HELIA, **28**, Nr. 42, p.p. 107-114, (2005) UDC 633.854.78:631.53.01.011

INFLUENCE OF AGING PROCESS ON BIOCHEMICAL CHANGES IN SUNFLOWER SEED

Balešević-Tubić, S.,[1*] Malenčić, Đ.,[2] Tatić, M.[1] and Miladinović, J.[1]

[1]*Institute of Field and Vegetable Crops, M. Gorkog 30, Novi Sad, Serbia and Montenegro*
[2]*Faculty of Agriculture, Novi Sad, Serbia and Montenegro*

Received: September 10, 2004
Accepted: May 22, 2005

SUMMARY

This investigation was conducted in order to determine the degree of biochemical changes during accelerated and natural aging of sunflower seed. Five sunflower lines developed in Novi Sad, submitted to accelerated aging for three and five days, and natural aging of six and twelve months under conventional storage and controlled conditions, were used in these trials. Malondialdehyde, superoxide dismutase contents and peroxidase activities were studied. Lipid peroxidation and superoxide dismutase and peroxidase activities (especially pronounced in accelerated aging variant) were caused by both types of aging. The degree of seed damage and the ability of seed to resist the negative consequences of aging were influenced, beside duration of aging period, by type of storage and characteristics of sunflower lines.

Key words: aging, lipid peroxidation, seed, sunflower, superoxide dismutase and peroxidase activities

INTRODUCTION

Autooxidation of lipids and increase in the content of free fatty acids during storage period are the main reasons for rapid deterioration of seed of oil plants. These processes cause enzyme inactivation, *i.e.,* denaturation of proteins and nucleic acids (Reuzeau *et al.*, 1992; Trawatha *et al.*, 1995). Damage of seed during storage is inevitable and decrease of quality greatly depends on temperature and relative air humidity in storage, seed moisture content, duration of storage, type of seed and initial quality of seed (Elias and Copeland, 1994; Fabrizius *et al.*, 1999).

In the study of biological oxidation, attention is paid to the development of oxygen radicals under stress conditions (Elstner, 1982; Bowler *et al.*, 1992; Hendry, 1993; Baisak *et al.*, 1994; Inzé and Van Montagu, 1995). Unfavorable environmen-

* corresponding author, Phone: +381 21 4898 481, Fax: +381 21 621 212, e-mail: ceca@ifvcns.ns.ac.yu

tal conditions and seed storage can cause oxidative stress in plant tissue and development of superoxide radical, hydrogen peroxide and hydroxyl radical, which are the most active, toxic and destructive products of oxidative stress (Smirnoff, 1993).

Lipid peroxidation is the primary cause of seed damage during storage (Hailstones and Smith, 1988; Gidrol *et al.*, 1989). Besides being the main cause of seed damage, lipid peroxidation causes initial biochemical changes in seed that can be observed during storage. Determination of malondialdehyde (MDA) content is the standard method for lipid peroxidation determination. Many authors observed increased content of MDA with prolonged storage period or with accelerated aging (Reuzeau and Cavalie, 1995; Ferguson *et al.*, 1990).

Plant cells contain numerous protective and recovery mechanisms against oxidative damage. Special attention is paid to enzyme activities due to their possible usage as significant indicators of seed vigor and longevity. Superoxide dismutase (SOD) is an enzyme belonging to the first group of protective mechanisms of plant cells against oxidative damage. Due to SOD activity low concentration of supeoxide is maintained in the cell, thus preventing the formation of harmful oxidative products (Bannister *et al.*, 1987). Level of SOD activity in stored seed can be a significant factor in determining the level of seed protection against oxidative stress (Bowler *et al.*, 1994).

Peroxidase is an enzyme catalyzing oxidation processes in different substrates using hydrogen peroxide and producing water and free radical. Peroxidase also participates in the lignification of cell wall, ethylene biosynthesis and defense against pathogens (Kvaratskhelia *et al.*, 1997). During natural and accelerated aging peroxidase activity gradually decreases (Puntarulo and Boveris, 1990; Dey and Mukherjee, 1986).

