

Seed priming as a method of preservation and restoration of sunflower seeds[☆]

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Abstract – The study focused on determining the preservative and restorative effects of seed priming with gibberellic acid (GA₃) and potassium nitrate (KNO₃) on sunflower seeds against deterioration. The seeds were immersed in different concentrations (0, 250, 500, and 1000 mg L⁻¹) of GA₃ and KNO₃ solutions before and after being subjected to accelerated aging (AA). Unprimed seeds (NT) were used as control. The results showed that AA led to a reduction in germination percentage, germination index, and seedling growth parameters. However, seed primed with GA₃ had a higher germination percentage after AA than NT. All seed primings shortened mean germination time and improved germination index. Seedling growth was stimulated by seed priming, and seed priming after AA produced more vigorous seedlings than primed seed before AA because they were severely affected by AA. In addition, hydration sufficiently induced the germination and seedling growth of aged seeds. A significant difference was found between GA₃ and KNO₃, and seeds were effectively protected from aging by GA₃, while hydration promoted germination of aged seeds. Consequently, the recovery effect of seed priming was more pronounced than the conservative effect, and sunflower seeds should be primed with 500-1000 mg L⁻¹ GA₃ before storage or, if this is not possible, hydration improves the vitality and subsequent growth of aged sunflower seeds.

Keywords: *Helianthus annuus* L / aging / germination / GA₃ / KNO₃

Résumé – Le priming des semences comme méthode de conservation et de restauration des semences de tournesol. Cette étude est consacrée à la détermination des effets conservateur et restaurateur du priming avec de l'acide gibbérellique (GA₃) et du nitrate de potassium (KNO₃) sur les semences de tournesol, pour contrer leur détérioration. Les semences ont été immergées dans différentes concentrations (0, 250, 500 et 1 000 mg L⁻¹) de solutions de GA₃ et KNO₃ avant et après avoir été soumises à un vieillissement accéléré (AA). Des semences non primées (NT) ont été utilisées comme témoins. Les résultats ont montré que l'AA entraînait une réduction du pourcentage de germination, de l'indice de germination et des paramètres de croissance des plantules. Cependant, les semences primées avec du GA₃ présentaient un pourcentage de germination plus élevé après le vieillissement accéléré que les témoins. Tous les primings de semences ont raccourci le temps moyen de germination et amélioré l'indice de germination. La croissance des plantules a été stimulée par le priming des semences, et le priming des semences après l'AA a produit des plantules plus vigoureuses que les semences primées avant l'AA, car elles ont été fortement affectées par le vieillissement accéléré. De plus, l'hydratation a induit la germination et la croissance des graines vieilles de manière satisfaisante. Une différence significative a été trouvée entre GA₃ et KNO₃, et les graines étaient efficacement protégées du vieillissement par GA₃, tandis que l'hydratation favorisait la germination des semences vieilles. Par conséquent, l'effet de restauration du priming des semences était plus prononcé que l'effet conservateur, et les semences de tournesol doivent être primées avec 500 à 1 000 mg L⁻¹ GA₃ avant le stockage ; si cela s'avère impossible, l'hydratation améliore la vitalité et la croissance ultérieure des semences de tournesol anciennes.

Mots-clés : *Helianthus annuus* L / vieillissement / germination / GA₃ / KNO₃

[☆] Contribution to the Topical Issue: "Sunflower / Tournesol".

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Highlights

- Seed priming is used to improve seed vigor against deterioration.
- It is necessary to have a decision on whether it is to be used before or after storage in sunflower.
- GA₃ priming should be applied to the seeds to be stored.
- The hydration should be advised for aged sunflower seeds.

1 Introduction

Sunflower (*Helianthus annuus* L.) is the most widely grown and consumed oilseed in Turkey with a highly qualified oil content of 40–50%. The demand for sunflower seed has increased in the last decade, and 34,000 tons of hybrid seeds were produced and approximately 26,000 tons were imported in 2022 (Anonymous, 2023). Depending on the changes in sowing areas, seed requirements may vary, and overproduction or unsold seeds are stored for long periods until they are planted in the next production season.

