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Effect of Pineapple Extract Concentration and Soaking Duration on Germination and Vigor of Seedless Watermelon (*Citrullus lanatus* L.) Seeds

Dwi Rahmawati ^{1*}, Zaki Afifah ²¹ Politeknik Negeri Jember, Indonesia; rahmawati@polije.ac.id² Politeknik Negeri Jember, Indonesia; zakiafifah567@gmail.com* Correspondence: rahmawati@polije.ac.id

Abstract: Seedless watermelon (*Citrullus lanatus* L.) seeds commonly exhibit physical dormancy due to a hard and impermeable seed coat that restricts water uptake and delays germination. Pineapple extract containing the proteolytic enzyme bromelain offers a natural alternative to mechanical cracking by facilitating seed coat softening. This study evaluated the effects of pineapple extract concentration and soaking duration on dormancy breaking and early seed performance of seedless watermelon. A factorial Completely Randomized Design was applied using three extract concentrations (25%, 50%, and 75%) and three soaking durations (5, 10, and 15 minutes). Germination percentage, radicle emergence, and vigor index were measured. The results showed that extract concentration and soaking duration significantly affected germination percentage and vigor index, while their interaction significantly influenced radicle emergence. The highest performance was consistently achieved at a 25% extract concentration with 5 minutes of soaking, resulting in 64.22% germination, 70.67% radicle emergence, and 53.56% vigor index. These findings indicate that moderate enzymatic treatment enhances dormancy breaking by promoting protein hydrolysis and improving early metabolic activation. The novelty of this study lies in identifying the optimal interaction between pineapple extract concentration and soaking duration for improving multiple physiological indicators of seed performance. Pineapple extract can therefore be considered a practical, natural, and environmentally friendly alternative for improving dormancy breaking and early seed performance in seedless watermelon.

Keywords: seed dormancy; pineapple extract; seed vigor

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1. Introduction

Seedless watermelon (*Citrullus lanatus* L.) is a high-value horticultural commodity with increasing market demand due to its desirable fruit quality and consumer convenience. However, its large-scale propagation is constrained by low and non-uniform germination, which reduces seedling establishment and production efficiency. This limitation is primarily due to physical dormancy caused by a hard, impermeable seed coat, which restricts water imbibition and delays the metabolic activation required for germination [2,5].

Conventional dormancy-breaking techniques, such as mechanical scarification, have been widely applied but present practical limitations, including the risk of embryo damage, lack of standardization, and low efficiency for large-scale seed handling [12,13,21]. Consequently, alternative approaches that are safer, more controllable, and environmentally friendly are needed to improve dormancy breaking and early seed performance.

Recent studies have highlighted the potential of enzyme-based seed treatments using natural bioactive compounds. Pineapple (*Ananas comosus*) extract contains bromelain, a proteolytic enzyme capable of hydrolysing structural proteins and facilitating seed coat

weakening, thereby enhancing water uptake and reserve mobilization during germination [3,25]. Bromelain-based treatments have been reported to improve germination and early seedling growth in several crops, indicating their potential as a biological agent for seed enhancement [16,17].

Nevertheless, the effectiveness of enzyme-based treatments is highly dependent on application conditions, particularly extract concentration and soaking duration. Excessive enzyme concentrations or prolonged soaking periods may cause seed tissue damage, respiratory disturbances due to hypoxic conditions, and reduced seed vigor. In contrast, suboptimal treatments may result in insufficient dormancy alleviation [4][22]. Therefore, determining an appropriate combination of extract concentration and soaking duration is a critical factor for treatment success.

However, existing studies predominantly evaluate bromelain or seed priming effects as single-factor treatments and rarely address the combined influence of enzyme concentration and soaking duration. In addition, limited information is available regarding how these factors interact to regulate key physiological indicators such as germination percentage, radicle emergence, and vigor index in seedless watermelon. This gap limits the development of optimized and scalable enzymatic treatments for dormancy breaking.

Therefore, this study aims to evaluate the effects of pineapple extract concentration and soaking duration, as well as their interaction, on dormancy breaking and early seed performance of seedless watermelon. The novelty of this study lies in: (i) the optimization of a biologically based dormancy-breaking approach using bromelain-rich pineapple extract, and (ii) the evaluation of interaction effects between extract concentration and soaking duration on multiple physiological seed parameters.

The findings are expected to provide a practical, environmentally friendly, and scalable alternative for improving seed performance, particularly for application in seed production systems and Teaching Factory (TEFA) units.

2. Materials and Methods

2.1. Study Site and Time

The study was conducted from August to November 2023 at the Laboratory of PT East West Seed Indonesia (EWINDO), Jember, East Java, Indonesia. The experimental site is located at an altitude of approximately 86 m above sea level, with an average temperature ranging from 29 to 32 °C, relative humidity of about 77%, and average monthly rainfall of 210.17 mm during the study period.

2.2. Materials

The materials used in this study included seedless watermelon (*Citrullus lanatus* L.) seeds, ripe pineapple (*Ananas comosus*) fruits, distilled water, germination paper (CD paper), and labelling materials. Pineapple fruits were selected based on full ripeness, indicated by yellow to reddish-brown skin colour, to ensure optimal bromelain enzyme content.