The aim of this investigation was to determine the effect of aging on biochemical changes in seed of the tested sunflower lines and assess possibilities of predicting the rate of seed deterioration during storage.

MATERIALS AND METHODS

Five sunflower lines were used in this investigation: *ocms*-74 (L1), *cms*-v-8931-3-4 (L2), *ocms*-22 (L3), *ocms*-98 (L4) and *cms*-ol-3 (L5). The tested lines had been developed at Institute of Field and Vegetable Crops in Novi Sad.

Accelerated aging. Seed was placed in metal dishes, on metal sieve and submerged in water bath at 42°C, and relative humidity of 100%. Testing was done at three- and five-day intervals.

Natural aging. Seed was stored in two ways. (1) - seed was kept in cool chamber (controlled conditions) at 4°C and relative humidity of 80 to 85%; (2) - seed was kept under conventional storage conditions (uncontrolled conditions). Testing was done after six and 12 months of storage.

Extraction of malondialdehyde (MDA) from hulled sunflower seeds was done using solutions of thiobarbituric acid (TBA), trichlor-acetic acid (CCl_3COOH) and perchloric acid ($HClO_4$), and its concentration was determined using spectrophotometer at 532 nm (Matkovich *et al.*, 1989). Hulled seeds (0.5 g) were homogenized in mortar with 4.5 ml extraction MDA reagents and incubated in water bath at 90°C for 20 minutes. After incubation, solutions were cooled to stop reaction and centrifuged for 10 min at 5500 r/min. MDA concentration *i.e.*, intensity of lipid peroxidation was expressed as nmol of MDA g^{-1} of fresh mass.

Determination of superoxide dismutase (SOD) and peroxidase (P) was obtained using extract of 1 g of fresh plant material (hulled seed) and 5 ml 0.1 M phosphate buffer pH 7. The same extract was used for determination of protein content in seed in order to express activities of tested enzymes. The obtained homogenate was centrifuged at 4500 r/min. at 5°C for 10 minutes.

SOD activity [U mg^{-1} (protein)] was determined using the spectrophotometric method according to Misra and Fridovich (1972) based on autooxidation of adrenaline to adenochrome. Change of adrenaline solution absorbance was measured at 480 nm in carbonate buffer pH 10.2.

The activity of P [U mg^{-1} (protein)] was determined on the basis of transformation of guaiacol into tetraguaiacol and by measuring change of absorbance at 436 nm in phosphate buffer (Matkovich *et al.*, 1989).

RESULTS AND DISCUSSION

Prolonged natural aging increased MDA content in seed of all tested sunflower lines, and it was mainly higher in seed kept under conventional storage (Figure 1). Gradual increase of MDA content in seed with prolonged storage period was confirmed by other authors (Halder and Gupta, 1980; Zhang andi Kirkham, 1996).

In seed of lines L1, L2 and L4, increase of MDA content was significant in all variants of natural aging, except in seed kept for six months under controlled conditions. These results confirmed assumptions that temperature and relative humidity during storage are important factors which, beside duration of storage period, affect the degree of biochemical changes in seed. In seed of lines L3 and L5, significant increase of MDA content was noticed in all variants of natural aging. It means that these lines were less tolerant to peroxidative lipid changes, since negative aging effects were more pronounced also in seed stored under controlled conditions. It can be observed that seed traits of individual lines of the same plant species affected peroxidative changes in seed during storage since oil content of these lines was the highest. These results were in accordance with results obtained by other investigators on seed damage during aging (Priestley *et al.*, 1980; St. Angelo and Ory, 1983).

