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Lipid Peroxidation and Antioxidant Responses during Seed Germination of *Jatropha curcas*

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ABSTRACT

Changes in malondialdehyde (MDA) content and the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in endosperms and cotyledons during *Jatropha curcas* seed germination were investigated in the present study. MDA content in endosperms increased during 10 day of germination, while in cotyledons it increased during six day of germination and then decreased. SOD activity in endosperms showed an increase at two day of germination and decreased gradually, while in cotyledons it increased gradually during germination. POD activity in endosperms increased gradually, peaking on 8 day of germination, while it peaked at 6 day in cotyledons. CAT activity in endosperms and cotyledons rose gradually, peaking on 8 day and 6 day of germination, respectively. Analysis by electrophoresis showed that the changes in patterns and intensities of SOD, POD and CAT isoenzymes in endosperms and cotyledons during germination appear to be depended on developmental processes and plant tissues. These results are also consistent with the changes of enzyme activities as assayed in extract solutions. Our findings indicate that the efficient participation of antioxidant mechanisms, including the synergistic activities of the different types of SOD, POD and CAT, might play an important role during seed germination.

Key Words: Antioxidant enzyme; Lipid peroxidation; *Jatropha curcas*; Seed germination

INTRODUCTION

Seed germination and post-germination seedling development are well-regulated process in plant physiology involving high metabolic activity and generation of reactive oxygen species (ROS) in the cell (Bailly, 2004). ROS affect various aspects of seed physiology, displaying two major functions: as a kind of cytotoxin and as a special role in seed development, dormancy breakage, and in defense against biotic and abiotic stresses (Apel & Hirt, 2004). A series of new roles for ROS has recently been identified: the control and regulation of biological processes, such as cell cycle, programmed cell death and hormone signaling (Gapper & Dolan, 2006). These studies extend our understanding of ROSs and suggest a dual role for ROS in plant biology as both toxic by products of aerobic metabolism and key regulators of growth, development and defense pathways.

ROS are produced in aerobic organisms within the cell and are normally in balance with antioxidant molecules. Oxidative stress arises from an imbalance between generation and elimination of ROS. These cytotoxic activated ROS can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Mittler *et al*., 2004). This can lead to changes in the selective permeability of bio-membranes, causing membrane leakage and changes in the activity of membrane-bound enzymes (Apel & Hirt, 2004). However, an elaborate and highly redundant plant ROS defence network, composed of antioxidant enzymes, antioxidants and ROS-producing enzymes, is responsible for maintaining ROS levels under tight control. In plant cells, antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), are considered to form a defensive team, whose combined purpose is to protect cells from oxidative damage during growth, development and senescence (Blokhina *et al*., 2003). Moreover, malondialdehyde (MDA) is considered sensitive marker commonly used for assessing membrane lipid peroxidation (Bailly *et al*., 1996; Goel & Sheoran, 2003).

Jatropha curcas L., commonly known as physic nut, belongs to the family *Euphorbiaceae* and has potent medicinal qualities and considerable commercial value. Various parts of the plant have been widely used for various purposes, such as energy source, therapeutic uses, charcoal production, fertilizer and animal feed (Kumar & Sharma, 2008). Increased cellular levels of ROS are known to occur

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during seed germination and development. Coordinated changes about antioxidant enzymes during seed germination have been established in several plant species, including wheat (Rogozhin *et al*., 2001), sunflower (Bailly *et al*., 2002), *Chenopodium rubrum* (Dučić *et al*., 2003) and pea (Wojtyla *et al*., 2006). Previous studies have also shown the behaviour and roles of the antioxidative system in *Jatropha curcas* seedlings under heavy metals stresses (Gao *et al*., 2008 & 2010). However, prior to these studies little were known of the changes of antioxidant activities and pattern of antioxidant isoenzymes with respect either to germination process or to a possible role during *Jatropha curcas* seed germination. The current study took a systematic approach to investigate whether there is a relationship between the changes in SOD, POD and CAT activities and germination process in the degrading endosperms and developing cotyledons.

