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Potential of two hydration treatments for improvement of sunflower seed vigour

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Abstract

Seed deterioration is an unavoidable process to which seeds of oilseed plant species such as sunflower are especially sensitive. This study aimed to determine whether different invigoration techniques can improve the attributes of germination in sunflower and alleviate the effects of seed ageing. Both aged and non-aged seeds were subjected to invigoration by pre-soaking with distilled water and with 3% of KH_2PO_4 for 24 hours at 25°C. Germination performance, seedling growth and SDS-PAGE protein profile were determined. The outcome of invigoration depended on the condition of the seeds: invigoration of seeds with both water and KH_2PO_4 reduced the percentage of germination in non-aged seeds, while in aged seeds there was an increase in germination compared to the control. The SDS-PAGE seed protein profiles revealed that the low molecular weight proteins produced high-intensity bands and the high molecular weight proteins were in low concentrations. After accelerated ageing followed by H_2O treatment, some bands of proteins appeared in the region of 2S albumins and were associated with a higher percentage of germination. Our results point out that invigoration treatments were more effective in low vigour seeds which can provide wide practical benefits.

Keywords: accelerating ageing, invigoration, seed storage, seed vigour, sunflower

Introduction

Seed germination and seedling emergence are considered the most important and vulnerable phases of crop production. Use of poor-quality seeds might have impacts on overall crop production performance, leading to re-sowing or reduced plant density and therefore reduced yield, lower crop competitiveness against weeds or development of favourable conditions for disease development (Lamichhane *et al*., 2019). The issue is further complicated by using oil seeds because the most frequent cause of deterioration and loss of seed viability is lipid peroxidation due to the higher content of polyunsaturated fatty acids (Schwember and Bradford, 2010). Seed deterioration is an unavoidable and unrestrainable process that leads to many physical, biochemical and structural changes (Nithya *et al*., 2017).

The oilcrops sector has been one of the most dynamic parts of world agriculture in recent decades. It is estimated that the production of vegetable oils will reach 282 MT in 2050 (Alexandratos and Bruinsma, 2012). The immense growth of the world population and the increasing use of vegetable oils in human nutrition are factors that will contribute most to this increase, but increase in the use of vegetable oils for biodiesel production will also contribute. On the global market, European seed exports reached €8.1 billion in 2020, making up about 19% of the total world market (ESCAA, 2021). Given these economic and practical circumstances, to improve the integration of the seed industry, new innovative biotechnology and molecular tools are necessary.

Seed invigoration involves pre-sowing seed treatments which improve overall field performance (seed germination, seedling emergence, early seedling growth). In general, invigoration techniques cover uncontrolled (presoaking) and controlled (seed priming) hydration, thermal treatments (chilling or drought treatment) and coating (Farooq *et al.*, 2009). Seed priming induces an overlap of various mechanisms, including efficient water absorption to an extent that allows activation of pre-germinative metabolism, but insufficient water to complete radicle emergence (Sivasubramaniam *et al*., 2011; Lechowska *et al*., 2019; Singh *et al*., 2020). In addition to the fact that these techniques can potentially have a positive effect on germination and early seedling growth, they can also alleviate the damage caused by seed ageing and various abiotic factors (Beckers and Conrath, 2007; Jisha *et al*., 2013). Since these techniques are considered a valuable strategy suitable to improve field performance, there is a great interest among farmers and seed companies to find appropriate, inexpensive treatments.

Considering that it is a significant source of edible oil, sunflower (*Helianthus annuus* L.) is a strategically important species. At the same time, due to the chemical composition of the seeds, it is considered an extremely sensitive species prone to intense changes during ageing and storage (Balesevic-Tubic *et al*., 2005).

This study aims to determine whether different invigoration techniques improve sunflower morpho-physiological attributes of seed germination and alleviate the damage incurred by accelerated seed ageing.

