Effect of Priming on Physiological and Chromosomal Changes of Aged Soybean Seeds

Phyu Sin Thant, Adam B Puteh, Uma Rani Sinniah and Mohd Firdaus Bin Ismail

***Abstract*—**Soybean seed ageing during storage is one of the major causes of seed deterioration. Seed priming is an effective technique to improve seed quality of various crops. This study was undertaken to examine changes in mechanisms involved in stored seeds following priming. The seeds of vegetative soybean cultivar AGS-190 were harvested at harvest maturity and stored at room temperature (25ºC). The seeds stored for 6, 9 and 12months were primed with water, 0.5% chitosan or -0.8MPa PEG. The results indicated that prolonged storage of the seeds under room temperature reduced seed germination performance. The activities of catalase (CAT) and superoxide dismutase (SOD) decreased and accumulation of malondialdehyde (MDA) content and chromosomal damage increased with longer storage period. Seed priming with -0.8MPa PEG improved the quality of 6 months stored seeds resulting in better germination percentage and germination index. Increase in SOD, CAT activities and decrease in the content of MDA and chromosomal aberrations were observed when 6 months stored seeds were primed with -0.8MPa PEG. The study indicates that loss of seed germination during storage is related to failure of CAT and SOD enzymes to protect reactive oxygen species (ROS) attack which resulted in auto-oxidation of lipid and nucleic acid. The results here also suggest that improvement of stored seed quality following priming is associated with reduction of lipid peroxidation and genetic damage through increase stimulation of antioxidants enzyme systems.

***Keywords*—**seed aging, storage, priming, seed quality, antioxidant enzymes, lipid peroxidation, chromosomal aberration

I. INTRODUCTION

SEED ageing during storage is one of the basic reasons for low productivity in soybean (Shelar et al., 2008). Seed ageing process in soybean is accelerated by storage conditions such as high temperature and high relative humidity, moisture content of seeds, storage period, and initial quality of seeds (Bewley et al., 2013). Seed deterioration is associated with biochemical and metabolic changes including increase lipid peroxidation and decrease in antioxidant enzyme activities such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) during ageing (Chen et al., 2013; Xin et al., 2014; Xia et al., 2015). On the other hand, loss in viability during seed storage is related to an increase in chromosomal aberrations in the seeds (Rao et al., 1987).

Seed priming is well known effective technique to improve seed quality and seedling establishment in a wide range of crops. It is a presowing seed treatment with natural or synthetic compounds before germination (Jisha et al., 2012). The beneficial effects of priming on the germination performance include induction of biochemical mechanisms such as the resumption of metabolic activity to restore cellular integrity through the synthesis of nucleic acids, proteins and the improvement of the antioxidant defense system (Bewley and Black, 1994). Seed priming enhances defensive antioxidant enzyme systems in aged seeds and consequently reduces cell membrane damage caused by accumulation of ROS (Siri et al., 2013). Improvement of seed viability in aged seeds following priming is related to reduction of chromosomal aberration (Sivritepe and Dourado, 1995).

It is needed to know how seed deterioration mechanisms such as seed germination process, defensive antioxidant enzymes, lipid peroxidation and genetic damage can be changed by priming. Therefore, this study was proposed to examine changes in mechanisms involved in storage seeds following priming.

II. MATERIALS AND METHODS

A. Seed Material and Priming

This experiment was conducted at Seed Technology laboratory, Department of Crop Science, Faculty of Agriculture, UPM. The seeds of vegetative soybean cultivar AGS-190 were harvested at harvest maturity and stored at room temperature (25ºC) for 6, 9 and 12months. Stored seeds were primed with water, 0.5% chitosan and -0.8MPa PEG (8000). Dry seeds were used as an unprimed control and water was used a primed control.

