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Seed priming with antioxidants improves sunflower seed germination and seedling growth under unfavorable germination conditions

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Abstract: The results of studying the effects of sunflower seed priming with an aqueous solution of ascorbic acid (A), tocopherol (T), and glutathione (G) performed prior to accelerated ageing and a cold test are presented in this paper. Germination, the percentage of abnormal seedlings, and the lengths of both roots and shoots were monitored. The results showed that the cold test caused a drastic drop in germination, an adverse effect on the shoot length, an increase in the percentage of abnormal seedlings, and no effect on the root length. Germination of seeds primed with the solution of A, T, and G (A+T+G) on which the cold test was then performed did not differ from seed germination of the control. Moreover, seed priming with the A+T+G solution of antioxidant substances prior to the cold test annulled the adverse effect of the cold test on shoot length, as well as on the percentage of abnormal seedlings. Seed priming with the A+T+G solution of antioxidants did not affect root length. Accelerated ageing resulted in a statistically significant decrease in seed germination and root length, but neither shoot length nor the percentage of abnormal seedlings was affected by accelerated ageing. The obtained results show that seed priming with the solution of antioxidant substances performed prior to accelerated ageing had a positive effect on the length of both roots and shoots. Seed priming with the solution of antioxidant substances performed prior to accelerated ageing did not affect germination, but it did increase the percentage of abnormal seedlings. The effect of antioxidant solution priming on the vigor of sunflower seeds exposed to the cold test was significantly more pronounced than that on seeds exposed to accelerated ageing.

Key words: Accelerated ageing, ascorbic acid, cold test, glutathione, tocopherol

Introduction

Seed vigor decreases at either a fast or slow rate after its physiological maturity, though it can be partially restored under certain circumstances (Pollock and Roos 1972). Nowadays, priming is widely applied with the aim to accelerate germination and to increase the uniformity of various crops, especially under unfavorable conditions during emergence (McDonald 2000; Halmer 2004). Seed priming affects the percentage of seed germination under different ecological conditions; it also affects germination rate, seed vigor, and seedling development. Many authors have shown that seed priming with water or solutions of various substances positively affects traits of seeds and seedlings (Burgass and Powell 1984; Bradford 1986; Taylor et al. 1998; McDonald 2000). Moreover, researchers have emphasized that seed priming mitigates the adverse effects of different

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stress factors (Chiu et al. 1995). Thus, hydropriming repairs the damage to sunflower seeds exposed to abiotic stresses such as salinity and drought, thereby improving germination performance and seedling growth (Kaya et al. 2006).

Sunflower seeds were mostly primed with water (Kaya et al. 2006) or a solution of polyethylene glycol. Bailly et al. (2002) determined that polyethylene glycol neutralizes the deterioration of seeds exposed to accelerated ageing. Namely, polyethylene glycol stimulates the activation of the antioxidant systems of catalase and glutathione reductase during seed imbibition and the initial seedling developmental stages (Bailly et al. 2002). Furthermore, priming of aged seeds progressively restores the initial germinative ability and reduces the level of lipid peroxidation (Bailly et al. 1998). Sunflower seed priming with a solution of polyethylene glycol has beneficial effects on germination even at lower temperatures (Bailly et al. 2000).

Apart from seed priming with water or a solution of polyethylene glycol, hormonal priming (Chen et al. 2005; Tiryaki and Buyukcingil 2009), priming with an aqueous solution of ascorbic acid (Basra et al. 2006), iron and boron (Mirshekari 2012), and α-tocopherol (Socrates et al. 1961) is also used. The strong effects of certain treatments on sunflower seed germination and their importance have been confirmed by Kibinza et al. (2011). These authors claim that 7 days of priming with polyethylene glycol (in the presence of 3-amino-1,2,4-triazole) following the ageing treatment improved the germination of aged seeds regardless of the duration of ageing.

The aim of this study was to observe whether priming with an aqueous solution of antioxidants (ascorbic acid, tocopherol, and glutathione) has an effect on sunflower seed performance under unfavorable germination conditions.

Material and methods

Hybrid seeds (moisture of 6.2%) of the F_1 generation of French high-oil sunflower Alvaro were used in these experiments. The seeds were treated with the pesticide metalaxyl-1 and stored at a temperature of 18 °C and a relative humidity of 75%.

