



Research Article

Improving germination of aged melon seed using garlic oil emulsion

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ABSTRACT

Seed storage is an important aspect of plant breeding. Aged seeds tend to lose their viability during storage, reducing their ability to grow. One effort to increase the viability of seed germination that has been stored for a long period is to provide priming treatment to the seeds before planting. The purpose of this study was to evaluate the effect of garlic oil emulsion on the germination of aged melon seed that has been stored for more than five years. The study was conducted using a factorial completely randomized design (CRD). The first factor consisted of two melon seed lines that had been stored for more than five years, namely Ougan makuwauri (V1) and Ginsen makuwauri (V2), and the second factor was the priming treatment consisting of P0 = Aquadest, P1 = 0.125% garlic oil emulsion, P2 = 0.25% garlic oil emulsion, and P3 = 0.5% garlic oil emulsion, each treatment was repeated three times with a soaking time of 20 hours. There is an interaction between the line and priming treatment that significantly affects the variable of cotyledon opening on the fifth day and hypocotyl length. Meanwhile, the single priming treatment has a significant effect on the observation variable of cotyledon opening on the third day and has a very significant effect on the variable of root length of germinated melon seeds.

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INTRODUCTION

Melon (*Cucumis melo* L.) is a seasonal fruit plant that is widely cultivated in Indonesia by farmers because it has high economic value and is quite popular with the community. Sustainable melon production requires a supply of high-quality seeds that are always available, obtained from seed production activities through plant breeding programs.

Seed storage is an important aspect of the world of agriculture, especially in the field of plant breeding. Seed storage activities will greatly determine the success of maintaining genetic diversity so that it can ensure the availability of genetic sources for agriculture in the future. The shelf life of melon seeds, which are included in the orthodox seed type, can be increased by reducing the water content of melon seeds and lowering the temperature during seed storage (Solberg et al., 2020). Non-ideal storage conditions are a major problem that can cause physiological degradation of seeds. Seeds tend to experience an aging process that causes loss of viability and reduces their ability to grow. This challenge is in line with seeds from plant breeding that usually undergo long-term storage (more than five years). The melon seeds tested in this study were oriental melon seeds from plant breeding that had been stored for more than five years. Based on these problems, innovation is needed to handle seeds from breeding that have been stored for a long time.

Priming is one of the efforts that can be done to increase the viability and germination of seeds even though they have been stored for a long time. This is possible because priming will help reduce the physiological non-uniformity of the seeds, thereby accelerating and synchronizing the germination process (Bouriou et al., 2020). Efficient seed germination will encourage the successful formation and deep root system of plants so it is very important to support the ability of plants to absorb water and nutrients and increase the stability of plant growth (Holz et al., 2024). Several studies related to melon seed priming that have been carried out, namely, osmopriming with PEG 6000 and halopriming treatment (NaCl, KNO₃, H₂PO₄, CaCl) have been shown to increase the percentage value of honeydew melon seed germination, in addition, priming of melon seeds (*Cucumis melo*) with potassium nitrate can modulate the development and early growth of seeds (Castañares & Bouzo, 2018; Mutetwa et al., 2023). The application of salicylic acid and gibberellin as priming agents aims to reduce the negative impact of water salinity and modulate the growth process, thereby increasing the vigor of melon seedlings (Silva et al., 2024).

Bioactive compounds found in garlic include *diallyl disulfide*, *diallyl trisulfide*, *allicin*, *ajoene*, and *S-allyl-cysteine*, which act as antioxidants, antibacterials, antifungals, and anti-inflammatories (Szychowski et al., 2018). Due to the presence of these organic compounds in garlic, previous studies have focused more on the potential of garlic as a natural biofungicide and post-harvest applications (Sarraz et al., 2020). Based on the report by Dahab et al. (2018), garlic (*Allium sativum* L.) contains higher phytohormones than shallots, namely IAA of (0.0312 mg 100 g⁻¹), ABA (0.3138 mg 100 g⁻¹), GA₃ (2.719 mg 100 g⁻¹), and zeatin (0.0149 mg 100 g⁻¹).

There are few studies examining the application of garlic as a seed priming agent. Several studies have reported that the application of garlic extract can improve certain physiological aspects of the recipient plants. The impact of the application of garlic extract will vary, depending on the level of concentration given and the type of plant (Adeleke, 2016). The use of garlic extract on tomato and cucumber commodities showed a significant effect on the growth of the tomato and cucumber plants tested (Hayat et al., 2018). Meanwhile, the application of garlic extract at a concentration of 20% had the effect of inhibiting seed germination and the growth of rice seedlings (Paiman & Hendrawan, 2023).