Materials and methods

The study was conducted on sunflower hybrid 'Fantazija' produced at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. For the germination assay, seeds were surface-sterilised using 5% sodium hypochlorite solution for five minutes, rinsed thoroughly in distilled water and dried at 25°C for 24 hours. Germination assays were performed at 25°C, in three replicates of 100 seeds placed in 90 mm-diameter Petri dishes on a layer of filter paper moistened with distilled water. Germination percentage (GP) was determined after 10 days, evaluating typical seedlings based on the criteria specified in the ISTA rules for sunflower. To determine the mean germination time (MGT), the number of seedlings whose radicle had reached 3 mm was counted every day for 10 days and the following formula was used (Coolbear *et al*. (1984) modified by Farooq *et al.* (2005)): $MGT = \sum (n \times d) / N$, where MGT is the average time between the start of imbibition and radicle

emergence, n is the number of seeds germinated on each day, d is the number of days from the beginning of the test and N is the total number of seeds germinated (3 mm radicle emergence). The formula from the same authors was used for the determination of the time taken to 50% germination (T50):

$$T50 = ti + [(N / 2 - ni) (ti - tj)] / ni - nj$$

where T50 – the time to reach 50% germination, N is the final number of emergence and ni, nj cumulative number of seeds germinated by adjacent counts at times ti and tj, respectively when $ni < N / 2 < nj$.

After four days, 10 seedlings per replicate of all treatments were transferred to a moistened filter paper towel and wrapped in a roll. Seedling growth parameters (length and fresh weight of the shout and root) were measured after 10 days.

Ageing treatment

The accelerated ageing test (AA test) was conducted according to Hampton and TeKrony (1995), with minor modifications as described by Jovicic *et al*. (2014). The seeds were exposed to 100% RH at 41°C for 72 hours in the water bath.

Invigoration treatments

Non-aged and aged seeds (after the accelerated ageing test) were subjected to invigoration by presoaking with distilled water (the seeds were soaked in water for 24 hours) and with 3% of KH_2PO_4 for 24 hours at 25°C. After that period, seeds were washed and re-dried near their original weight. Seeds were identically subjected to germination tests as described above and all parameters (GP, MGT, T50, length and fresh weight of the shoot and root) were determined.

Non-aged seeds without any treatment (invigoration) were used as a control, as well as aged seeds after the AA test, but without invigoration treatments.

Protein analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli *et al*. (1970) and Chanyou *et al*. (2006). Seeds of all treatments (controls, aged and invigorated samples) were ground into fine powder to extract the seed storage proteins. Ground seed material (50 mg) was homogenised with 0.5 ml of 0.1 M Tris buffer (pH 8.0) for two hours at room temperature, followed by centrifugation at 14,000 rpm for 10 minutes. The supernatant was mixed with denaturing buffer (0.125 M Tris ph 6.8 1% SDS, 5% glycerol, 2.5% mercaptoethanol and 0.01% bromophenol blue) in ratio 1:4 and boiled for four minutes at 90°C. Resolving gel (13%) and stacking gel (5%) were prepared according to ISTA (2020). The same amount of each extract (12 µl) was loaded and run at 50 mM for one hour and 20 minutes and at 70 mM for five hours and 10 minutes. The marker Page Ruler Prestained Protein Ladder (Thermo Scientific, #26616) was used for the determination of protein molecular weight in electrophoretograms. The proteins were simultaneously fixed and stained overnight with 0.23% Coomassie Blue R-250, dissolved in a solution of 3.9% TCA, 6% acetic acid and 17% methanol. Gel destaining was performed with 10% of acetic acid and 30% methanol.

Data Analysis

Statistical analysis of the data was performed using Statistica (version 12). The data were subjected to 2-way ANOVA to determine the effects of invigoration technique, seed ageing and their interaction. Tukey's test ($P \leq 0.05$) was used to differentiate between treatments.

Results

Seed invigoration did not affect germination, while seed ageing and interaction between invigoration and ageing had a significant impact (figure 1). However, both main factors and their interaction had a significant effect on the mean germination time and the time needed to reach 50% of germination. Although the initial germination of sunflower seeds under optimal conditions was quite high (96%), simultaneous stress conditions of elevated temperature and relative humidity during the AA test reduced seed germination (by 11.3%). At the same time, they slightly prolonged the time necessary for germination and did not significantly affect the required time to reach 50% germination.

