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Changes in the growth and antioxidant enzyme activity of seedlings originating from wheat seeds subjected to accelerated ageing test

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Abstract. *The objective of this study was to investigate the reaction processes of seedlings originating from wheat seeds after they were subjected to accelerated ageing, which imitated low-temperature storage. Germination, vigor, morphological characteristics, relative chlorophyll content and antioxidant enzyme activity were measured in seedlings and young plants of wheat (Triticum aestivum L.) after processes of accelerated ageing of the seeds. The ageing procedures of the seeds were done according to the standard conditions (40±0.5°C and 100% air relative humidity) of the International Seed Testing Association for 72 and 120 hours of treatments of the Bulgarian varieties Geya-1 and Sadovo 772. For the control were used seeds, which were not subjected to the accelerated ageing conditions. The coleoptile of 5 days seedlings and second leaf of 10 and 15 days plants in seedling stage, following the seed accelerated ageing procedure, was measured in the laboratory and the greenhouse. The seed germination rate and vigor, fresh and dry weight of growing coleoptiles were inhibited after different periods of ageing exposure and well correlated with increased accumulation of total hydrogen peroxide, malondialdehyde content, guaiacol peroxidase activity and rate of cells membrane stability index. The negative changes of chlorophyll content index, fresh and dry weight and leaf area of the leaves of young plants descending from aged seeds sowed in pots were found. It was established that the modern variety Geya-1 was more tolerant to the applied ageing condition than the older Sadovo 772. In conclusion, we considered that accelerated ageing could be used as a model for estimation of seed deterioration in wheat after long-term storage.*

Keywords: accelerated ageing test, antioxidant enzyme activities, common winter wheat, reaction, treatments, seed germination

Abbreviations: AA- accelerated ageing test, MDA – malondialdehyde, MSI – membrane stability index, FW- fresh weight, DW- dry weight, GPOD- guaiacol peroxidase activity, H_2O_2- hydrogen peroxide, CCI- Chlorophyll content index, DAS- days after sowing, ISTA- International Seed Testing Association.

Introduction

The loss of seeds viability during their ex situ storage is a major issue for both the seed industry and for germplasm conservations (Agaska-Moldoch et al., 2016). Some 1750 *ex situ* genebanks have been established over the last decades to combat the continuing erosion of genetic variation experienced by crop plants. They are estimated to currently curate over 7.4 million accessions, of which ~45% are cereal species by FAO (2010). Although there is a well-recognized genetic contribution to seed longevity, for example, seeds of pea, alfalfa and wheat can remain viable for many decades, while those of other species (e.g., lettuce, onion, parsnip and rye) are short-lived (Walters et al., 2005; Nagel and Börner, 2010), there are non-genetic determinants which are the ambient environment during seed development, the seed's moisture content and maturity of the seed harvest, the presence of pathogenic microflora on the surface of, or within the seed, the extent of mechanical damage to the seed and the post-harvest storage conditions (Copeland and McDonald, 2001). The deterioration in viability experienced during long-term low-temperature storage is due to damage to the membranes, to the DNA and to the action of a variety of enzymes and other proteins (Coolbear, 1995; McDonald, 1999). Among the agents responsible for seed ageing identified to date, lipid peroxidation is the most well documented (Davies, 2005), but oxidative damage to DNA and proteins has also been identified as being casual (Rao et al., 1987; Bailly et al., 2008). Standard experimental ageing procedures have been

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elaborated by the International International Seed Testing Association (ISTA) to speed the assessment of the viability loss. The accelerated ageing (AA) has been used to reveal the genetic basis of longevity in wheat (Black et al., 2006). The purpose of AA test was to mimic long-term storage and, hence, allow for the evaluation of longevity. The tests subject the seeds to a combination of high temperature and high relative humidity, and this reflects longevity as seeds with greater longevity deteriorate to a lesser extent than those with poor longevity (Hampton and TeKrony, 1995; Walters, 1998).

The objective of this study was to investigate the reaction processes of seedlings originating from wheat seeds subjected to accelerated ageing, which imitates low-temperature storage.