A laboratory seed germination (control) was tested, the cold test was performed, and the seeds were exposed to accelerated ageing. Laboratory germination of seeds primed with the solution of antioxidants was tested. Finally, the effects of seed priming with the solution of antioxidant substances performed prior to accelerated ageing and the cold test were observed. According to the results obtained for germination (%), percentage of abnormal seedlings (%), and lengths of roots (cm) and shoots (cm), the effects of accelerated ageing, cold test, and priming on observed seeds have been determined. Seedlings were classified as normal or abnormal depending on the presence or absence of all essential structures (ISTA 2009). The experiment was carried out during 2010 in an ISTA accredited laboratory (Maize Research Institute Zemun Polje, Belgrade).

Seed priming

Seeds were primed with an aqueous solution of ascorbic acid, tocopherol, and glutathione. The tocopherol used in the experiment was dl-α-tocopheryl acetate (DSM Nutritional Products) in powder form (1 g = 500 IU). The ascorbic acid and glutathione were manufactured by SERVA Electrophoresis.

Seed priming with the aqueous solution of antioxidants consisted of the following steps: 1) dissolving the antioxidant substances in distilled water, 2) priming the seeds for 2 h with the obtained aqueous solution, and 3) taking the seeds out of the solution and drying for 5 h at 28 °C.

Seed priming was performed using solutions of the following 7 concentrations and combinations: 1) ascorbic acid (A) at 0.5% and 2.5%, 2) tocopherol (T) at 0.3% and 0.9%, 3) glutathione (G) at 0.05% and 0.1%, and 4) the combination of ascorbic acid at 0.5%, tocopherol at 0.3%, and glutathione at 0.05% (A+T+G).

Laboratory seed germination

A total of 500 (5 × 100) seeds were used for the germination test, while 5 × 10 randomly selected normal seedlings were used for the measurement of root and shoot lengths. Germination was tested at temperatures of 20 °C for 16 h and 30 °C for 8 h. Floragard compost of type floradur b-fine (pH = 6.16, moisture 68.8%) was used as a medium. The seedling evaluation and the measurement of root and

shoot lengths were completed on day 10. Germination of the initial material (control) and primed seeds was tested.

Cold test

A total of 500 (5 × 100) seeds were used for the germination test, while 5 × 10 randomly selected normal seedlings were used for the measurement of root and shoot lengths. Prior to carrying out the cold test, the water and compost necessary for the experiment were stored in a refrigerator for 24 h at 10 °C. The tests were performed in 2 stages. First, seeds were exposed to the temperature of 10 °C for 7 days during the cold stage. Next, in the warm stage, seeds were exposed to temperatures of 20 °C and 30 °C (16/8 h) for 6 days (ISTA 1995; Murcia et al. 2002). The germination determination, seedling evaluation, and measurements of root and shoot lengths were performed on day 6 of the warm stage.

Accelerated ageing

A total of 500 (5 × 100) seeds were used for the germination test, while 5 × 10 randomly selected normal seedlings were used for the measurement of root and shoot lengths. Accelerated ageing at the temperature of 40 °C under conditions of high air humidity (95%-100%) lasted 72 h (Gay et al. 1991), after which the laboratory seed germination was tested.

Statistical analysis

In order to test the effects of seed priming with the aqueous solutions of ascorbic acid, tocopherol, and glutathione prior to the laboratory germination, accelerated ageing, and cold tests, we compared each case with the laboratory seed germination (control). We cannot presume that the variables (germination, the percentage of abnormal seedlings, and the lengths of both roots and shoots) are normally distributed. Therefore, for each comparison we performed 2 sample Wilcoxon tests, also known as the Mann-Whitney test (Hollander and Douglas 1999). In cases where $P < 0.05$, we report differences in the median values as statistically different. We used the R package (Ihaka and Gentleman 1996) (version 2.9.2) to calculate the statistics.

Results

Seed priming and laboratory germination

Seed priming with the A+T+G aqueous solution led to a decreased germination of seeds tested under optimum temperature conditions, though this was not statistically significant (Table 1). Furthermore, seed priming with the A+T+G solution resulted in a slight increase in root length, a decrease in shoot length, and a slight increase in the percentage of abnormal seedlings, though this was not statistically significant (Table 1).

Seed priming and cold test

Germination under cold test conditions was drastically reduced in the observed seeds, whereas the germination of seeds that were primed with the A+T+G solution prior to the performance of the cold test was almost the same as the seed germination of the control (Table 1). The obtained results show that the cold test had no effect on root length (Table 1). Seed priming with the A+T+G antioxidant solution performed prior to the cold test led to a small increase in root length in relation to the control, but

Table 1. Median values of germination, root length, shoot length, and percentage of abnormal seedlings in the case of priming and cold test are given. Statistically significant differences between the laboratory seed germination (C) and other effects are denoted with an asterisk (*). For effect abbreviations, see the legend of Figure 1.

