

Influence of Seed Storage Period on the Germination Performance of Pepper Seeds

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Abstract

Seed longevity is a critical determinant of crop performance and germplasm conservation, however genotype-dependent variability remains poorly documented in pepper (*Capsicum annuum* L.). This study investigated the impact of a decade of storage on the germination performance of three Tunisian genotypes (Nabeul, Mahdia, and Kairouan). Seed lots harvested in 2013 and 2023 were evaluated under controlled conditions using viability, germination speed and vigor parameters. Long-term storage resulted in significant reductions in viability, germination rate, and vigor across genotypes indicating pronounced physiological deterioration. Fresh seeds exhibited consistently higher germination percentage, capacity, speed index, coefficient of velocity, and germination vigor index values, together with a markedly lower mean germination time. Clear genotype-specific responses were observed: Nabeul maintained high germination and vigor regardless of storage duration, whereas Mahdia showed excellent performance when fresh but severe deterioration after 10 years; Kairouan displayed intermediate sensitivity. These findings highlight substantial genetic variation in seed aging tolerance and underscore the value of long-lived genotypes such as Nabeul for seed conservation, breeding programs, and long-term germplasm management.

1. Introduction

Seed quality is a key determinant of successful crop establishment, influencing germination performance, seedling vigor, and ultimately field productivity. Among horticultural crops, pepper (*Capsicum annuum* L.) holds a prominent position worldwide due to its economic, nutritional, and cultural value (Arif et al., 2023). Ensuring the availability of high-quality seeds is therefore critical for growers, breeding programs and germplasm conservation. Seed quality, however, declines progressively during storage as seeds undergo physiological and biochemical deterioration, including membrane degradation, oxidative stress,

reduced enzyme activity, and decreased mitochondrial efficiency (Arif et al., 2023; Bissoli et al., 2022; Yıldırım et al., 2020). These changes lead to delayed germination, reduced vigor, and lower viability. Numerous studies have shown that the longevity of pepper seeds can vary widely between genotypes, with some maintaining high viability for many years, while others deteriorate rapidly despite similar storage conditions (Arif et al., 2023). Understanding such genotype-dependent responses is crucial for optimizing seed conservation strategies, especially for landraces and locally adapted cultivars (Bissoli et al., 2022; Pirredda et al., 2023). Long-term studies (≥10 years) on seed longevity remain scarce, yet are

essential to inform seed conservation strategies, particularly for landraces and locally adapted cultivars. In Tunisia, traditional pepper landraces such as Nabeul, Mahdia, and Kairouan represent valuable genetic resources for breeding programs targeting resilience, productivity and adaptability. However, limited information is available regarding the effect of long-term storage on the germination performance of these local genotypes. Evaluating their behavior under aging conditions is essential to guide seed management decisions and ensure the long-term preservation of national pepper germplasm.

The specific objectives of the study were (i) compare fresh and long-term stored seed lots, evaluate genotype-dependent responses to seed aging, and (iii) assess germination and vigor parameters used to quantify these effects. The findings contribute to a better understanding of seed aging dynamics in *Capsicum annuum* and offer practical implications for seed conservation and breeding efforts.

2. Material and Methods

The experiments were conducted at the Horticulture Laboratory of the National Institute of Agronomic Research of Tunisia. Pepper seeds from three Tunisian genotypes (Nabeul, Mahdia and Kairouan) harvested in 2013 and 2023 were sown under controlled laboratory conditions. Seeds were stored for 10 years in a cold storage chamber at a constant temperature of 5°C and a relative humidity of approximately 80%. Seeds were maintained in closed containers under cold-room conditions. Previous agronomic studies (Silva et al., 2017; Verma et al., 2018) report that the optimal temperature for pepper seed germination generally lies between 25 and 30°C, depending on storage conditions, pre-treatments, and experimental objectives. Germination tests were conducted in Petri dishes incubated at 28°C with a relative humidity maintained between 70% and 90%. For each genotype and harvest year, 30 seeds were placed per Petri dish with three replicates, resulting in a total of 90 seeds per genotype per year. This experimental setup ensured uniform environmental conditions to accurately compare the germination performance and vigor of the freshly harvested seeds (2023) with those stored for a decade (2013).