The vigorous and healthy seeds provide high germination percentage, emergence, and uniform seedling emergence, which results in an increase in seed yield and quality of sunflower. Due to their high oil content, sunflower seeds are easily deteriorated by inappropriate storage conditions such as high humidity and temperature (Sisman, 2005). Changes in the physiological and biochemical properties of the seeds during storage cause a gradual decline in germination ability and vigor over time (Bailly *et al.*, 2000; Balešević-Tubić *et al.*, 2005). In order to inhibit seed deterioration during storage or to improve germination performance after storage, several seed priming methods have been developed in maize (Siadat *et al.*, 2011), bean (Amanpour-Balaneji and Sedghi, 2012), cotton (Santhy *et al.*, 2014), sunflower (Kausar *et al.*, 2009; Jovičić *et al.*, 2022), soybean (Miladinov *et al.*, 2021), and safflower (Tonguç *et al.*, 2023). Depending on the priming methods, species, and even varieties, seeds germinate more uniformly than non-primed seeds, even if they have been stored for a long time (McDonald, 1999). The most commonly used priming agents are PEG (Polyethylene glycol m.w. 6000 and 8000) (Arif *et al.*, 2010; Kadir *et al.*, 2023), which are non-toxic high-molecular-weight compounds, inorganic salts such as magnesium, potassium, and sodium (Sher *et al.*, 2019) and low-molecular-weight organic compounds such as glycerol, mannitol, and sucrose. In addition, plant growth regulators (PGR) such as gibberellic acid (GA₃) and ethylene are used alone or in combination (Kaya, 2008; Zhu *et al.*, 2021; Zhang *et al.*, 2023). Rouhi and Sepehri (2020) found more pronounced effects of GA₃ after accelerated aging in groundnut and they suggested 100 ppm of GA₃ dose. Kaur *et al.* (2023) reported that a considerable improvement in germination percentage of high and low vigor seeds (constituted by accelerated aging) in brinjal with priming of 1% KNO₃ and 100 ppm GA₃. However, there is no information on whether seed priming should be preferred before or after

storage. Therefore, this study was undertaken to determine the restorative and conservative effects of seed priming with different doses of gibberellic acid (GA₃) and potassium nitrate (KNO₃) on seed viability and subsequent seedling growth of sunflower seeds.

2 Materials and methods

This study was conducted at the Seed Science and Technology Laboratory, Department of Field Crops, Eskisehir Osmangazi University, Turkey. Seeds of sunflower hybrid P64LE119 from Pioneer Seed Company were used as material. To determine the conservation and recovery effects of seed priming, seeds were divided into two groups.

2.1 Seed treatments

In the first group (priming + AA), seeds were soaked with increasing levels of GA₃ and KNO₃ and then subjected to accelerated aging as described below.

In the second group (AA + priming), seeds subjected to accelerated aging were primed with the same concentrations of GA₃ and KNO₃.

Accelerated aging (AA) was performed in aging boxes (11 × 11 × 4 cm) filled with 40 mL of distilled water. The seeds were uniformly spread on a wire mesh tray with dimension 10 × 10 × 3 cm and covered plastic boxes. The boxes were incubated for 96 h at 45°C and approximately 100% relative humidity as described in sunflower by Kaya *et al.* (2018).

For priming applications, seeds were soaked in solutions of 0 (distilled water, referred to as hydration), 250, 500, and 1000 mg L⁻¹ GA₃ (Sigma) and KNO₃ (Merck, 1.05063) at 20°C for 16 h in the dark. After completion of the seed treatments, seeds were rinsed three times with tap water and were carefully surface-dried with paper towels. They were allowed to dry at room temperature for 2 days until they reached approximately the original moisture content as determined by change in seed weight. After the priming and aging treatments, the seeds were left in room conditions to allow the moisture content of the seeds to equilibrate. Unprimed and unaged (NT) seeds were used as a control.

2.2 Germination conditions

The germination test was performed at 25°C for 10 days with 200 seeds (4 × 50, replication × seed) according to ISTA (2018) rules. Fifty seeds were spread between three layers of filter paper (20 × 20 cm) and moistened with 21 mL of distilled water including 1.5 g L⁻¹ fungicide (80% thiram). After rolling, the papers were placed in a zippered plastic bag to prevent water evaporation. Germinated seeds were counted every 24 h to determine the germination rate by calculating the mean germination time (MGT) as a mean of four replicates. A visible radicle protrusion was evaluated as a germination criterion. On the last day of counting, growth parameters such as shoot length, root length, fresh weight, and dry weight (determined at 80°C for 24 h) were examined on ten randomly selected normal, uncrushed, and healthy seedlings. The germination index (GI) was calculated with the following

Table 1. Changes in seed germination and subsequent growth parameters of sunflower seeds after accelerated aging.

