods

The study was conducted in October 2025 at the Hasanuddin University Teaching Farm, South Sulawesi, Indonesia (approximately 9 m above sea level), which represents a lowland coastal agroecosystem typical of shallot production systems. A two-factor factorial arrangement was implemented within a randomised complete block design (RCBD) to control field variability and improve inferential precision. The first factor was the dormancy-breaking agent, comprising the control, GA_3 at 100 mg L^{-1}, and 20% H_2O_2. The second factor was vernalization temperature, including room temperature (control), 5°C, and 10°C. Nine treatment combinations were randomized within each block, resulting in 27 experimental plots.

Bulbs of the Lokana shallot cultivar were uniformly selected and subjected to a 14-day vernalization period at specified temperatures. Following vernalization, approximately one-quarter of each bulb tip

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

was transversely excised and assigned to one of three dormancy-breaking treatments: no treatment (control), immersion in 100 mg L^{-1} GA_3 for 6 hours, or immersion in 20% H_2O_2 for 2 hours (Nurfaida et al., 2024). The 100 mg L^{-1} GA_3 concentration was selected based on Marlin et al. (2021), who demonstrated its efficacy in enhancing shallot growth and morphological traits without adverse effects. The 20% H_2O_2 treatment for 2 hours followed the protocol of D'Angelo & Goldman (2019), who reported effective dormancy release without phytotoxic symptoms.

Treated bulbs were planted in a homogenized 1:1 mixture of native soil and compost, with a spacing of 20 cm by 15 cm in accordance with standard agronomic practice. Each experimental plot contained 45 plants; five plants were randomly selected and tagged as observation samples, and their measurements were averaged to yield a single value per plot. The experimental plot was designated as the unit of analysis for the analysis of variance, while the five tagged plants constituted the observation (sampling) unit. Across all plots, a total of 1,215 plants were included, ensuring a sufficient sample size for robust statistical analysis.

Sprouting performance was evaluated through daily records of individual bulb sprouting, utilizing germination indices adapted to bulb physiology. These indices included time to 10% sprouting (T10), time to 50% sprouting (T50), mean sprouting time (MSpT), mean sprouting rate (MSpR), coefficient of variation of sprouting time (CVT), variance of sprouting time (VarSpR), coefficient of velocity of sprouting (CVG), sprouting speed index (SSI), synchronization index (SINC), uniformity of sprouting (UnifSp), and uncertainty of sprouting (UNC). Collectively, these indices characterize the timing, speed, uniformity, and synchronization of sprouting.

Data were analyzed using factorial analysis of variance (ANOVA) to determine the main and interaction effects of vernalization temperature and dormancy-breaking agents. Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$ was applied for mean comparisons. Relationships among variables were assessed using Pearson's correlation in R.

3. Results and Discussion

Table 1. Sprouting speed index, coefficient of velocity of sprouting, and coefficient of variation of time values in various treatment combinations

Treatment Combinations	Parameters		
	SSI	CVG (%)	CVT (%)
Control	2.82 ±0.14^{e}	5.94 ±0.25^{e}	2.12 ±0.24^{bc}
No Plant Growth Regulator + Vernalization 5°C	4.24 ±0.07^{cd}	7.94 ±0.17^{cd}	3.31 ±0.13^{ab}
No Plant Growth Regulator + Vernalization 10°C	4.37 ±0.15^{cd}	8.27 ±0.16^{cd}	2.94 ±0.23^{ab}
Gibberellic Acid (GA_3) + No Vernalization	3.28 ±0.06^{de}	7.05 ±0.15^{de}	1.72 ±0.14^{c}
Gibberellic Acid (GA_3) + Vernalization 5°C	4.74 ±0.05^{bc}	9.20 ±0.20^{c}	3.09 ±0.19^{ab}
Gibberellic Acid (GA_3) + Vernalization 10°C	5.83 ±0.43^{ab}	10.92 ±0.56^{b}	3.42 ±0.37^{a}
Hydrogen Peroxide (H_2O_2) + No Vernalization	3.31 ±0.01^{de}	7.23 ±0.00^{de}	1.28 ±0.12^{c}
Hydrogen Peroxide (H_2O_2) + Vernalization 5°C	6.91 ±0.40^{a}	12.78 ±0.59^{a}	3.83 ±0.44^{a}
Hydrogen Peroxide (H_2O_2) + Vernalization 10°C	4.88 ±0.37^{bc}	9.60 ±0.49^{bc}	3.01 ±0.40^{ab}
$p < 0.05$	1.17	1.70	1.20