2.1. Germination percentage (GP)

The germination percentage (GP) of pepper seeds refers to the proportion of seeds that successfully sprout and develop into seedlings under given conditions. It is a key measure of seed viability. It is calculated at the end of the test period.

$$GP (\%) = \frac{\text{Number of seed germinated}}{\text{Total seeds tested}} \times 100$$

2.2. Germination capacity (GC)

Germination capacity (GC) is the proportion of seeds in a lot that are capable of germinating and producing normal seedlings under ideal conditions. It is calculated after 14 days of germination.

$$GC (\%) = \frac{\text{Number of normal seedling}}{\text{Total seeds tested}} \times 100$$

2.3. Germination speed index (GSI)

Germination speed index (GSI) measures how fast the seeds germinate. GSI was calculated by the following formula (Ellis and Roberts, 1981).

$$GSI = \sum \frac{\text{Number of seed germinated on each day}}{\text{Days since sowing}}$$

2.4. Mean germination time (MGT)

Mean germination time (MGT) shows the average time needed for seeds to germinate. MGT was calculated according to Ellis and Roberts (1980) as follows:

$$MGT = \frac{\sum(n \times D)}{\sum n}$$

Where n is the number of seeds germinated at D days and D is the number of days from the start of the germination test.

A seed was considered germinated when the radicle had visibly emerged to a length of at least 2 mm. Germination was recorded at 24 h intervals throughout the test. The germination test was conducted for a total duration of 14 days, after which no further germination was observed.

2.5. Vigor index (VI)

It is indicative of both the process of germination and the subsequent growth of seedlings.

$$VI = GP \times SL$$

Where: GP: Germination percentage, and SL: Seedling length (cm).

2.6. Coefficient of velocity of germination (CVG)

The coefficient of velocity of germination (CVG) indicates the rapidity of germination. CVG was calculated using the following formula (Scott et al., 1984):

$$CVG = \frac{\sum ni}{\sum (ni \times ti)} \times 100$$

n_i = number of seeds germinated on day i

t_i = number of days from the start of the test to day i .

2.7. Data analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 25.0 (IBM Corp., Armonk, NY, USA). The experiment followed a factorial design with two fixed factors: genotype (Nabeul, Mahdia, and Kairouan) and harvest year (2013 and 2023). Data were subjected to two-way analysis of variance (ANOVA) to assess the main effects of genotype and harvest year, as well as their interaction, on all germination and vigor parameters. When significant effects were detected ($P < 0.05$), mean separation was carried out using the least significant difference (LSD) test at the 5% probability level. Results are presented as means values, and significant genotype \times harvest year interactions were interpreted to explain differential aging responses among genotypes.

3. Results and Discussion

3.1. Germination percentage

The germination percentage varied significantly among genotypes and between the two storage periods ($P < 0.05$) (Figure 1 and Figure 2). Seeds harvested in 2023 exhibited a markedly higher germination percentage compared to the 2013 seed lot, indicating that prolonged storage negatively affected seed viability. Among genotypes, Nabeul maintained the highest germination percentage in both years (100% and 97%, respectively), while Kairouan showed the greatest decline after 10 years of storage (50% and 43%, respectively). Recent studies have clearly demonstrated that seed longevity in *Capsicum annuum* is strongly genotype-dependent and that certain accessions

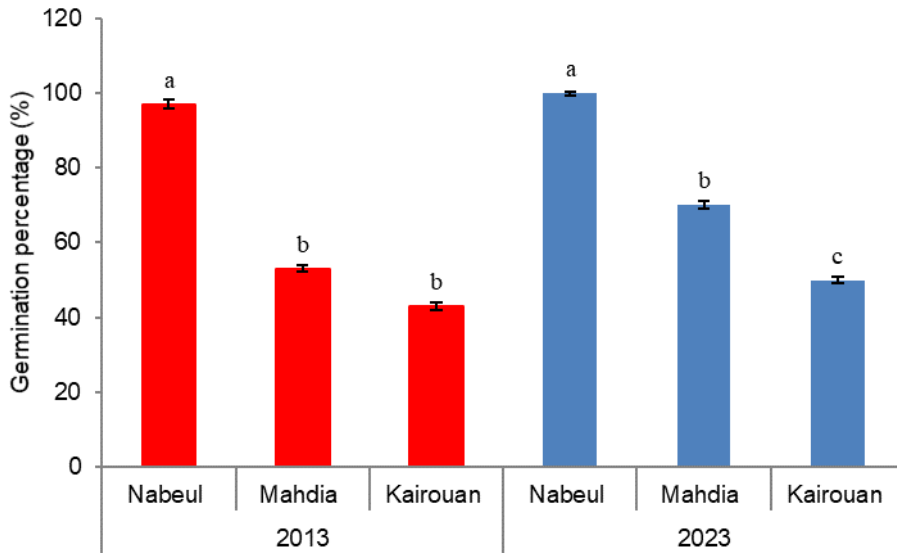


Figure 1. Germination percentage of three Tunisian *Capsicum annuum* genotypes (Nabeul, Mahdia, Kairouan) for seeds harvested in 2013 and 2023 (Values are means \pm SD of three replicates, $n = 3$, 30 seeds per replicate. Bars with the same color marked with the same letters are not significantly different, LSD Test, $p < 0.05$).