Based on the reports presented above, the application of garlic preparations has the potential as a priming agent for seeds that have been stored for a long time. Although several studies have addressed the priming effect of garlic extracts, the use of garlic oil emulsion as a priming agent is still very limited, especially for melon seeds that have been subjected to long-term storage. Highlighting the challenges of seed deterioration during long-term storage, investigating the potential of garlic oil emulsion as a priming agent is expected to contribute insights into improving seedling vigor and viability. Various concentrations of garlic oil emulsion were used to evaluate the effect of garlic oil emulsion on the germination of aged melon seed that has been stored for more than five years.

MATERIALS AND METHODS

Research location and experimental design

This research was conducted at Plant Laboratory 1, Food Crops Cultivation Department, Lampung State Polytechnic, Bandar Lampung City, Lampung Province, Indonesia. The research was conducted from early March to June 2024.

This experiment was arranged using a factorial completely randomized design. The first factor consisted of two seed lines, namely Ougan makuwauri (V1) and Ginsen makuwauri (V2), and the second factor was the priming treatment consisting of P0 = priming with distilled water as a control, P1 = priming with 0.125% garlic oil emulsion, P2 = 0.25% garlic oil emulsion, and P3 = 0.5% garlic oil emulsion, each treatment was repeated three times so that there were 24 experimental units.

Preparation of garlic oil emulsion

The formation of garlic oil emulsion was made using a low-energy method with an inversion phase approach (Nuryanti et al., 2023). A 100% pure garlic essential oil (produced by Distiler, Indonesia) and tween 80 as a surfactant were mixed in a ratio (1:1; v:v) until homogeneous using a magnetic stirrer. The formation of garlic oil emulsion was carried out by slowly dripping aquadest at a drip rate of 4 mL min⁻¹ and stirring continuously using a magnetic stirrer at a speed of 600 rpm for 2 hours at room temperature. The emulsion that had been made at this stage was referred to as a 1% concentration garlic oil emulsion solution as stock solution. By using the dilution process, priming concentrations of garlic oil of 0.5%, 0.25%, and 0.125% were made.

Seed source and priming procedure

The seeds used in this study were the result of plant breeding carried out in a greenhouse using a net vine planting system and bracket pots (Wahyudi et al., 2022). The two inbred melon seed lines, namely, Ougan makuwauri (V1) and Ginsen makuwauri (V2), have been stored in cold storage for 5 years at 3-5 °C with a seed moisture content of 7%.

The priming procedure followed the protocol Alam et al. (2022) with slight modifications, i.e., the melon seeds were soaked in a prepared solution for 20 hours at room temperature. The ratio of seed weight to priming solution volume was 1:5 (g mL⁻¹). After soaking for 20 hours, the seeds were then rinsed using distilled water and then drained. Each melon seed that had been treated was germinated on rice straw paper and placed in a plastic container of 750 mL (Length: 16.5 cm, width: 11 cm, height: 5.5 cm).

Observation

The observed characteristics of melon germination include seed germination ability (%), root length (cm), hypocotyl length (cm), and the number of opened cotyledons observed on the third and fifth days after sowing the seeds. The percentage of melon seed germination was observed after seven days of sowing, the results were calculated as a percentage of the number of seeds that germinated in the plastic container. The calculation of the percentage of germination was carried out using the following formula:

$$\text{Germination \%} = \frac{\sum \text{Normal seedlings at final count}}{\sum \text{Seed tested}} \times 100\%$$

Hypocotyl and root length data collection was carried out on the 7th day after priming treatment. Melon hypocotyls were measured in centimeters using a ruler from the base of the root to the end point of hypocotyl growth. Each replication was taken as many as 5 normal sprout samples randomly.

The length of the melon roots that had germinated on the 7th day was measured with a ruler, measurements were taken at the part between the top of the primary root to the base of the hypocotyl. The cotyledons will open after the melon seed coat begins to break and then comes off. Observation of the opening of the cotyledons was carried out by recording the number of cotyledons that had opened on the third and fifth days. Observations were made by visual check at both parts of the melon seed cotyledons that had opened completely and were seen moving away from the hypocotyl. Seedlings with two cotyledons open at an angle >45° are classified as open (Lin et al., 1998).

Data analysis

The data obtained were analyzed using the 5% F test, then continued with the LSD test at the 5% level. The software used for data analysis was SPSS version 16.0.