Remark: Values are presented as mean ± standard deviation (SD). SSI denotes sprouting speed index, CVG denotes coefficient of velocity of sprouting, and CVT denotes coefficient of variation of sprouting time. Values followed by the same letter do not differ significantly at the 5% significance level according to Tukey's Honestly Significant Difference (HSD) test.

The interaction between dormancy-breaking treatments and vernalization significantly affected SSI, CVG, and CVT ($p < 0.05$, Table 1). The combination of H_2O_2 and 5°C vernalization produced the highest SSI (6.91 ± 0.40) and CVG (12.78 ± 0.59),

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

both of which were significantly greater than those observed in all other treatment combinations. For CVT, H_2O_2 without vernalization (1.28 ± 0.12%) and GA_3 without vernalization (1.72 ± 0.14%) resulted in the lowest and statistically similar values. In contrast, H_2O_2 with 5°C vernalization yielded the highest CVT (3.83 ± 0.44%), which did not differ significantly from GA_3 with 10°C vernalization (3.42 ± 0.37%) or from the absence of plant growth regulator with 5°C vernalization (3.31 ± 0.13%), but was significantly different from the remaining treatments.

Table 2. Mean sprouting rate, mean sprouting time, and synchronization index values in various treatment combinations

Treatment Combinations	Parameters		
	MSpR (days^{-1})	MSpT (days)	Sinc
Control	0.06 ±0.00^{e}	16.90 ±0.74^{a}	0.10 ±0.02
No Plant Growth Regulator + Vernalization 5°C	0.08 ±0.00^{cd}	12.60 ±0.26^{bcd}	0.07 ±0.01
No Plant Growth Regulator + Vernalization 10°C	0.08 ±0.00^{de}	12.10 ±0.24^{cde}	0.08 ±0.01
Gibberellic Acid (GA_3) + No Vernalization	0.07 ±0.00^{ef}	14.21 ±0.32^{b}	0.14 ±0.03
Gibberellic Acid (GA_3) + Vernalization 5°C	0.09 ±0.00^{cd}	10.88 ±0.23^{def}	0.10 ±0.01
Gibberellic Acid (GA_3) + Vernalization 10°C	0.11 ±0.01^{b}	9.21 ±0.48^{fg}	0.08 ±0.01
Hydrogen Peroxide (H_2O_2) + No Vernalization	0.07 ±0.00^{ef}	13.82 ±0.00^{bc}	0.17 ±0.02
Hydrogen Peroxide (H_2O_2) + Vernalization 5°C	0.13 ±0.01^{a}	7.86 ±0.35^{g}	0.10 ±0.01
Hydrogen Peroxide (H_2O_2) + Vernalization 10°C	0.10 ±0.01^{bc}	10.47 ±0.53^{ef}	0.08 ±0.01
$p < 0.05$	0.021	1.86	-

Remark: Values are presented as mean ± standard deviation (SD). MSpR denotes mean sprouting rate, MSpT denotes mean sprouting time, and Sinc denotes synchronization index. Values followed by the same letter do not differ significantly at the 5% significance level according to Tukey's Honestly Significant Difference (HSD) test.

Table 3. Time to 10% sprouting, time to 50% sprouting values for various treatment combinations

Treatment Combinations	Parameters	
	T10 (days)	T50 (days)
Control	11.63 ±1.41	16.86 ±0.74^{a}
No Plant Growth Regulator + Vernalization 5°C	6.28 ±0.40	12.09 ±0.74^{bcd}
No Plant Growth Regulator + Vernalization 10°C	6.50 ±0.58	11.92 ±0.38^{bcd}
Gibberellic Acid (GA_3) + No Vernalization	10.39 ±0.18	13.86 ±0.22^{b}
Gibberellic Acid (GA_3) + Vernalization 5°C	5.81 ±0.59	10.85 ±0.62^{cde}
Gibberellic Acid (GA_3) + Vernalization 10°C	4.38 ±0.62	8.84 ±0.64^{ef}
Hydrogen Peroxide (H_2O_2) + No Vernalization	10.71 ±0.29	13.62 ±0.03^{bc}
Hydrogen Peroxide (H_2O_2) + Vernalization 5°C	3.42 ±0.25	7.06 ±0.57^{f}
Hydrogen Peroxide (H_2O_2) + Vernalization 10°C	5.47 ±0.62	9.94 ±0.74^{de}
$p < 0.05$	-	2.81