Figure 2. Pepper seed germination progression in petri dish tests.

are capable of maintaining very high viability for extended periods when stored under optimal conditions. In the present study, seeds were stored under cold conditions (5°C, closed containers), which are known to substantially slow down deterioration processes in orthodox seeds by limiting oxidative damage and membrane degradation.

3.2. Germination capacity

The germination capacity varied significantly between storage periods and among genotypes (Figure 3). Fresh seeds harvested in 2023 exhibited a markedly higher germination capacity than seeds harvested in 2013, confirming the negative impact of prolonged storage on seed viability. Among genotypes, Nabeul maintained relatively high germination capacity in both years (53% and 63% in 2013 and 2023, respectively), indicating a moderate tolerance to aging. In contrast, Mahdia exhibited a severe reduction in germination capacity after 10 years of storage, with only 3% germination in the 2013 seed lot compared to 63% in freshly harvested seeds. These results confirm that the impact of seed aging on germination capacity is strongly genotype-dependent.

3.3. Germination speed index

The germination speed index (GSI) showed clear differences among genotypes and between storage periods (Table 1). Seeds harvested in 2023 exhibited significantly higher GSI values compared to those from 2013, confirming that prolonged storage markedly slows down the germination process. Nabeul displayed the highest GSI in both years, indicating strong physiological vigor and

good tolerance to seed aging. In contrast, Mahdia recorded the lowest GSI in the 2013 seed lot (0.78), reflecting severe loss of vigor after long-term storage, while fresh seeds of this genotype showed a substantial improvement (7.89). Kairouan presented intermediate values, suggesting moderate sensitivity to seed aging.

3.4. Mean germination time

Mean germination time (MGT) showed a clear effect of storage period and genotype (Table 2). Seeds harvested in 2023 exhibited significantly lower MGT values compared to those from 2013, confirming that prolonged storage slows down the germination process. Mahdia recorded the highest MGT in the 2013 seed lot, indicating very slow germination and strong sensitivity to aging, whereas its fresh seeds germinated much faster. Nabeul and Kairouan showed more stable MGT values across years, with Nabeul maintaining relatively rapid germination even after long-term storage. These results demonstrate that the impact of seed aging on germination speed is strongly genotype-dependent.

3.5. Germination vigor index

The germination vigor index (GVI) showed pronounced differences among storage periods and genotypes (Table 3). Fresh seeds harvested in 2023 exhibited markedly higher GVI values compared to the 2013 seed lot, indicating that long-term storage substantially reduced seed vigor. Mahdia displayed the lowest GVI in the 2013 seeds, confirming high sensitivity to aging, but reached the highest vigor when freshly harvested. Nabeul maintained consistently high GVI values in both

