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Germination and Seedling Growth Response on Sunflower Seeds to Priming and Temperature Stress

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Abstract: The present paper shows results obtained on effects of priming of sunflower seeds, subjected to accelerated ageing and the cold test, on seed vigour. Seeds were primed with distilled water, the potassium nitrate solution (0.2%) and the gibberellic acid solution (0.04%). The following parameters were tested: energy of germination, germination, proportion of abnormal seedlings, lengths of roots and shoots of normal seedlings. Accelerated ageing in the course of 3 and 5 days resulted in a statistically significant reduction in energy of germination and germination; it adversely affected the length of roots and shoots and it increased the proportion of abnormal seedlings. Seed priming with all three solutions mitigated adverse effects of 3-day accelerated ageing on energy of germination. Furthermore, seed priming with gibberellic acid prior to 3-day accelerated ageing positively affected seed germination and neutralized a negative effect of accelerated ageing on the number of abnormal seedlings, as well as on lengths of shoots and roots of normal seedlings. The cold test (at 5°C for 7 days) negatively affected energy of germination and the root length, increased the proportion of abnormal seedlings and did not affect seed germination. Priming of seeds with distilled water prior to the cold test completely neutralized the adverse effect of low temperatures on energy of germination. Finally, priming of seeds with all three solutions completely neutralized the adverse effect of cold test on the root length.

Keywords: accelerated ageing, cold test, gibberellic acid, potassium nitrate, seed enhancement

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Introduction

Priming is a process in which seeds are allowed to imbibe sufficient water to enable the early events in the germination process to occur, but not sufficient to permit radicle emergence through the seed coat (Heydecker *et al*., 1975). Seed priming increases germination, germination speed and uniformity (at low and high temperatures), and improves seed vigour (Basu *et al*., 1979). Therefore, there is a strong interest in the seed industry to find suitable priming agent(s) that might be used to increase the tolerance of plants under adverse field conditions (Job *et al*., 2000). In recent decades, different hydration treatments on seeds have been applied (good reviews: Taylor *et al*., 1998; McDonald, 2000).

The majority of studies on *Helianthus annuus* L. seed have been related to seed priming with the polyethylene glycol solution (Bailly *et al*., 1998; Mwale *et al*., 2003). Numerous studies have confirmed that priming increased germination of *H. annuus* L. seed even under conditions of accelerated ageing (Bailly *et al*., 1998, 2002; Kibinza *et al*., 2011). Furthermore, it has been shown that priming increased resistance of seeds of many species to low temperatures: *H. annuus* L. (Chojnowski *et al*., 1997; Bailly *et al*., 2000), *Allium porrum* L. (Corbineau *et al*., 1994), *Momordica charantia* L. (Chen and Sung, 2001; Wang *et al*., 2003), *Solanum lycopersicon* L., *Capsicum annuum* L., *Beta vulgaris* L. (Nelson and Sharples, 1980a), *Cucumis melo* L., *Cucumis sativus* L. (Nelson and Sharples, 1980b) and sweet maize (*Zea mays* var. *saccharata*) (Sung and Chang, 1993). Positive effect of priming is reflected in the increase of germination, speed of germination and uniformity (Dahal *et al*., 1990; Mauromicale and Cavallaro, 1996).

Besides hydropriming (Fujikura *et al*., 1993; Kaya *et al*., 2006) and priming with the polyethylene glycol solution (Dell'Aquilla and Tritto, 1990) seeds are primed with an aqueous solutions of ascorbic acid, tocopherol and glutathione (Draganić *et al*., 2011; Draganić and Lekić, 2012), potassium nitrite solution (Demir and Van de Venter, 1999) and solutions of plant growth regulators, most often gibberellic acid solutions (Naidu *et al*., 2000; Chen *et al*., 2005; Liopa-Tsakalidi and Barouchas, 2011). Gibberellins have a specific role in the induction *de novo* synthesis of hydrolytic enzymes (Chen *et al*., 2005), including α-amylase, a key enzyme during germination and a later growth of cereal seedlings (Mitsunaga *et al*., 2007). Kaur *et al*. (1998) have emphasized that seed treatments with gibberellins induce germination by enhancing the availability of gibberellins contained in the seed.

The present study was designed to investigate the possibility of sunflower seeds invigoration by seed priming with distilled water, potassium nitrate solution (KNO_3) and the gibberellic acid solution (GA_3). The accelerated ageing

protocol (Gay *et al.*, 1991) and cold test were used to induce seed deterioration evaluated by the decrease in energy of germination, germination percentage, lengths of roots and shoots of normal seedlings and by the increase in proportion of abnormal seedlings.

