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Full Length Research Paper

Hydropriming effects on carbohydrate metabolism, antioxidant enzyme activity and seed vigor of maize (*Zea mays* L.)

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Two cultivars of seeds from the National Corn and Sorghum Research Center (NCSRC) and a Private Company (PC) were hydroprimed for 3 to 12 h, then artificially aged at 42°C, 100% RH for 96 h. The germination of NCSRC unprimed seeds after accelerated aging was greater than the PC seeds caused by its initial ascorbate peroxidase (APX) activity. Hydropriming for 3 to 9 h prevented any loss in germination due to the aging process. However, this effect was inhibited by hydropriming for a longer duration. The amelioration of the aging process may be due to a reduction in oxidative stress by antioxidant enzymes. Superoxide dismutase (SOD) and APX may play a role in NCSRC, but APX only contributed in PC seeds. The high amylase activity with remaining glucose content may associate to improve germination in the hydroprimed with aged seeds. Hydropriming periods of greater than 9 h resulted in loss of seed germination after accelerated aging. This result was caused by a reduction in sucrose and raffinose content. The sharply increased amylase activity for both cultivars and the decreased activities of SOD and APX in NCSRC and APX in PC may also indicate the loss of primed seed vigor.

Key words: Priming, seed vigor, carbohydrate metabolism, antioxidant enzymes.

INTRODUCTION

Maize (*Zea mays* L.) is an important worldwide cereal crop (FAOSTAT, 2008). It is used as a raw material for food and feed processing and recently as a bio-fuel (OECD-FAO of The United Nations, 2008). Thus, the demand for maize plants and grains is steadily increasing. Seeds are the most important material for producing crops and they must have the capacity to germinate in a wide range of environments. The biochemical processes of seed germination initiate after seed imbibitions. Seeds activate a number of repair mechanisms during imbibition, including repairing membranes of cells and organelles, as well as protein and enzyme activation to breakdown food reserve (McDonald, 1999). α-Amylase activity was significantly increased in *Araucaria araticana* (Mol.) seed after the start of imbibitions for 12 to 24 h (Reinero et al., 1983) and until 64 h of imbibition in maize seeds (Vashisth and Nagarajan, 2010). An accumulation of *RAmy1A* mRNA started at 12 h of imbibitions was found in the rice embryo (Kaneko et al., 2002). Reducing sugars

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Abbreviations: **APX**, Ascorbate peroxidase; **SOD**, superoxide dismutase; **NCSRC**, National Corn and Sorghum Research Center; **PC**, Private Company.

increased rapidly after 36 h of imbibitions in *Euphorbia heterophylla* embryo (Suda and Giorgini, 2000). The markedly declined raffinose and stachyose during the first 24 h of imbibitions can be accounted for the utilization of these sugars for germination in cotton seeds (Doman et al., 1982). This evidence, confirmed by Blochl et al. (2006), suggests that the raffinose family (RFOs) play an important role for early germination in pea seeds.

At the same time, the respiration rate of the imbibed seeds is accelerated to produce ATP, and essential biochemicals for germination, such as protein (Rajjou et al., 2004) or DNA (Bino et al., 1996). As a result, reactive oxygen species (ROS) are also produced in cells during the germination process (Bailly, 2004; Mittler, 2002). If with the produced ROS, superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), the detoxification system is not fully balance in the imbibed seeds, the ROS can initiate injurious degradation causing lipid peroxidation, membrane deterioration, and protein and nucleic acid degradation (McDonald, 1999) leading to poor germination. Hence, the antioxidant system plays a crucial role in balancing free radicals displayed during imbibitions and germination in the cell (Bailly, 2004). Superoxide dismutase (SOD) is the specific enzyme that converts O_2^- into H_2O_2 (Halliwell and Gutteridge, 1984). SOD activity of sunflower seeds gradually increased during imbibitions for 3 to 6 h (Bailly et al., 2000) and the highest SOD activity was found at final stages of germination and early seedling development in *Chenopodium murale* (Bogdanovic et al., 2008). Ascorbate peroxidase (APX) is a key enzyme to scavenge H_2O_2 in the ascorbate-glutathione cycle (Asada, 1992). This enzyme was detected in embryos excised from germinating seeds within 12 to 24 h from the onset of imbibition in wheat (De Gara et al., 1997) and pea (Pallanca and Smirnoff, 1999).

Priming techniques are pre-germination seed treatments in which seeds are held at water potential that allows imbibition, but prevents radical extension (Bradford and Bewley, 2002). Hydropriming was reported to increase yield in maize (Afzal et al., 2002; Harris et al., 1999), to improve seedling emergence in rice (Farooq et al., 2010), and to decrease the germination time and increase the seedling vigor in wheat (Basra et al., 2003). Chiu et al. (2003) showed that an increased percent germination in primed sweet corn seeds was due to the greater activity of antioxidant enzymes that reduced oxidative stresses compared with unprimed control seeds. The activity of SOD and APX were observed to enhance germination of primed bitter gourd seeds (Hsu et al., 2003). Moreover, their activities were related in the enhanced low temperature tolerance of *Zea diploperennis* (Janhke et al., 1991). More also, increased peroxidase (POD) and catalase (CAT) activities were found to enhance chilling tolerant of maize seedling after priming (Guan et al., 2009). Hydropriming for 2 h partially maintained germination and the activities of various antioxidant enzymes under artificial aging (Goel et al., 2003). Some authors suggest that priming has been shown to repair and build up nucleic acids and membranes, and also speeds up the synthesis of proteins in tomato (Van Pijlen et al., 1996; Gurusinghe et al., 2002) and sunflower (Wahid et al., 2008) seeds. Thus, the increase in antioxidant enzymes, and repair or synthesis of protein induced by priming may play an important role in enhanced seed vigor in either normal or stress conditions.