Remark: Values are presented as mean ± standard deviation (SD). T10 denotes time to 10% sprouting, and T50 denotes time to 50% sprouting. Values followed by the same letter do not differ significantly at the 5% significance level according to Tukey's Honestly Significant Difference (HSD) test.

The interaction also significantly influenced MSpR and MSpT ($p < 0.05$, Table 2), but did not affect SINC. H_2O_2 with 5°C vernalization resulted in the highest MSpR (0.13 ± 0.01 days^{-1}) and the lowest MSpT (7.86 ± 0.35 days). The MSpT for this treatment was not significantly different from GA_3 with 10°C vernalization (9.21 ± 0.48 days), but was significantly lower than all other treatments. The control exhibited the

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

lowest MSpR (0.06 ± 0.00 days^{-1}) and the highest MSpT (16.90 ± 0.74 days). SINC values ranged from 0.07 ± 0.01 to 0.17 ± 0.02, with no significant differences among treatments, indicating no effect on sprouting synchronization.

T10 was not significantly affected by the interaction, with values ranging from 3.42 ± 0.25 to 11.63 ± 1.41 days. In contrast, T50 was significantly influenced ($p < 0.05$, Table 3). H_2O_2 with 5°C vernalization produced the lowest T50 (7.06 ± 0.57 days), which was not significantly different from GA_3 with 10°C vernalization (8.84 ± 0.64 days), but was significantly lower than all other treatments. The control had the highest T50 (16.86 ± 0.74 days). H_2O_2 with 10°C vernalization (9.94 ± 0.74 days) did not differ significantly from GA_3 with 10°C (8.84 ± 0.64 days) or GA_3 with 5°C vernalization (10.85 ± 0.62 days), but differed significantly from the control and from non-vernalized treatments.

Table 4. Uncertainty value, uniformity of sprouting, variance of sprouting in various treatment combinations

Treatment Combinations	Parameters		
	Unc (bits)	UnifSp (days)	VarSpr (days^2)
Control	3.44 ±0.22 ^{ab}	9.13 ±1.19	12.74 ±2.29
No Plant Growth Regulator + Vernalization 5°C	3.69 ±0.11 ^{a}	11.36 ±0.52	17.53 ±2.05
No Plant Growth Regulator + Vernalization 10°C	3.43 ±0.14 ^{ab}	9.32 ±0.67	12.90 ±2.21
Gibberellic Acid (GA_3) + No Vernalization	2.96 ±0.19 ^{bc}	6.62 ±0.92	6.10 ±1.21
Gibberellic Acid (GA_3) + Vernalization 5°C	3.31 ±0.05 ^{ab}	8.62 ±0.71	11.41 ±1.38
Gibberellic Acid (GA_3) + Vernalization 10°C	3.45 ±0.17 ^{ab}	8.77 ±1.25	10.25 ±2.52
Hydrogen Peroxide (H_2O_2) + No Vernalization	2.61 ±0.12 ^{c}	4.68 ±0.55	3.18 ±0.56
Hydrogen Peroxide (H_2O_2) + Vernalization 5°C	3.18 ±0.14 ^{abc}	7.78 ±0.44	9.10 ±1.57
Hydrogen Peroxide (H_2O_2) + Vernalization 10°C	3.41 ±0.12 ^{abc}	8.74 ±0.77	9.80 ±1.63
$p < 0.05$	0.59	-	-

Remark: Values are presented as mean ± standard deviation (SD). Unc denotes uncertainty of sprouting, UnifSp denotes uniformity of sprouting, and VarSpr denotes variance of sprouting time. Values followed by the same letter do not differ significantly at the 5% significance level according to Tukey's Honestly Significant Difference (HSD) test.