Materials and methods

Hybrid seed of the F_1 generation of the medium early KWS sunflower hybrid (moisture 5.78%) was used in the studies. Seeds were treated with the insecticide "CRU" and stored at the temperature of 18°C and relative humidity of 65%. The experiment was carried out at the ISTA Accredited Laboratory (Maize Research Institute "Zemun Polje", Belgrade).

Seed vigour of the initial material (control) was tested, and then effects of accelerated ageing and the cold test on sunflower seeds were observed. Finally, the effect of priming on vigour of seeds subjected to accelerated ageing and the cold test was studied. The parameters of seed vigour are as follows: energy of germination (%), germination (%), proportion of abnormal seedlings (%), root length (cm) and shoot length (cm) of normal seedlings. Energy of germination is the percentage of germinating seeds 4 days after sowing relative to the total number of seeds tested (Ruan *et al.*, 2002). Seedlings were classified as normal or abnormal depending on the presence or absence of all essential structures, respectively (ISTA, 2009).

Seed priming

Seeds were primed with (a) distilled water, (b) aqueous solution of potassium nitrate (0.2%) and (c) aqueous solution of gibberellic acid GA_3 (0.04%). Seeds were primed, dried and subjected to the low and high temperature treatments in the following three ways: (a) priming + drying + accelerated ageing, (b) accelerated ageing + drying + priming and (c) priming + cold test. Priming lasted 6 h, while drying was performed at 28°C in the course of 24 h.

Seed germination

A total of 400 seeds (4×100) were used for the germination test, while 4×10 randomly selected normal seedlings were used to measure the root and shoot

length. Germination was tested at the temperature of 25°C, while compost was used as a substrate (Floragard, type: floradur b-fine, pH = 6.16, moisture 68.8%). Energy of germination and the measurement of the root and shoot length were estimated on the fourth day. The final count of germination was estimated on the 10th day.

Accelerated ageing

A total of 400 seeds (4×100) were used for the test, while 4×10 randomly selected normal seedlings were used to measure the root and shoot length. Seeds were subjected to the temperature of 40°C (Gay *et al.*, 1991) under conditions of high air humidity (95–100%) in the course of 48, 72 and 120 h (2, 3 and 5 days). Seed vigour was determined after accelerated ageing.

Cold test

The test consists of cold and warm phases. In the cold phase, seeds were subjected to the temperatures of: a) 9°C for 7 days; b) 9°C for 9 days and c) 5°C for 7 days. The worm phase at the temperature of 25°C lasted 6 days. A total of 400 seeds (4×100) were used for the test, while 4×10 randomly selected normal seedlings were used to measure the root and shoot length. Water and a substrate (compost), used in the test, were cooled down to 10°C for 24 h prior to the beginning of the cold test. After the performance of the cold test, seed vigour was determined; energy of germination and the length of roots and shoots of normal seedlings were estimated on the fourth day of the warm phase, while the final count and the number of abnormal seedlings were determined on the sixth day of the warm phase.

Statistical analysis

Descriptive and analytical statistics were performed with the statistic package SPSS 10.0 for Windows. Significance of differences between mean values of observed factors (energy of germination, germination, abnormal seedlings, root and shoot lengths) was tested by the application of the analysis of variance (ANOVA). All estimates of significance were derived from *F* and least significant difference (LSD) tests at the 5% probability level. Coefficient of correlations between studied traits was also calculated.

Results and discussion

The single-factor ANOVA points out to the existence of statistically very significant differences ($p \leq 0.01$) in energy of germination, germination, the number of abnormal seedlings, shoot and root lengths between control and all applied treatments (Tables 1 and 2).

Table 1: Analysis of variance – accelerated ageing and priming.

Source of variation	Df	Mean squares (MS)				
		Energy of germination (%)	Germination (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)
Replications	3	29.53	20.133	11.83	0.06	0.23
Treatments	21	2,878.44**	1,733.58**	64.79**	18.96**	8.00**
Error	63	108.60	79.01	12.484	2.21	0.57
Total	**87**					

Note: **$p \leq 1\%$.

Table 2: Analysis of variance – cold test and priming.