2.3. Preparation of Pineapple Extract

Fresh pineapple flesh was peeled, cut into small pieces, and blended without the addition of water. The resulting slurry was filtered using a fine mesh sieve to obtain crude pineapple extract. The extract was then diluted with distilled water to obtain three concentration levels: 25%, 50%, and 75% (v/v). All extracts were prepared immediately before use to maintain enzymatic activity.

2.4. Experimental Design and Treatments

The experiment was arranged in a factorial Completely Randomized Design (CRD) with two factors. The first factor was pineapple extract concentration, consisting of three levels: 25% (K1), 50% (K2), and 75% (K3). The second factor was soaking duration,

consisting of three levels: 5 minutes (P1), 10 minutes (P2), and 15 minutes (P3). The combination of these factors resulted in nine treatment combinations (K1P1–K3P3). Each treatment was replicated three times, yielding a total of 27 experimental units. Each experimental unit consisted of 25 seeds, resulting in a total of 675 seeds used in the experiment. This sample size was selected to ensure adequate representation and statistical reliability.

2.5. Seed Soaking and Germination Test

Seedless watermelon seeds were soaked in the respective pineapple extract solutions according to the designated soaking durations. After soaking, the seeds were removed and briefly air-dried on sterile tissue paper. Germination tests were conducted using the rolled paper method. Seeds were placed on moist germination paper and incubated in a germinator at a constant temperature of 25 ± 2 °C, under 12-hour light and 12-hour dark photoperiod, for 7 days. Moisture was maintained by adding distilled water as needed to keep the paper uniformly moist throughout the test period.

2.6. Observed Parameters

The parameters observed in this study included germination percentage, radicle emergence, and vigor index.

- Germination percentage was determined based on the proportion of normal seedlings at the final count (day 7).
- Radicle emergence was recorded as the percentage of seeds showing visible radicle protrusion during the early germination phase (day 3).

The vigor index was calculated using the following formula:

$$\text{Vigor Index (\%)} = \frac{\text{Number of normal seedlings at first count}}{\text{Total number of seeds}} \times 100$$

The first count was conducted on day 3 to represent early and uniform seedling emergence, reflecting seed vigor and establishment potential.

2.7. Statistical Analysis

The collected data were analysed using analysis of variance (ANOVA) appropriate for a factorial Completely Randomized Design. When significant differences were detected, mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at significance levels of 1% and 5%. The analysis was conducted to evaluate the effects of pineapple extract concentration, soaking duration, and their interaction on dormancy breaking and early seed performance.

3. Results and Discussion

3.1. Germination Percentage

The analysis of variance indicated that pineapple extract concentration and soaking duration significantly affected germination percentage, while their interaction was not significant. Based on DMRT at the 1% level (Table 1), treatment K1 (25%) produced the highest germination (64.22%), followed by K2 (50%) (54.44%), and K3 (75%) (45.11%). Similarly, soaking for 5 minutes (P1) resulted in higher germination (62.22%) compared to longer durations. These results indicate that moderate extract concentration and shorter soaking duration are optimal for improving germination.

Table 1. Results of DMRT on the effects of pineapple extract concentration (K) and soaking duration (P) on germination percentage.

Treatment	Germination percentage (%)
K3	45.11 a
K2	54.44 ab
K1	64.22 b
P3	43.11 a
P2	58.44 b
P1	62.22 b

Note: Means followed by the same letter within the same column are not significantly different according to DMRT at the 1% level.

The improvement in germination under moderate treatment conditions is associated with the proteolytic activity of bromelain, which partially degrades structural proteins in the seed coat. This process enhances permeability, allowing faster water imbibition and oxygen diffusion, both of which are critical for activating metabolic pathways during early germination [3][19]. In addition, protein hydrolysis contributes to the release of amino acids that support embryo growth and enzymatic activation.

Similar results have been reported in enzymatic seed priming studies, where mild treatments improved germination by enhancing membrane repair and metabolic readiness during imbibition [4][15]. Pérez et al. [16] also demonstrated that bromelain-based priming significantly increased germination performance in pepper seeds through improved protein reserve mobilization.

The decline in germination at higher extract concentrations and longer soaking durations may be attributed to excessive enzymatic activity, which can damage seed tissues and disrupt cellular integrity. Prolonged soaking may also induce hypoxic conditions that limit oxygen availability for respiration, thereby reducing germination efficiency [13][22].

3.2. Radicle Emergence

A significant interaction between pineapple extract concentration and soaking duration was observed for radicle emergence (Table 2). The highest radicle emergence (70.67%) was recorded under K1P1, while the lowest value (27.33%) was observed under K3P3. These findings confirm that the interaction between enzyme concentration and soaking duration plays a critical role in early germination processes.

Table 2. Results of DMRT on the interaction effects of pineapple extract concentration (K) and soaking duration (P) on radicle emergence.