Characteristics	Control	Aged seed
Germination percentage (%)	94.0 ^a ± 1.41	79.5 ^{b*} ± 2.63
Germination index	24.2 ^a ± 0.44	12.5 ^b ± 0.74
Mean germination time (day)	2.00 ^b ± 0.02	3.57 ^a ± 0.05
Shoot length (cm)	6.30 ^a ± 0.37	2.73 ^b ± 0.26
Root length (cm)	7.08 ^a ± 0.53	3.90 ^b ± 0.21
Seedling fresh weight (mg plant ⁻¹)	386 ^a ± 15.0	268 ^b ± 13.1
Seedling dry weight (mg plant ⁻¹)	50.8 ± 3.14	50.7 ± 1.96
Vigor index	1257 ^a ± 83.3	529 ^b ± 44.4

* Means followed by the different letters are significant at $p < 0.05$, ±: Standard error.

equation 1 (Salehzade *et al.*, 2009):

$$GI = \frac{\text{Number of germinated seeds} / \text{days of first count} + \dots + \text{Number of germinated seeds}}{\text{days of final count}} \quad (1)$$

The vigor index was calculated according to the following equation 2 (Sadeghi *et al.*, 2011):

$$\text{Vigor index} = \text{Germination}(\%) \times \text{Seedling length}(\text{root} + \text{shoot})(\text{cm}) \quad (2)$$

The experiment was set up with a two-factor factorial in a completely randomized design (CRD) with four replications for each seed application, and the data were analyzed with the computer statistic program MSTAT-C (Michigan State University, v. 2.10).

3 Results

The germination and seedling growth parameters of control and aged seeds are shown in Table 1. Accelerated aging decreased germination percentage from 94.0% to 79.5%, the germination index from 24.2 to 12.5, while the mean germination time was prolonged from 2.00 to 3.57 days. Apart from seedling dry weight, lower seedling growth parameters, and vigor index were observed in aged seeds.

The percentage of germination was negatively affected by aging (Tab. 1), but seed priming significantly promoted it (Fig. 1). All levels of GA₃ and KNO₃ resulted in higher germination rates in primed seeds after aging (Fig. 1A). It is evident that primed seeds are less affected by aging than NT seeds. However, sunflower seeds primed with distilled water, 1000 mg L⁻¹ GA₃ or KNO₃ germinated better than control seeds before and after AA (Fig. 1B). Hydration did not significantly differ with chemical treatments (GA₃ and KNO₃). Mean germination time was shortened by primed seeds. The seeds primed with 250 mg L⁻¹ GA₃ or KNO₃ achieved the shortest germination time in seeds primed after AA, while the highest value was observed in NT seeds (Fig. 1E and 1F). The hydration of the aged seeds caused a remarkable increase in the germination index, but the aged seeds had the maximum value of the index after priming with 1000 mg L⁻¹ of KNO₃ (Fig. 1C and 1F).

Seed priming with different concentrations of GA₃ and KNO₃ stimulated shoot length of aged and NT seeds. The

longest shoots were measured in aged seeds primed with 500 mg L⁻¹ GA₃ followed by 1000 mg L⁻¹ GA₃ (Fig. 2A). Seed treatments increased shoot length produced by the seeds aged after priming with GA₃ levels without significant differences (Fig. 2B). Moreover, root length was positively affected by priming with KNO₃ and GA₃, but the longest root was recorded in seeds primed with 1000 mg L⁻¹ GA₃ after AA. Also, significant differences were determined between GA₃ and KNO₃, and the advantage of GA₃ was apparent in seed priming after AA (Figs. 2C and 2D). Seedling fresh weight was boosted by seed primings and heavier fresh weight in aged seeds was obtained from seed priming with 500 mg L⁻¹ and 1000 mg L⁻¹ GA₃. However, the maximum seedling fresh weight was achieved in hydrated seeds before AA (Figs. 2E and 2F). The vigor index of aged sunflower seeds increased, especially priming with 500 and 1000 mg L⁻¹ GA₃ (Figs. 2G and 2H).