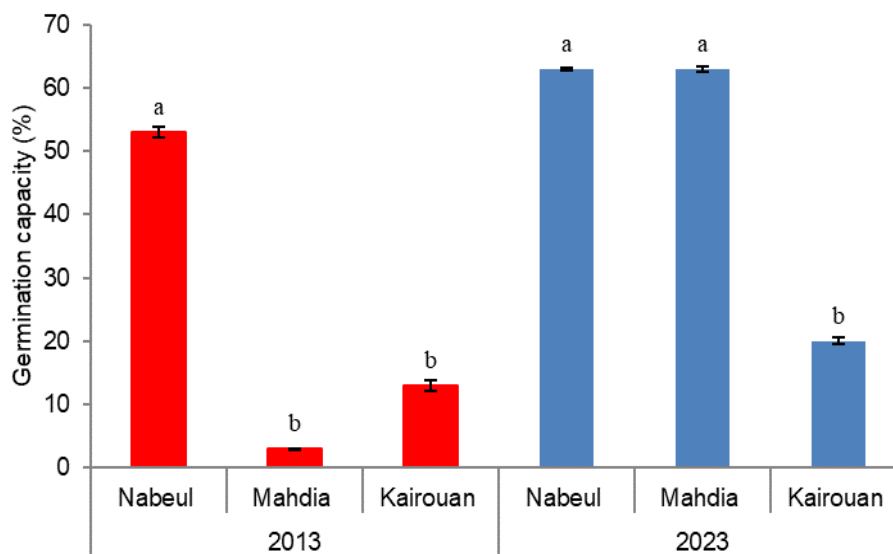


Figure 3. Germination capacity of three Tunisian *Capsicum annum* genotypes (Nabeul, Mahdia, Kairouan) for seeds harvested in 2013 and 2023 (Values are means \pm SD of three replicates, $n = 3$, 30 seeds per replicate. Bars with the same color marked with the same letters are not significantly different, LSD Test, $p < 0.05$).

Table 1. Germination speed index of pepper (*Capsicum annuum* L.) seeds from three genotypes (Nabeul, Mahdia, and Kairouan) harvested in 2013 and 2023.

Genotype	Seeds harvested in 2013	Seeds harvested in 2023
Nabeul	8.68±0.1 a	11.34±0.2 a
Mahdia	0.78±0 c	7.89±0.3 b
Kairouan	5.02±0.01 b	5.78±0.01 c

Values represent means of three independent replicates (30 seeds per replicate). Different letters indicate significant differences among genotype × harvest year combinations based on LSD mean separation at $P < 0.05$.

Table 2. Mean germination time of pepper (*Capsicum annuum* L.) seeds from three genotypes (Nabeul, Mahdia, and Kairouan) harvested in 2013 and 2023.

Genotype	Seeds harvested in 2013	Seeds harvested in 2023
Nabeul	16.35±1 b	15.45±1 a
Mahdia	21.82±2 a	11.5±1 b
Kairouan	15.97±2 b	15.28±1 a

Values represent means of three independent replicates (30 seeds per replicate). Different letters indicate significant differences among genotype × harvest year combinations based on LSD mean separation at $P < 0.05$.

Table 3. Germination vigor index of pepper (*Capsicum annuum* L.) seeds from three genotypes (Nabeul, Mahdia, and Kairouan) harvested in 2013 and 2023.

Genotype	Seeds harvested in 2013	Seeds harvested in 2023
Nabeul	203.7±3.01 a	210±4 ab
Mahdia	53±1.33 c	217±2.54 a
Kairouan	164.3±1.23 b	200.1±0 b

Values represent means of three independent replicates (30 seeds per replicate). Different letters indicate significant differences among genotype × harvest year combinations based on LSD mean separation at $P < 0.05$.

Table 4. Coefficient of velocity of germination of pepper (*Capsicum annuum* L.) seeds from three genotypes (Nabeul, Mahdia, and Kairouan) harvested in 2013 and 2023.

Genotype	Seeds harvested in 2013	Seeds harvested in 2023
Nabeul	0.061±0.002 a	0.065±0.003 b
Mahdia	0.046±0.002 b	0.089±0.001 a
Kairouan	0.063±0.001 a	0.061±0.002 b

Values represent means of three independent replicates (30 seeds per replicate). Different letters indicate significant differences among genotype × harvest year combinations based on LSD mean separation at $P < 0.05$.

years, reflecting strong physiological stability and good tolerance to seed aging. Kairouan showed intermediate vigor, with a moderate improvement in the 2023 seed lot. The results highlight that the decline in seed vigor due to aging is strongly genotype-dependent.

3.6. Coefficient of velocity of germination

The coefficient of velocity of germination (CVG) revealed clear differences among genotypes and storage periods ($P < 0.05$) (Table 4). Fresh seeds harvested in 2023 exhibited higher CVG values, indicating faster germination, whereas the 2013 seed lot showed reduced velocity. Mahdia exhibited the lowest CVG value in the 2013 seed lot and the highest value in 2023, reflecting a pronounced improvement in germination speed when seeds were freshly harvested. This pattern is consistent with the strong reduction in MGT observed for Mahdia and indicates a biologically meaningful response to seed aging. In contrast, Nabeul and Kairouan showed only small numerical differences in CVG between years. Although these differences were statistically significant according to the LSD test, the absolute variation was limited and likely reflects minor shifts in germination timing rather than major biological changes. For these genotypes, CVG and MGT values indicate relatively

stable germination behavior across storage periods. CVG results, which closely mirror MGT patterns, confirm that the impact of seed aging on germination speed is genotype-dependent, with Mahdia being the most affected genotype.