However, a disadvantage of priming is that it may reduce the longevity of primed seeds, the cause of which is still unclear. A decrease in DNA repair activity during hydration may cause reduced longevity of primed seeds (Van Pijlen et al., 1996). Accumulated free radical stimulating lipid peroxidation during hydration and subsequent drying may also be involved in reducing the longevity of primed seeds (Leprince et al., 1994). Previous research has reported that the decreased antioxidant enzyme activities caused an increase in ROS mediated lipid peroxidation, leading to a diminished longevity of primed sweet corn (Chiu et al., 2002; Chang and Sung, 1998) and bitter gourd seeds (Yeh et al., 2005). Furthermore, reductions in seed longevity associated with reduced oligosaccharide content were found after priming and post-priming longevity restoration treatments in bell pepper and tomato seeds (Buitink et al., 2000; Gurusinghe and Bradford, 2001). This decrease could be responsible for the reduced longevity of the primed seeds by decreasing the glassy state and increasing the molecular mobility within the intracellular glass (Hoekstra et al., 1994). In addition to the effects of hydropriming, seed deterioration in general is associated with various biochemical and biophysical changes including the loss of antioxidant enzymes and membrane integrity (Hendry, 1993), genetic alteration (Saracco et al., 1995), changes in soluble carbohydrates (Bernal-Lugo and Leopold, 1992, 1995) and accumulation of Maillard reaction products (Wettlaufer and Leopold, 1991; Murthy and Sun, 2000). Till date, only few studies have looked into the relationships between the priming conditions and seed vigor in terms of seed germination and biochemistry.

Two cultivars of maize seeds were used in this experiment. The NCSRC is a commercial cultivar from a Thai government office, the National Corn and Sorghum Research Center (NCSRC). It is an open pollinating cultivar and is recommended due to its high yield. The Private Company (PC) is distributed from a local private seed company. It is a famous F1-hybrid cultivar among farmers due to its high productivity and resistance to downy mildew and rust. This study was focused on maize seed germination enhancement using hydropriming. The biochemical mechanism, antioxidant enzymes, and soluble carbohydrate involved with hydroprimed seed vigor were investigated. The results obtained can introduce suitable hydropriming times to improve maize

seed performance.

MATERIALS AND METHODS

Seed materials

The NCSRC is an open pollinating cultivar commercialized by a Thai government office, NCSRC. The PC is distributed from local private seed company. It is a famous F1-hybrid cultivar among farmers due to its high productivity and resistance to downy mildew and rust. Their initial biochemical properties were monitored.

Moisture contents

Moisture contents before and after hydropriming were determined following the methods of the International Seed Testing Association (ISTA) 2007.

Hydropriming treatment

For the hydropriming process, 200 seeds of each cultivar were placed on a metal wire sieve container laying on a distilled water surface for 3, 6, 9, or 12 h at ambient temperature (26 ± 2°C). The imbibed seeds were immediately dried at 40°C for 24 h in hot air oven to reduce their moisture content. Four replications were applied in this experiment and unprimed seeds were used as a control.

Germination test

50 unprimed and primed seeds were placed on paper towels saturated with distilled water. The towels and seeds were then individually enclosed in plastic bags and incubated at ambient temperature (26 ± 2°C) by day-light for seven days. Normal seedlings were counted on the fourth day (first count) and seventh day (final count) after incubation according to the rules of ISTA (2007).

Accelerated aging test (AA-test)

The unprimed and primed seeds were artificially aged following the method of Duangpatra (1986) with a minor modification. 50 seeds were placed on a wire mesh tray. The tray was then placed above distilled water in a sealed aging chamber to obtain a relative humidity (RH) of about 100% and incubated at 42°C for 96 h. After aging, the seed germination was tested as described above.

Malondialdehyde (MDA) assay

The MDA content of unprimed and primed seeds was measured using a modified method proposed by Tamagnone et al. (1998). Briefly, 150 mg of milled seed was homogenized in 2 ml of extraction buffer (0.1 M potassium phosphate, pH 6.8) in a cold pestle and mortar. The homogenate was centrifuged (Sorvall RC 5C Plus, Kendro Laboratory Products, Newtown, USA) at 13,000 ×g for 15 min at 2 to 5°C and stored on ice. Two microliters of the supernatant was then mixed with 800 µL of water, 500 µL of 20% (w/v) trichloroacetic acid and 1 ml of 10 mM thiobarbituric acid. This mixture was incubated for 30 min at 100°C and then centrifuged at 13,000 ×g for 10 min. The absorbance of the supernatant was measured at 532 nm by spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan) and the concentration of MDA was calculated from its molar extinction coefficient (Σ = 156 $\mu mol^{-1}cm^{-1}$).