	Germination	Root length	Shoot length	Abnormal seedlings
C	86	11.7	10.2	7
P + CT	85	12.9	10.5	7
CT	75*	12	7.9*	14*
P + LG	83	12	9.3	8

this difference was not statistically significant (Table 1). The cold test of seeds resulted in an extreme decrease in shoot length. However, seed priming with the A+T+G solution completely neutralized the negative effect of the cold test on shoot length (Table 1). The cold test increased the percentage of abnormal seedlings, while seed priming with the A+T+G antioxidant solution completely neutralized the negative effect of the cold test on the percentage of abnormal seedlings (Table 1).

Seed priming and accelerated ageing

Accelerated ageing resulted in a statistically significant reduction in germination of the observed sunflower seeds (Table 2). In addition, seed priming with the solution of antioxidant substances carried out prior to accelerated ageing (in all applied concentrations) resulted in the seed germination decreasing in relation to the control (Table 2). Accelerated ageing negatively affected root length. Nevertheless, root length after priming and accelerated ageing did not show a statistically significant reduction in relation to the control (Table 2). Accelerated ageing did not affect shoot length. Furthermore, seed priming with the 0.5% solution of ascorbic acid did not affect the shoot length of seeds that underwent accelerated ageing (Table 2). However, seed priming with solutions of ascorbic acid (2.5%), tocopherol (both concentrations), glutathione (both concentrations), and A+T+G resulted in a statistically significant increase in the shoot length of normal seedlings in relation to the control (Table 2). Accelerated ageing did not affect the percentage of abnormal seedlings. In addition, seed priming with the solutions of tocopherol (both concentrations) and glutathione (the higher concentration) did not affect the percentage of abnormal seedlings (Table 2). However, seed priming with the solutions of ascorbic acid (both concentrations), glutathione (the lower concentration), and the A+T+G antioxidant performed prior to accelerated ageing increased the percentage of abnormal seedlings (Table 2).

Discussion

Since Heydecker et al. (1973) developed the term 'seed priming', many researchers have been studying the positive and negative effects of priming on traits of seeds and seedlings of many species. In this study, in order to establish the effect of priming on the traits of their seeds and seedlings, sunflower seeds were primed with a solution of antioxidant substances for the first time.

Seed priming with the A+T+G solution of antioxidant substances performed prior to the laboratory germination testing (20/30 °C) did not affect germination, the percentage of abnormal seedlings, or the lengths of roots and shoots in relation to the seeds of the control (Figures 1a-1d). Similar results have been obtained for other crop species (Demir and Van de Venter 1999; Amooaghaie et al. 2010).

Table 2. Median values of germination, root length, shoot length, and percentage of abnormal seedlings in the case of priming and accelerated ageing are given. Statistically significant differences between the laboratory seed germination (C) and other effects are denoted with an asterisk (*). For effect abbreviations, see the legend of Figure 1.

	Germination	Root length	Shoot length	Abnormal seedlings
C	86	11.7	10.2	7
AA	73*	10.3*	10.9	7
a05	69*	9.7	10.6	11*
a25	74*	10.8	12.0*	16*
t03	74*	11.6	11.4*	10
t09	76*	12.8	12.9*	9
g005	69*	12.6	14.8*	14*
g01	68*	12.3	11.7*	9
A+T+G	74*	12.8	16.9*	14*

In this study, the cold test of sunflower seeds resulted in a statistically significant reduction in germination and shoot lengths while it increased the percentage of abnormal seedlings, but it did not affect root lengths. Seed priming with the A+T+G solution performed prior to the cold test annulled the negative effects of this test on germination. Moreover, priming neutralized the adverse effect of the cold test on both shoot length and percentage of abnormal seedlings. However, seed priming did not affect root length (Figures 1a-1d).