The interaction significantly affected UNC ($p < 0.05$; Table 4) but did not affect UnifSp or VarSpr. The lowest UNC was observed with H_2O_2 without vernalization (2.61 ± 0.12 bits), which was not significantly different from GA_3 without vernalization (2.96 ± 0.19 bits), the absence of plant growth regulator with 10°C vernalization (3.43 ± 0.14 bits), or H_2O_2 with 10°C vernalization (3.41 ± 0.12 bits), but was significantly different from the control (3.44 ± 0.22 bits) and the remaining treatments (3.31 to 3.69 bits). UnifSp ranged from 4.68 ± 0.55 to 11.36 ± 0.52 days, and VarSpr ranged from 3.18 ± 0.56 to 17.53 ± 2.05 days^2, with no significant differences among treatments.

The combined application of H_2O_2 and short-duration vernalization resulted in a greater acceleration of sprouting than either treatment alone. This finding indicates that both interventions likely target the same rate-limiting step in dormancy release, specifically the abscisic acid/gibberellic acid (ABA/GA) balance, through complementary redox and thermal signals. This occurs through two complementary mechanisms, a redox signal and a thermal signal. This interpretation aligns with Bailly (2019), who reports that accumulation of reactive oxygen species (ROS) promotes ABA degradation and enhances GA signaling, thereby lowering the physiological threshold for reactivation. When this threshold is already reduced by cold exposure, a lower exogenous H_2O_2 input is sufficient to induce sprouting. This accounts for the disproportionately large response observed

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

with the combined treatment compared to a simple additive effect.

The reduced sprouting performance observed after 10°C of vernalization compared to 5 days, despite identical H_2O_2 treatment, suggests that prolonged cold exposure shifted the internal redox balance beyond its optimal range rather than further enhancing it. This result aligns with the oxidative window for germination described by Bailly et al. (2008), in which ROS levels within a specific range promote reactivation, while levels outside this range do not. Extended vernalization likely increased antioxidant enzyme activity, thereby partially neutralizing the H_2O_2 signal and shifting the redox status outside the productive window. These findings confirm that the interaction is genuinely optimal-dependent, rather than merely reflecting the fact that increased cold exposure yields better outcomes.

The comparatively weaker effect of GA_3 relative to H_2O_2, particularly in the absence of vernalization, indicates that GA_3 likely acts downstream of ROS-mediated dormancy alleviation rather than serving as an independent trigger. This interpretation is supported by Shu et al. (2016), who describe germination as governed by two interacting hormonal phases, with GA-driven germination proceeding only after the ABA-dominated phase begins to dismantle. Oracz & Stawska (2016) further note that the effectiveness of phytohormone signaling depends on tissue ROS status. Therefore, GA_3 is most effective as an accelerant in a system already primed for dormancy release, rather than as a primary dormancy-breaking agent.

The significant effect of treatment on T50, but not on T10, indicates that interventions influenced the majority of the bulb population, while the least dormant fraction sprouted early regardless of treatment. This finding is consistent with Finch-Savage & Footitt (2017), who describe dormancy status as being continuously adjusted by environmental signals, resulting in individual differences in proximity to dormancy release. Krzyszton et al. (2022) also demonstrate considerable transcriptional heterogeneity among genetically identical seeds, supporting the concept that population-level variability, rather than a uniform threshold, underlies differential sprouting timing. Therefore, T50 and the coefficient of variation in time (CVT) are more reliable indicators of treatment efficacy than T10 for commercial-scale evaluation.

The absence of treatment effects on synchronization, uniformity, and variance, despite an increased mean sprouting rate, suggests that accelerating the average response does not reduce variability among bulbs regarding maturity, carbohydrate reserves, and hormone levels, which is typical of storage organ populations (Finch-Savage & Footitt, 2017). In shallot, Cennawati et al. (2025) report that storage duration strongly determines the percentage of sprouting and rooting, as well as their synchrony, with near-uniform responses occurring only within a narrow storage window. Ansar et al. (2022), indicating that the pre-treatment condition of bulbs, rather than the dormancy-breaking agent alone, determines sprouting uniformity. This observation represents a boundary condition rather than a methodological flaw. Achieving true synchronization likely requires upstream control of bulb uniformity, such as standardized harvest timing, curing, and size grading before chemical treatment, which was not addressed in this study.