Determination of antioxidant enzyme activities

SOD activity was analyzed in primed and unprimed seeds. Five grams of seeds were ground and homogenized in a cold mortar and pestle with 10 ml of 50 mM phosphate buffer (pH 7.0) containing 3 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 g of polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 × g for 20 min at 0°C and the supernatant was used for the enzyme activity assay following the method of Beauchamp and Fridovich (1971). The reaction mixture contained 2.3 ml of 50 mM Na_2CO_3-$NaHCO_3$ buffer (pH 10.2), 0.1 ml of 1.0 mM nitrotetrazolium blue chloride (NBT; Sigma-Aldrich, Inc., USA), 0.1 ml of 4.0 mM xanthine (Sigma-Aldrich Chemie GmbH P.O., Steinheim, Germany), 0.1 ml of 3.0 mM EDTA, 0.15% bovine serum albumin (BSA; Fluka Chemicals Ltd., Gilingham, UK), 0.1 ml of homogenate and 0.1 ml of xanthine oxidase (from bovine milk; Sigma-Aldrich Chemie GmbH P.O., Steinheim, Germany). The mixture was incubated at 30°C for 20 min, after which 0.2 ml of 8 mM $CuCl_2$ was added and the activity of the enzyme was measured using the absorbance at 560 nm. One unit of SOD was defined as the level of enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50% (expressed as units SOD ($kernel^{-1}$)).

APX activity was determined according to the method of Nakano and Asada (1987). Seed samples (2 g fresh weight) were homogenized with 4 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (PVP) using a pestle and mortar kept on ice. The homogenate was centrifuged at 4°C for 20 min at 15,000 × g and the supernatant was collected for assay. APX activity was measured using the decrease in absorbance at 290 nm due to ascorbate oxidation in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.1 mM EDTA for 1 min. Enzyme activity was expressed in units with one unit defined as 1 nmol substrate metabolized at 1 mg^{-1} protein min^{-1}. The concentration of APX protein was determined by the Bradford method (Bradford, 1976).

Analysis of soluble sugar and activities of amylase and invertase

One gram of milled seed was mixed with 2 ml of 80% ethanol (high performance liquid chromatography (HPLC) grade; Carlo Erba Reagent, Italmar Co., Ltd., France) and homogenized in a cooled mortar and pestle. The homogenate was centrifuged at 14,000 × g for 10 min. The solution was filtered through a cellulose nitrate membrane filter (0.45 µM pore size, Whatman GmbH P.O., Dassel, Germany) (Bhowmik et al., 2001). A twenty microliter aliquot of filtered solution was injected into HPLC (Chromatopac C-R6A, Shimadzu Corporation, Kyoto, Japan) fitted with an Asahipak NH_2P-504E column (Shodex Packed Column for HPLC, Shoko Co., Ltd., Japan) (4.6 mm ID × 250 mm) and eluted with 25/75 (v/v) of H_2O/CH_3CN (HPLC grade; RCI Labscan Ltd., Bangkok, Thailand) at a flow rate of 1 ml min^{-1} at 30°C. The retention times and concentrations of glucose, sucrose (HPLC grade; TCI-GR, Tokyo Kasei Kogyo Co., Ltd., Japan) and raffinose (Fluka Chemicals Ltd., Gilingham, UK) were compared and determined from standard solutions of these sugars.

Amylase activity was investigated in primed and unprimed seeds samples. Briefly, 500 mg of milled seeds were ground in a chilled mortar and pestle with 0.1 M sodium citrate buffer (pH 5.5) and centrifuged at 10,000 × g at 4°C for 15 min. The supernatants were collected and amylase activity was analyzed following the method of Miller (1959). The assay mixture contained 0.5 ml of 1% soluble

Table 1. Germination percentage, germination after accelerated aging, malondialdehyde (MDA) content and superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities of maize seeds from the NCSRC and PC cultivars either hydroprimed for 3–12 h or not hydroprimed.

Seed cultivars	Priming times (h)	Germination (%)		MDA	SOD	APX
		unaged	aged	□ □ mol/g DW)	(U/kernel)	(U/mg protein)
NCSRC	unprimed	100	92±3^{b}	5.09±0.03^{a}	4.43±0.15^{b}	11.52±1.16^{bc}
	3	100	96±2^{ab}	4.03±0.03^{b}	4.80±0.17^{b}	15.97±0.93^{b}
	6	100	99±1^{a}	3.89±0.05^{c}	4.56±0.64^{b}	13.30±1.64^{bc}
	9	100	96±2^{ab}	3.72±0.06^{d}	2.81±0.64^{c}	16.88±1.43^{ab}
	12	100	86±1^{c}	2.64±0.05^{fg}	1.46±0.24^{d}	14.38±2.07^{b}
PC	unprimed	100	76±2^{d}	5.05±0.04^{a}	7.24±0.51^{a}	8.14±1.90^{c}
	3	100	96±2^{ab}	3.01±0.05^{e}	7.24±0.23^{a}	21.40±1.08^{a}
	6	100	94±3^{ab}	2.93±0.04^{e}	6.74±0.21^{a}	16.48±1.62^{ab}
	9	100	92±3^{b}	2.75±0.05^{f}	7.29±0.35^{a}	14.30±2.78^{b}
	12	100	84±2^{c}	2.60±0.01^{g}	7.18±0.15^{a}	12.23±1.18^{bc}
Cultivar (C)		-	**	**	**	ns
Priming time (P)		-	**	**	**	**
C □ P		-	**	**	**	**

Means ± SE (n = 4) within a column followed by the same letter are not different according to Duncan's New Multiple Range tests at *P* = 0.05. For the ANOVA, ns = non significance and ** = significant at *P* = 0.01.

starch (Univar Reagent, Asia Pacific Specialty Chemicals Limited, Seven Hills, Australia) and 0.25 ml of 0.1 M citrate buffer (pH 5.5) to which 100 µL of supernatant was added, after which the reaction mixture was incubated at 50°C for 20 min. The reaction was terminated by adding 3 ml of 1% 3,5-dinitrosalicylic acid (DNS) (Sigma-Aldrich Chemie GmbH P.O., Steinheim, Germany) solution and boiling for 5 min. The boiled solution was measured with a spectrophotometer at 550 nm. One unit of amylase activity was defined as the amount of enzyme that produced a reducing sugar equivalent to 1 µmol of glucose from starch per minute at 50°C.