MATERIALS AND METHODS

Mature *Jatropha curcas* seeds were collected in August, 2008 from more than 10 individual wild trees in Panzhihua, Sichuan provice, China. Seeds were oven dried at 30°C for 24 h and then selected and stored at 4°C until processing. *J. curcas* seeds were surface sterilized in 70% ethanol for 30 sec, and then in 0.1% mercuric chloride for 8 min. Seeds were rinsed with distilled water soaked for 24-36 h at room temperature and sown in trays filled with sand for germination and growth. Trays were arranged as for a complete randomized design on a table kept in the laboratory with an even light supply and an average temperature of 30°C. Trays containing seeds were supplied with ample water to maintain approximately 100% relative humidity within each tray before germination. Distilled water was used to maintain the 70% relative humidity during germination and early seedlings growth. Seeds were considered as having germinated when seed coat dehiscence, occurred usually after 44–48 h of incubation. The moment two cotyledons of seedlings had developed (10 days) is referred to as "developed seedling". Ten germinated seeds or developed seedlings were selected from the trays every two day, and endosperms and cotyledons were separated immediately frozen in liquid nitrogen and stored at -80°C for further analysis. Germination experiment was performed three repetitions with 500 seeds.

MDA content was determined by the thiobarbituric acid (TBA) reaction (Bailly *et al*., 1996). Endosperms or cotyledons were homogenized with 0.1% trichloroacetic acid (TCA) (m/v, 1/10) and the homogenates were centrifuged at 15294×g for 15 min. To a 1.0 mL aliquot of the supernatant, 3.0 mL of 0.5% TBA in 5% TCA was added. The mixture was heated at 95°C for 30 min and then cooled immediately in an ice bath. The reaction mixture was centrifuged at 15294×g for 10 min and the observance of supernatant was recorded at 532 nm and 600 nm. Lipid peroxidation was expressed as MDA content in μM per gram fresh weight, by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

For protein content and assaying antioxidant enzymes, endosperms and cotyledons were homogenized with 50 mM sodium phosphate buffer (pH 7.0) (m/v, 1/10) including 150 mM NaCl and 1 mM EDTA. The homogenate was centrifuged at 15294×g for 10 min at 4°C. Protein was quantified by Lowry method using bovine serum albumin as a standard.

All assays of enzyme activities were performed using a UV-Vis spectrophotometer (TU-1901, Purkinje General, Beijing, China). All assays and measurements were repeated three times at room temperature. SOD activity was determined by the method of Chen and Pan (1996). The reaction mixtures included 50 mM sodium phosphate buffer (pH 7.0), 10 mM methionine, 1.17 mM riboflavin, 56 mM NBT and 100 μL protein extract. The changes of absorbance were read at 560 nm using a UV/vis spectrophotometer (TU-1901, Purkinje General, Beijing, China). One SOD unit was defined as the enzyme activity that reduced the photoreduction of nitroblue tetrazolium to blue formazan by 50%. POD activity was measured by Sakharov and Aridilla (1999) method with slight modifications. A 3 mL mixture consisted of 2.85 mL of guaiacol (3%), 0.1 mL H_2O_2 (2%) and 50 μL enzyme extract and the changes of absorbance at 470 nm were measured using a UV/vis spectrophotometer (TU-1901, Purkinje General, Beijing, China). One unit of POD activity was expressed as 1.0 change in absorbance per minute. CAT activity was measured following the change in the absorbance of the reaction mixture at 240 nm (Philippe *et al*., 2007). The assay was detected in a 3 ml reaction mixture containing 2.8 mL phosphate buffer (50 mM, pH 7.0), 100 μL H_2O_2 (1%) and 100 μL of crude extract. One CAT unit is defined as the amount causing the decomposition of 1 μM H_2O_2 per min. Polyacrylamide gel electrophoresis (PAGE) for isoenzymes assay was performed with 10% acrylamide gel at 4°C. A vertical Mini-Protein II electrophoresis system (Bio-Rad, Herculus, CA) was used for gel electrophoresis. The electrophoretic run was carried out 80V in the stacking gel followed by 120V in the separating gel. SOD activity was detected by the Beauchamp and Fridovich (1971) method. Gels were equilibrated with 50 mM phosphate buffer (pH 7.5) containing 2.8×10^{-5} M riboflavin, 28 mM *N*,*N*,*N*,*N*-tetramethyl ethylenediamine (TEMED) for 30 min and then washed in distilled water for several times and resubmerged in the same buffer containing 2.45 mM NBT for 10-20 min with gentle agitation in the presence of light. Isoenzyme bands displayed as colorless bands on a purple background. POD isoenymes were shown by the Ros Barcelo (1987) method. Gels were rinsed in water and then stained in staining solution containing 0.06% (v/v) H_2O_2, 0.2% benzidine and 0.1% (v/v) acetic acid till brown color bands appear. CAT isoenzyme was assayed by the Woodbury (1971) method. Gels were first incubated in 0.03% H_2O_2 for 5-10 min, and then transferred into 4% soluble starch

solution for 1 h. Gels was stained in 2% $FeCl_3$ and 2% $K_3Fe(CN_6)$ solution for 5-10 min until the colorless bands were clear visible on the deep blue gel.

