

The Dormancy of Natural and Unnatural Aging in Pistacia khinjuk Stocks Seeds

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Abstract

The concept of physiological maturity refers to the period during which seeds reach their maximum germination capacity. Accurately determining this stage facilitates efficient seedling production and helps balance supply and demand. Although seed storage conditions and duration are known to affect physiological dormancy, obtaining the desired number of rootstocks from *Pistacia khinjuk* Stocks (buttum) remains constrained by currently low germination rates. In this study, we assessed the effects of natural (25°C) and unnatural (4°C) aging storage conditions on physiological dormancy by evaluating the germination of newly harvested seeds alongside seeds stored for 1, 2, 3, and 4 years at 4°C and 25°C. To elucidate the underlying mechanisms, germination data were correlated with endosperm starch content, β -amylase activity, and the levels of abscisic acid (ABA) and gibberellic acid (GA₃) in the embryo. Our results indicated that the highest germination rate (81%) was achieved in newly harvested seeds without any dormancy-breaking pretreatment. Prolonged storage at 25°C over 4 years led to a significant decrease in endosperm starch content (from 56 to 29.71 mg/g) and a concurrent increase in β -amylase activity (from 10.95 to 26.86 EU/g). Furthermore, the ABA/GA₃ ratio dropped remarkably from 5.05 ng/g in newly harvested seeds to 0.89 ng/g in 3-year-old seeds. Storage temperature did not significantly affect germination rates. Interestingly, although the enzymatic and hormonal shifts observed during storage typically favor dormancy release, germination rates did not increase. These findings suggest that newly harvested *P. khinjuk* seeds do not exhibit deep physiological dormancy, and that natural aging, despite inducing favorable biochemical changes, does not enhance germinability.

Introduction

Adaptations that enable survival during periods when adverse environmental factors reach extreme levels for living organisms are critical for the continuation of species. "Dormancy," a behavior when vital functions are reduced to minimum levels, ensures avoiding extreme environmental factors. Dormancy refers to a natural state, which occurs in all life forms in the living world and in which development is stopped (Willis et al., 2014). Seed dormancy expresses a period during which development stops and many mature seeds do not germinate despite favorable conditions (Einali & Valizadeh, 2017). Seed germination requires the removal of physiological, morphological or physical obstacles related to the embryo or the tissues that cover the embryo (Finch-Savage & Leubner Metzger, 2006).

Primary and secondary dormancy cycles that originate from physiological barriers within the seed are usually controlled by the synthesis and signaling of key hormones, such as gibberellin, ethylene, cytokinin, and abscisic acid (Baskin & Baskin, 2004). While gibberellin, cytokinin, and ethylene stimulate seed germination, abscisic acid inhibits germination and stimulates dormancy mechanisms (Bewley et al., 2013). Furthermore, diverse environmental factors such as light, oxygen, moisture, and temperature regulate these processes. After moisture, temperature is the most critical limiting factor affecting seed germination. Although the intrinsic regulatory components in dormant seeds have not been completely elucidated, the impacts of temperature on the induction and elimination of dormancy in seeds are definitively accepted (Finch-Savage & Leubner-Metzger, 2006). Seeds maintain metabolic activity

throughout the period of storage and energy requirements are met through cellular respiration (Chidananda et al., 2014). This prolonged metabolic activity leads to reserve depletion, significantly reducing soluble carbohydrates and thus depleting the substrates essential for respiration during eventual germination (Sharma et al., 2007). In endosperm seeds, the seed's energy source is produced from starch by α -amylase into soluble oligosaccharides, which are then hydrolyzed by β -amylase to release maltose (Nomura et al., 1969). Finally, α -glucosidase breaks down maltose into glucose. It is known that β -amylase plays an active role in the seed's life cycle during this dormant period, when metabolic activity is minimized, thus helping the organism conserve energy (Farashah et al., 2011)

Seed senescence represents a natural and irreversible process that gradually decreases germination potential and degrades seed quality, finally leading to the death of all seeds (Pérez-García et al., 2008). The impact of seed age on germination success varies depending on diverse factors, including plant species, seed storage conditions, physiological characteristics and dormancy status. Seeds generally tend to lose their viability and their germination rates decrease with aging. This process is defined as "seed senescence" or "physiological deterioration" and is characterized by a series of biochemical changes, e.g., slowing of metabolic activity, inactivation of enzymes, and deterioration in DNA and protein structures (McDonald, 1999; Walters et al., 2010).