Material and methods

Two Bulgarian wheat (*Triticum aestivum L.)* varieties Geya-1 and Sadovo 772 were used for investigation of a reaction to accelerated ageing (AA) of their seeds. Geya-1 and Sadovo 772 are created in the Institute of Plant Genetic Resources (IPGR), Sadovo, Bulgaria. These varieties have different characteristics by duration of the vegetation period, physical qualities of grains and year of origin, as Geya-1 is newer and with longer vegetation period. The seed material was provided from the Bulgarian National Genebank, which is located in IPGR.

Accelerated ageing test (AA): For the AA test, two replicates (two glasses) of 100 grains per accession were laid on a rack within a sealed glass exicator, which contained 200 ml deionized water to ensure the maintenance of near 100% relative humidity. The glass vessel was exposed to 40±0.5°C for 72 h and for 120 h, following International Seed Testing Association protocols (ISTA, 2008).

Germination and vigor of seeds after AA test: The grains ability to germinate was tested by a standard germination test (SGT) (ISTA, 2008). Germination was evaluated at 22±0.5°C, with two replicates of 50 seeds for experimental treatment. Seeds were sown on two 14.0 cm diameter Petri dishes, between two discs of filter paper. Afterwards each Petri dish was watered with 20 ml of distilled, sterile water. The germination and vigor were measured on 3rd and 5th day, respectively, as a seed was considered to have germinated when the coleoptile reaches a length over 2 mm. The fresh and dry weight of the coleoptiles and coleoptiles and roots length were measured on the 5th day from the germ development.

Fresh weight of the coleoptiles and plant parts were measured as soon as after cut from plant or seedlings. The dry weight was obtained after drying the plant parts at 104°C (Beadle, 1993). For that purpose the Analytical balance Kern EW 220-3NM with precision ±0.001 g or 1 mg was used.

Membrane stability index (MSI) of the coleoptiles-MSI was determined following the method of Premachandra et al. (1990). The 100 mg samples of coleoptiles were cut into discs and kept in test tubes containing 10 ml of Bi-distilled water in two sets. One set was kept at 40°C for 30 min and another set at 100°C in boiling water bath for 15 min and their respective electric conductivities C1 and C2 were recorded. The calculation was done by the following formula: MSI = [1− (C1/C2)] × 100, %.

The lipid peroxidation in the coleoptiles was determined by the method reported by Heath and Packer (1968). The results are presented as an accumulation of malondialdehyde (MDA) per unit fresh weight of the mean sample (nmol MDA/g^{-1} FW). The absorbance of the sample was measured at 600 and 532 nm. The extinction coefficient of 155 mM^{-1} cm for calculating the concentration of MDA was used.

Guaiacol peroxidase activity (GPOD) in coleoptiles was determined spectrophotometrically, according to the methodology of Mocquot et al. (1996). The activity of guaiacol peroxidase (GPOD) (EC 1.11.1.7) is measured at 436 nm wave-length according to Bergmeyer et al. (1974). In a cuvette are placed 2.3 ml of KH_2PO_4 buffer (pH 7.0), 300 µl of H_2O_2, 300 µl of 8 mM guaiacol and 100 µl of extract. Absorbance at 436 nm wave-length is measured against a “blank” sample with the same components without enzyme extraction (E = 26.6 mM^{-1}cm^{-1}). The values obtained are expressed as U mg/g^{-1}FW.

The hydrogen peroxide content (H_20_2) was measured spectrophotometrically according to Alexieva et al. (2001).

Soil pot experiment: Vegetation pot trial was made with seedlings, which descended from seeds that endured AA test conditions for 72 h. For comparison, controls not subjected to AA test conditions were used. The seeds were sown in pots, which contained 1 kg dry soil and grown for 15 days in greenhouse under natural conditions of light and temperature 25°C day (15°C night and 40-60 % RH). On the 10th and 15th day after seed germination second fully developed leaves were used for the analyses. Chlorophyll content index (CCI) of the leaves was measured using a portable apparatus Chlorophyll Content Meter-CCM 200 plus manufactured by Opti-Sciences, Inc., NH, USA. Measurements were made on two dates for every variant. From each variant 20 leaves were analyzed by readings on the central part of the leaf (n=20). The morphological assessment of the leaves was carried out on the greenhouse. For determination of the mean values of fresh leaf weight, dry leaf weight and leaf area 10 leaves (n=10) were used.