We performed three independent experiments in duplicate for each condition. Statistical significance was evaluated with a Student's t-test, and considered to be significant when the *P* value was less than 0.05.

RESULTS

The changes of MDA content in endosperms and cotyledons during germination are shown in Fig. 1. MDA contents in endosperms increase gradually during the 10 day germination. In cotyledons, MDA content increased sharply during the first six day of germination and then decreased gradually. The peak content is about 8.5 times that of the control.

The changes of SOD activity in endosperms and cotyledons during germination are demonstrated in Fig. 2. As shown in Fig. 2, SOD activity in endosperms increased sharply at early (two days) germination, and the maximum activity increased 1.79 fold compared to the control. Then, SOD activity decreased gradually during germination, and at 10 day it is only 77% of that of the control. SOD activity in cotyledons increased during seed germination, and at 8 day of germination it reached the highest activity which was about 2.2 times that of the control. Native gel electrophoresis analysis reveal that at least four SOD isoenzymes are observed in endosperms during germination, and the highest intensity of isoenzymes is found at 2 days of seed germination (Fig. 3A). In cotyledons, at least four SOD isoenzymes were detected and one new isoenzyme appeared at 2 and 4 day of germination. Moreover, the intensities of two SOD isoenzyme bands (I & IV) in cotyledons enhance gradually during germination (Fig. 3B).

The changes of POD activities in endosperms and cotyledons during germination are shown in Fig. 4. POD activities in endosperms increased gradually during 8 days of germination, the highest value being about 4.49 times that the control. POD activity in cotyledons increased greatly during the first 6 days of germination, and the highest activity was 4.44 times that of the control. Patterns of POD isoenzymes in endosperms and cotyledons during germination are shown in Fig. 5. In endosperms, POD isoenzymes showed at least seven isoenzymes (Fig. 5A). New POD isoenzymes are synthesized/activated upon endosperms degradation (I, IV, V, VI & VII) whereas one isoenzyme (III) disappeared upon endosperms degradation. During germination cotyledons had at least seven POD isoenzymes (Fig. 5B). Five new isoenzymes (I, IV, V, VI & VII) are observed during germination and early seedlings development. Ungerminated cotyledons contained only two POD isoenzymes (II & III) and they remained in the developing cotyledons.

Fig. 1: Changes of malondialdehyde (MDA) contents in endosperms and cotyledons during seed germination. The values and standard errors (vertical bars) of three replicates are shown

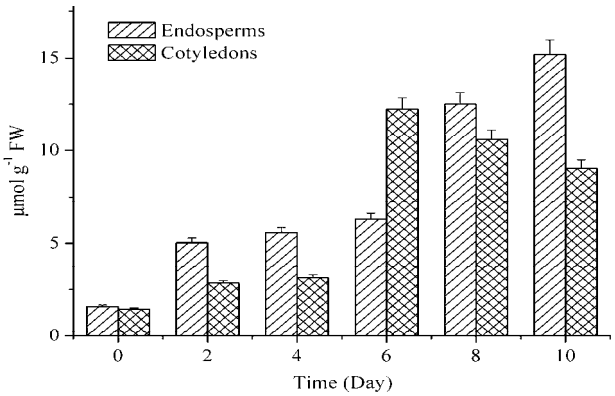


Fig. 2: Changes of superoxide dismutase (SOD) activities in endosperms and cotyledons during germination. The values and standard errors (vertical bars) of three replicates are shown