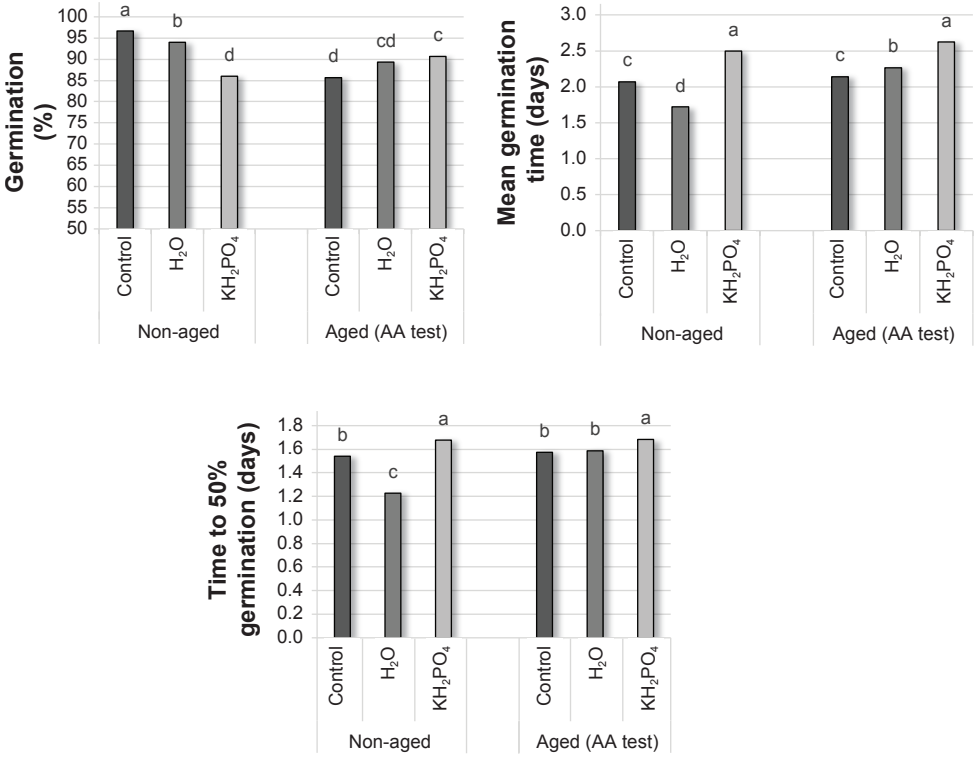

Figure 1. Effect of different invigoration techniques (water, KH_2PO_4) on germination characteristics of sunflower seeds cv. 'Fantazija' with and without accelerated ageing. Different letters at the top of the columns within each graph indicate significant differences ($P < 0.05$).

The different invigoration techniques had contrasting effects on the germination in non-aged and aged sunflower seeds. For instance, seed invigoration with water reduced the percentage of germination in non-aged seeds, while in aged seeds there was an increase in germination compared to the control. The trend was the same with seeds invigorated by KH_2PO_4. In other words, invigoration positively affected the germination process of aged seeds, in contrast to non-aged seeds where invigoration methods caused germination reduction in comparison to control, by 4.12% (water) and 9.3% (KH_2PO_4).

Analysing MGT and T50, common parameters used to evaluate germination speed, the same tendency was observed. Although the artificial ageing conditions did not retard germination, which might have been expected, the invigorating agents affected the change in germination rate in different ways. Also, the influence of invigorating agents differed depending on the condition of the seed. Water, as an invigoration agent, significantly decreased the germination rate of non-aged seeds, while it slowed down the germination of aged seeds. Invigoration with KH_2PO_4 prolonged the germination of both non-aged and aged sunflower seeds. The minimum time of T50 and the lowest MGT (1.3 days, 1.6 days, respectively) were recorded during the germination of non-aged seeds invigorated with water, while aged seeds and non-aged seeds invigorated with KH_2PO_4 germinated at the slowest rate.

Although it might have been expected, the unfavourable conditions during the AA test did not affect the length of shoots and the roots in control seeds (figure 2). In the case of non-aged seed, invigoration with water had a significant effect on the increase of these two parameters, while KH_2PO_4 affected the growth of only the roots. On the contrary, in aged seeds, KH_2PO_4 significantly increased the length of shoots and roots, and water only increased the roots compared to the control. The result also revealed that conditions causing seed deterioration were responsible for the differences observed in fresh shoots and roots weight of seedlings. A decline in fresh shoot weight was observed for both invigoration techniques, but the lowest degree of reduction was recorded in water invigoration.