Invertase was extracted from 500 mg of milled seeds in a chilled mortar and pestle using 0.02 M sodium phosphate buffer (pH 7.0) (Krishnan et al., 1985). The extract was centrifuged at 10,000 × g, 4°C for 10 min and invertase activity in the supernatant was determined by measuring the liberated reducing sugars by DNS (Chaplin, 1987) with a minor modification. The assay mixture comprised 0.1 ml supernatant, 0.5 ml of 0.1 M sucrose and 0.5 ml of 10 mM sodium acetate buffer pH 5.0 and was incubated at 37°C for 30 min. The reaction was stopped by boiling the mixture with 0.5 ml of 1% DNS solution for 10 min. The liberated reducing sugars were determined at 540 nm and calibrated with a sucrose standard (TCI-GR, Tokyo Kasei Kogyo Co., Ltd., Japan) (0 to 0.2 mM). One unit of invertase activity was defined as the amount of enzyme that hydrolyzed sucrose to 1 µmol of glucose per minute at 37°C and pH 4.6. In this experiment, all chemicals used were of analytical grade or otherwise would be addressed.

Statistical analysis

The experiment was arranged as a factorial in completely randomized design with four replicates. Data were subjected to analysis of variance (ANOVA) and significant differences among means were determined by Duncan's new multiple range tests at *P* = 0.05 using the SAS statistical software (SAS, 1986). Means, standard errors and correlation coefficients (*r*) were calculated from replicates within the experiments and analyzed using Microsoft Excel 2007.

RESULTS

Germination and vigor

The germination of both cultivars before and after hydro-priming treatments was 100% (Table 1) and the seed moisture content after priming ranged from 8.7 to 9.5% in both cultivars (data not shown). Seed germination after accelerated aging, which indicated seed vigor, is shown in Table 1. The vigor of NCSRC seeds was greater than that of the PC seeds particularly in the controls (unprimed seeds). There was a trend for both the NCSRC and PC seeds to have higher percentage germination than the control when primed for 3 to 9 h. However, priming for 12 h reduced germination of the NCSRC seeds below the control level. Priming of the PC seeds for 12 h reduced germination below that observed at shorter priming times; although germination was still higher than the control.

MDA content

The initial MDA content of unprimed seeds was not different between the NCSRC and PC varieties (Table 1) and decreased with duration of priming time for both cultivars. The MDA content of both sources of seed primed for 12 h dropped to approximately half of initial