B. Germination Performance

Standard germination test of aged seeds was done according procedure described by ISTA (2006). A total of 50 seeds from each priming treatment were germinated in the box (300 × 230 × 100 mm) containing oven dried sterilized sand media at 25ºC. Seedlings were evaluated and counted daily for seven consecutive days. Standard germination was recorded based on three replications. Germination index was calculated as described by (Zhang et al., 2007) using the formula: Germination index (GI) =Σ (Gt/Tt) where Gt is the number of germinated seeds on day t, Tt is time corresponding to Gt in

Phyu Sin Thant Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia
Adam B Puteh, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia
Uma Rani Sinniah Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia
Mohd Firdaus Bin Ismail Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia

https://doi.org/10.17758/URUAE.DIR0917207

days.

C. Antioxidant Enzyme Activities

Three samples per replicate were prepared for all priming treatments applied at 6, 9and 12 months storage period to measure antioxidant enzyme activities. The activity of CAT was detected as described by Aebi (1984) using spectrophotometer. The 3 mL reaction mixture contained 1.5mL 50 mM potassium phosphate buffer (pH 7), 0.5mL 75 mM H_2O_2, 0.05mL extracted enzyme and distilled water. The mixture without enzyme extract was used as a blank. Catalase activity was detected in absorbance at 240 nm for two minutes. SOD activity was measured according to the method of Gupta *et al*. (1993). The reaction mixture contained 0.1mL 200mM methionine, 0.01mL 2.25mM nitro blue tetrazolium (NBT), 0.1 mL 3 mM EDTA, 1.5 mL 100mM potassium phosphate buffer, 1mL distilled water, 0.1 mL 60 µM riboflavin and 0.05 mL enzyme extract. Absorbance was recorded at 560 nm by measuring decrease in absorbance of superoxide nitro blue tetrazolium complex by the enzyme.

D. Lipid Peroxidation (MDA assay)

The level of lipid peroxidation was determined as malondialdehyde (MDA) content by the method described by Stewart and Bewley (1980) with a little modification. Three samples per replicate were prepared for all priming treatments applied at 6, 9and 12 months storage period. A total of 3 mL reaction mixture contained 1mL extracted sample and 2 mL 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid solution. MDA was detected at 450, 532 and 600 nm.

E. Chromosomal Aberrations

The chromosomal aberration was detected at anaphase of mitotic cell division in 6, 9 and 12 months aged seeds following priming according to the procedure of Sivritepe (1992). The 10mm of 3-4 day-old seedling were fixed with (1:3) glacial acetic acid: absolute alcohol mixture for 24 hours in the refrigerator. After fixation, the radicles are washed with distilled water and hydrolyzed with 1M HCl in a water bath at 60 °C for 10 minutes. The radicles were placed in a Petri dish and one drop of 1% aceto-carmine stain was added. They were then warmed on a hot plate at 50-60°C before transfer to a glass slide and the 0.8-1.0 mm from the radicle-tip was excised for examination. One drop of 1% aceto-carmine was added and radicle-tip squashes were made. Each slide was covered with a cover slip and examined under the light microscope with high power magnification.

F. Data Analysis

The data were subjected to ANOVA using Statistical Analysis System software, version 9.4. The least significant differences (LSD) test was applied at $P<0.05$ to compare the means between different priming treatments at different storage periods.

III. Results and Discussion

A. Germination Performance

The results showed that seed germination percentage and germination index performed very well in the seeds stored for 6 months in comparison with stored seed for 9 and 12 months (Table 1). When seed priming was applied on 6 months stored seeds, the highest germination percentage of 82.67% was recorded in -0.8MPa PEG. Germination index was also improved when primed with -0.8MPa PEG and 0.5% chitosan. The improvements of PEG primed seeds may be quantitative changes in biochemical content of the seeds and improved membrane integrity and enhanced physiological activities at seed germination (Sung and Chang, 1993).

TABLE I.
GERMINATION PERCENTAGE AND GERMINATION INDEX OF AGS-190 AS AFFECTED BY STORAGE PERIOD AND PRIMING TREATMENTS

Storage Period (month)	Priming	Germination %	Germination Index
6	Control	75.33b	33.46b
	Water	69.33c	26.81b
	0.5% chitosan	76.67b	42.28a
	-0.8Mpa PEG	82.67a	46.47a
9	Control	62.00ab	24.52a
	Water	42.00c	13.01b
	0.5% chitosan	53.33b	23.28a
	-0.8Mpa PEG	64.67a	26.26a
12	Control	44.00a	18.19a
	Water	42.00a	16.51a
	0.5% chitosan	40.67a	15.20a
	-0.8Mpa PEG	46.00a	16.44a

Means followed by same letters are not significantly different ($P \leq 0.05$, LSD Test).