The accelerated aging test showed that the MDA content in seed was significantly increased. The highest MDA content was found after five days of accelerated

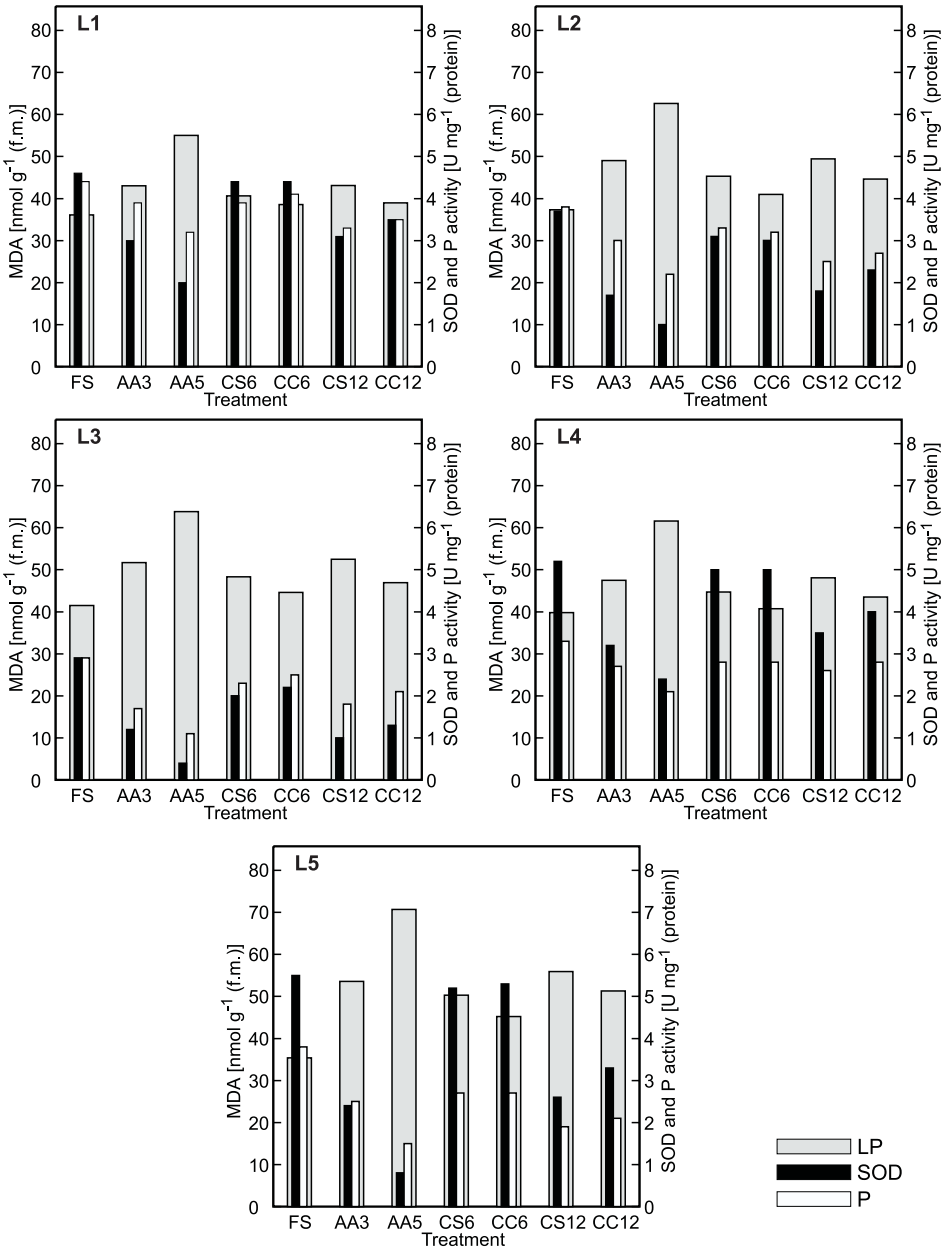

Figure1: Lipid peroxidation (LP) and activity of superoxide dismutase (SOD) and peroxidase (P) during accelerated and natural aging of sunflower seed (FS-fresh seed; AA-accelerated aging for 3 and 5 days; CC-controlled condition and CS-conventional storage for 6 and 12 months)
Sunflower lines: L1 - ocms-74, L2 - cms-v-8931-3-4, L3 - ocms-22, L4 - ocms-98 and L5 - cms-ol-3