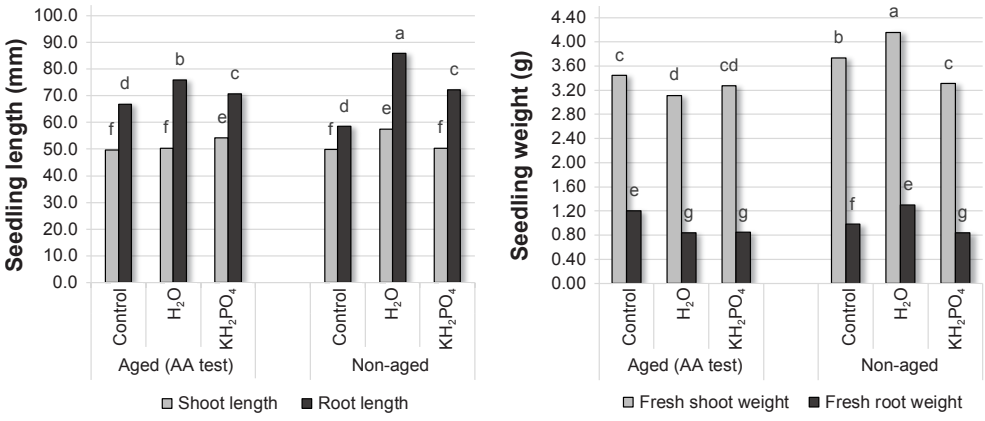

Figure 2. Effect of different invigoration techniques (water, KH_2PO_4) on shoot and root lengths of sunflower seedlings with and without accelerated ageing of the seeds. Different letters at the top of the columns within each graph indicate significant differences ($P < 0.05$).

Aiming to better understand the accelerated ageing mechanism of seed invigoration, SDS-PAGE was performed (figure 3). There was a similar number of protein bands in all treatments. The lowest molecular weight protein observed was 10 kDa, while the highest was 95 kDa. The low molecular weight proteins (lower than 34 kDa) produced high-intensity bands; in contrast, the high molecular weight proteins were in low concentrations.

In both seed samples invigorated with distilled water, the bands of 11 kDa were present (figure 3, samples 2 and 5), while in the aged seed invigorated with distilled water there was also bands of 13 and 17 kDa and absence of band of 15 kDa (figure 3, sample 5). Furthermore, there was a difference in thickness and relative intensity of bands between some treatments.

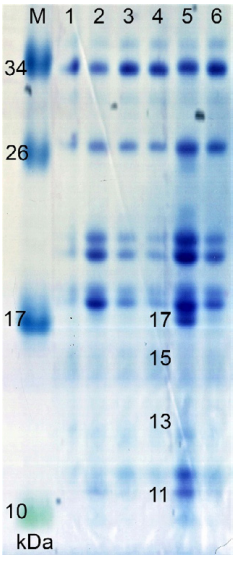

Figure 3. SDS PAGE profile of sunflower: M = molecular marker Page Ruler Prestained Ladder, #26616 (10-180 KDa); 1 = control non-aged; 2 = non-aged invigorated with water; 3 = non-aged invigorated with KH_2PO_4; 4 = control aged; 5 = aged invigorated with water; 6 = aged invigorated with KH_2PO_4.

Discussion

The positive effect of seed invigoration is attributed to the acceleration of the germination process upon sowing. Priming affects the lag phase of germination causing a series of events such as DNA replication (Thornton *et al*., 1993), promoting the activation of enzymes that mobilise reserve proteins (Di Girolamo and Barbanti, 2012), ATP levels and repair of mitochondria (Nie *et al*., 2020), and faster embryo growth (Pandita *et al*., 2007). However, the reduction of germination percentage of non-aged seeds after pre-soaking and the positive effect on germination percentage of aged seeds in this experiment (figure 1) suggests a different outcome of the invigoration agent depending on the condition of the seeds. At the same time, it indicates invigoration's impact on numerous metabolic processes, and therefore the complexity of this process. Thus, germination decrease after invigoration in the fresh seed can be explained by inadequate temperature or invigoration

duration. Yari *et al*. (2010) reported that *Triticum aestivum* L. subsp. *aestivum* seeds soaked in KH_2PO_4 for 12 hours at 20°C resulted in improved germination percentage and seed vigour, pointing out the importance of temperature in the success of the invigoration process. The importance of the interaction of primer treatment time and initial seed quality in determining the subsequent effects on seed viability and storage potential is also emphasized by Cheng and Bradford (1999) and Powell *et al.* (2000).