In agricultural practice, germination and rooting problems in pistachio rootstock seeds present considerable obstacles to efficient propagation (Rahemi & Baninasab, 2000). The impermeable and hard seed coats of wild *Pistacia* species may prevent or delay germination. To overcome these physical barriers, mechanical and chemical scarification, hot water immersion, and chilling treatments are commonly utilized (Hashim, 2018). However, while such coat-breaking methods address physical dormancy, successful germination can only be achieved if physiological dormancy is simultaneously broken. Previous germination experiments testing dormancy-breaking methods on newly harvested seeds of *Pistacia khinjuk*, *Pistacia mutica* Fisch. & C.A.Mey., *Pistacia vera* L., *Pistacia terebinthus* L., and *Pistacia atlantica* Desf. have largely focused on physical barriers, while physiological dormancy has frequently been overlooked (Baninasab & Rahemi, 2001; Hashim, 2018).

To optimize seedling production, it is essential to determine the precise conditions under which seeds germinate uniformly and at high rates. Despite significant progress, especially in the fields of genetic control, molecular mechanisms and physiological and environmental factors that impact germination and dormancy, numerous issues remain unclear (Nautiyal et al., 2023). Therefore, the primary objective of the current study is to determine the age-dependent physiological dormancy levels and overall germination potential of *P. khinjuk* seeds. By comparing germination rates, starch utilization, β -amylase activity, and the ABA and GA3 contents of newly harvested, 1-, 2-, 3-, and 4-year-old *P. khinjuk* seeds, this study aims to elucidate the impact of natural seed aging on physiological activity and dormancy release.

Material and methods

Plant Material and Storage Conditions

Pistacia khinjuk Stocks seeds were randomly harvested in October of 2019, 2020, 2021, 2022, and 2023 from trees located in Çimenli village private land Hakkari province, Türkiye (37°29'06" N latitude, 43°37'57" E longitude; altitude 1144 m), with permission obtained from the garden owner. Based on the experimental timeline, the harvest years corresponded to 4-year-old (2019), 3-year-old (2020), 2-year-old (2021) and 1-year-old (2022) seeds. These aged seeds were stored either at 4°C in a refrigerator or at 25°C in a greenhouse. Newly harvested seeds (2023) were stored in a greenhouse for approximately five days prior to the experiments. To facilitate the removal of the pericarp, all seeds were soaked in tap water for 24 hours. Subsequently, germination assays and biochemical analyses were conducted. Germination assays were performed using three replicates of 100 seeds each, while all biochemical analyses were carried out in triplicate. The seeds were not subjected to any dormancy-breaking treatments prior to germination. For the germination assay, seeds were placed in 11 × 11 × 4 cm aluminum containers on a moist cotton medium and kept in the dark. They were irrigated with 100 mL of tap water every other day for 45 days.

Methods

Seed Viability Assessment via Triphenyl Tetrazolium Chloride (TZ) Test

Seed viability test in 1% 2,3,5 triphenyl tetrazolium chloride (TZ) Assay (Verma and Majee 2013) method was modified to assess the viability of *P. khinjuk* seeds. As a negative control, approximately 100 seeds were devitalized by heat treatment (100°C for 1 hour) in a drying oven. To prepare 1% Tetrazolium (TZ) solution, added 1 g 2,3,5 triphenyl tetrazolium chloride in 100 ml autoclaved distilled water and dissolved. The pH of TZ was adjusted 6. Firstly, 100 seeds were incubated in distilled water for hydration 30°C for 24 h. Secondly the seeds were sterilized with 20% 100 ml commercial sodium hypochlorite for 10 minutes than washed at least three times with sterile distilled water to remove sodium hypochlorite. After sterilisation, excess water was removed and the seeds incubated with 1% TZ solution at 30°C for 24 h in dark.