B. Antioxidant Enzyme Activities

Prolonged storage of soybean seeds under room temperature (25 °C) reduced CAT and SOD activities (Figure 1-A, B). Seed priming increased CAT and SOD activities of AGS-190 especially in the seeds stored for 6 months.

It was visible that higher CAT and SOD activities were obtained when six months old seeds were primed with water, 0.5% chitosan and -0.8MPa PEG. Furthermore, priming with -0.8MPa PEG improved both CAT and SOD activities of 9 months stored seeds. For 12 months storage, slightly higher activities in CAT were observed in water and chitosan primed seeds. However, all the priming treatments exhibited lower SOD activities in 12 months stored seeds than control. It indicated that seed priming allows the initiation of enzymatic antioxidant systems such as SOD, CAT in responsible to ROS

accumulation during seed ageing. Involvement of antioxidant enzyme in seed recovery has been reported in sunflower in which osmopriming with PEG induce the synthesis of catalase which play a key role in protection and repair systems during ageing (Kinbiza et al., 2011).

C. Lipid Peroxidation

As a symptom of lipid peroxidation, MDA content in control seeds of AGS-190 linearly increased with longer storage period (Figure 1-C). Seed priming with -0.8MPa reduced the content of MDA in 6 months stored seeds although priming with water and 0.5% chitosan exhibited almost the same level of MDA to unprimed aged seeds. It was clearly seen that osmopriming also decreased the MDA content in 9 and 12 months stored seeds. Lower level of MDA in primed seeds suggested that osmopriming improved seed deterioration by repairing cell membrane indicating reduction of lipid peroxidation with increased rate of antioxidant system synthesis responsible for eliminating ROS from the cells. Chen and Arora (2011) also reported that MDA accumulation was reduced during osmopriming.

D. Chromosomal Aberrations

Seed ageing increased genetic damage indicating that chromosomal damage increased 6% in 9 months and 8% in 12 months stored AGS-190 seeds compared to control (Figure 1-D). Seed priming reduced genetic damage and it was clearly seen in 9 and 12 months stored seeds. Priming with water and -0.8MPa PEG showed lower accumulation of chromosomal damage in seeds stored for 6 months. Lower accumulation of chromosomal aberrations in primed seeds indicated seed priming can repair genetic damage of aged seeds. Improvement of seed quality could be because of completion of DNA repair during priming (Osborne, 1983) and consequent activation of many enzymatic processes (Moosavi et al., 2009).