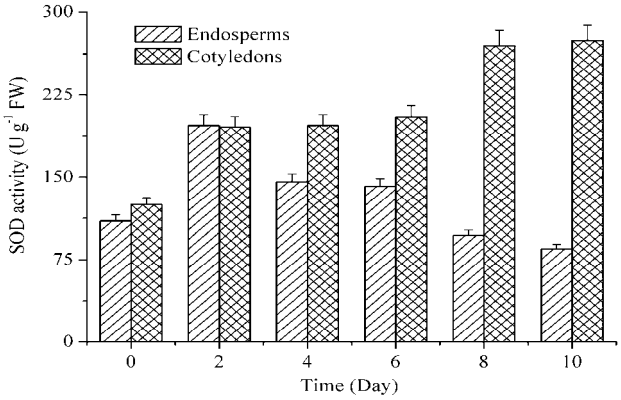
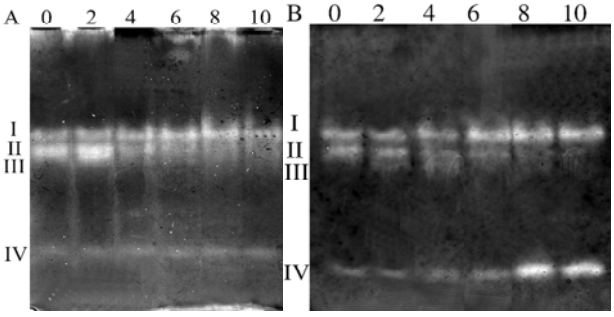


Fig. 3: Native PAGE for SOD isoenzymes in endosperms and cotyledons during germination. A: endosperms; B: cotyledons. About 25 µL extract from each sample was loaded



The changes of CAT activity in endosperms and cotyledons during germination are showed in Fig. 6. CAT activity in endosperms increased gradually during 6 days of germination, and the highest observed activity was about

1.83 times that of the control. Similarly, CAT activity in cotyledons increased gradually during 8 days of germination, the highest activity was 5.08 times that of control. However, CAT activity decreased significantly at 10 day of the germination, being 3.73 times that of the control. Only one CAT isoenzyme band in endosperms and cotyledons was observed during germination. The intensities of CAT isoenzyme bands increased gradually during 6 and 8 days of germination. Moreover, the staining intensities of these bands in cotyledons are considerably higher than that in endosperms, especially in the later stage of development.

DISCUSSION

It is well known that ROS induced lipid peroxidation of membranes is a reflection of stress induced damage at the cellular level. The change in MDA contents, especially in oil rich seeds, is often used as an indicator of oxidative damage (Sung, 1996). In the present study, oxidative damage to tissue lipid was estimated by MDA content. Increased MDA contents in endosperms and cotyledons during seed germination suggest that lipid peroxidation increases during germination process. In this study, we noted that MDA content increased in parallel with the increase in activities of SOD, POD and CAT (Figs. 2, 4 & 6). Elevated MDA contents mediated by free radicals and peroxides are considered to be one of the likely explanations for lipid peroxidation during germination (Schopfer *et al*., 2001). It is generally recognized that plants can protect themselves by inhibiting lipid peroxidation by activated antioxidant enzymes (Bailly *et al*., 1996; Bailly, 2004). Our findings indicate that lipid peroxidation occurred during seed germination and early seedlings growth.

The control of steady-state ROS levels by SOD is an important protective mechanism against cellular oxidative damage, since $O_2^{\bullet-}$ acts as a precursor of more cytotoxic or highly relative ROS (Mittler *et al*., 2004). SOD has been established to work in collaboration with POD and CAT which act in tandem to remove $O_2^{\bullet-}$ and H_2O_2, respectively (Blokhina *et al*., 2003). Early reports showed that increased SOD activities and cellular ROS levels are involved in many life of plant including developmental course such as seed germination (Rogozhin *et al*., 2001; Dučić *et al*., 2003; Wojtyla *et al*., 2006). SOD activity in the degrading endosperms was higher than that of the control except for at 10 day of germination, and the activity in the developing cotyledons increased progressively (Fig. 2). Therefore, enhanced SOD activity can be triggered by increased production of ROS or it might be a protective measure adopted by *J. curcas* plants against oxidative damage. Moreover, the changes of SOD activity in the degrading endosperms and developing cotyledons are correlated to those of POD and CAT activities (Figs. 4 & 5). Our findings are also in agreement with previous reports suggesting the participation of SOD in the defense mechanism during germination and early seedlings development (Dučić *et al*.,

Fig. 4: Changes of peroxidase (POD) activities in endosperms and cotyledons during germination. The values and standard errors (vertical bars) of three replicates are shown