Starch extraction and analysis

Using 0.1 M phosphate buffer (pH 7.5) at 4°C, endosperm tissues were extracted from each seed and homogenized in a cooled mortar. The pellet that resulted from centrifuging the homogenate at 12,000 × g for 15 minutes was then collected for starch analysis. After that, a 4:1 volume ratio of dimethyl sulfoxide and 8 M hydrochloric acid was used to dissolve this particle. The samples were continuously shaken at 60 rpm while being incubated at 60°C for 30 minutes in order to aid in starch dissolution. Following a second centrifugation step at 12,000 × g for 15 minutes, 100 µl of the resulting supernatant was combined with 1 ml of distilled water and 100 µl of iodine–HCl reagent (0.06% KI and 0.003% I₂

produced in 0.05 M HCl). The absorbance was measured at 600 nm using the method described by Barka et al. (2006) after the reaction mixture was allowed to sit at room temperature for 15 minutes.

Determination of β -Amylase Enzyme Activity

The Betamyl-3® assay kit (Megazyme International Ireland Ltd., Bray, Ireland) was used to measure β -amylase activity in the endosperm of *Pistacia khinjuk* seeds, using the ICC (1998) procedure with minor adjustments. In order to extract the enzymes, 5 mL of extraction buffer comprising 1 M Tris–HCl (pH 8.0), 20 mM EDTA, 0.02% (w/v) sodium azide, and 100 mM cysteine–HCl was combined with 0.5 g of finely powdered endosperm tissue. After allowing the combination to stand for 60 minutes to guarantee effective enzyme extraction, insoluble debris was removed by centrifuging the liquid. The enzyme extract was made from the supernatant that was left behind after centrifugation. A pre-equilibrated chromogenic substrate solution containing p-nitrophenol- β -D-maltotriose (PNP- β -G3) was mixed with 0.2 mL of the extract to measure enzyme activity. After 10 minutes of incubation at 40°C, the reaction mixture was stopped by adding 3 mL of 1% Tris solution (pH 8.5). Spectrophotometric monitoring of p-nitrophenol release was conducted at 400 nm. One unit (U) represents the amount of enzyme needed to release 1 μ mol of p-nitrophenol per minute at 40°C under the specified test conditions. Enzyme activity was quantified in terms of the amount of p-nitrophenol produced

Phytohormone Extraction and Analysis

For phytohormone extraction, 1 g of embryo tissue was finely ground in chilled methanol. The homogenate was maintained at 4°C for 24 h in darkness with continuous agitation using an orbital shaker to ensure efficient extraction. After incubation, the suspension was filtered through Whatman No. 1 filter paper and the liquid phase was collected. The remaining solid material was subjected to a second extraction under identical conditions, and both filtrates were combined to maximize hormone recovery. The pooled extract was clarified by passing it through 0.45 μ m PTFE membrane filters. Methanol was subsequently evaporated under reduced pressure at 35°C. The dry residue was reconstituted in 0.1 M phosphate buffer (pH 8.5) and centrifuged at 10,000 rpm for 60 min at 4°C to remove insoluble particles. The supernatant was treated with 1 g polyvinylpyrrolidone (PVPP) to eliminate interfering phenolic compounds, followed by filtration through Whatman No. 1 paper. The purified solution was then applied to Sep-Pak C18 solid-phase extraction cartridges. After cartridge loading, retained hormones were eluted with 80% (v/v) methanol and collected for analysis. High-performance liquid chromatography (HPLC) was employed for hormone quantification. Prior to sample analysis, calibration curves were generated using different concentrations of commercial standards of gibberellic acid (GA₃) and abscisic acid (ABA). Subsequently, embryo extracts of *Pistacia khinjuk* were injected into the HPLC system, and detection was carried out at 245 nm. Hormone levels were calculated by comparing sample peak areas with those derived from the standard calibration curves (Kuraishi et al. 1998; Battal and Tileklioğlu, 2001).

Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA) to evaluate treatment effects. When significant differences were detected, means were compared using Duncan’s multiple range test as a post hoc procedure. Differences were considered statistically significant at a probability level of $P \leq 0.05$. Results are presented as mean values accompanied by their standard errors (SE). All statistical computations were carried out using SPSS software (version 16.0 for Windows).