For commercial shallot production, combining H_2O_2 with 5°C vernalization offers a practical strategy to shorten the conventional four-month post-harvest dormancy period, which restricts planting calendar flexibility, as documented by Nurfaida et al. (2024) and Cennawati et al. (2025). H_2O_2 is cost-effective and does not require specialized storage, unlike synthetic regulators such as GA_3, making it more suitable for smallholders once concentration and exposure duration are standardized. However, Nurfaida et al. (2024) report that efficacy varies with concentration and variety. As this trial utilized a single cultivar

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

under screen house conditions and did not include direct ABA/GA or antioxidant enzyme assays, the proposed mechanism remains the most biologically plausible explanation rather than a confirmed pathway. Validation across multiple cultivars, field conditions, and physiological assays is necessary before recommending this combination as a standard commercial practice.

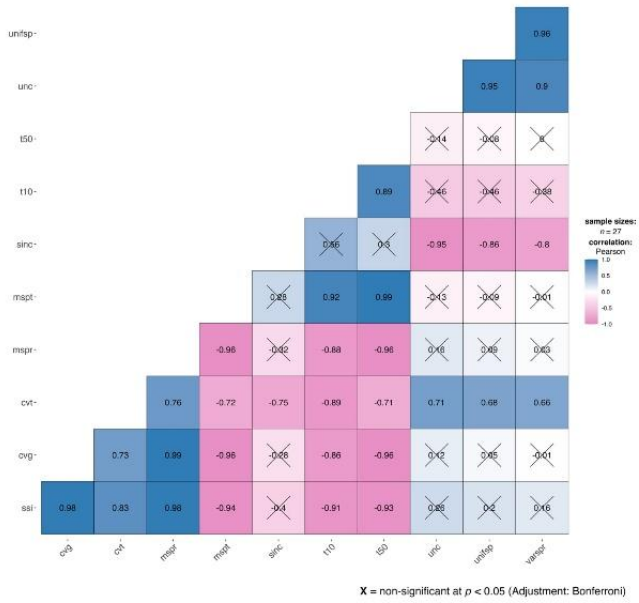

Figure 1. Correlation matrix of sprouting traits.

Pearson correlation analysis (n = 27) identified three distinct parameter groups with internal relationships (Figure 1). The germination rate group (SSI, CVG, CVT, MSpR) showed strong to very strong positive correlations (r = 0.73 to 0.99; $p < 0.05$). Similarly, the germination time group (MSpT, T10, T50) demonstrated strong positive correlations (r = 0.89 to 0.99; $p < 0.05$). A strong to very strong negative correlation was observed between the germination rate and germination time groups (r = 0.71 to 0.96; $p < 0.05$), consistent with the expectation that higher germination rates correspond to shorter times to reach a given germination percentage. The dispersion group (UNC, UnifSp, VarSpr) also exhibited very strong positive correlations (r = 0.90 to 0.96; $p < 0.05$).

The acceleration of bulb sprouting observed under H_2O_2 treatment is primarily attributed to its function as a reactive oxygen species (ROS) signaling molecule, rather than to its oxidative properties alone. At regulated concentrations, H_2O_2 activates hydrolytic enzymes, promotes reserve mobilization, stimulates meristematic cell division, suppresses abscisic acid (ABA) activity, and enhances gibberellic acid (GA) signaling. This interpretation is consistent with Wojtyla et al. (2016), who identify H_2O_2 as a central signaling hub connecting ABA, GA, and other regulators during the transition from dormancy to active growth. Anand et al. (2019) also report that H_2O_2 signaling directly integrates with phytohormone pathways to promote germination. Cheng et al. (2022) provide molecular evidence that H_2O_2 counteracts ABA-induced inhibition and shifts the ABA/GA_3 balance toward germination in a calcium-dependent manner. These findings indicate that the promotive effect of H_2O_2 arises from active hormonal modulation rather than from oxidative stress alone.