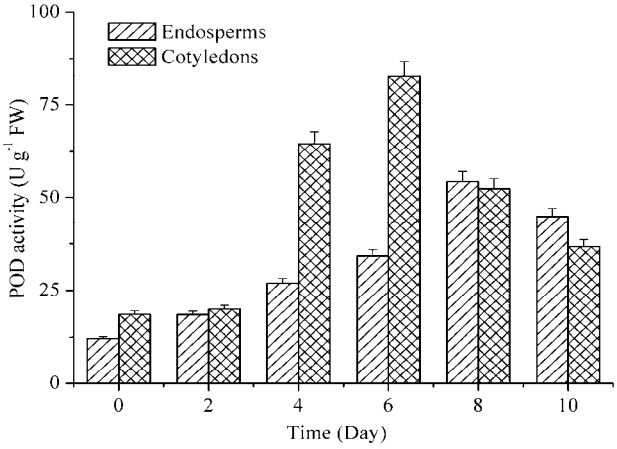
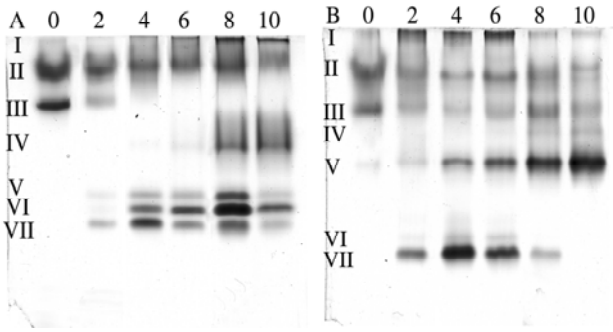


Fig. 5: Native PAGE for peroxidase (POD) isoenzymes in endosperms and cotyledons during germination. A: endosperms; B: cotyledons. About 10 µL extract from each sample was loaded



2003; Wojtyla *et al*., 2006). SODs are generally organized into multi-gene families. Multiple SOD isoenzymes reported for some plant species are differentially expressed in different organs and at distinct developmental and physiological conditions (Blokhina *et al*., 2003; Mittler *et al*., 2004). Thus, the changes in SOD isoenzyme patterns reflect a complex defense against oxidative stress (Blokhina *et al*., 2003; Mylona *et al*., 2007). New SOD isoenzymes are synthesized at the early stage of germination, and the intensities of two SOD isoenzyme bands (I & IV) in the developing cotyledons show a progressive increase by the germination process (Figs. 3A & B). The increase in SOD isoenzyme activity might be an important mechanism for avoiding the oxidative stress during seed germination and early seedling development. These results suggest that they carry specific functions and contribute to its unique properties during germination and early seedlings development. Our findings could be also used as a basis for elucidating the mechanisms by which SOD transcripts are induced during germination process.

Fig. 6: Changes of catalase (CAT) activities in endosperms and cotyledons during germination. The values and standard errors (vertical bars) of three replicates are shown

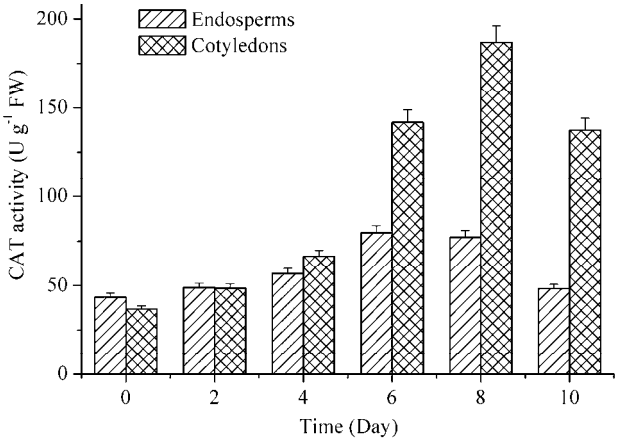
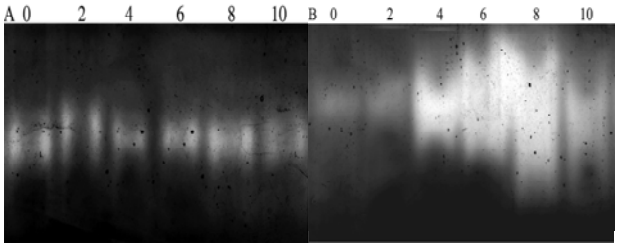