4 Discussion

Seed priming is a valuable method for improving germination and emergence under both optimal and stress conditions. It is also useful for restoring the adverse effects of aging after storage. Abreu *et al.* (2013) showed that a longer storage period led to a reduction in germination percentage and oil content in sunflower. In the present study, germination percentage and index were diminished from 94.0% to 79.5% by AA. Therefore, the optimum concentration of GA₃ and KNO₃ priming on sunflower seed was tested to understand whether they should be applied before or after storage. Our results showed that seed priming resulted in improved germination performance of both control and aged seeds, with the positive effects being particularly evident in aged seeds. This result is consistent with the findings of Chojnowski *et al.* (1997), who found that the germination capacity of primed seed declined more rapidly during AA than that of non-primed seed, depending on priming duration. According to the result of the study, priming agents also play a crucial role in overcoming seed deterioration in sunflower. Thus, GA₃ was more suitable agent for the restoration of aged sunflower seeds than KNO₃, while hydration was superior for the preservation against seed deterioration due to aging. All primed seeds before or after AA germinated better than NT seeds. Similar results were reported by Kausar *et al.* (2009) and Jovičić *et al.* (2022) in sunflower and Abdolahi *et al.* (2012) in canola, who

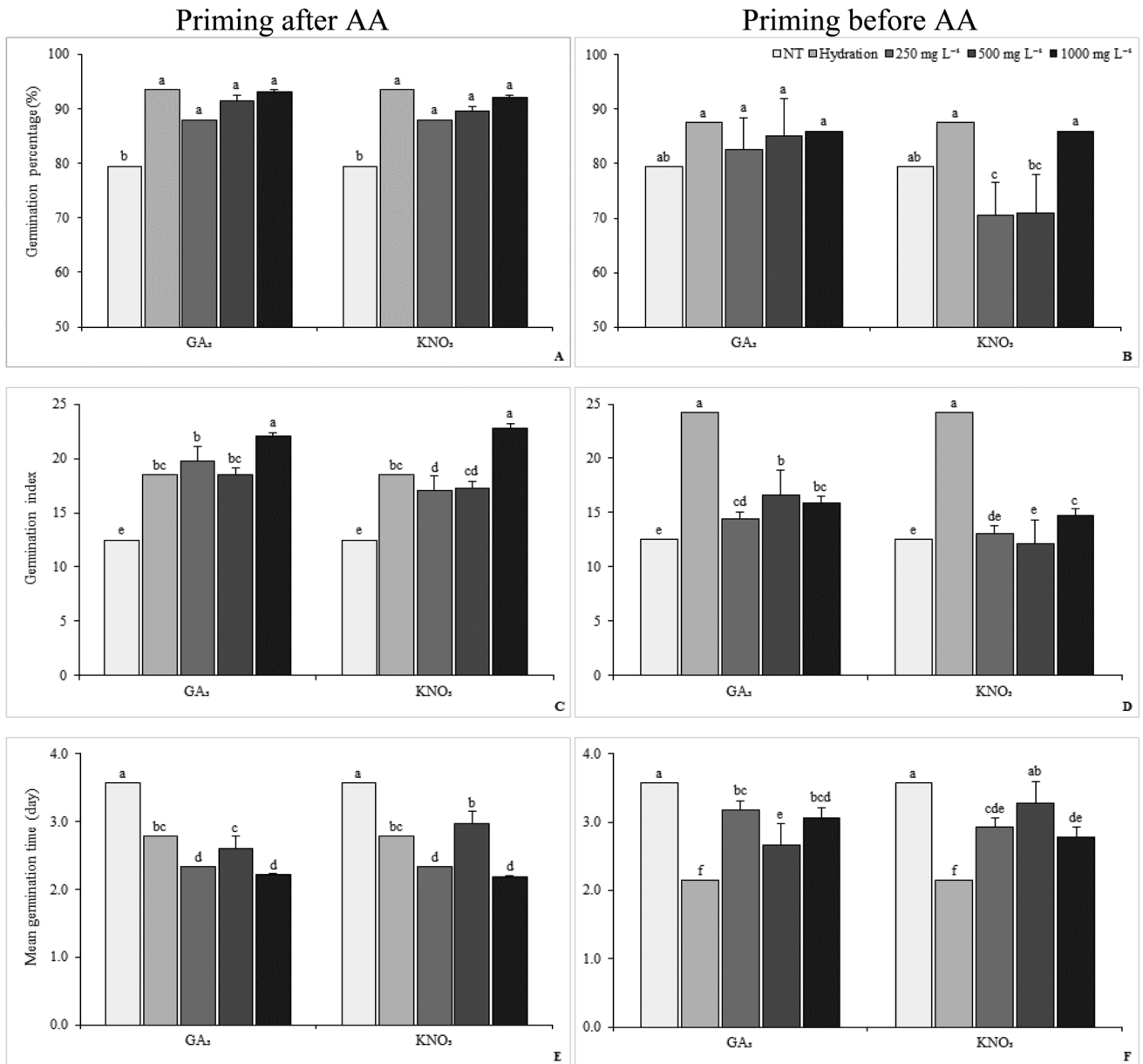


Fig. 1. Effects of seed priming with different concentrations of GA₃ and KNO₃ on sunflower seeds' germination percentage (A, B), and germination index (C, D), and mean germination time (E, F) before and after AA. NT: No treatment.