Treatment	Germination percentage (%)
K3P3	27.33 a
K3P2	33.33 ab
K1P3	36.67 abc
K2P3	40.00 bcd
K2P2	42.67 bcde
K1P2	46.00 cde
K3P1	50.00 de
K2P1	54.00 e
K1P1	70.67 f

Note: Means followed by the same letter within the same column are not significantly different according to DMRT at the 5% level.

Radicle emergence is strongly associated with the efficiency of reserve mobilization and cell elongation processes. During germination, protein reserves are mobilized earlier than carbohydrates and lipids, providing essential substrates for respiration and

structural development [25]. Bromelain enhances this process by accelerating proteolysis, thereby increasing the availability of soluble proteins and amino acids required for radicle protrusion.

In seedless watermelon, the hard seed coat imposes a physical constraint on radicle emergence. Bromelain-mediated weakening of seed coat proteins reduces this mechanical resistance, facilitating radicle penetration.

These results are consistent with previous studies reporting that bromelain-based treatments improved radicle emergence and seedling establishment in horticultural crops [16][17]. Wazeer et al. [24] further explained that plant-based biostimulants, including proteolytic enzymes, enhance early metabolic activation and stress tolerance in seeds. In addition, bromelain has been shown to function as a proteolytic enzyme that softens the seed coat and breaks down storage proteins, thereby accelerating germination and radicle protrusion [11][14][17]. Overall, these results support the hypothesis that enzymatic treatments can effectively stimulate early germination when applied under optimal conditions.

3.3. Vigor Index

The analysis showed that pineapple extract concentration and soaking duration significantly affected the vigor index as independent factors, while their interaction was not significant. The highest vigor index was obtained at 25% extract concentration (53.56%) and 5 minutes of soaking (51.33%).

These results indicate that moderate enzymatic treatment improves seed vigor by promoting rapid and uniform germination.

Table 3. Results of DMRT on the effects of pineapple extract concentration (K) and soaking duration (P) on the Vigor index of seedless watermelon seeds.

Treatment	Vigor index (%)
K3	37.33 a
K2	45.56 ab
K1	53.56 b
P3	38.00 a
P2	47.11 ab
P1	51.33 b

Note: Means followed by the same letter within the same column are not significantly different according to DMRT at the 5% level.

Seed vigor is closely related to membrane integrity, enzyme activation, and efficient utilization of stored reserves. Bromelain treatment likely enhances these processes by facilitating protein degradation and improving metabolic efficiency during imbibition [6][9][10]. Improved membrane repair and enzyme activation under optimal conditions contribute to faster seedling establishment.

Paparella et al. [15] reported that priming treatments enhance vigor through improved metabolic synchronization, while Liu et al. [9] highlighted the role of biochemical activation in accelerating germination performance. Similar findings were also reported by Gao and Shi [6], emphasizing the importance of environmental and biochemical factors in regulating seed vigor.

However, the vigor index values obtained in this study were still lower than those of non-dormant seeds. Several studies on seed soaking using natural growth regulators have shown that improvements in seed Vigor do not always reach the performance level of non-dormant seeds, indicating inherent physiological limitations related to seed structure [1][18][23]. This suggests that bromelain treatment alone was insufficient to fully overcome physical dormancy. The limitation may be associated with the relatively low protein content in the seed coat, which restricts the extent of enzymatic hydrolysis. Since bromelain primarily targets protein substrates, its effectiveness depends on

substrate availability and tissue composition [20]. Furthermore, the structural rigidity of the seed coat may require combined treatments (e.g., enzymatic and mechanical approaches) to achieve complete dormancy alleviation.

3.4. Implications for Seed Technology

The results demonstrate that pineapple extract is a promising natural agent for dormancy breaking in seedless watermelon. Compared with conventional mechanical scarification, enzymatic treatment using bromelain provides a safer and more controllable approach, minimizing the risk of embryo damage while improving seed performance. The effectiveness of this treatment is strongly influenced by application conditions, particularly extract concentration and soaking duration. In this study, the optimal treatment (25% pineapple extract with 5 minutes of soaking) consistently produced the best germination and vigor responses, indicating its practical applicability in seed technology. These findings highlight the importance of optimizing enzymatic treatments to achieve maximum efficiency. Further research is recommended to explore combined approaches, such as enzymatic treatment integrated with other priming techniques, as well as detailed biochemical analysis of seed coat composition to better understand and enhance dormancy-breaking mechanisms.

4. Conclusions

This study demonstrates that pineapple extract concentration and soaking duration influence dormancy breaking and early performance of seedless watermelon seeds. Treatment with 25% pineapple extract and a soaking duration of 5 minutes produced the best results in improving germination percentage, radicle emergence, and seed vigor index. The interaction between both factors significantly affected radicle emergence, whereas their effects on the vigor index were independent. Nevertheless, the overall vigor values obtained were still lower than those of non-dormant seeds, indicating that bromelain activity was not sufficient to fully overcome the physical dormancy of seedless watermelon seeds. Practical implication for Teaching Factory (TEFA): Pineapple extract treatment has the potential to be applied as a simple, natural, and environmentally friendly technology during the early stages of seedless watermelon seed production in TEFA units.

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