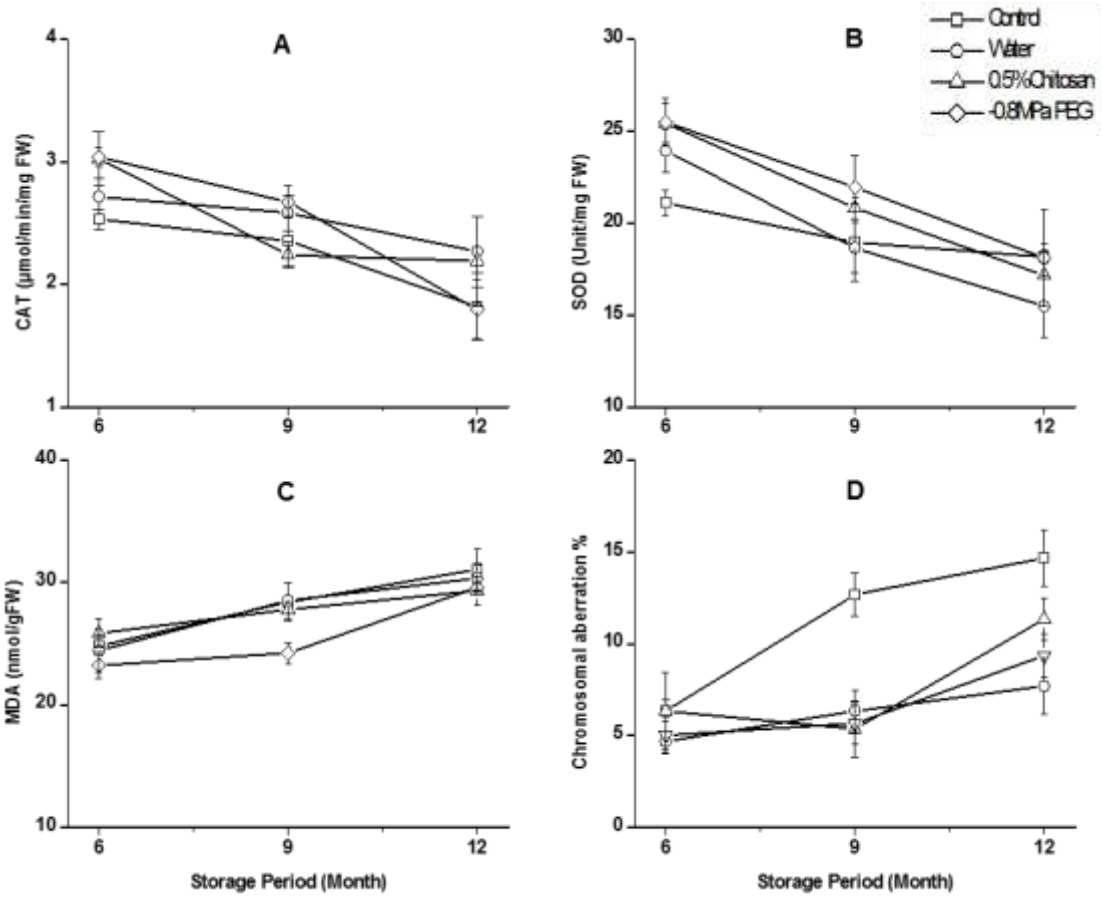

Fig. 1: Changes in CAT(A), SOD (B), MDA (C) and chromosomal aberration (D) of AGS-190 as affected by storage period and priming. The seeds stored in room temperature after 6, 9 and 12 months were primed with water, 0.5% chitosan and -0.8MPa PEG. Dry seeds were used as control. The vertical bars above mean represent standard error.

IV. Conclusion

It is recommended that priming with -0.8MPa PEG should be applied to enhance seed quality soybean stored for less than one year. The results here indicate that lipid peroxidation occurred in stored seeds were reduced with sufficient amount of antioxidant enzymes promoted by priming. Reduction of genetic damage in stored seeds might be associated with stimulation of enzymatic and non- enzymatic antioxidants during priming. The study suggests that priming improves the activities of antioxidant enzymes by repairing the cell membrane and genetic damage occurring during storage.

ACKNOWLEDGMENT

The authors express the appreciation to Universiti Putra Malaysia for giving Phyu Sin Thant the opportunity to work on this research project. Financial support by Organization of Women Scientists for Developing World (OWSD) to conduct this research at Universiti Putra Malaysia is truly grateful.

REFERENCES

[1] H. Aebi. 1984. Catalase in vitro. *Methods of Enzymol.*,105. pp. 21-126. https://doi.org/10.1016/S0076-6879(84)05016-3

[2] J. D. Bewley, K. J. Bradford, H. W. M. Hilhorst, and H. Nonogaki. 2013. Seeds: *Physiology of development, germination and dormancy*, 3rd ed. *Seed Sci. Res.*, 23. pp. 289.

[3] J. D. Bewley, and M. Black, "Seeds", in *physiology of development and germination*, 2nd ed., New York, USA: Plenum Press,1994, pp. 167-171.