Source of variation	Df	Mean squares (MS)				
		Energy of germination (%)	Germination (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)
Replications	3	2.38	0.77	0.89	0.60	0.33
Treatments	12	39.02**	11.01**	7.74**	10.18**	19.19**
Error	36	1.86	1.91	0.36	0.22	0.22
Total	**51**					

Note: **$p \leq 1\%$.

Accelerated ageing led to a significant reduction in the shoot length (Table 3). Three- and 5-day accelerated ageing resulted in a statistically significant decrease of energy of germination, germination, root length of normal seedlings and in the increase in the number of abnormal seedlings. The cold test at 5°C in the course of 7 days did not affect germination, but it led to the decrease of energy of germination of tested seeds. Furthermore, the cold test carried out at 9°C and 5°C for 7 days resulted in the increased number of abnormal seedlings (Table 4). Seed priming, especially with the gibberellic acid solution, differently affected the observed traits (Tables 3 and 4).

Table 3: Energy of germination, germination, proportion of abnormal seedlings and lengths of roots and shoots of normal seedlings of seeds in control, seeds after accelerated ageing (2, 3 and 5 days, seeds primed before and after accelerated ageing (2, 3 and 5 days).*

Treatment	Traits				
	Energy of germination (%)	Germination (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)
C	93.7 a	95.7 a	0.75 i	14.07 a	5.71 defg
AA2	84.0 abcd	89.0 abcd	4.25 fghi	12.46 abcde	4.58 hijk
$H_2O + AA2$	90.0 ab	92.5 ab	2.25 hi	12.84 abc	6.50 cd
$KNO_3 + AA2$	89.7 abc	92.0 abc	2.25 hi	11.97 abcde	6.46 cde
$GA_3 + AA2$	85.7 abcd	88.7 abcd	4.25 fghi	12.24 abcde	7.95 a
$AA2 + H_2O$	65.0 ef	79.7 cde	8.00 cdef	13.17 ab	5.44 efgh
$AA2 + KNO_3$	74.5 def	87.5 abcd	6.50 defgh	13.13 ab	5.23 fghi
AA3	47.7 g	65.0 f	10.00 bcde	9.53 fg	3.67 kl
$H_2O + AA3$	69.0 ef	77.2 def	5.75 defgh	10.65 def	4.67 ghijk
$KNO_3 + AA3$	65.2 ef	73.0 ef	7.75 def	11.15 bcdef	4.87 ghij
$GA_3 + AA3$	78.7 bcde	83.0 bcde	2.75 ghi	13.30 a	7.84 ab
$AA3 + H_2O$	62.5 f	78.0 de	6.00 defgh	11.99 abcde	5.08 fghi
$AA3 + KNO_3$	63.2 f	77.5 def	4.50 fghi	10.41 efg	4.51 hijk
AA5	24.7 hi	47.0 g	12.75 abc	7.29 hi	3.21 l
$H_2O + AA5$	32.7 h	47.2 g	10.25 bcd	9.71 fg	3.89 jkl
$KNO_3 + AA5$	15.7 i	38.5 gh	10.00 bcde	7.34 hi	3.08 l
$GA_3 + AA5$	18.5 hi	48.0 g	16.25 a	6.23 i	3.78 kl
$AA5 + H_2O$	24.2 hi	36.5 gh	8.75 cdef	8.41 gh	3.23 l
$AA5 + KNO_3$	28.2 hi	44.0 g	9.75 bcde	9.60 fg	4.35 ijk
$AA5 + GA_3$	15.2 i	31.0 h	14.25 ab	9.63 fg	5.14 fghi
$LSD_{0.05}$	**14.7**	**12.6**	**5.0**	**2.1**	**1.06**

Note: *C, control; AA2, 2-day accelerated ageing; AA3, 3-day accelerated ageing; AA5, 5-day accelerated ageing; $H_2O + AA2$, AA3 or AA5, seed priming with distilled water prior to 2-, 3- or 5-day accelerated ageing; $KNO_3 + AA2$, AA3 or AA5, seed priming with aqueous solution of potassium nitrite prior to 2-, 3- or 5-day accelerated ageing; $GA_3 + AA2$, AA3 or AA5, seed priming with aqueous solution of gibberellic acid prior to 2-, 3- or 5-day accelerated ageing; AA2, AA3 or $AA5 + H_2O$, seed priming with distilled water after 2-, 3- or 5-day accelerated ageing; AA2, AA3 or $AA5 + KNO_3$, seed priming with aqueous solution of potassium nitrite after to 2-, 3- or 5-day accelerated ageing; AA2, AA3 or $AA5 + GA_3$, seed priming with aqueous solution of gibberellic acid after 2-, 3- or 5-day accelerated ageing.