RESULTS AND DISCUSSION

Based on the data presented in Table 1, the application of garlic oil emulsion as a priming treatment significantly affected all observed variables, except for melon seed germination. In this study, the performance of melon seed germination (Figure 1) showed a better germination effect from all priming treatments using garlic oil emulsion when it

was compared to the performance of the control treatment (aquadest). The interaction between line and priming showed a significant effect on the emergence of cotyledons on the 5th day and the length of the hypocotyl.

Table 1. Summary of the analysis of the effects of priming treatment and its interaction on melon seed viability.

Observation parameters	Line (V)	Priming treatment (P)	VxP
Germination percentage	ns	ns	ns
Root length	ns	**	ns
Hypocotyl length	ns	**	*
Cotyledon emergence day 3	ns	*	ns
Cotyledon emergence day 5	*	**	**

Note: * = significant effect at the 5%, ** = significant effect at the 1%, ns = no significant effect.

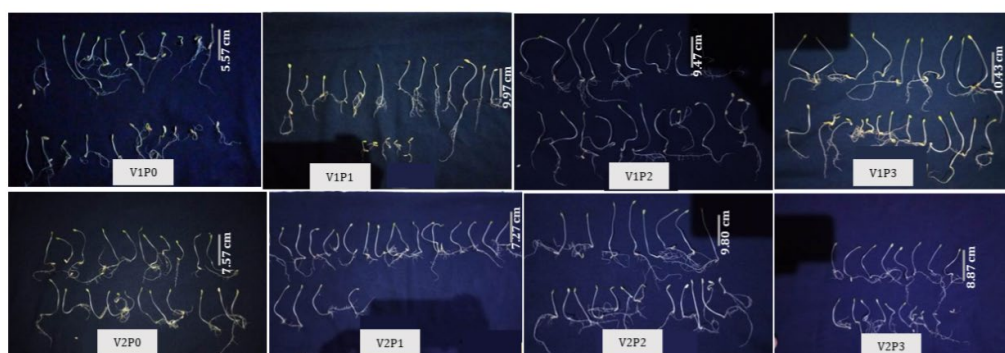


Figure 1. Performance of priming seeds (hypocotyl length): V1P0= Ougan makuwauri with aquadest, V1P1 = Ougan makuwauri with 0.125% emulsion, V1P2 = Ougan makuwauri with 0.25% emulsion, V1P3=Ougan makuwauri with 0.5% emulsion, V2P0 = Ginsen makuwauri with aquadest, V2P1 = Ginsen makuwauri with 0.125% emulsion, V2P2 = Ginsen makuwauri with 0.25% emulsion, V2P3 = Ginsen makuwauri with 0.5% emulsion.

Germination percentage

High-quality seeds meet three main aspects, namely, high physical, genetic, and physiological quality (Costa et al., 2021). Based on the observation results, the seed viability of the two inbred melon lines tested showed no significant difference. Even though they had been stored for 5 years, the germination rate remained high with a germination rate of 92% in the control treatment (Table 2). This indicates that melon seeds still have very good physiological abilities after long-term storage at 3-5 °C, and keeping the seed moisture content at 7%. The level of loss of vigor and viability in orthodox seeds during storage is mainly influenced by temperature and seed moisture content, where the lower the storage temperature and initial moisture content, the longer the shelf life (Corbineau, 2024). The present study strengthens the previous finding that storing seeds at low temperatures will help slow down the rate of seed respiration compared to seeds stored at high temperatures (> 25 °C) (Mahlangu et al., 2024). Low storage temperatures reduce seed metabolic activity, thereby extending shelf life and maintaining the viability of the seeds (El-Maarouf-Bouteau, 2022). Low temperatures and low seed water content will help limit the cellular metabolic activity of seeds by forming an intracellular glass layer, thus supporting long-term seed viability (Sano et al., 2016).

Table 2. Seed germination from different melon varieties and priming solutions.

Treatment	Seed germination (%)
V1P0	92
V2P0	97
V1P1	100
V2P1	97
V1P2	95
V2P2	95
V1P3	97
V2P3	99

Note: ns = not significant; V1P0= Ougan Makuwauri with aquadest, V1P1 = Ougan makuwauri with 0.125% emulsion, V1P2 = Ougan makuwauri with 0.25% emulsion, V1P3 = Ougan makuwauri with 0.5% emulsion, V2P0 = Ginsen makuwauri with aquadest, V2P1 = Ginsen makuwauri with 0.125% emulsion, V2P2 = Ginsen makuwauri with 0.25% emulsion, V2P3 = Ginsen makuwauri with 0.5% emulsion.