Results

Tetrazolium Test

No staining was observed in the control group; thus all seeds in this said group were considered dead. The formation of a distinct reddish-pink color was observed in the embryo tissues of the other groups and the seeds were determined to be viable to a significant extent according to AOSA (2005) data (Fig. 1). Consistent with the findings of Ersali (2024), higher viability was detected in our seeds stored at 25°C for 3 years, while the lowest viability rates were observed in newly harvested seeds and those stored at 4°C. It is estimated that these results were obtained due to contamination caused by microorganism activity during germination. Thus, França-Neto and Krzyzanowski (2022) and Ersali (2024) stated that germination rates under the TZ test and natural germination conditions during storage and germination might yield results inconsistent with the test.

Germination

Newly harvested (0-year-old) seeds had the highest germination rate (81%). For the stored seeds (1, 2, 3, and 4 years old), storage temperature did not significantly affect germination rates. Although germination rates were higher in 3-year-old seeds than in 1- and 2-year-old seeds this result appears to be statistically insignificant (Table 1). No significant difference was identified in germination rates between 1- and 2-year-old seeds (Table 1). Storage temperature (4°C and 25°C) and year (1, 2, 3, and 4 years) did not affect the germination rate in *P. khinjak* seeds substantially.

Table 1 The germination of *P. khinjak* seeds in storage time and temperature

Storage Time (Year)	Germination Rate (%) [*]
New Harvest	81 ± 5.1 ^a
4°C	
1	41 ± 1.55 ^b
2	42 ± 2.00 ^b
3	52 ± 3.18 ^b
4	45 ± 2.05 ^b
25°C	
1	45 ± 2.40 ^b
2	40 ± 1.43 ^c
3	51 ± 2.26 ^b
4	44 ± 1.70 ^b

^{*}The means ± standard deviation (n = 3) are used to express the results. According to Duncan's multiple range test, different letters denote significant differences ($p \leq 0.05$) between samples of *P. khinjak* seeds.

Starch content in *P. khinjak* Endosperm

The highest starch content (56 mg/g) was determined in newly harvested seeds, while the lowest (29 mg/g) was found in 4-year-old seeds stored at 25°C. Seeds stored at 4°C had higher starch values, which were close to those of newly harvested seeds (Table 2). With an increase in storage time, a decrease in starch content varies significantly by year. Since starch content in the endosperm decreases faster at 25°C, storage at 4°C is more advantageous. It can be stated that starch values gradually decrease over the years.

Table 2
Starch content in *P. khinjuk* endosperm

Storage Time (Year)	Starch Content (mg/g)*
New Harvest	56 ± 4.10 ^a
4°C	
1	54.63 ± 3.12 ^a
2	55.25 ± 4.05 ^a
3	51.20 ± 3.25 ^b
4	48.52 ± 2.15 ^b
25°C	
1	46.52 ± 2.10 ^b
2	37.45 ± 1.82 ^c
3	35.75 ± 1.92 ^c
4	29.71 ± 1.70 ^c

*The means ± standard deviation (n = 3) are used to express the results. According to Duncan's multiple range test, different letters denote significant differences ($p \leq 0.05$) between samples of *P. khinjuk* seeds.

β -Amylase Enzyme Activity Content in *P. khinjuk* Endosperm

The highest β-amylase enzyme activity (26.86 EU/g) was in 4-year-old seeds stored at 25°C, whereas the lowest enzyme activity (10.95 EU/g) was determined in newly harvested seeds. Across all storage durations, β-amylase activity was consistently higher in seeds stored at the elevated temperature of 25°C (Table 3). Overall, storing *P. khinjuk* seeds at this higher temperature led to a clear, duration-dependent increase in enzyme activity. Conversely, when seeds were stored at a lower temperature (4°C), year-over-year differences in enzyme activity were not statistically significant; however, a gradual upward trend was still observed as storage time increased.