found a significant improvement in germination percentage of aged seeds primed with water and KH₂PO₄. Similarly, Kaya *et al.* (2006) determined the positive effect of priming with KNO₃ and water on germination under salt and drought stresses, and Bailly *et al.* (2000) reported the recovery effect of priming with PEG 8000 on aged sunflower seeds, which increased germination along with a decrease in 50% germination (T_{50}).

Successful emergence and uniform crop stand are determined by germination capacity and subsequent seedling growth performance. Therefore, it is crucial to consider the growth of seedlings from primed and unprimed seeds. In our study, the root and shoot growth of primed seeds were better than that of unprimed seeds both before and after AA. The highest lengths of root and shoot were achieved through

priming with 500 mg L⁻¹ and 1000 mg L⁻¹ GA₃ following AA. This result aligns with the findings of Siadat *et al.* (2011), who indicated that immersing corn seeds in gibberellin at a concentration of 400 mg L⁻¹ for 12 h was an effective approach to enhancing the growth of low-quality corn seeds. Also, Jovičić *et al.* (2022) found that KH₂PO₄ significantly promoted root and shoot growth in aged sunflower seeds, whereas hydration increased root length compared to unprimed seeds. Correspondingly, these results are consistent with the findings of Rouhi and Sepehri (2020) on aged peanut kernels. Catiempo *et al.* (2021) found that dry weight of seedlings was higher in primed sunflower seeds than in unprimed seeds, and that dry weight of shoots and fresh weight of leaves and stems increased with increasing duration of priming. The benefits of seed priming were approved by