Root length

The results of the analysis of variance of priming treatments using garlic oil emulsion at various concentration levels had a significant effect on the root length (Table 3). Melon seeds that were given priming treatment with garlic oil emulsion showed higher values as compared to the control treatment. Priming treatment with garlic oil emulsion at a concentration of 0.5% gave the best results with an average root length of 10.3 cm.

Table 3. Effect of priming on root length parameters.

Priming treatment	Root length (cm)
Aquades/control (P0)	7.6a
Garlic oil emulsion 0.125% (P1)	9.5b
Garlic oil emulsion 0.25% (P2)	9.3b
Garlic oil emulsion 0.5% (P3)	10.3b

Note: Numbers followed by the same letter in the same column are not significantly different in the 5% LSD test.

Seed metabolism will be active when the imbibition process occurs, where good metabolism will help the seeds to continue the root formation phase (Qu et al., 2019). A study by Pompelli et al. (2023) verified that the seed imbibition process will be greatly influenced by the different osmotic potentials between the seeds and the surrounding media, in addition to other factors such as seed hardness, seed weight, and contact surface also have an important role in seed water absorption. The report by Arthawijaya et al. (2022) states that inappropriate invigoration treatment with polyethylene glycol causes the amount of water absorbed during the imbibition process to be limited so that it can slow down the process of enzyme activity and metabolism for root growth of a plant. In addition, Zhang et al. (2022) state that the formation of seed roots is closely related to the contribution of the auxin hormone. Organic compounds obtained from garlic essential oils are thought to have a similar role to auxin so that they can stimulate the elongation of melon seedling roots.

The effect of administering *diallyl disulfide* (DADS) compounds from garlic at low concentrations (0.01-0.62 mM) increases root length and mitotic activity, while at higher concentrations it will inhibit root growth by affecting the length and division activity of meristematic cells (Cheng et al., 2016). This explains why the treatment of garlic oil emulsion at a concentration of 0.5% gave the best results because at this concentration, the active compounds in garlic work optimally in stimulating root growth. This finding is in line with the results of a study by Ali et al. (2019) which reported that administering various concentrations of garlic affected the germination process and early growth of seedlings, including root length. Another study by Ahmed (2023) also showed that priming with garlic extract was shown to increase the germination rate and seedling vigor, including root length in corn seeds. Administration of garlic extract also had a positive

effect, namely being able to increase the root length of bougainvillea cuttings (Meidodga et al., 2021).

Hypocotyl length

Application of garlic oil emulsion stimulated higher hypocotyl length of melon seeds than the control (Table 4). The interaction between lines and priming treatments showed significant variations in growth responses. V1 (Ougan makuwauri) showed a higher response to priming treatment, especially at a concentration of 0.5% (P3), with a hypocotyl length reaching 10.43 cm, longer than V2 (Ginsen makuwauri) 8.87 cm. This was also seen in the P1 treatment, where the hypocotyl length of V1 reached 9.97 cm, higher than V2 which only reached 7.27 cm. The treatment of priming with garlic oil at a concentration of 0.25% showed no interaction.

Table 4. Interaction between line and priming treatment on hypocotyl length.

Line (V)	Hypocotyl length (cm)			
	P0	P1	P2	P3
V1 (Ougan makuwauri)	5.57aA	9.97bB	9.47aB	10.43bB
V2 (Ginsen makuwauri)	7.57bA	7.27aA	9.80aB	8.87aB

Note: P0 = control, P1 = Priming at 0.125%, P2 = 0.25%, P3 = 0.5%; numbers followed by the same lowercase letter in the same row are not significantly different in the 5% LSD test; numbers followed by the same uppercase letter in the same column are not significantly different in the 5% LSD test.

The present study indicates that priming treatment with garlic oil emulsion at various concentrations has varying effects on the hypocotyl length of the two oriental melon genotypes tested (Table 4). The difference in hypocotyl length is due to genetic differences between the two oriental melon genotypes. This is in accordance with Sadjad's statement (1993), that genetic vigor will greatly determine the difference in seed vigor between varieties. Oriental melon seeds V1 (Ougan makuwauri) are thought to have better genetic and physiological abilities in maximizing the response to priming treatment to increase imbibition efficiency and nutrient mobilization capabilities during the germination process. On the other hand, when viewed from the control data (P0) genotype V2 (Ginsen makuwauri) has a stronger initial growth potential but does not show a significant increase in response after being given priming treatment when compared to V1 (Ougan makuwauri). The difference in response is an indication that V2 (Ginsen makuwauri) has reached its maximum growth capacity without the need for additional treatment, or that priming treatment at concentration levels of 0.5% and 0.125% is less appropriate to be applied to these genotypes. This statement of results is in line with the results of previous studies which stated that the effectiveness of a priming material can produce a response that varies greatly according to the genotype of the plant seeds tested (Ekren et al., 2024). The compound *diallyl disulfide* (DADS) from garlic is known to affect the balance of hormones such as IAA (*Indole-3-Acetic Acid*) and GA (*Giberellin*), which are related to cell growth and shoot elongation (Cheng et al., 2016). The administration of garlic extract in previous studies was reported to increase shoot length, root length, and wet and dry weight in corn seedlings under salt stress (Kousar et al., 2023).