Vernalization treatment at 5°C functioned as an environmental trigger, preparing the bulb for subsequent ROS-

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

mediated activation. Exposure to low temperatures induces metabolic and hormonal changes that increase meristem sensitivity to internal signals, thereby priming the tissue for H_2O_2 application. This interpretation aligns with Li et al. (2022), who indicate that ROS serve as key intermediaries between environmental cues and hormonal reprogramming during dormancy release in storage organs. Liu et al. (2024) further demonstrate that tuber dormancy release is governed by coordinated hormonal and gene-regulatory shifts induced by temperature. Jurdak et al. (2022) support this priming role by tracing intracellular ROS movement during dormancy alleviation, showing that ROS accumulation at the onset of radicle protrusion is a consistent feature of dormancy-breaking treatments. Collectively, these findings reinforce the concept of an oxidative window during which ROS signaling is most effective. The observed synergistic effect, where the combined treatment exceeded the effects of either factor alone, suggests coordinated activation rather than independent additive responses.

In contrast, the combination of GA_3 with 10°C vernalization produced a weaker and less consistent response, suggesting that GA_3 efficacy depends on the bulb's prior physiological state rather than acting independently. Anand et al. (2019) support this conclusion, noting that hormone-driven germination responses are less coordinated in the absence of sufficient ROS signaling. Nurfaida et al. (2024) further demonstrate that dormancy-breaking efficacy in shallot varies with treatment type and concentration, indicating non-uniform chemical responses under different physiological conditions. Overall, these findings suggest that H_2O_2 is most effective when combined with an appropriate vernalization temperature, resulting in a faster and more physiologically coordinated sprouting response than GA_3 under the tested conditions.

4. Limitations and Future Directions

This study evaluated only morphological sprouting parameters and did not incorporate direct assays for abscisic acid (ABA), gibberellic acid (GA), or reactive oxygen species (ROS). Consequently, the proposed mechanism remains speculative. The experiments were limited to a single cultivar, conducted under screen-house conditions. They utilized a fixed range of hydrogen peroxide (H_2O_2) concentrations and vernalization durations, which constrains the generalizability of the findings. Although the combined treatment accelerated sprouting and reduced the time to sprouting, it did not enhance synchronization or uniformity, nor did it decrease variance. This outcome indicates that bulb-to-bulb heterogeneity remains a significant source of variability.

Future research should confirm the underlying mechanisms through direct assays of ABA, GA, and ROS, validate these results across multiple cultivars and field conditions, and examine a broader spectrum of H_2O_2 concentrations, vernalization temperatures, and durations to optimize treatment protocols. Additionally, improving sprouting uniformity may require upstream bulb management strategies, such as modifications to harvest timing, curing, and size grading. The establishment of standardized protocols will be essential to enable the commercial-scale application of H_2O_2 and vernalization in shallot seed production.

5. Conclusion

The interaction between dormancy-breaking treatment and vernalization temperature significantly affected most germination rate and germination time parameters, indicating that treatment efficacy depended on the combination of both factors. Among all treatment combinations, H_2O_2 combined with 5°C vernalization consistently produced the best performance by accelerating dormancy release and improving germination rate, whereas the untreated control showed the poorest germination performance. In contrast, germination synchrony and uniformity were not significantly affected by the interaction, indicating that the treatments primarily increased the rate of dormancy release rather

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

than the consistency of sprouting among bulbs. Pearson correlation analysis further demonstrated strong positive associations within germination rate parameters and within germination time parameters. At the same time, these two groups were strongly negatively correlated, confirming their complementary roles in evaluating dormancy-breaking responses.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the author(s) used Grammarly exclusively for proofreading and language refinement. No generative artificial intelligence tools were used in the development of scientific content. The author(s) have reviewed and approved the final version and take full responsibility for its content.

Authorship Contribution Statement

Masrinda Oktavia collected, analyzed, and interpreted the data, drafted the manuscript, and reviewed and approved the final manuscript. Elkawakib Syam'un conceived and designed the study, analyzed and interpreted the data, drafted the manuscript, served as the corresponding author, and reviewed and approved the final manuscript. Tigin Dariati conceived and designed the study, drafted the manuscript, and reviewed and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

References

Alamar, M. C., Anastasiadi, M., Lopez-Cobollo, R., Bennett, M. H., Thompson, A. J., Turnbull, C. G. N., Mohareb, F., & Terry, L. A. (2020). Transcriptome and phytohormone changes associated with ethylene-induced onion bulb dormancy. *Postharvest Biology and Technology, 168*, 111267. https://doi.org/10.1016/j.postharvbio.2020.111267