Data are presented as mean values±standard error (SE). T-test was used to establish significance of the difference between means of the variants.

Results and discussion

Germination and coleoptile growth of wheat seeds subjected to AA treatment

The AA test conditions applied for 3 days and 5 days on wheat grains resulted in decrease of their vitality. The germination and vigor of the grains, as well growth characteristics of the coleoptiles and roots were significantly reduced in comparison with the control variant (Figures 1 and 2). The minimum values of the ratio between AA-3 days variant and control were reported for the parameter length of the coleoptiles for Sadovo 772 - 36.1% and for Geya-1 - 45.2% (Figure 3). In both varieties more tolerant reaction to AA test of the roots was established

(Figure 2 and Figure 3). The duration of 120 h of AA conditions led to more pronounced increase of growth inhibition (over 50%), except for seed germination. The 5 days of AA procedure was negative on the coleoptile length, for Sadovo 772 - 28.7% of control, on the other hand the average root length was more stable (Figure 3). Based on these results, it could be concluded that the variety Geya-1 is a more tolerant variety to the AA test than Sadovo 772 with relation to growing characteristics. However, the variety Sadovo 772 expressed better response to AA conditions for indices germination and vigor of seeds.

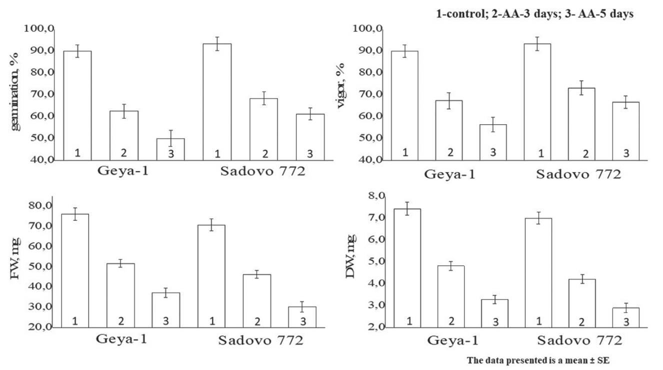

Figure 1. Germination and coleoptile growth characteristics of wheat seeds subjected to AA test

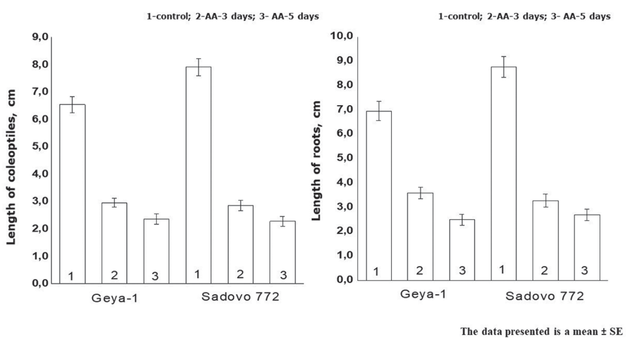

Figure 2. Average length of coleoptiles and roots following AA seeds test

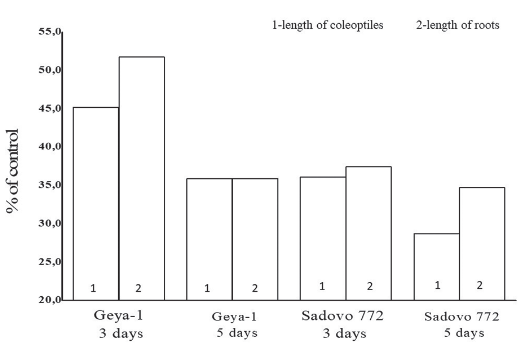

Figure 3. Mean value of the ratio between coleoptiles and roots length of germs following AA seeds test