[4] H. Chen, D. Osuna, L. Colville, O. Lorenzo, K. Graeber, H. Kuester, ... and I. Kranner. 2013. Transcriptome-wide mapping of pea seed ageing reveals a pivotal role for genes related to oxidative stress and programmed cell death. *PLoS One*, 8(10). pp. e78471. https://doi.org/10.1371/journal.pone.0078471 https://doi.org/10.1371/journal.pone.0078471

[5] K. Chen, and R. Arora. 2011. Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in Spinach (*Spinacia oleracea*). *Plant Sci.*, 180. pp. 212-220. https://doi.org/10.1016/j.plantsci.2010.08.007

[6] A.S. Gupta, R. P., Webb, A. S. Holaday, and R. D. Allen.1993. Overexpression of superoxide dismutase protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants). *Plant Physiol.*, 103(4). pp. 1067-1073. https://doi.org/10.1104/pp.103.4.1067

[7] ISTA. 1999. International rules for seed testing. *Seed Sci. Technol.*, 27. pp. 340.

[8] ISTA. 2006. *International rules for seed testi*ng, International Seed Testing Association, Bassersdorf, Switzerland.

[9] K. C. Jisha, K. Vijayakumari, and J. T. Puthur. 2012. Seed priming for abiotic stress tolerance: an overview. ***Acta Physiol. Plant.***, 35(5). pp.1381-1396. https://doi.org/10.1007/s11738-012-1186-5

[10] S. Kibinza, J. Bazin, C. Bailly, J. M. Farrant, F.Corbineau, and H. El-Maarouf-Bouteau. 2011. Catalase is a key enzyme in seed recovery from ageing during priming. *Plant Sci*. 181(3). pp. 309-315. https://doi.org/10.1016/j.plantsci.2011.06.003

[11] A. Moosavi, R.Tavakkol Afshari, F. Sharif-Zadehand, and A. Aynehband. 2009. Seed priming to increase salt and drought stress tolerance during germination in cultivated species of Amaranth. *Seed Sci. Technol.*, 37*(*3). pp. 781-785. https://doi.org/10.15258/sst.2009.37.3.26

[12] D. J. Osborne. 1983. Biochemical control systems operating in the early hours of germination. *Can. J. Bot*., 61(12). pp. 3568-3577. https://doi.org/10.1139/b83-406

[13] N. K. Rao, E. H Roberts, and R. H. Ellis. 1987. Loss of viability in lettuce seeds and the accumulation of chromosome damage under different storage conditions. *Ann. Bot.*, 60. pp. 85-96. https://doi.org/10.1093/oxfordjournals.aob.a087425

[14] V. R. Shelar. 2008. Role of mechanical damage in deterioration of soybean seed quality during storage-a review. *Ag Review*, 29(3). pp. 177-184.

[15] B. Siri, K. Vichitphan, P. Kaewnaree, S. Vichitphan, and P. Klanrit. 2013. Improvement of quality, membrane integrity and antioxidant systems in sweet pepper (*Capsicum annuum* Linn.) seeds affected by osmopriming. *Australian J. Crop Sci.*, 7 (13). pp. 2068-2073.

[16] H. O. Sivritepe, and A. M. Dourado. 1995. The effect of priming treatments on the viability and accumulation of chromosomal damage in aged pea seeds. *Ann. Bot.*, 75(2). pp.165-171. https://doi.org/10.1006/anbo.1995.1008

[17] H.O. Sivritepe, "Genetic Deterioration and Repair in Pea (*Pisum sativum* L.) Seeds During Storage," PhD Thesis, University of Bath, 1992.

[18] R. R. Stewart and J. D. Bewley. 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.*, 65(2). pp. 245-248. https://doi.org/10.1104/pp.65.2.245

[19] J. M. Sung and Y.H. Chang. 1993. Biochemical activities associated with priming of sweet corn seeds to improve vigor. *Seed Sci. Technol.*, 21. pp. 97-105.

[20] F. Xia, L. Chen, Y. Sun, and P. Mao. 2015. Relationships between ultrastructure of embryo cells and biochemical variations during ageing of oat (*Avena sativa* L.) seeds with different moisture content. ***Acta Physiol. Plant.,*** 37(4). pp. 1-11. https://doi.org/10.1007/s11738-015-1825-8