Table 3
-amylase activity in *P. khinjuk* endosperm

β	
Storage Time (Year)	β -Amylase Activity (EU/g)*
New Harvest	10.95 $\pm$ 0.5 ^c
4°C	
1	11.21 $\pm$ 0.30 ^c
2	12.90 $\pm$ 0.53 ^c
3	15.01 $\pm$ 0.85 ^b
4	16.45 $\pm$ 0.92 ^b
25°C	
1	14.12 $\pm$ 0.75 ^b
2	19.72 $\pm$ 1.12 ^a
3	21.49 $\pm$ 1.54 ^a
4	26.86 $\pm$ 1.50 ^a

*The means $\pm$ standard deviation (n = 3) are used to express the results. According to Duncan's multiple range test, different letters denote significant differences ($p \leq 0.05$) between samples of *P. khinjuk* seeds.

Hormone content in *Pistacia khinjuk* Embryos

The highest ABA/GA₃ ratio (5.05) was in the embryo of newly harvested seeds. In seed embryos stored at 25°C, this ratio generally exhibited a decreasing trend over the 4-year storage period, with the lowest ratio (0.89) observed in the third year. Conversely, in seed embryos stored at 4°C, the highest ABA/GA₃ value was obtained in seeds stored for 1 and 2 years (3.09 and 3.03, respectively), and the lowest value (1.35) was observed in seeds stored for 4 years. It is remarkable that the ABA/GA₃ value in seed embryos stored at 4°C decreased significantly in seeds stored for 3 and 4 years (Table 4). The rate of decrease in ABA/GA₃ values by storage years was higher in seed embryos stored at 25°C.

Table 4
ABA and GA₃ contents in *P. khinjak* embryos

Storage Time (Year)	ABA(ng/g)	GA ₃ (ng/g)	ABA/GA ₃
New Harvest	141.55 ± 8.9 ^b	28.20 ± 1.3 ^d	5.05 ^a
4°C			
1	48.44 ± 2.60 ^e	15.67 ± 0.5 ^d	3.09 ^b
2	38.79 ± 2.25 ^e	12.78 ± 0.2 ^d	3.03 ^b
3	133.03 ± 8.80 ^b	88.68 ± 4.1 ^b	1.50 ^c
4	97.03 ± 4.55 ^c	71.44 ± 3.1 ^b	1.35 ^c
25°C			
1	120.67 ± 8.84 ^c	20.24 ± 0.86 ^d	2.99 ^b
2	186.9 ± 9.20 ^a	119.63 ± 8.75 ^a	1.56 ^c
3	106.45 ± 5.60 ^c	118.92 ± 8.50 ^a	0.89 ^d
4	74.69 ± 3.75 ^d	60.94 ± 3 ^c	1.22 ^c

* The means ± standard deviation (n = 3) are used to express the results. According to Duncan's multiple range test, different letters denote significant differences ($p \leq 0.05$) between samples of *P. khinjak* seeds.

Discussion

Previous studies have reported varying effects of storage duration and temperature on seed germination across different species. For instance, Kalmbacher et al. (1999) found that the germination of *Paspalum atratum* seeds was only slightly impacted after being stored at 3°C for 5 years. However, in a study conducted on rice seeds stored at room temperature, Hu et al. (2022) stated that seed germination rates decreased by 40% at the end of two years. In another study, wheat seeds were stored at 40°C and 25°C for 12 months. At the end of the storage time, the germination ability of seeds stored at 40°C decreased by 55–94%, while that of seeds stored at 25°C decreased by 15–22% (Strelec et al., 2010). Likewise, in our research, it can be assumed that the lower limit of the optimal factors (nutrients and regulatory agents) required for seed germination was not reached after 4 years in *P. khinjak* seeds stored for a maximum of 4 years. As specified by Díaz et al. (2015), endosperm size impacts the duration of seed viability and germination rate, and the embryo-to-endosperm ratio in *P. khinjak* seeds is at a level to support our assumption. The researchers emphasized that storage conditions significantly affect germination and viability changes, and that seed viability varies depending on species (Strelec et al., 2010).