Antioxidant enzyme activity of wheat coleoptiles with origin from seeds subjected to AA test

Determination of lipid peroxidation, MTS and enzyme activity of GPOD can reveal the deterioration of cells vitality due to AA test conditions (Bailly, 2004). After the 3 days and 5 days of AA treatment different reactions of GPOD activity, MDA and H_2O_2 content (only 3 days AA treatment presented), MSI of membranes results in both varieties were found. Application of 3 days of AA test did not reveal larger difference between both varieties, but had significant difference between variants. For example, the average values for increase of the malondialdehyde (MDA) content in AA conditions variants was about 300% toward the AA test conditions control variant (Figure 4). These results gave a strong correlation between the data of H_2O_2 content, MSI of coleoptiles cells and enzyme activity of GPOD. Lehner et al. (2008) presented similar results related to AA conditions in wheat seeds. The variety Sadovo 772 showed higher MDA content but less GPOD activity in comparison with Geya-1. At the same time Sadovo 772 had less accumulated H_2O_2 and significantly increased MSI for both variants of AA treatment than Geya-1. These values could be discussed as decreased protective mechanism of the cells of coleoptiles. To some extent, the better morphological growth characteristics of coleoptiles of variety Geya-1 correspond well to the above mentioned measurements (Figures 2 and 3). On the basis of these results it could be concluded that 3 days treatment of AA could best reveal genetic and physiological differences between the varieties.

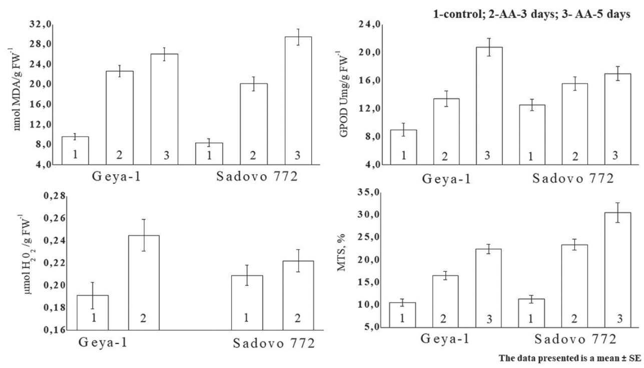

Figure 4. Antioxidant enzyme activity of wheat coleoptiles with origin from seeds subjected to AA test

Morphological characteristics and chlorophyll content index of transferred to pots wheat seeds surviving previously 3 days of AA

The results for growth characteristics and chlorophyll content index of the re-growth from the aged seeds transplanted to pots seedlings indicated similar negative growth reactions as were shown to germs and coleoptiles. On Table 1 are presented the results for the seedlings grown in pots under natural greenhouse conditions with duration of 10 days and 15 days. The maximal reduction of fresh and dry weight and development of second leaf was reached for Sadovo 772 (75.0% of control) on the 15th day than for Geya-1 (85.1% of control) on the 10th day. The chlorophyll content index of the second leaf reduced in lesser degree for Geya-1 (94.1% of control) than for Sadovo 772 (84.5% of control) on the 10th day. There was a different reaction between both varieties towards reductions of estimated parameters for AA condition variants. Sadovo 772 showed a stronger negative reaction at first reading, followed by a more intense growth reaction on the second readings, whereas variety Geya-1 had gradually decreased the values at two points of readings, but all of them showed more tolerant reaction. The results indicated that Sadovo 772 was found to be more sensitive than Geya-1 comparing these reactions.