The findings of this study clearly demonstrate that seed storage duration has a significant impact on the germination performance of pepper seeds. Seeds harvested in 2023 exhibited markedly higher germination percentage, faster germination, and greater vigor compared to the 2013 seed lot. These differences reflect the well-known physiological and biochemical deterioration associated with seed aging, including oxidative damage, membrane degradation, and reduced metabolic efficiency, as widely reported in recent studies (Arif et al., 2023; Ariyaratna et al., 2022; Bissoli et al., 2022; Nickas et al., 2025; Yıldırım et al., 2021). The substantial reduction in germination percentage and capacity observed in the 2013 seeds confirms that prolonged storage negatively affects seed viability. Similar declines in viability have been reported in pepper seeds stored for more than 6-10 years, even under controlled conditions (Arif et al., 2023; Pirredda et al., 2023; Yıldırım et al., 2020). Among the studied genotypes, Nabeul maintained the highest germination percentage and capacity in both years, suggesting a greater inherent tolerance to aging. Seed longevity in *Capsicum* is highly genotype-

dependent and influenced by structural and biochemical characteristics, such as seed coat lignification and antioxidant capacity, which may contribute to a slower decline in viability during storage (Bissoli et al., 2022; Pirredda et al., 2023). Recent research highlights that certain *Capsicum* accessions possess natural genetic mechanisms that slow down the deterioration of membranes and reserve materials during storage (Arif et al., 2023; Bissoli et al., 2022). In contrast, Mahdia and Kairouan showed pronounced declines, indicating a higher sensitivity to long-term storage. This genotype-dependent aging response aligns with findings in seed longevity studies (Arif et al., 2023; Bissoli et al., 2022). Germination speed indices were also strongly influenced by storage duration. Seeds from 2023 germinated faster, as indicated by higher germination speed index and coefficient of velocity of germination and lower mean germination time. These patterns confirm that aging slows down physiological processes essential for germination. Recent studies attribute this decline to reduced activity of hydrolytic enzymes, impaired mitochondrial function, and decreased ATP production in aged seeds (Małecka et al., 2021; Pirredda et al., 2023; Van Aken, 2021; Zhang et al., 2022). The extremely high mean germination time observed in Mahdia 2013 seeds indicates severe physiological deterioration. Conversely, Nabeul displayed stable and relatively fast germination across both years, confirming its better physiological resilience. The germination vigor index additionally confirmed the adverse effects of seed aging. The 2023 seeds displayed substantially higher vigor, whereas the 2013 seeds exhibited significant reductions. Several recent studies support the use of germination vigor index as a highly sensitive indicator of early-stage seed deterioration (Oballim et al., 2023; Wu et al., 2024; Xing et al., 2023). Interestingly, Mahdia showed the lowest germination vigor index in 2013 but the highest germination vigor index in 2023, indicating that it performs very well when fresh but is strongly affected by long-term storage. This behavior corresponds to the “high-performing but low-longevity” genotype category described in pepper seeds (Bissoli et al., 2022; Granata et al., 2025; Nickas et al., 2025; Pirredda et al., 2023; Solberg et al., 2020). Nabeul consistently maintained higher viability and vigor across both storage periods, suggesting a potential tolerance to long-term storage. Nevertheless, these genotype-specific responses should be considered preliminary observations that warrant further investigation to confirm their broader applicability.

4. Conclusion

This study suggests that seed storage duration has a substantial influence on the germination performance and vigor of pepper (*Capsicum*

annuum) seeds. Seeds harvested in 2023 generally exhibited higher germination percentage, faster germination, and greater vigor than those harvested in 2013, indicating that prolonged storage is associated with physiological deterioration. The magnitude of deterioration, however, was strongly genotype-dependent. Nabeul maintained the highest viability and vigor across both storage periods, suggesting a greater tolerance to seed aging. In contrast, Mahdia exhibited excellent performance when fresh but a pronounced decline after ten years of storage, fitting the profile of a “high-performing but low-longevity” genotype. Kairouan showed intermediate behavior, with moderate but noticeable reductions under long-term storage. These results indicate that genetic background may play an important role in determining seed longevity in pepper. While the observed genotype-dependent responses provide useful indications for seed conservation and breeding programs, further studies involving a broader range of genotypes, storage conditions, and physiological markers would be necessary to confirm the general applicability of these findings.

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