Fig. 7: Native PAGE for CAT isoenzymes in endosperms and cotyledons during germination. A: endosperms; B: cotyledons



In plants, POD is considered to be associated with a number of essential metabolic processes, such as cell elongation, lignification, phenolic oxidation, pathogen defense and defense against stress (Passardi *et al*., 2005). Moreover, PODs probably play important roles in seed germination, growth, morphogenesis, and even in the final stage of senescence and death (Kawano *et al*., 2003). The present results indicate that POD activity in the degrading endosperms and developing cotyledons are considerably greater than those of the control (Figs. 4 & 5). Changes in POD activities occur during developmental process in tissue specific manner and differential regulation in response to germination process and plant species has been reported (Omidiji *et al*., 2003; Dučić *et al*., 2003; Wojtyla *et al*., 2006). Thus, increased POD activity might be involved in the defense system during seed germination and early seedlings development. Plant PODs are generally organized into multi-gene families. Multiple isoenzymes of POD reported for some plants were detected in the same tissue at different developmental stages (Duroux & Welinder, 2003). Analysis by electrophoesis show that the presence of different POD isoenzymes with distinct activities in different tissues of *J. curcas* during germination process. For instance, there were at least seven POD isoenzymes in the degrading endosperms and developing cotyledons, but different patterns are observed (Figs. 5A & B). Our findings indicate that the expression of certain POD isoenzymes may be regulated and influenced during seed germination and early seedlings development. Thus, patterns of isoenzymes here show a complex regulation of steady-state POD activities at a temporal and spatial level, as well as provide evidence for the developmentally programmed turnover of POD. Moreover, the intensities of POD isoenzyme bands in the developing endosperms and developing cotyledons are well correlated with the changes as assayed in solution (Figs. 4 & 5). These findings suggest that isoenzyme may play different roles and be involved in the defense mechanism of plant tissues against oxidative damage during seed germination and early seedlings development.

CAT plays an integral role in the removal of ROS produced under various stress conditions and then for the avoidance of oxidatant damage. CAT and POD, are often considered to keep H_2O_2 balance in plant tissues (Blokhina *et al*., 2003). In oily seeds, CAT is particularly important in the early events of seedling growth, because it removes H_2O_2 produced during β-oxidation of the fatty acids (Bailly, 2004). In the present study, CAT activity was also examined, and a trend similar to that observed for POD activity was recorded (Figs. 4 & 6). Increased CAT activity could be an indication of the cellular evaluated ROS, since the amount of CAT present in aerobic cells is directly proportional to the oxidative state of the cells (Apel & Hirt, 2004). The induction of CAT expression has been studied intensively during seed germination and post-germination seedling growth in maize and sunflower, and displays a complex regulation mechanism (Bailly *et al*., 1996; Mylona *et al*., 2007). The present results lend further support to those findings. CAT is presented as multiple isoenzymes encoded by a small gene family in many plants (Willekens *et al*., 1995). However, our results indicate only one CAT isoenzyme band in the degrading endosperms and developing cotyledons (Figs. 7A & B). Its intensity varies during germination and is consistent with the changes of the activities assayed in solutions. Taken together these data indicate that increased CAT activity is probably involved in the defense mechanism of *J. curcas* plant against oxidative stress during germination.

In conclusion, the present findings suggest that the changes of MDA content and antioxidant enzymes activity in the degrading endosperms and developing cotyledons of *J. curcas* observed appear to be more closely related to germination process and plant tissues. Changes in antioxidant enzymes activity might be regulated by different responses of SOD, POD and CAT, not only by changing enzyme activities, but also by alteration in isoenzyme patterns. The timing of appearance or disappearance of different POD, SOD and CAT isoenzymes suggests that some of them could play a key role during seed germination. These different responses may be attributed to differences in gene expression and protein function in

different germination stages and plant tissues. Thus, our findings strongly support the hypothesis that POD, SOD and CAT activities are up-regulated as an antioxidant defense system against endogenous oxidant radicals generated during seed germination. The whole biological consequences of these alterations, in particular, low molecular mass antioxidants as well as altered antioxidant defense mechanisms during seed germination, are unclear and should be further investigated.