Table 1. Fresh and dry weight, leaf area and chlorophyll content index of wheat seedlings grown in pots originating from seeds subjected to 3 days AA test

Variants	FW mg 10 DAS n=10	FW mg 15 DAS n=10	DW mg 10 DAS n=10	DW mg 15 DAS n=10	LA cm^2 10 DAS n=10	LA cm^2 15 DAS n=10	CCI index 10 DAS n=20	CCI index 15 DAS n=20
Geya-1 control	64.0±5.6[a]	80.3±6.2[a]	7.82± 1.1[a]	10.22±0.7[a]	2.29±0.13[a]	2.55±0.15[a]	2.14±0.18[a]	2.36±0.17[a]
Geya-1 AA-3 days	63.5 ±4.8[a]	68.7±3.7[b]	7.70±0.8[a]	8.89±0.6[b]	1.95± 0.15[b]	2.19 ±0.22[b]	2.05±0.19[a]	2.22±0.21[a]
% of control	99.2	85.5	98.5	86.9	85.1	85.9	95.8	94.1
Sadovo 772 control	66.5± 5.1[a]	70.5 ±3.5[a]	8.10±1.0[a]	9.33±0.6[a]	2.07±0.18[a]	2.32±0.31[a]	2.19±0.13[a]	2.35±0.11[a]
Sadovo 772 AA-3 days	51.9±3.9[b]	62.8±3.0[b]	6.11± 0.7[b]	7.77± 0.5[b]	1.60±0.22[b]	1.74± 0.25[b]	1.85±0.14[b]	2.11± 0.10[b]
% of control	78.0	89.1	75.4	83.3	77.3	75.0	84.5	89.8

*mean±standard error (SE); the same letters indicate for no significance difference (P>0.05); different letters in the same column indicated for significant difference (p<0.05).

The reported values of biochemical analysis and MTS confirmed the presence of stress in the cells of coleoptiles originating from seeds subjected of AA treatment. The results showed a gradual increase in MTS, hydrogen peroxide content and enzyme activity and decrease in germination rate during accelerated ageing. Qin et al. (2011) reported such a result during AA conditions treatment at 40°C and 45°C for 2 and 4 days. These relationships also lead to a reduction in seed viability expressed by inhibited seedling growth. This is in accordance with the study of Ghahfarokhi et al. (2014). It could be seen that our experiments with wheat seeds showed a better response to accelerated ageing in Geya-1 than in Sadovo 772. One of the reasons for this difference could be the chemical composition of seeds. Geya-1 showed greater weight of dry seeds before and after water inbibition of seeds. The total content of proteins in dry spell seeds was found to be higher in Geya-1 than in Sadovo 772, as a result of multi-year trials on the field of IPGR. The result confirmed correlation with better growing characteristics of the coleoptiles, although there was no positive relation with germination and seed vigor in variety Geya-1. Bolegon et al. (2011) reported similar dependencies in a study of wheat seedlings during accelerated ageing process. Though lipids were known as more responsible for the ageing processes of seeds because of their higher rate oxidation than proteins, it suggested that proteins could play a protective vital role in preservation of seeds vitality. There is little information about the protective role of proteins in ageing especially in wheat. However, our data are in support of the results of several authors who indicated such relationships (Coolbear, 1995; McDonald, 1999; Davies, 2005). One of the reasons for this suggestion is that except for reserve, proteins can play a vital role in the metabolic reaction in germination in the embryo, including generating antioxidant power.

Conclusion

The germination rate and vigor of seeds were inhibited after different periods of ageing exposure. The fresh and dry weight of coleoptiles following ageing treatments were decreased and well correlated with increased accumulation of total hydrogen peroxide, malondialdehyde content, guaiacol peroxidase activity and cells membrane stability index. Negative changes of chlorophyll content index, fresh and dry weight and the leaf area of the leaves of young plants descending from aged seeds sowed in pots were found. It was established that the modern variety Geya-1 was more tolerant to the applied ageing condition than the older Sadovo 772. In conclusion, we consider that accelerated ageing could be used as a model for estimation of seed deterioration in wheat after long-term storage.

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References

Agaska-Moldoch M, Abdul Rehman Arif M, Lohwasser U, Doroszewska TC, Alexieva V, Sergiev I, Mapelli S and Karanov E, 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Cell Environmental, 24, 1337-1344.

Bailly C, 2004. Active oxygen species and antioxidants in seed biology. Seed Science Research, 14, 93-107.