β -amylase activity increased, while starch content decreased on a yearly basis for 4 years in *P. khinjak* seeds used in our research. β -amylase activity was higher, and a decrease in starch content was greater in seeds stored at 25°C. As highlighted by Nomura et al. (1969), the primary energy source in endospermic seeds is the hydrolysis of starch into maltose by β -amylase, directly linking starch depletion to increased amylase activity. Results regarding starch content and amylase activity, similar to those obtained in the present study, were achieved by Sharma et al. (2010) in soybeans. The researchers reported that the starch content in the endosperm of soybeans decreased during storage, while amylase activity increased, and storage at room temperature further reduced starch content while increasing amylase activity further. Likewise, it was stated that when dormancy was broken in *Cyclocarya paliurus* seeds, approximately 45% of the starch was depleted, whereas amylase activity increased by 60% (Fang et al., 2007). On the contrary, Hu et al. (2022) stressed that starch content did not change in Indica rice seeds stored for two years. It was indicated that the seeds maintained their metabolic activity status during storage, the expenditure of energy met through respiration continued, and the respiration rate was related to temperature, moisture content, and the seed's structural integrity (Chidananda et al., 2014). Heightened metabolic activity during storage deplete starch reserves, potentially exhausting the vital respiratory substrates needed for subsequent germination (Sharma et al., 2010). Interestingly, enzyme activity does not always directly correlate with viability; Paravar et al. (2023) reported that α -amylase and β -amylase activities decreased but persisted even when seed germination was 0%. Moreover, Marques et al. (2014) indicated that changes in α -amylase activity during storage did not reflect in germination rates. Thus, this study determined that the germination rate was the highest when β -amylase activity was the lowest; on the contrary, germination was not the highest when β -amylase activity was the highest. The result above demonstrates that, in *P. khinjak* seeds, fat and protein reserves, in addition to starch, may also be utilized for germination. It is not precisely known how long β -amylase activity or the activity of other hydrolytic enzymes can be maintained; in other words, how long the reserves can feed *P. khinjak* seeds. Conversely, it can be stated that a 4-year storage period is insufficient for the depletion of starch reserves.

Whereas high ABA/GA₃ ratios and low germination rates were observed in newly harvested *Caryocar brasiliense* seeds (Pinto et al., 2025) and maize seeds (Yue et al., 2024), it was reported that storing *Caryocar brasiliense* seeds for a year and maize seeds for eight months resulted in reduced ABA/GA₃ ratios and increased germination. On the other hand, while the ABA/GA₃ ratio did not change in the maize plant after eight months of storage, germination decreased by 50% (Wang et al., 2022). In the present study, a rather intriguing dynamic was observed: although newly harvested *P. khinjak* seed embryos exhibited the highest ABA/GA₃ ratio, they also demonstrated the highest germination rate. Additionally, the lowest ABA/GA₃ ratio was detected in 3-year-old seeds among the stored seeds, which was reflected in germination despite the absence of a significant difference. Similar deviations from the typical ABA/GA₃ paradigm have been documented in *Idesia polycarpa* Maxim. (Yanmei et al., 2018) and soybean seeds (Shuai et al., 2017). The occurrence of high germination rates despite a high ABA/GA₃ ratio suggests that germination capacity in *P. khinjak* cannot be determined solely by this hormonal balance. It is highly probable that other factors impacting the embryo, triggered by variables such as

storage duration, induce distinct genetic and biochemical responses that override this ratio. This assumption is strongly supported by Malviya and Gayen (2025), who emphasized the necessity of understanding the complex interactions between genes, hormone signaling pathways, and broader subsystems regulating the seed life cycle to fully grasp the mechanisms maintaining seed viability and quality.

Conclusion

In the present study, newly harvested *P. khinjak* seeds exhibited an impressive 81% germination rate in a moist, dark cotton medium without requiring any pretreatment. While prolonged storage (1 to 4 years) adversely affected germination rates, storage temperature did not exert a statistically significant impact. Biochemically, β -amylase activity increased and starch content decreased over the storage period. However, because these metabolic shifts did not directly correlate with changes in germination capacity, the specific role of endosperm starch reserves and β -amylase activity in driving germination could not be conclusively established based solely on these parameters.