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REFERENCES

Apel, K. and H. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 55: 373–399

Bailly, C., 2004. Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.*, 14: 93–107

Bailly, C., A. Benamar, F. Corbineau and D. Dome, 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seed as related to deterioration during accelerated aging. *Physiol. Plant.*, 97: 104–110

Bailly, C., R. Bogatek-Leszczynska, D. Come and F. Corbineau, 2002. Changes in activities of antioxidant enzymes and lipoxygenase during growth of sunflower seedlings from seeds of different vigour. *Seed Sci. Res.*, 12: 47–55

Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, 44: 276–287

Blokhina, O., E. Virolainen and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.*, 91: 179–194

Chen, C.N. and S.M. Pan, 1996. Assay of superoxide dismutase activity by combining electrophoresis and densitometry. *Bot. Bull. Acad. Sin.*, 37: 107–111

Dučić, T., I. Lirić-rajlić, A. Mitrović and K. Radotić, 2003. Activities of antioxidant systems during germination of *Chenopodium rubrum* seeds. *Biol. Plant*, 47: 527–533

Duroux, L. and K.G. Welinder, 2003. The peroxidase gene family in plants: a phylogenetic overview. *J. Mol. Evol.*, 57: 397–407

Gapper, C. and L. Dolan, 2006. Control of plant development by reactive oxygen species. *Plant Physiol.*, 141: 341–345

Gao, S., R. Yan, M. Cao, W. Yang, S. Wang and F. Chen, 2008. Effects of copper on growth, antioxidant enzymes and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedling. *Plant Soil Environ.*, 54: 117–122

Gao, S., C. Ou-yang, L. Tang, J. Zhu, Y. Xu, S. Wang and F. Chen, 2010. Growth and antioxidant responses in *Jatropha curcas* seedling exposed to mercury toxicity. *J. Hazard. Mater.*, 182: 591–597

Goel, A. and L.S. Sheoran, 2003. Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. *Biol. Plant*, 46: 429–434

Kawano, T., 2003. Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. *Plant Cell Rep.*, 21: 829–837

Kumar, A. and S. Sharma, 2008. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): a review. *Ind. Crop Prod.*, 28: 1–10

Mittler, R., S. Vanderauwera, M. Gollery and F. Van Breusegem, 2004. Reactive oxygen gene network of plants. *Trends Plant Sci.*, 9: 490–498

Mylona, P.V., A.N. Polidoros and J.G. Scandalios, 2007. Antioxidant gene responses to ROS-generating xenobiotics in developing and germinated scutella of maize. *J. Exp. Bot.*, 58: 1301–1312

Omidiji, O., J. Okpuzor and O. Otubu, 2003. Peroxidase activity of germinating *Sorghum bicolor* grains: effect of some cations. *J. Sci. Food Agric.*, 82: 1881–1885

Passardi, F., C. Cosio, C. Penel and C. Dunand, 2005. Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep.*, 24: 255–265

Philippe, M., R.K. Koraljka and B. Karlheinz, 2007. A simple method to measure effective catalase activities: optimization, validation, and application in green coffee. *Anal. Biochem.*, 360: 207–215

Rogozhin, V.V., V.V. Verkhoturov and T.T. Kurilyuk, 2001. The antioxidant system of wheat seeds during germination. *Biol. Bull.*, 28: 126–133

Ros Barcelo, A., 1987. Quantification of lupin peroxidase isoenzymes by densitometry. *Ann. Biol.*, 14: 33–38

Sakharov, I.Y. and G.B. Ardila, 1999. Variation of peroxidase activity in cacao beans during their ripening, fermentation and drying. *Food Chem.*, 65: 51–54

Schopfer, P., C. Plachy and G. Frahry, 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide & hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol.*, 125: 1591–1602

Sung, J.M., 1996. Lipid peroxidation and peroxide-scavenging in soybean seeds during aging. *Physiol. Plant*, 97: 85–89

Willekens, H., D. Inze, M. Van Montagu and W. Van Camp, 1995. Catalases in plants. *Mol Breed.*, 1: 207–228

Wojtyla, Ł., M. Garnczarska, T. Zalewski, W. Bednarski, L. Ratajczak and S. Jurga, 2006. A comparative study of water distribution, free radical production and activation of antioxidative metabolism in germinating pea seeds. *J. Plant Physiol*., 163: 1207–1220

Woodbury, W., A.K. Spencer and M.A. Stahmann, 1971. An improved procedure using ferricyanide for detecting catalase isozymes. *Anal. Biochem.*, 44: 301–305

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