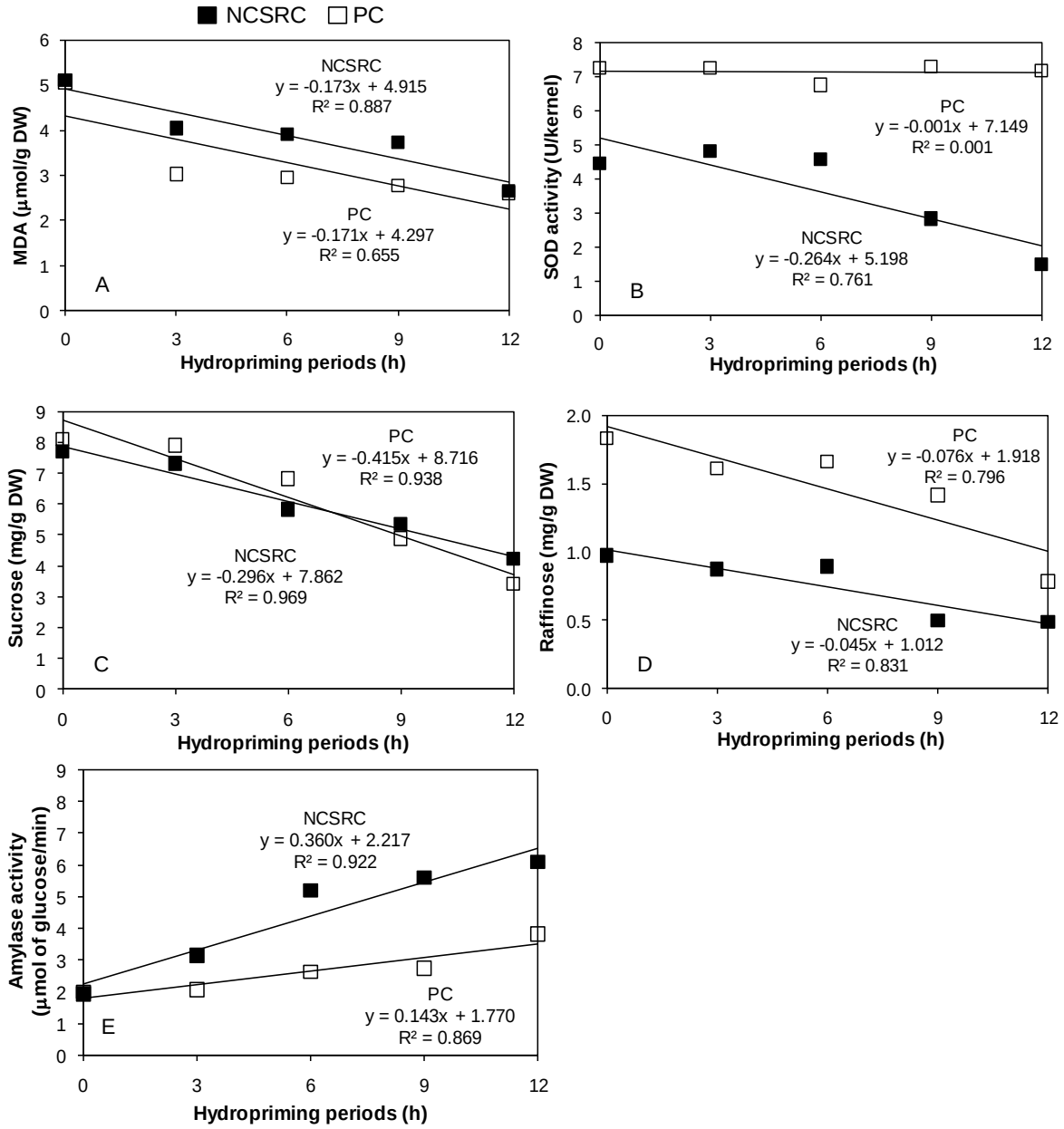

Figure 1. Relationship between hydropriming periods and malondialdehyde (MDA) content (A), superoxide dismutase (SOD) activity (B), the content of sucrose (C) and raffinose (D) and amylase activity (E) of maize unprimed and primed seeds from the cultivars, NCSRC (■) and PC (□).

value; however, the rate of reduction was faster in the PC seeds resulting in a significant interaction between seed source and priming time. Moreover, strong negative correlation was found between priming periods and MDA for NCSRC seeds (r = -0.942) and for PC seeds (r = -0.810) at P = 0.05 (Figure 1A). The relationship between germination after accelerated aging and MDA was not observed for both cultivars (Figure 2A). Besides, MDA levels may not be an appropriate indicator for maize primed seed vigor.

Antioxidant enzyme activities

The activities of SOD and APX of unprimed and primed seeds in both cultivars were determined to investigate their relationship with germination after accelerated aging (Table 1). The SOD activity of unprimed PC seeds (6.74 to 7.29 U/kernel) was significantly higher than that from NCSRC (1.46 to 4.80 U/kernel). SOD activity in the NCSRC seeds decreased significantly in proportion to the priming time and reduced to approximately ⅓ of its

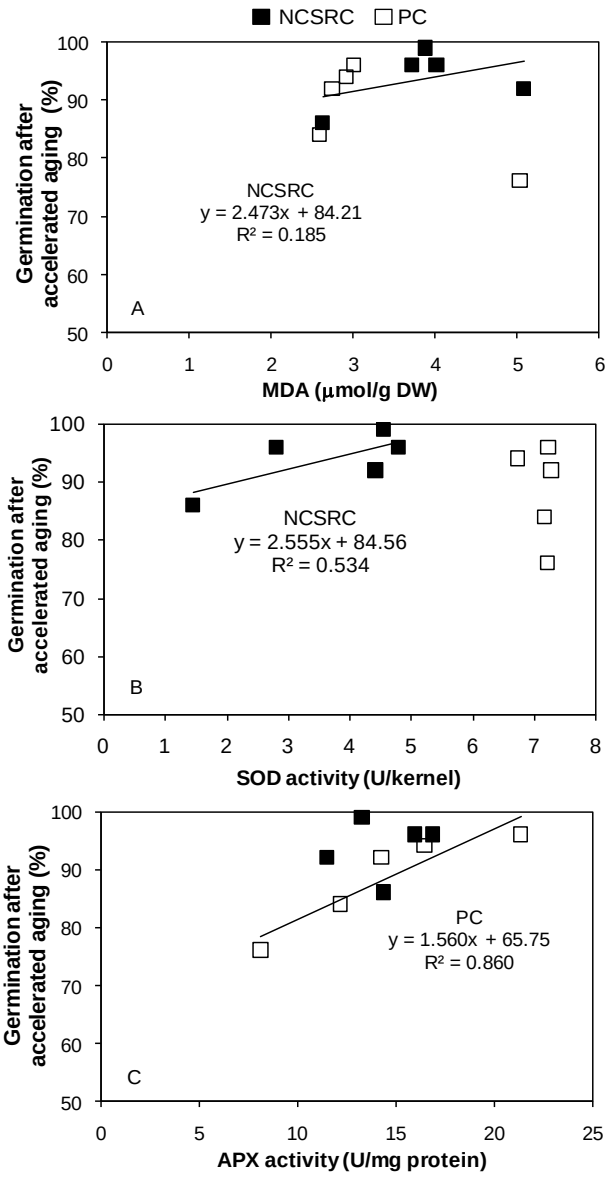

Figure 2. Relationship between germination after accelerated aging and malondialdehyde (MDA) content (A) and the activities of superoxide dismutase (SOD) activity (B) and ascobate peroxidase (APX) (C) of maize unprimed and primed seeds from the cultivars, NCSRC (■) and PC (□).

original value. However, no decline in SOD activity was seen in the PC seeds given any duration of priming. The data for APX was highly variable and overall there was no difference between the two sources of seeds. However, there was a trend for primed seeds to have higher APX activity than unprimed seeds.