aging. Seed exposure to extreme conditions (40°C and relative humidity of 100%) led to highly pronounced peroxidative lipid degradation in seed. These results are in accordance with those obtained by other authors who determined high content of free radicals in seed exposed to accelerated aging, and who assumed that reaction of lipid peroxidation was important for accumulation of harmful products in aged seed (Stewart and Bewley, 1980; Buchvarov and Gantcheff, 1984).

Extreme aging conditions such as accelerated aging make processes of lipid peroxidation in seed more intensive than natural aging. The results obtained in this study showed it was possible to predict degree of lipid peroxidation in seed during storage by applying accelerated aging test. The increase of MDA content in seed of the tested sunflower lines after three days of accelerated aging was the same as in seed stored for 12 months under conventional storage except of line L5. The MDA content in seed after three days of accelerated aging was the same as value for MDA content in seed kept for 12 months under controlled conditions. The obtained relations among the results of the accelerated aging test and natural aging in seed of line L5 showed that this line was less tolerant to peroxidative changes than the other tested sunflower lines. This is in agreement with the results of Kruse (1999), that, after a period of accelerated aging, seed lots of the same initial quality responded differently to oxidative stress. According to some researchers, the technique of accelerated aging itself causes changes in seed not equal to changes occurring in seed during natural aging (Priestley and Leopold, 1983; Wilson and McDonald, 1986).

SOD and P activities decreased during natural aging of sunflower seed of lines L2, L3, L4 and L5 12 months after natural aging in comparison with SOD and P activities in fresh seed. The accelerated aging test decreased significantly the SOD and P activities in seed in all tested lines. Enzyme activity in aged seed was decreased due to harmful changes in their structure during seed aging. Decreased SOD and P activities in seed during accelerated and natural aging were observed by many researchers (Anderson and Baker, 1983; Puntarulo and Boveris, 1990; Sung and Jeng, 1994; Zhang and Kirkham, 1996).

Exposure of sunflower seed to accelerated and natural aging caused significant negative correlation between MDA content and SOD activity in seed, with highly significant correlation coefficient in line L3 (r=-0,71) and line L5 (r=-0,86). Aged seed of these lines had the highest MDA content and the lowest SOD activity in relation to the other tested sunflower lines. The obtained correlations revealed that lipid peroxidation was most intensive in sunflower seed of lines L3 and L5, which led to significant changes and damage of seed especially in the case of line L5. Significant negative correlations between MDA content and P activity were determined in aged seed of lines L2 (r=-0,65), L3 (r=-0,68) and L5 (r=-0,76). High correlation was noticed between level of lipid peroxidation and SOD activity.

CONCLUSIONS

Content of MDA in seed was increased by prolonged storage of sunflower seed indicating that lipid peroxidation was more intensive in aged seed. The most intensive lipid peroxidation was observed after the application of the accelerated aging test for five days. MDA content in seed exposed three days to accelerated aging was almost identical to the MDA content in seed stored for 12 months under conventional storage. The activities of superoxide dismutase and peroxidase decreased during sunflower seed aging and it was especially pronounced when accelerated aging was applied.

ACKNOWLEDGEMENTS

The principal author expresses her gratitude to Prof. Dr. Dragan Škorić who initiated these investigations and provided plant material.

REFERENCES

Anderson, J.D. and Baker, J.E., 1983. Deterioration of seed during aging. Phytopathology 53: 321-325.