Bailly C, El-Maarouf-Bouteau H and Corbineau F, 2008. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. Comptes Rendus Biologies, 331, 806-814.

Beadle CL, 1993. Growth Analysis. In: Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual (eds. D.O. Hall, J.M.O. Scurlock, R. Bolharnordenkampfh, R.C. Leegood and S.P. Long), pp. 36-46, Chapman and Hall, London.

Bergmeyer HU, Gawehn K and Grassl M, 1974. Enzymes as biochemical reagents. In: Methods of Enzymatic Analysis (ed. H.U. Bergmeyer), Academic Press, New York, 1, pp. 727-733.

Black M, Bewley JD and Halmer, 2006. The encyclopedia of seeds: science, technology and uses. Wallingford, UK: CABI Publishing, p. 900.

Boligon AA, Dal'Col Lúcio A, Lopes SJ, Filho AC and Garcia DC, 2011. Wheat seedlings emergence estimated from seed analysis. Scientia Agricola, 68, 3, 336-341.

Coolbear P, 1995 Mechanisms of seed deterioration; In: Seed Quality: Basic mechanisms and agricultural implications (ed. A.S. Basra), pp. 223-277, Food Product Press, New York.

Copeland LO and McDonald MB, 2001. Principles of seed science and technology. 4th edn. Norwell, Massachusetts: Kluwer Academic Publishers, p. 488.

Davies MJ, 2005. The oxidative environment and protein damage. Biochimica et Biophysica Acta, 1703, 93-109.

FAO, 2010. The second report on the state of the world's plant genetic resources for food and agriculture. Commission on Genetic Resources for Food and Agriculture, p. 399, Food and Agriculture Organization of the United Nations, Rome.

Goodarzian GM, Elahe G, Mohsen S and Kazafi Zei H, 2014. The effect of accelerated aging on germination characteristics, seed reserve utilization and malondialdehyde content of two wheat cultivars. Journal of stress physiology and biochemistry, 10, 15-23.

Hampton JG, TeKrony DM and the Vigour Test Committee, 1995. Handbook of vigour test methods, 3rd edition, International Seed Testing Association, Zurich, p. 117.

Heath RL and Packer L, 1968. Photoperoxidation in isolated chloroplasts I: Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 125,189-198.

ISTA, 2008. International rules for seed testing. International Seed Testing Association, Bassersdorf.

Lehner A, Mamadoua N, Poelsb P, Comea D, Baillya C and Corbineaua F, 2008. Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. Journal of Cereal Science, 47, 555-565.

McDonald MB, 1999. Seed deterioration: Physiology, repair and assessment. Seed Science and Technology, 27, 177-237.

Mocquot B, Vangronsveld J, Clijsters H and Mench M, 1996. Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant Soil, 182, 287-300.

Nagel M and Börner A, 2010. The longevity of crop seeds stored under ambient conditions. Seed Sciences Research, 20, 1-12.

Opti-Sciences, Inc. www.optisci.com/ccm-200.html

Peng Q, Kong Z, Liao X and Liu Y, 2011. Effects of accelerated aging on physiological and biochemical characteristic of waxy and non-waxy wheat seeds. Journal of Northeast Agricultural University (English edition).

Premachandra GS, Saneoka H and Ogata S, 1990. Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soyabean. Journal of Agricultural Science, 115, 63-66.

Qualset CO and Börner A, 2016. The inheritance of wheat grain longevity: a comparison between induced and natural ageing. Journal of Applied Genetics 57, 477-481

Rao NK, Roberts EH and Ellis RH, 1987. Loss of viability in lettuce seeds and the accumulation of chromosome damage under different storage conditions. Annual Botany, 60, 85-96

Walters C, 1998. Understanding the mechanisms and kinetics of seed aging. Seed Science Research, 8, 223-244.

Walters C, Wheeler LM and Grotenhuis JM, 2005. Longevity of seeds stored in a genebank: species characteristics. Seed Sciences Research, 15, 1-2.