Anand, A., Kumari, A., Thakur, M., & Koul, A. (2019). Hydrogen peroxide signaling integrates with phytohormones during seed dormancy and germination. *Scientific Reports, 9*, 1–12. https://doi.org/10.1038/s41598-019-45102-5

Ansar, M., Bahruddin, Maemunah, & Paiman. (2022). Effect of harvest age and storage duration on viability and vigor of shallot (*Allium cepa* L.) tubers. *Research on Crops, 23*(4), 815–822. https://doi.org/10.31830/2348-7542.2022.ROC-909

Bailly, C. (2019). Active oxygen species and antioxidants in seed biology. *Biochemical Journal, 476*(20), 3019–3032. https://doi.org/10.1042/BCJ20190159

Bailly, C., El-Maarouf-Bouteau, H., & Corbineau, F. (2008). From intracellular signaling networks to cell death: The dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies, 331*(10), 806–814. https://doi.org/10.1016/j.crvi.2008.07.022

Center for Agricultural Data and Information Systems. (2025). Analysis of shallot trade performance in 2025. Ministry of Agriculture, Republic of Indonesia. https://satudata.pertanian.go.id/details/publikasi/851

Cennawati, C., Faried, M., Syam'un, E., Putri, R. W., & Wijaya, P. (2025). Optimal storage duration for dormancy break and early growth in shallot bulbs. *Advances in Horticultural Science, 39*(4), 293–303. https://doi.org/10.36253/ahsc-18383

Cheng, M., Guo, Y., Liu, Q., Nan, S., Xue, Y., Wei, C., Zhang, Y., Luan, F., Zhang, X., & Li, H. (2022). H_2O_2 and Ca^{2+} signaling crosstalk counteract ABA to induce seed germination. *Antioxidants, 11*(8), 1594. https://doi.org/10.3390/antiox11081594

D'Angelo, C. J., & Goldman, I. L. (2018). Temporal aspects of vernalization and flowering in long-day storage onion. *Journal of the American Society for Horticultural Science, 143*(6), 446–453. https://doi.org/10.21273/JASHS04495-18

D'Angelo, C. J., & Goldman, I. L. (2019). Breaking onion bulb endodormancy with

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

hydrogen peroxide. *HortScience, 54*(10), 1694–1702. https://doi.org/10.21273/HORTSCI14118-19

Dong, Y., Fang, H., Hou, Y., Zhao, Y., Sun, X., & Liu, S. (2022). Transcriptome analysis reveals differential gene expression in garlic aerial bulbs in response to gibberellin application. *Journal of Plant Growth Regulation, 41*, 2967–2979. https://doi.org/10.1007/s00344-021-10488-y

Farooq, M. A., Zhang, X., Zafar, M. M., Ma, W., & Zhao, J. (2021). Roles of reactive oxygen species and mitochondria in seed germination. *Frontiers in Plant Science, 12*, 781734. https://doi.org/10.3389/fpls.2021.781734

Finch-Savage, W. E., & Footitt, S. (2017). Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. Journal of Experimental Botany, 68(4), 843-856. https://doi.org/10.1093/jxb/erw477

Grainge, G., Nakabayashi, K., Steinbrecher, T., Kennedy, S., Ren, J., Iza, F., & Leubner-Metzger, G. (2022). Molecular mechanisms of seed dormancy release by gas plasma-activated water technology. *Journal of Experimental Botany, 73*(12), 4065–4078. https://doi.org/10.1093/jxb/erac150

Ishibashi, Y., Aoki, N., Kasa, S., Sakamoto, M., Kai, K., Tomokiyo, R., Watabe, G., Yuasa, T., & Iwaya-Inoue, M. (2017). The interrelationship between abscisic acid and reactive oxygen species plays a key role in barley seed dormancy and germination. *Frontiers in Plant Science, 8*, 275. https://doi.org/10.3389/fpls.2017.00275

Indonesia Central Bureau of Statistics. (2024). Horticulture statistics 2023. Central Bureau of Statistics. https://www.bps.go.id/id/publication/2024/06/10/790c957ba8892f9771aeefb7/statistik-hortikultura-2023.html