Obtained results indicate that the increase in the duration of accelerated ageing led to a gradual, but statistically significant decrease in energy of germination and germination. After 5 days of accelerated ageing, the drop in energy of germination was higher that after 3 days of accelerated ageing (statistically significant difference) in relation to the control (Table 3). Similar results have

Table 4: Energy of seed germination, germination, number of abnormal seedlings and lengths of roots and shoots of seeds in control, seeds after cold test and seeds primed before cold test*.

Treatment	Traits				
	Energy of germination (%)	Germination (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)
C	93.7 def	95.7 cd	0.75 gh	14.57 bc	5.71 i
CT1	94.5 cde	95.0 cd	2.25 def	11.91 e	11.88 b
H_2O + CT1	92.7 ef	95.0 cd	2.75 cde	10.84 f	11.16 cd
KNO_3 + CT1	96.7 b	97.7 ab	1.50 fg	11.51 ef	10.04 f
GA_3 + CT1	94.7 cd	95.0 cd	2.75 cde	12.16 e	13.48 a
CT2	89.5 g	96. 0 bc	3.00 bcd	13.33 d	8.42 g
H_2O + CT2	92.0 f	94.5 cd	2.50 de	14.07 c	7.24 h
KNO_3 + CT2	89.0 g	94.5 cd	3.75 b	15.07 b	8.56 g
GA_3 + CT2	88.0 g	92.0 e	5.00 a	14.61 bc	11.80 bc
CT3	98.7 a	98.7 a	0.50 h	14.60 bc	10.91 de
H_2O + CT3	93.2 def	95.0 cd	3.50 bc	14.27 c	10.60 def
KNO_3 + CT3	96.2 bc	95.7 cd	2.00 ef	14.37 c	10.26 ef
GA_3 + CT3	93.2 def	94.0 d	5.00 a	16.28 a	12.45 b
$LSD_{0.05}$	**1.95**	**1.98**	**0.86**	**0.68**	**0.67**

Note: *C, control; CT1, cold test at 9°C for 7 days; CT2, cold test at 5°C for 7 days; CT3, cold test at 9°C for 9 days.

been obtained by other researchers who had studied seeds of *H. annuus* L. (Torres *et al.*, 1997) and *Cuc. melo* L. (Nascimento and Souza de Aragão, 2004).

Seed priming with all three solutions mitigated the adverse effect of the 3-day accelerating ageing on energy of germination (Table 3). Nevertheless, the positive effect of seed priming on germination was detected in seeds primed with the gibberellic acid solution prior to the 3-day accelerating ageing and in hydroprimed seeds after the 3-day accelerated ageing (Table 3). However, other species differently respond to priming. Fujikura *et al.* (1993) have established that hydropriming of accelerated-aged *Brassica oleracea* L. seeds did not affect their germination.

Anyhow, priming of sunflower seeds tested prior or after the 3-day accelerated ageing with the potassium nitrate solution did not affect their germination (Table 3), because mechanisms of activities of gibberellins and nitrites are different. Nascimento and Souza de Aragão (2004) have established the positive effect of priming of aged *Cuc. melo* L. seeds with the potassium nitrate solution on their germination, which indicates that this solution does not equally affect seeds of different species.

The greater duration of accelerated ageing is the greater number of abnormal seedlings is (Table 3), which is confirmed by studies carried out on seeds of *H. annuus* L. (Torres *et al.*, 1997), *Phaseolus vulgaris* L., *Pisum sativum* L., *Lens*

culinaris L. and *Panicum miliaceum* L. (Chhetri *et al*., 1993). Gibberellic acid and potassium nitrate applied prior and after 3-day accelerated ageing, respectively, completely neutralized a negative effect of accelerated ageing on the number of abnormal seedlings (Table 3).

Finally, priming of seeds with all three solutions before and after 5-day accelerated ageing did not alleviate its negative effect on energy of germination and the number of abnormal seedlings (Table 3). Nonetheless, some researchers have determined that priming of aged sunflower seeds (45°C for 5 days at relative humidity of 100%) with other solutions (polyethylene glycol) completely neutralized an adverse effect of 5-day accelerated ageing on germination of sunflower (Bailly *et al*., 1998, 2002). Furthermore, Kibinza *et al*. (2011) have stated that 7-day priming (in the presence of 3-amino-1,2,4-triazol) following the ageing (3, 5, 7 and 9 days at 35°C) treatment improved germination of aged sunflower seeds whatever the duration of ageing.