Priming of *Brassica oleracea* L. *var. capitata* seeds using KH_2PO_4 significantly increased germination, seedling strength, biomass accumulation and reduced mean germination time (Batool *et al*., 2015). Further, priming of sunflower seeds significantly increased germination, germination rate, shoots and roots dry weights and reduced mean germination time compared to control, and that priming with water was more effective in increasing all parameters compared to priming with KH_2PO_4 (Pirmani *et al*., 2013). However, in our study, the treatments with water and KH_2PO_4 in non-aged seeds affected the decrease of germination, but the water was effective in reducing MGT while KH_2PO_4 prolonged this parameter (figure 1). Regarding the aged seeds, both water and KH_2PO_4 solution caused a significant increase in germination percentage, compared to the control. The same results with *Brassica napus* L. seed were confirmed by Abdolahi *et al.* (2012), explaining that KH_2PO_4 seed priming influences membrane stability and mitigation of the adverse effects of artificial ageing. On the other hand, the prolongation of MGT and T50 caused by both invigoration agents in this experiment may be attributed to the delayed absorption of water during the invigoration process in conditions of limited available water due to the presence of salt affecting the slowed metabolic processes and the germination process (Ghassemi-Golezani *et al.*, 2008). Lower concentrations of KH_2PO_4 had a positive effect on germination parameters of *Cucumis sativa* L. seeds that were exposed to accelerated ageing for a shorter time, while seeds that are aged longer react positively to invigoration only at higher concentrations of primer agent (Krainart *et al.*, 2015). Furthermore, their study showed that KH_2PO_4 invigoration significantly reduced the lipid peroxidation intensity and the amount of total peroxide compared to the accelerated ageing seed before invigoration, simultaneously activating the antioxidant system.

Since the loss of vigour is a process that occurs gradually, MGT and T50 are imperative indicators, especially when it comes to germination and emergence under adverse environmental conditions. MGT represents the average delay between imbibition start and radicle protrusion, and it was found to be prognostic of emergence performance in different plant species: maize (Khajeh-Hosseini *et al*., 2009), pepper (Demir *et al*., 2008), oilseed rape (Amirmoradi and Feizi, 2017) and rice (Chinnasamy *et al.*, 2021). Furthermore, one of the first consequences of seed ageing is an increase in MGT (Bishoni and Santos, 1996). The results of this study are in agreement with Catiempo *et al.* (2021) who also confirmed that water-primed seeds germinate and emerge faster and more uniform, which is reflected in the decrease of T50. The reason for this may be the fact that the primed seeds are partially hydrated, which initiates early metabolic activities, thus the positive effect is maintained after re-drying (Chen and Arora, 2011). Since water is the main initiator of all activities in living cells, all metabolic processes occur much faster after the seed re-swelling during germination of the invigorated seed (Catiempo *et al*. 2021).

The positive effect of invigoration might be because it activates the cell cycle and mobilizes the storage proteins and increases nuclear replication at the seedling shoot and root cells (Ghiyasi *et al*., 2008; Salehzade *et al*., 2009). Hydropriming hastened seedling shoot and root growth in seeds stored for two years (Hussein, 2019). Sunflower seed invigoration with KH_2PO_4 improved root and shoot seedling length in non-aged seeds, while in aged seeds it showed almost no effect (figure 2). Although deeply damaged due to unfavourable conditions of artificial ageing, the aged seed is still able to germinate but is not able to regain its vigour after invigoration (Kausar *et al.*, 2009).

The water-soluble proteins found in storage vacuoles of sunflower seeds and whose molecular mass is between 10 and 20 kD seed are named 2S albumins (Franke *et al*., 2016). They are a major group of seed storage proteins widespread in both monocotyledonous and dicotyledonous plants (Li *et al*., 2001). An important role of these proteins is reflected in the protection against fungal attacks (Agizzio *et al.*, 2006). These proteins accumulate in the protein bodies of seeds, and plants use them as a source of nutrients during germination and seedling growth (Moreno and Clemente, 2008). In this study, after the accelerated ageing followed by water treatment in this study, some bands of proteins appear in the region of 2S albumins (figure 3). Taking into consideration the significantly higher germination percentage in this treatment, it is possible that changes in protein profile under these conditions positively affected the germination process. In contrast, Kausar *et al*. (2009) found that proteins with approximately 26, 48, 49, 69, 90, 118, 121, 150 and 199 kDa were affected by adverse conditions during accelerated ageing, which was not confirmed by this study.

When taken together, the results show that invigoration treatments were more effective in the low-vigour seed (aged), which can provide wide practical benefits, especially when seeds that have been stored for a long period are used.

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