Jurdak, R., Launay-Avon, A., Paysant-Le Roux, C., & Bailly, C. (2022). Intracellular reactive oxygen species trafficking participates in seed dormancy alleviation in *Arabidopsis* seeds. *New Phytologist, 234*(3), 850–866. https://doi.org/10.1111/nph.18038

Kiran, P. R., Aradwad, P., Kumar, A. T. V., Nayana, N. P., Ramya, C. S., Sahoo, M., Urhe, S. B., Yadav, R., Kar, A., & Mani, I. (2024). A comprehensive review on recent advances in postharvest treatment, storage, and quality evaluation of onion (*Allium cepa*): Current status, and challenges. *Future Postharvest and Food, 1*(1), 124–157. https://doi.org/10.1002/fpf2.12009

Krzyszton, M., Yatusevich, R., Wrona, M., Sacharowski, S. P., Adamska, D., & Swiezewski, S. (2022). Single seeds exhibit transcriptional heterogeneity during secondary dormancy induction. *Plant Physiology, 190*(1), 211–225. https://doi.org/10.1093/plphys/kiac265

Li, W., Niu, Y., Zheng, Y., & Wang, Z. (2022). Advances in the understanding of reactive oxygen species-dependent regulation of seed dormancy, germination, and deterioration in crops. *Frontiers in Plant Science, 13*, 826809. https://doi.org/10.3389/fpls.2022.826809

Liu, S., Yang, J., Zhang, N., & Si, H. (2024). Genome-wide analysis of non-coding RNA reveals the role of a novel miR319c for the tuber dormancy release process in potato. *Horticulture Research, 12*(2), uhae303. https://doi.org/10.1093/hr/uhae303

Marlin, M., Hartal, H., Romeida, A., Herawati, R., & Simarmata, M. (2021). Morphological and flowering characteristics of shallot (*Allium cepa* var. *aggregatum*) in response to gibberellic acid and vernalization. *Emirates Journal of Food and Agriculture, 33*(5), 373–380. https://doi.org/10.9755/ejfa.2021.v33.i5.2697

Moldovan, C., Frumuzachi, O., Babotă, M., Barros, L., Mocan, A., Carradori, S., & Crişan, G. (2022). Therapeutic uses and pharmacological properties of shallot (*Allium ascalonicum*): A systematic review. *Frontiers in Nutrition, 9*, 903686.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i2.2770

https://doi.org/10.3389/fnut.2022.903686

Nurfaida, N., Syam'un, E., Ulfa, F., Mantja, K., & Faried, M. (2024). Breaking dormancy of shallot (*Allium ascalonicum* L.) bulb using hydrogen peroxide. *Jurnal Teknik Pertanian Lampung, 13*(1), 205–212. https://doi.org/10.23960/jtep-l.v13i1.205-212

Oracz, K., & Stawska, M. (2016). Cellular recycling of proteins in seed dormancy alleviation and germination. *Frontiers in Plant Science*, *7*, 1128. https://doi.org/10.3389/fpls.2016.01128

Puccio, G., Crucitti, A., Tiberini, A., Mauceri, A., Taglienti, A., Piccionello, A. P., Carimi, F., van Kaauwen, M., Scholten, O. E., Sunseri, F., Vosman, B., & Mercati, F. (2022). WRKY gene family drives dormancy release in onion bulbs. *Cells*, *11*(7), 1100. https://doi.org/10.3390/cells11071100

Sano, N., & Marion-Poll, A. (2021). ABA metabolism and homeostasis in seed dormancy and germination. *International Journal of Molecular Sciences, 22*(10), 5069. https://doi.org/10.3390/ijms22105069

Shu, K., Liu, X. D., Xie, Q., & He, Z. H. (2016). Two faces of one seed: Hormonal regulation of dormancy and germination. *Molecular Plant, 9*(1), 34–45. https://doi.org/10.1016/j.molp.2015.08.010

Wojtyla, Ł., Lechowska, K., Kubala, S., & Garnczarska, M. (2016). Different modes of hydrogen peroxide action during seed germination. *Frontiers in Plant Science, 7*, Article 66. https://doi.org/10.3389/fpls.2016.00066