Accelerated ageing negatively affected the seedling root and shoot lengths. Priming of seeds with the gibberellic acid solution prior to 3-day accelerated ageing and hydropriming of seeds after 3-day accelerated ageing completely neutralized negative effects of accelerated ageing on the root length (Table 3). Other solutions also positively affect the root length of different species. Priming of *Lactuca sativa* L. seeds with water and polyethylene glycol prior to the controlled deterioration test positively affects the root length (Tarquis and Bradford, 1992). Furthermore, priming of seeds with ascorbic acid weakens the negative effect of accelerated ageing on root and shoot lengths of *Pha. vulgaris* L., *Pis. sativum* L., *L. culinaris* L. and *Pan. miliaceum* L. seedlings (Chhetri *et al*., 1993).

The strongest effect on the shoot length of seeds subjected to 2- and 3-day accelerated ageing was expressed by gibberellic acid (Table 3). A similar effect determined by Chhetri *et al*. (1993) showed that seed priming of French bean, peas, lentil and millet with the solution of ascorbic acid significantly reduced negative effects of accelerated ageing on the length of both roots and shoots.

Seed hydropriming neutralized a negative effect of the cold test (5°C for 7 days) on energy of germination (Table 4).

Seed priming with solutions of potassium nitrite and gibberellic acid had no effect on energy of germination of seeds subjected to the cold test at 5°C for 7 days (Table 4). Similar results have been reported by Tiryaki and Buyukcingil (2009), who have emphasized that plant hormones, first of all gibberellic acid, had no effect on germination and energy of germination of *Sorghum bicolor* L. Moench seeds at low temperatures. However, some researches claim that seed priming with the gibberellic acid solution positively affected seeds of *S. lycopersicon* L., *Cap. annuum* L., *B. vulgaris* L. (Nelson and Sharples, 1980a), *Cuc. sativus* L. and *Cuc. melo* L. (Nelson and Sharples, 1980b) at low temperatures.

Seed priming with the gibberellic acid solution before the cold test (9°C for 7 days) successfully neutralized an adverse effect of low temperatures on the number of abnormal seedlings (Table 4).

Finally, seed priming with all three solutions completely neutralized a negative effect of the cold test (5°C for 7 days) on the root length (Table 4).

Results obtained in the present study point out that low temperatures affect less shoot lengths than root lengths of normal sunflower seedlings, which is in accordance with the results gained by Gay *et al.* (1991).

Coefficient of correlations between studied traits in the regime of accelerated ageing is given in Table 5. Strong positive correlations were found between germination and root and shoot length (Table 5). Furthermore, correlations between energy of germination, as well as germination, and abnormal seedlings were negative and statistically very significant. Also negative and very significant correlations were found between abnormal seedling and both root length and shoot length (Table 5).

Coefficient of correlations between studied traits in the regime of cold test is given in Table 6. Strong and negative correlation was found between germination and abnormal seedlings in the regime of cold test (Table 6). But correlations between germination and both root and shoot length were weak and negative (Table 6).

Table 5: Coefficient of correlations between studied traits in the regime of accelerated ageing.

Studied trait	Germination (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)
Energy of germination (%)	0.971**	−0.800**	0.802**	0.736**
Germination (%)		−0.761**	0.762**	0.679**
Abnormal seedlings (%)			−0.655**	−0.599**
Root length (cm)				0.717**

Note: $**p \leq 1\%$

Table 6: Coefficient of correlations between studied traits in the regime of cold test.

Studied trait	Germination (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)
Energy of germination (%)	0.661**	−0.651**	−0.270	0.164
Germination (%)		−0.644**	−0.167	−0.121
Abnormal seedlings (%)			0.344**	0.370**
Root length (cm)				−0.106

Note: $**p \leq 1\%$

Conclusions

Two-day accelerating ageing did not affect the observed seed traits, while 3- and 5-day accelerating ageing of seed negatively influenced seed vigour. Besides, accelerated ageing affected more deterioration of observed traits of sunflower seeds than the cold test. Gained results indicate that gibberellic acid most strongly neutralized activities of accelerated ageing on the proportion of abnormal seedlings, lengths of roots and shoots, but only if changes were not irreversible.

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