Numerous researchers have established that seed priming with an osmotic solution, especially under suboptimal temperature conditions, stimulates seed germination of sunflower (Smok et al. 1993), maize, wheat, barley, soya bean (Bodsworth and

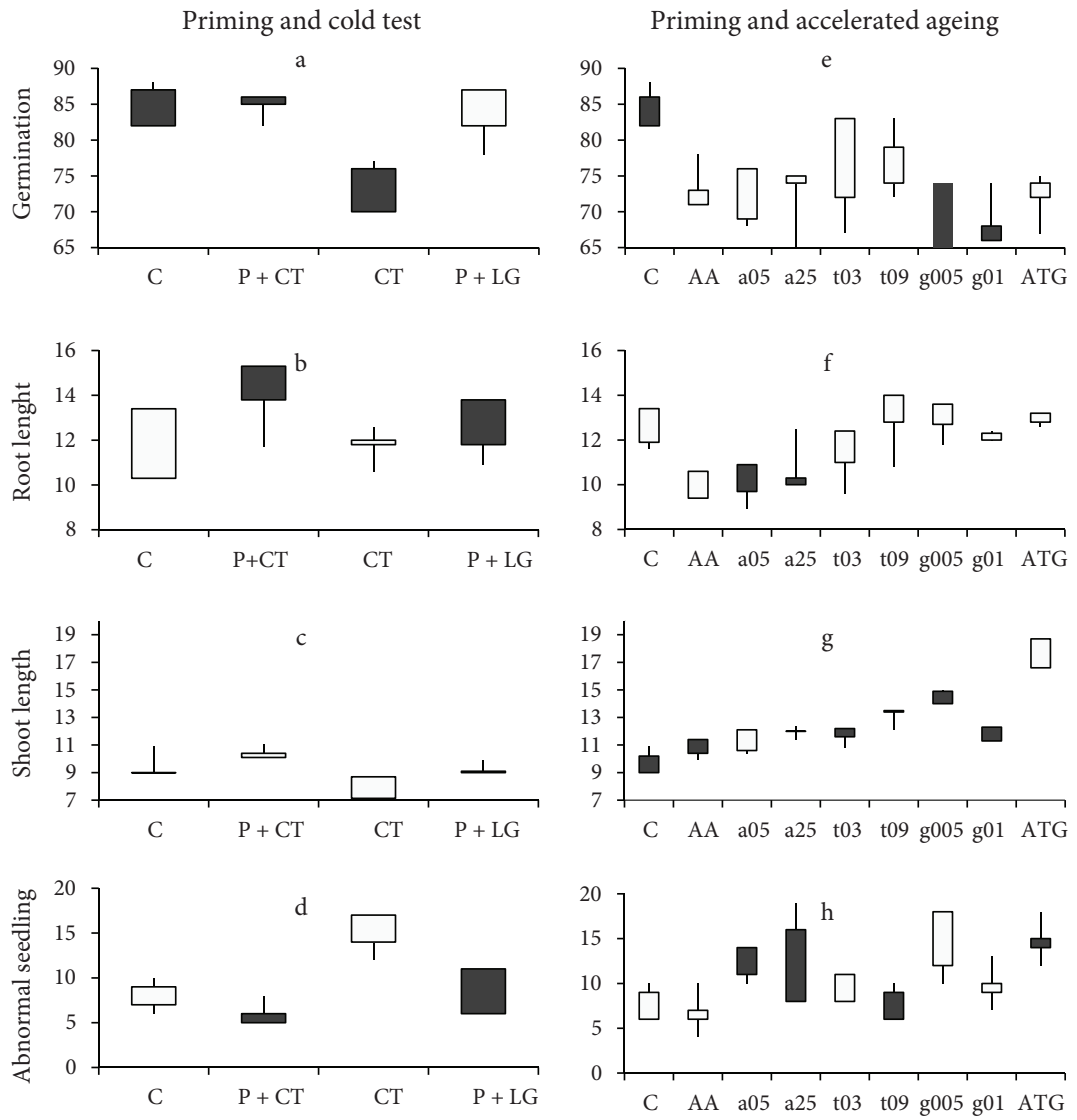

Figure 1. The boxplot matrix gives the results for germination, root length, shoot length, and percentage of abnormal seedlings in cases of seed priming prior to laboratory germination, accelerated ageing, and cold test. The rows of the boxplot matrix represent monitored characteristics and the columns represent different effects of priming. C = control, P + CT = priming + cold test, CT = cold test, P + LG = priming + laboratory germination, AA = accelerated ageing, a05 = ascorbic acid 0.5%, a25 = ascorbic acid 2.5%, t03 = tocopherol 0.3%, t09 = tocopherol 0.9%, g005 = glutathione 0.05%, g01 = glutathione 0.1%, and A+T+G = solution of antioxidant substances.

Bewley 1981), and sweet maize (Sung and Chang 1993). Furthermore, Chen and Sung (2001) showed that priming of *Momordica charantia* L. seeds with an aqueous solution of sodium selenite (Na_2SeO_3) positively affected seed germination under suboptimal temperatures.