Baisak, R., Rana, D., Archarya Patel, B.B. and Kar, M., 1994. Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Plant Cell Physiol. 35: 489-495.

Bannister, J.V., Bannister, W.H. and Rotilo, G., 1987. Aspects of the structure, function and applications of superoxide dismutase. CRC Crit. Rev. Biochem. 22: 111-180.

Bowler, C., Van Montagu, M. and Inzé, D., 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43: 83-116.

Bowler, C., Van Camp, W., Van Montagu, M. and Inzé, D., 1994. Superoxide dismutase in plants. Crit. Rev. Plant Sci. 13: 199-218.

Buchvarov, P. and Gantcheff, Ts., 1984. Influence of accelerated and natural aging on free radical levels in soybean seeds. Physiol. Plant. 60: 53-56.

Dey, G. and Mukherjee, R.K., 1986. Deteriorative change in seeds during storage and its control by hydration-dehydration pretreatments. Seed Research 14: 49-59.

Elias, S.G. and Copeland, L.O., 1994. The effect of storage conditions on canola (*Brassica napus* L.) seed quality. J. of Seed Technol. 18: 21-22.

Elstner, E.F., 1982. Oxigen activity and oxigen toxicity. Annu. Rev. Plant Physiol. 33: 73-96.

Fabrizius, E., TeKrony, D., Egli, D.B. and Rucker, M., 1999. Evaluation of a viability model for predicting soybean seed germination during warehouse storage. Crop Sci. 39: 194-201.

Ferguson, J.M., TeKrony, D.M. and Egli, D.B., 1990. Changes during early soybean seed and axes deterioration: II. Lipids. Crop Sci. 30: 179-182.

Gidrol, X., Serghini, H., Noubhani, A., Mocoquot, B. and Mazliak, P., 1989. Biochemical changes induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane damage. Physiol. Plant. 76: 591-597.

Hailstones, M.D. and Smith, M.T., 1988. Lipid peroxidation in relation to declining vigour in seeds of soya (*Glycine max* L.) and cabbage (*Brassica oleracea* L.). J. Plant Physiol. 133: 452-456.

Halder, S. and Gupta, K., 1980. Effect of storage of sunflower seeds in high and low relative humidity on solute leaching and internal biochemical changes. Seed Sci. and Technol. 8: 317-321.

Hendry, G.A.F., 1993. Oxigen free radical processes and seed longevity. Seed Sci. Res. 3: 141-153.

Inzé, D. and Van Montagu, M., 1995. Oxidative stress in plants. Plant Biotech. 6: 153-158.