Furthermore, contrary to prevailing literature—which typically associates a decrease in the ABA/GA₃ ratio with enhanced germination—our findings revealed that a reduction in this ratio during storage, despite coinciding with increased β -amylase activity and starch degradation, did not result in higher germination rates. This highlights that physiological events in *P. khinjak*, particularly reserve mobilization during germination, are complex and cannot be evaluated solely through starch metabolism or standard ABA/GA₃ balances. Given the high lipid content of these seeds, we hypothesize that future evaluations should incorporate other hydrolytic enzymes, specifically emphasizing fatty acid content and lipase activity. Identifying exactly which primary reserve (starch, lipid, or protein) predominantly sustains these dormant seeds is crucial. Ultimately, we propose that investigating protein degradation, protease enzyme activity, and their complex interactions with other phytohormones—such as auxins (indole-3-acetic acid), ethylene, and brassinosteroids—could serve as valuable alternative parameters for comprehensively understanding the physiological dormancy of *P. khinjak* seeds.

Declarations

Ethics approval and consent to participate

This study does not involve human participants or animal subjects. The plant species examined in this research is not under protection and is not listed as an endangered species. Therefore, ethical committee approval was not required. The research was conducted in accordance with relevant scientific and ethical principles.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author Contribution

Y.E., İ.S.K., and M.A.S.–Analysis, interpretation, literature review, writing. All authors have read and agreed to the published version of the manuscript.

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Data Availability

All data generated or analysed during this study are included in this published article.

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Figures

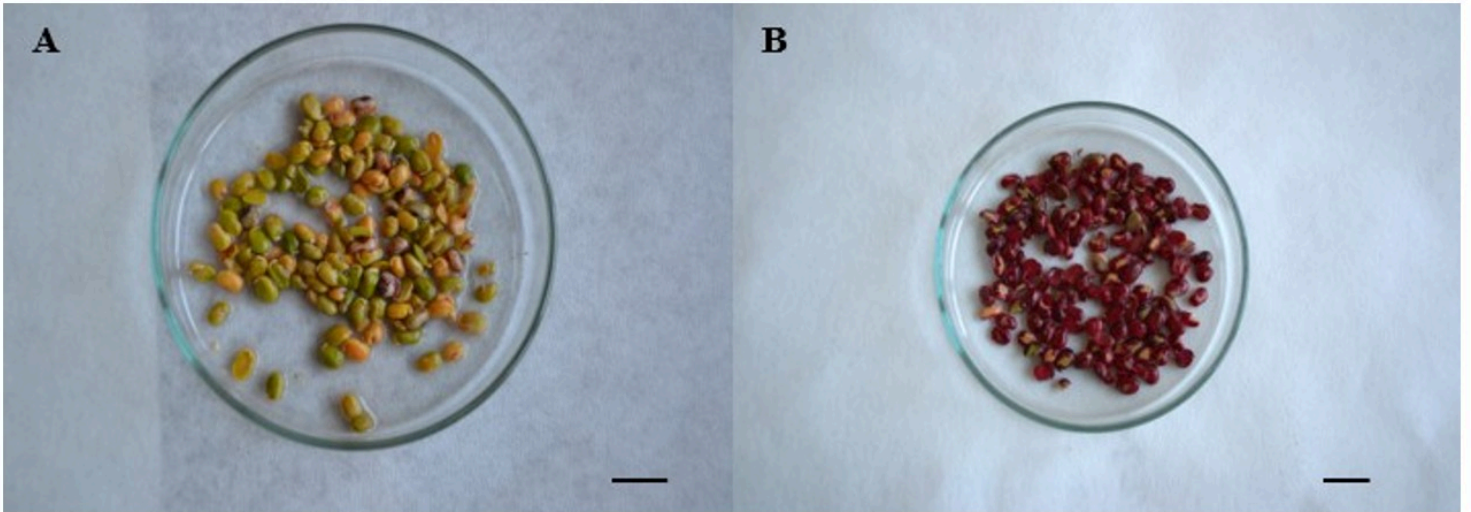


Figure 1

Application of tetrazolium and stained *P. khinjak* seeds. A; all non-viable seeds (Bar: 14 mm), B; mostly viable seeds (Bar: 13 mm)



Figure 2

A sample of germinated *P. khinjak* seeds with endocarp (Bar: 10 mm)