Moreover, SOD activity negatively correlated with priming periods in NCSRC seeds (r = -0.873), but this correlation was not observed in PC seeds (Figure 1B). A moderately positive correlation was found between germination after accelerated aging and SOD activity for NCSRC seeds (r = 0.726), but this relationship was very weak in PC seeds (Figure 2B). A correlation between APX and priming periods did not exist for both cultivars but there was a moderately positive correlation with germination after accelerated aging in PC seeds (r = 0.513) (Figure 2C). Therefore, the activated antioxidant enzyme activity (APX for the PC, and APX and SOD for the NCSRC), caused by hydropriming, would reduce ROS accumulation induced by accelerated aging. As a result, the germination after accelerated aging of hydro-primed seeds for 3 to 9 h was greater for both cultivars.

Table 2. Glucose, sucrose and raffinose contents and activities of amylase and invertase in maize seeds from the NCSRC and PC cultivars either hydroprimed for 3–12 h or not hydroprimed.

Seed cultivars	Priming times (h)	Glucose	Sucrose (mg/g DW)	Raffinose	Amylase (μmol of glucose/min)	Invertase
NCSRC	unprimed	1.82±0.13^{bc}	7.69±0.88^{ab}	0.97±0.03^{c}	1.91±0.17^{d}	1.02±0.04^{ab}
	3	1.65±0.11^{c}	7.32±0.15^{ab}	0.87±0.05^{c}	3.13±0.21^{c}	0.92±0.02^{bc}
	6	1.62±0.04^{c}	5.83±0.23^{bcd}	0.89±0.12^{c}	5.19±0.15^{a}	0.94±0.11^{abc}
	9	1.67±0.06^{c}	5.36±0.33^{cd}	0.49±0.07^{d}	5.58±0.23^{a}	1.11±0.05^{a}
	12	1.91±0.14^{bc}	4.23±0.42^{d}	0.48±0.07^{d}	6.10±0.08^{a}	1.14±0.13^{a}
PC	unprimed	2.17±0.14^{ab}	8.12±0.34^{a}	1.83±0.14^{a}	1.98±0.10^{d}	0.86±0.02^{bc}
	3	2.35±0.19^{a}	7.90±0.44^{a}	1.61±0.09^{ab}	2.05±0.17^{d}	0.84±0.01^{c}
	6	2.38±0.14^{a}	6.82±0.12^{abc}	1.66±0.16^{ab}	2.62±0.22^{c}	0.89±0.03^{bc}
	9	2.21±0.24^{ab}	4.88±0.63^{de}	1.41±0.08^{b}	2.72±0.12^{c}	0.79±0.03^{c}
	12	2.15±0.17^{ab}	3.40±0.17^{e}	0.78±0.08^{c}	3.80±0.32^{b}	0.88±0.09^{bc}
Cultivar (C)		**	ns	**	**	**
Priming time (P)		ns	**	**	**	ns
C × P		**	**	**	**	*

Means ± SE (n = 4) within a column followed by the same letter are not different according to Duncan's New Multiple Range tests at $P = 0.05$. For the ANOVA, ns = non significance, * = significant at $P = 0.05$ and ** = significant at $P = 0.01$.

Soluble carbohydrate content and activities of amylase and invertase

The observed changes in soluble carbohydrates were similar in both cultivars from the data shown in Table 2. There was no effect of priming on the glucose content of the two sources of seeds; however, glucose was higher in the PC seeds than in the NCSRC seeds. With respect to sucrose, concentrations declined in proportion to the duration of priming, with the rate of decline being greater in the PC seeds. Raffinose also declined in both cultivars in proportion to the duration of the priming time, and it reduced by approximately 2-fold. In the control and all treatments, raffinose was higher in the PC seeds than in the NCSRC seeds.

The rates of amylase activity in the unprimed seeds from both sources were the same. Priming increased amylase activity in both seed sources with activity increasing in proportion to the duration of priming. However, amylase activity increased more in the NCSRC seeds than in the PC seeds with the former showing a three-fold increase and the latter only a two-fold increase indicating a significant interaction between seed source and priming time. There was less change in invertase activity in response to priming. However, invertase activity was higher in the seeds from NCSRC than that from the PC.

In addition, a highly negative correlation between priming periods and sucrose and raffinose was found for both cultivars that was -0.985 and -0.912 for NCSRC and -0.969 and -0.892 for PC seeds (Figures 1C and D). Amylase activity strongly correlated with the priming periods of NCSRC at 0.960 and PC at 0.932 (Figure 1E). The priming times showed the non significant difference to glucose content and invertase activity (Table 2). Hence, an analysis of a relationship between priming periods and these parameters were not carried out. However, a correlation between germination after accelerated aging with sucrose and raffinose and the amylase activity for both cultivars was not observed in this experiment (data not shown). Our results show that the increased amylase activity and decreased sucrose and raffinose content during the hydropriming process may reduce germination after accelerated aging of hydroprimed seeds for both cultivars.