Accelerated ageing resulted in a statistically significant decrease in seed germination, which had been previously determined by other authors studying sunflower seed (Torres et al. 1997). However, seed priming with the antioxidant solutions (all applied concentrations) performed prior to accelerated ageing neither reduced nor neutralized the drop in germination, but did neutralize the negative effect of accelerated ageing on root length (Figures 1e-1f). There was no statistically significant difference between the root length of the control and the root length of seedlings from seeds on which priming followed by accelerated ageing was applied. The same effect of short-duration water prehydration and osmopriming (polyethylene glycol) on the root length of lettuce seeds performed prior to a controlled deterioration test was established by Tarquis and Bradford (1992).

On the other hand, seed priming with the antioxidant solution varied greatly in terms of its effect on shoot length. First of all, accelerated ageing did not affect the shoot length. Secondly, priming with the solution of ascorbic acid of a lower concentration prior to accelerated ageing did not affect shoot length. Thirdly, seed priming with the solutions of glutathione (both concentrations), tocopherol (both concentrations), ascorbic acid (the higher concentration), and the A+T+G solution performed prior to accelerated ageing led to an increase in shoot length. Finally, the action of the A+T+G solution showed the strongest positive effect on seed shoot length (Figure 1g).

Previous studies carried out by Chhetri et al. (1993) showed that seed priming of French bean, peas, lentil, and millet with a solution of ascorbic acid significantly reduced the negative effects of accelerated ageing on the length of both roots and shoots, which is supported by the results obtained in the present study.

Accelerated ageing did not result in an increase in the percentage of abnormal seedlings. However, in certain cases, the priming performed prior to accelerated ageing increased the percentage of abnormal seedlings, while, as discussed below, in other cases it did not increase this percentage. Namely, seed priming with the ascorbic acid (both concentrations), glutathione (the lower concentration), and A+T+G solutions performed prior to accelerated ageing increased the percentage of abnormal seedlings, while in the remaining cases the percentage of abnormal seedlings did not increase (Figure 1h).

Many authors have revealed different effects of seed priming performed prior to accelerated ageing on seed germination. The negative effect of priming with a solution of polyethylene glycol on sunflower seed germination was established in Chojnowski et al. (1997). Seed priming adversely affected germination of annual ryegrass (Hacisalihoglu and Ross 2010) and of leek and carrot (Dearman et al. 1987; Corbineau et al. 1994). However, some researchers claim that seed priming of onion (Dearman et al. 1986) and pepper (Georghiou et al. 1987) reduces the negative effects of accelerated ageing, while Hacisalihoglu (2008) shows that seed priming of switchgrass performed prior to accelerated ageing positively affects germination.

The results obtained in this study are supportive of the view that priming increases sunflower seed susceptibility to accelerated ageing. Greater susceptibility of primed seeds to accelerated ageing is related to the effect of priming and drying on the protection mechanisms encompassing free radical and peroxide-scavenging enzymes, such as superoxide dismutase, catalase, and glutathione reductase (Chojnowski et al. 1997). This is also in agreement with the finding that a strong relationship exists between the reduction of activities of the stated enzymes and sunflower seed deterioration during accelerated ageing (Bailly et al. 1996)

The results of this study are in agreement with the opinion given by McDonald (2000), which states that different types of seed priming should be evaluated individually for each type and lot of seeds. Therefore, it is important to always determine the effects of a certain type of priming on a given trait of seeds, i.e. whether these effects are positive, negative, or neutral (Argerich and Bradford 1989).

The results obtained in the present study show that sunflower seed priming performed prior to a cold test completely neutralized the decrease in germination, shoot length, and the percentage of abnormal seedlings. In addition, priming neutralized the negative effect of accelerated ageing on root length. Finally, priming performed prior to accelerated ageing (except in the case of the lower concentration of ascorbic acid) increased the shoot length in relation to the control.

Based on the achieved results, it can be concluded that the effect of priming with solutions of antioxidant substances on the vigor of sunflower seeds exposed to low temperatures (cold test) was significantly greater than that of seeds exposed to both high temperatures and high humidity (accelerated ageing). This can be explained by a lower accumulation of deterioration when the cold test is applied, as opposed to cases when accelerated ageing is performed, which provides the opportunity for the seeds to restore vigor after priming. The subsequent studies on sunflower seed priming should be aimed at priming duration, combinations of antioxidant substances, and their effects on seed longevity.

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