Cotyledon opening

Data on the opening of the cotyledons on the third day (Table 5) showed that the priming treatment at a concentration of 0.25% (P2) gave the best response. The significant increase in cotyledon opening is in line with research by Hayashi et al. (2022) which reported that the average opening of cotyledons in lettuce seedlings (*Lactuca sativa* L. var. Longifolia) given nutrients was faster, namely 66.2 hours, while seedlings that were only given water were 68 hours. In kalak seeds (*Mitrephora polypyrene*), the opening of the seed cotyledons was grouped in the fourth phase (Handayani, 2021). The faster cotyledon opening response can also help seedlings cope with suboptimal environmental conditions. Priming with brassinosteroid hormones (EBT) can significantly accelerate cotyledon opening, thereby increasing the chances of plant survival (Chakma et al., 2021).

Table 5. Opening of cotyledons on the third day of observation.

Priming treatment	Cotyledon opening on the third day (%)
Aquades/control (P0)	0.3a
Garlic oil emulsion 0.125% (P1)	4.8ab
Garlic oil emulsion 0.25% (P2)	5.3b
Garlic oil emulsion 0.5% (P3)	3.0b

Note: Numbers followed by the same lowercase letter in the same column are not significantly different in the 5% LSD test.

Observation data on the fifth day (Table 6), showed an interaction between the line and priming treatment on the opening of the cotyledons. In V1 (Ougan Makuwauri), priming treatment with a concentration of 0.5% (P3) produced the highest cotyledon opening (24%), significantly different from the control (P0). Likewise, at concentrations of 0.25% (P2) and 0.125% (P1), V1 showed consistently better results than the control. In contrast, in line 2 (Ginsen Makuwauri), although there was an increase in P1 (0.125%) and P2 (0.25%) compared to the control, the responses shown were much more varied. In the P3 treatment, there was no significant difference compared to the control, indicating that V2 was less responsive to higher priming concentrations compared to V1. However, at lower concentrations such as the P2 treatment, V2 (Ginsen makuwauri) showed an increase, although not as good as V1 (Ougan Makuwauri). This indicates a difference in sensitivity between lines to priming treatment. Line V1 showed more responsiveness to various priming concentrations, resulting in faster and more even cotyledon opening.

Table 6. Interaction between line and priming treatment on cotyledon opening on the fifth day (%).

Line (V)	Cotyledone opening on fifth day (%)			
	P0	P1	P2	P3
V1 (Ougan makuwauri)	10.00aA	23.67bB	21.33bB	24.00bB
V2 (Ginsen makuwauri)	9.00aA	10.33aC	15.00aB	8.33aA

Note: P0 = control, P1 = Priming at 0.125%, P2 = 0.25%, P3 = 0.5%; numbers followed by the same lowercase letter in the same row are not significantly different in the 5% LSD test; numbers followed by the same uppercase letter in the same column are not significantly different in the 5% LSD test.

The opening of cotyledons is one of the important seed vigor parameters. Cotyledons play an important role in the early growth of plants. The role of cotyledons includes providing food during the germination process until the formation of real leaves to be able to start photosynthesis and distributing the energy stored in the seed to the growing parts, namely the plumula and roots (Cao et al., 2022). Based on a research report by Yang et al. (2020), the opening of cotyledons in *A. membranaceus* plants is influenced by several external factors including light, where there are rapid metabolic changes during the opening stage and the greening of the cotyledons. Unopened cotyledons are thought to be caused by the absence or undeveloped of one of the cotyledons of a plant which will then cause the role of the cotyledon as a prephotosynthesis to be inactive or not yet active (Haryanti & Budihastuti, 2015).

CONCLUSIONS

The findings in this study suggest that garlic oil emulsion can be a useful priming agent to improve seed vigor and germination yield in melon seeds, which highlights the importance of optimizing priming treatments in seed management practices.

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