DISCUSSION

The priming of seeds in water known as hydropriming, improves the seed germination of rice, maize, and chickpea (Harris et al., 1999). For the two sources of seeds used in this study, germination before hydro-priming was high (100%) and was not reduced by this treatment (Table 1). Unprimed seeds showed a significant reduction in vigor after being subjected to an artificial aging process; the NCSRC seeds presented more vigor compared to the PC seeds (Table 1). The different response could be caused by their seed history and genotype (Copeland and McDonald, 1985), and in

particular, the difference of the APX activity (Table 1). Although SOD activity of the PC seeds was higher compared to the NCSRC, the germination after accelerated aging was significantly reduced. This means ROS produced during accelerated aging was not completely detoxified by only SOD. It also suggests that the higher vigor of NCSRC compared to the PC seeds would be influenced by greater initial APX activity, rather than SOD activity. The differences of gene expression with responses to stimulants or the environment has been documented. In pigeon pea seeds, the SOD activity was varied among seed cultivars (Kalpana and Madhava Rao, 1994). The activity of SOD of the maize hybrid 351 line was increased by atrazine treatment, but the activity was decreased in Giza 2 line (Nemat Alla and Hassan, 2006).

In the case of initial soluble sugar (Table 2), raffinose content in the unprimed PC seeds was significantly higher, but its germination after accelerated aging was lower compared to NCSRC (Table 1). The larger amount of initial raffinose content may not point to high or low vigor of maize seeds. This research revealed that the initial soluble sugar content in the unprimed maize seeds for both cultivars might not play an important role in indicating declined germination after the aging process. This result is different from previous research, which suggested that sugars, sucrose, raffinose, and stachyose are essential for the cowpea seed germination process, and higher sugar cultivars were found with higher germination (Islam et al., 2008). Therefore, the amelioration of the aging process of unprimed maize seeds of NCSRC was greater than the PC seeds, and this may be caused by a reduction in oxidative stress by APX activity, thus leading to greater germination. The soluble sugar content in the maize seeds may not play any important role in this evidence.

Maize seeds responded differently to hydropriming treatment, thereby resulting in different biochemical changes and their germination after accelerated aging (Tables 1 and 2). Hydropriming for up to 9 h maintained the ability of the seed to germinate after the aging process; hydropriming for a longer period reduced germination (Table 1). The results suggest that the vigor of PC seeds is more greatly affected by the aging process than that of the NCSRC seeds, and that short priming times can prevent this decline (Table 1). It should be noted that the optimal period for maize seeds hydropriming is for 3 to 9 h. This research found a significant reduction in MDA in the primed seeds for 3 to 9 h of both cultivars. Similar reductions in MDA content have also been shown to enhance seed germination in primed seeds of tomato (El-Araby and Hegazi, 2004) and purple coneflower (Chiu et al., 2002, 2006). However, decreased MDA value was observed in the maize seeds priming for 12 h together with a reduction of germination after accelerated aging of the primed seeds (Table 1). The reduction of MDA was not associated with decreasing germination after accelerated aging of the maize primed seeds (Figure 2A). Therefore, the relationship between MDA content and germination of primed seed during imbibitions and re-drying processes was intensively monitored. Recent research found that MDA content was significantly increased during imbibitions step, but the MDA content declined after re-drying the imbibed seeds (unpublished data).

The amelioration of the aging process in the seeds hydroprimed for 3 to 9 h may be due to a reduction in oxidative stress. Evidence for this comes from two sources. First, the levels of the antioxidant enzymes, APX and SOD, assayed in this study were increased (Table 1). The APX presented a positive relationship and may be crucial in influencing the high vigor of PC seeds for 3 to 9 h (Figure 2C). However, SOD and APX played a major role that enhanced the 3 to 6 h NCSRC primed seed vigor, but the APX impacted the 9 h primed seed vigor. The enhancement of SOD and APX activities may balance ROS produced during aging condition. As a result, germination of the hydroprimed seeds for 3 to 9 h would be greater compared to the unprimed seeds (Table 1). According to Goel et al. (2003), the activities of the SOD, POD, CAT, APX, and glutathione reductase (GR) of cotton seeds, activated by 2 h hydropriming, were higher than unprimed seeds and the germination after accelerated aging was improved. In contrast, the percent emergence and CAT and APX activities of primed bitter gourd seeds was decreased after accelerated aging more rapidly than unprimed seeds (Hsu et al., 2003). However, the levels of these enzymes (SOD and APX) should be measured during the aging and germination process. This would give a more comprehensive indication of their relationship between antioxidant enzyme activities and vigor. The second line of evidence suggest that the increased amylase activity caused by hydropriming treatment for 3 to 9 h may support high respiration rate and speed up germination after aging condition compared to unprimed seeds for both cultivars (Table 2). The amylase activity of NCSRC seeds was induced two to four-fold by hydropriming compared to the control samples. This evidence is accompanied with reduction of glucose and sucrose content in the NCSRC seeds during priming for 3 to 9 h. This implied a higher respiration rate for the primed NCSRC seeds. The similar pattern of PC seeds was observed; however, the respiration rate of the hydroprimed seeds for 3–9 was lower than that of the NCSRC seeds (Table 2).