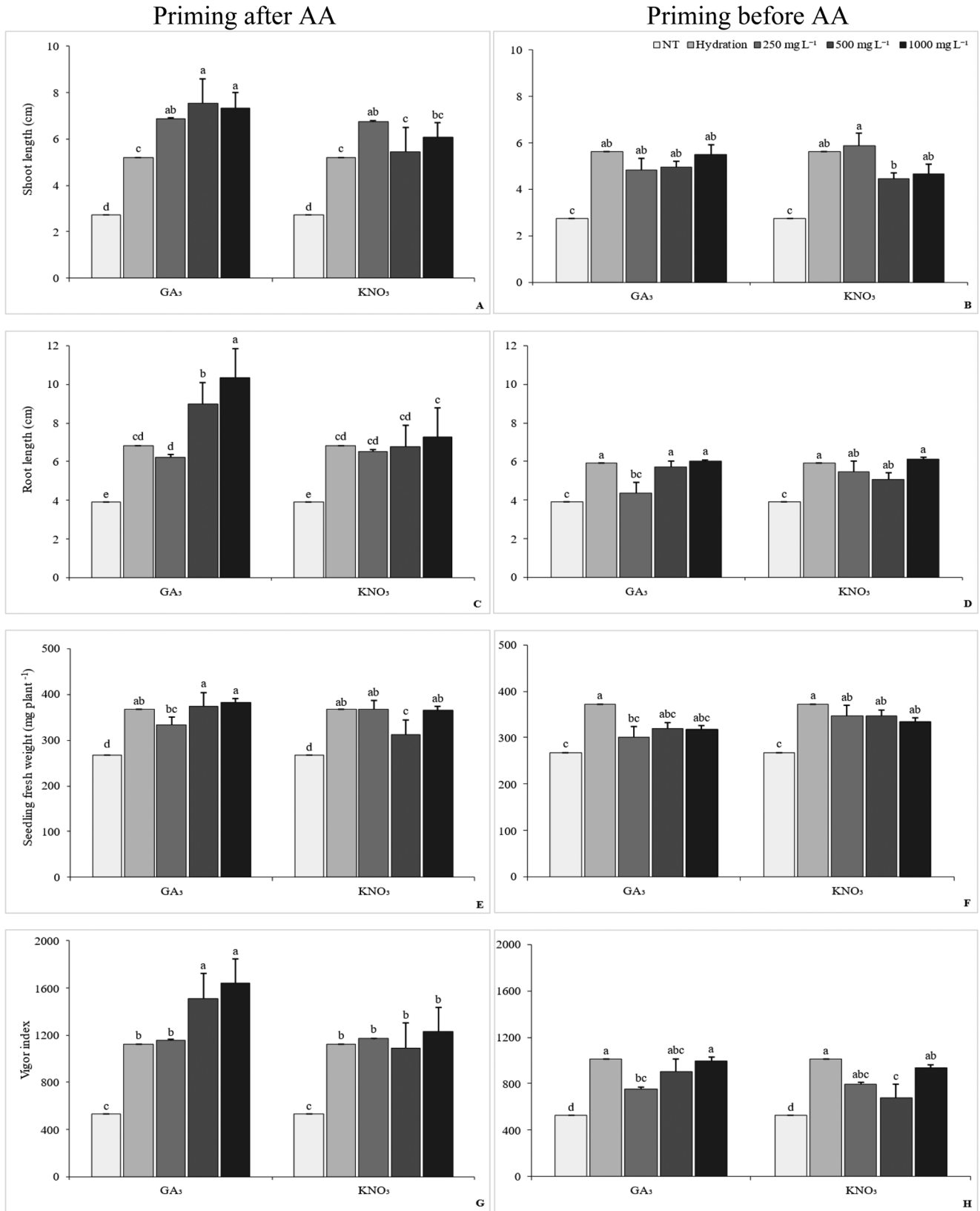


Fig. 2. Effects of seed priming with different concentrations of GA₃ and KNO₃ on shoot length (A, B), root length (C, D), seedling fresh weight (E, F), and vigor index (G, H) of sunflower seeds before and after AA. NT: No treatment.

Amanpour-Balaneji and Sedghi (2012) in field beans, and they found that priming with 5 mg L⁻¹ GA₃ had the highest dry and fresh weights in aged bean seeds compared to kinetin and IAA. In contrast, we could not find a significant decrease in dry weights produced by aged seeds compared to control seed. Shekari *et al.* (2015) reported that accelerated aging reduced dry weight of wheat seedlings and that heavier seedlings were obtained from salicylic acid and GA₃. In addition, Prabha *et al.* (2018) determined that aged bean seeds treated with 5 mg L⁻¹ GA₃ induced germination and seedling growth parameters.

5 Conclusion

Sunflower seeds are sensitive to prolonged storage or unsuitable storage conditions and easily lose their viability during storage due to their high oil content. In this study, we investigated whether priming seeds with GA₃ and KNO₃ could prevent seed deterioration during storage or promote germination of sunflower seeds after storage. The results indicate that seed priming improves germination and seedling growth of aged seeds and protects sunflower seeds from seed deterioration during aging. Furthermore, it is clearly revealed that the benefits of priming after aging outweigh those of priming before aging. It was concluded that priming with 500 mg L⁻¹ or 1000 mg L⁻¹ GA₃ should be advised before storage or that hydration can be applied to improve germination of aged sunflower seeds.

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Conflicts of Interest

The authors declare that they have no conflicts of interest in relation to this article.

Author contribution statement

Mehmet Demir Kaya: Conceptualization, methodology, supervision, validation, writing-review & editing. Nurgül Ergin: Investigation, visualization, writing-original draft preparation. Engin Gökhan Kulan: Investigation. Pınar Harmancı: Investigation.

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