Kruse, M., 1999. Application of the normal distribution for testing the potential of the controlled deterioration test. Crop Sci. 39: 1125-1129.
Kvaratskhelia, M., Winkel, C. and Thorneley R.N.F., 1997. Purification and Characterization of a Novel Class III Peroxidase Isoenzyme from Tea Leaves. Plant Physiol. 114: 1237-1245.
Matkovich, B., Gašić, O., Varga, Sz.I., Štajner, D. and Kraljević-Balalić, M., 1989. The antioxidant enzyme activities in wheat seeds and their F_1 hybrids. Cereal Res. Commun. 17: 113-119.
Misra, H.D. and Fridovich, I., 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple measurement for superoxide dismutase. J. Biol. Chem. 247: 3170-3175.
Priestley, D.A., McBride, M.B. and Leopold, A.C., 1980. Tocopherol and organic free radical levels in soybean seeds during natural and accelerated aging. Plant Physiol. 66: 715-719.
Priestley, D.A. and Leopold, A.C., 1983. Lipid changes during natural aging of soybean seeds. Physiol. Plant. 59: 467-470.
Puntarulo Susana and Boveris, A., 1990. Effect of natural and accelerated aging on the hydroperoxide metabolism of soybean embryonic axes. Plant Sci. 68: 27-32.
Reuzeau, C., Goffner, D. and Cavalie, G., 1992. Relations between protein composition and germination capacity of sunflower seeds. Seed Sci. Res. 2: 223-230.
Reuzeau, C. and Cavalie, G., 1995. Activities of free radical processing enzymes in dry sunflower seeds. New Phytol. 130: 59-66.
Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 125: 27-58.
St. Angelo, A.J. and Ory, R.L., 1983. Lipid degradation during seed deterioration. Phytopathology 53: 315-317.
Stewart, R.R.C. and Bewley, J.D., 1980. Lipid peroxidation associated with accelerated ageing of soybean axes. Plant Physiol. 65: 245-248.
Sung, J.M. and Jeng, T.L., 1994. Lipid peroxidation and peroxide-scavening enzymes associated with accelerated aging of peanut seed. Physiol. Plant. 91: 51-55.
Trawatha, S.E., TeKrony, D.M. and Hildebrand, D.F., 1995. Relationship on soybean quality to fatty acid and C_6-aldehyde levels during storage. Crop Sci. 35: 1415-1422.
Wilson, D.O. and McDonald, M.B., 1986. The lipid peroxidation model of seed ageing. Seed Sci. and Technol. 10: 269-300.
Zhang, J. and Kirkham, M.B., 1996. Lipid peroxidation in sorghum and sunflower seedlings as affected by ascorbic acid, benzoic acid, and propyl gallate. J. Plant Physiol. 35: 489-493.

INFLUENCIA DEL PROCESO DE ENVEJECIMIENTO EN LOS CAMBIOS BIOQUÍMICOS EN LA SEMILLA DE GIRASOL

RESUMEN

La investigación fue realizada con el objetivo de determinar el grado de los cambios bioquímicos durante el envejecimiento acelerado y natural de la semilla de girasol. Para la investigación fueron utilizadas cinco líneas de girasol creadas en Novi Sad, y que fueron sometidas al envejecimiento acelerado de tres a cinco días y al envejecimiento natural de seis a doce meses en las condiciones de almacenamiento convencional y almacenamiento bajo las condiciones controladas. Fueron investigados los contenidos de malondialdehido y superóxido dismutasa, tanto como la actividad de peroxidasa. Los dos tipos de envejecimiento causaron los procesos bioquímicos, es decir, la peroxidación de lípidos y la disminución de actividad de superóxido dismutasa y peroxidasa (lo que fue especialmente destacado en el crecimiento acelerado). El grado de deterioro de la semilla y la aptitud de la semilla de resistir a las consecuencias negativas de envejecimiento, eran bajo la influencia no sólo de duración del período de envejecimiento sino también del tipo de almacenamiento y características de las líneas de girasol.

EFFET DU PROCESSUS DE VIEILLISSEMENT SUR LES CHANGEMENTS BIOCHIMIQUES DANS LA GRAINE DE TOURNESOL

RÉSUMÉ

Nous avons fait cette recherche dans le but de déterminer le degré des changements biochimiques au cours des vieillissements accéléré et naturel de la graine de tournesol. Nous avons utilisé cinq lignées de tournesol développées à Novi Sad et les avons soumises à un vieillissement accéléré de trois à cinq jours et à un vieillissement naturel de six à douze mois dans des conditions d'entreposage conventionnelles et dans des conditions d'entreposage contrôlées. Nous avons analysé les contenus de malondialdéhyde, de dismutase superoxyde et l'activité du peroxydase. Les deux types de vieillissement avaient provoqué des processus biochimiques, à savoir la peroxydation des lipides ainsi qu'une diminution de l'activité des dismutase superoxyde et peroxydase (ce qui était particulièrement marqué dans le cas du vieillissement accéléré). Le degré d'endommagement de la graine et son aptitude à résister aux conséquences négatives du vieillissement ont été influencés non seulement par la durée de la période de vieillissement mais aussi par le type d'entreposage et les caractéristiques des lignées de tournesol.