The respiratory metabolism was also stimulated during accelerated aging (Sung and Jeng, 1994) and the germination process (Copeland and McDonald, 1985). In this process, ROS was consequently produced; and would be degraded by greater antioxidant enzyme activities induced by hydropriming. As a result, the greater germination after accelerated aging of the hydroprimed maize seeds for 3 to 9 h would be improved compared to the unprimed seeds. The causes of this evidence could be explained by biochemical reactions, high respiration

rate, and greater antioxidant enzyme activities induced by hydropriming for 3 to 9 h. It is suggested that the levels of amylase activity of the hydroprimed seeds should be monitored during the aging and germination process. This would give a more comprehensive explanation of its impact to maize seed germination after accelerated aging. In contrast, germination of hydroprimed seeds for 12 h subsequently accelerated aging significantly declined compared to the maize seeds with shorter priming times (Table 1). A loss of maize seed vigor by hydropriming for 12 h was also observed with a reduction of MDA (Table 1). Recently, our study found that MDA content in the imbibed seed was significantly increased and declined after re-drying the imbibed seeds (Wattanakulpakin et al., to be submitted). In addition, amylase activity was markedly increased, accompanied by no change in glucose in the seeds primed for 12 h. The more or less constant glucose level and lower sucrose content suggests the production and consumption of glucose may be balanced in the maize primed seeds for both cultivars (Table 2). This means the high respiration rate and oxidative stress would be extremely increased, leading to an accumulation of ROS in the maize seeds hydroprimed for 12 h. Meanwhile, the antioxidant enzymes of maize seeds with hydropriming for 12 h were reduced (APX and SOD in the NCSRC, and APX in the PC seeds). Therefore, the germination process of hydroprimed seeds for 12 h after accelerated aging was not completely developed, resulting in low germination percentages (Table 1). It should be mentioned that the markedly increased amylase activity, and decreased antioxidant enzyme activities at 12 h of priming may partially reduce germination after accelerated aging.

Furthermore, changes in soluble sugar composition in seeds may be an important determinant of seed longevity (Horbowicz and Obendorf, 1994). In this research, the vigor reduction of seeds after aging is possibly related to this phenomenal. Previous studies on pea, maize, and rape seeds assume that soluble sugars, mainly oligosaccharides of the RFOs, may be involved in the protection against the deleterious effects occurring during dehydration and/or aging (Obendorf, 1997). Moreover, various authors (Bernal-Lugo and Leopold, 1992; Horbowicz and Obendorf, 1994; Piotrowicz-Cieslak, 2005) demonstrated that sugar content, particularly the ratio of oligosaccharide to sucrose contents, may be used as an indicator for seed storability or aging. For example, a value of this ratio higher than 0.2 in maize corresponds to a good seed storability (Bernal-Lugo and Leopold, 1995), but loss of wheat grain viability at 45°C and 100% humidity is associated with the increase of the raffinose/sucrose ratio (Lehner et al., 2006). Similarly, a decrease of longevity at 20±8°C in primed seeds may be attributed to further reduction of non-reducing sugar content (Williams and Leopold, 1995). Changes in soluble carbohydrates are reported to be associated with a loss of seed storability (Horbowicz and Obendorf, 1994). This study shows a reduction in raffinose and sucrose in proportion to the duration of priming (Figures 1C and D). Even though correlation between raffinose or sucrose and germination after accelerated aging was not observed, their contents in the seeds primed for 12 h were reduced approximately two-fold and were associated with a significant decline in germination after accelerated aging compared with seeds primed for 3 to 9 h (Table 2). A decrease in sucrose in proportion to the duration of priming was observed despite no change in invertase activity. The reduction of sucrose may be due to the activity of other enzymes, such as sucrose synthase (Sturm, 1999; Bhowmik et al., 2001). Also, the decline in raffinose may be due to its conversion to reducing sugars by α-galactosidase (Gurusinghe and Bradford, 2001), although this enzyme was not investigated in this study. Hence, the reduction of sucrose and raffinose may point to a decrease in primed seed vigor.

A positive correlation between raffinose content and seed longevity was revealed in various maize genetic lines during storage (Bernal-Lugo and Leopold, 1995). Gurusinghe and Bradford (2001) also found a significant correlation between a decline in sucrose and raffinose in hydroprimed lettuce seeds with a reduction in vigor. Sucrose may also stabilize membranes, as it has a good ability to form hydrogen bonds with membrane components (Wolker et al., 1998a, b). The sucrose and raffinose in this study may have reached sufficiently low concentrations in the seeds primed for 12 h to affect membrane stability. As a result, ion leakage and ROS may have been stimulated and caused further cell and seed deterioration as found by Leprince et al. (1994). In bell pepper seeds, osmo-priming affected the decrease in longevity involved with loss of oligosaccharide content, but this sugar did not affect the stability of the intracellular glassy state (Buitink et al., 2000). Therefore, the increased amylase activity and decreased sucrose and raffinose content accompanied with declined anti-oxidation enzyme activities could be noted to reduce maize primed seed vigor.

In conclusion, the higher germination of NCSRC unprimed seeds after accelerated aging compared to the PC seeds was caused by its initial APX activity. Hydropriming for short durations (3 to 9 h) may prevent reductions in the vigor of primed seeds as they age. The amelioration of the aging process is likely due to reduction in oxidative stress by antioxidant enzymes (SOD and APX in this study). SOD and APX may play a role in NCSRC, but APX contributed in PC. The high amylase activity with remaining glucose content may be a partial response to germination improvement in the hydroprimed seeds subjected to accelerated aging. Meanwhile, priming periods of greater than 9 h did not result in as much protection from aging as those for shorter durations, and this may be due to a loss of

membrane stability caused by a reduction in sucrose and raffinose. The extremely high activity of amylase for both cultivars, and the decreased activities of SOD and APX in NCSRC and APX in PC may also indicate the vigor loss of primed seeds. Further study should be done to investigate change in non enzymatic antioxidants, ROS level, or plant hormones of these two cultivars after hydropriming to link the enhanced germination after accelerated aging.

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