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Full Length Article

Drought Stress Induced the Expression Level of Ascorbate Peroxidase in the Late Seedlings of Melinjo (*Gnetum gnemon*)

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Abstract

Melinjo is the family of Gnetaceae that contains high antioxidant compound withthe potentialsof being medicinal plant useful for human health. In plant, antioxidant functions as defense system against reactive oxygen species (ROS) highly accumulated by unfavorable environment such as drought stress. Ascorbate peroxidase (APX) is one of the antioxidant enzymes needed for defense system against ROS. In this study, drought stress was stimulated by polyethylene glycol (PEG) in various concentrations (0 to 15%). The results showed drought stress decreased several morphological characters and chlorophyll content of Melinjo seedlings. On the contrary, drought stress increased the level of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and the expression of APX. The H_2O_2 was increased from 40.31 µmol/g FW to 87.24 µmol/g FW at 15% PEG. While MDA level was increased from18.55 nmol/g FW to 38.20 nmol/g FW at 15% PEG. The expression of APX in the RNA level and the enzyme activity of 15% PEG were increased 2 folds higher than the control. Thus, APX can be infered to be an inducible agent as the key enzyme which plays a pivotal role in the defense mechanism of Melinjo seedlings against drought stress.

Keywords: Melinjo; Antioxidant; Acorbate peroxidase; Drought stress

Introduction

Drought is one primary unfavorable condition which influences most crops in the world (Dorota *et al*., 2016). Plant responses drought stress through high reactive accumulation of oxygen species (ROS) such as superoxide anion (O^{2-}), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) (Caverzan *et al.*, 2012; Sharma *et al*., 2012). It causes an oxidative damage at the cellular level (Yoshimura *et al*., 2000) that disrupts protein, lipid, and fatty acid (Jiang *et al.*, 2010). Furthermore, the damage may cause cell death and suppress the plant growth and development that eventually impacts reduction of crop production. There are many mechanisms in plant defense against ROS one of which is through antioxidant system. The antioxidant is used to avoid a large amount of ROS by preventing the cellular damage and keep the cellular metabolism in balance (Racchi, 2013).

Recently, researchers have discovered the antioxidant compoundsof the medicinal plant for pharmaceutical and nutraceutical purpose namely Melinjo (*Gnetum gnemon*). Melinjo seed extract contains reserveratol which contains antibacterial and antioxidant activity (Hisada *et al*., 2005). The ethanol extract of Melinjo seed has known contain stilbenoids which have 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, one is gnemonoside A which has antibacterial activity to depress the multiplication of enterobacteria to prevent it from making negative impacts on human health (Kato *et al*., 2009). The seed extract of Melinjo also contains active gnetin C that possess an antitumor activity in human and murine tumor cell (Narayanan *et al.*, 2011).

Siswoyo *et al*. (2011) suggested that 9–10% protein in the Melinjo seed has high antioxidants which will likely actas the scavenging agent against free radical. Siswoyo *et al.* (2017) recently reported that the fermented flour extract from Melinjo seeds increased the capability to resist the oxidative damage. Terangpi *et al.* (2013) also reported this tree has potentials to be multiple benefits-food supply. Melinjo is the family of Gnetaceae closely related to *Ginkgo biloba*, as the ancient tree. It is widely spread in Southeast Asia and Melanesia as an important Agroforestryspecies which might betolerant in various unfavorable conditions including several months of drought stress (Manner and Craig, 2006).

Many antioxidants play a pivotal role in hindering the oxidative damage caused by unfavorable conditions one of which is ascorbate peroxidase (APX). The expression of APX may be activated by various factors one of which is water deficit (Dabrowska *et al.*, 2007). It acts as the key enzyme to catalyze the reaction of H_2O_2 into H_2O (Correa-Aragunde *et al*., 2013). According to Tarchoune *et al.*

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(2010), APX used 2 molecules of ascorbateto reduce H_2O_2 into the water in a variety of cellular compartments.

This study was aimed at evaluating whether or not drought stress was able to trigger the expression of APX in Melinjo seedling. Its expression may deal with the capability for scavenged ROS accumulated by drought stress. Many researches have been conducted dealing with the use of antioxidant for Melinjo medicinal purposes, but its role as the defense mechanism under drought stress has not yet been explored.The study on the expression of antioxidant and its capacity to scavenging ROS accumulation not only enables to figured out the role of APX in alleviating the ROS accumulated by drought stress, but also show the potential use as germplasm enhancement of Melinjo.

Materials and Methods

Plant Preparation and Drought Stress Treatment

This study was carried out using 11-month melinjo (*Gnetum gnemon L.*, *Gnataceae*) seedlings, which taken from the collection of Laboratory of Plant Analysis, University of Jember, East Java, Indonesia. The seedlings were transplanted in polybag containing soil, compost, and sand (1:1:1). The plants were watered every three days in field capacity for each polybag. The experiment consisted of four treatments such as 0% PEG (control), 5% PEG (5 g/100 mL H_2O), 10% PEG (10 g/100 mL H_2O), and 15% PEG (15 g/100 mL). Prior to treatment with PEG, all seedlings have been adapted for 1 month after transplantation. Five replications were provided for each treatments and designed in a random block for all treatments. The leaf samples and the data of morphological characters were taken after6 weeks of treatment.

Determination of Chlorophyll Content

The chlorophyll content was determined by Lichtenthaler and Wellburn (1983). The leaf samples (0.05 g) were homogenized in 80% acetone and centrifuged at 6000 rpm for 10 min at 4°C. The remaining pellet was added by 80% acetone and re-centrifuged at 6000 rpm for 10 min at 4°C. The supernatant after centrifugation was collected and added with 80% acetone up to 20 mL. The absorbance was measured at 646 nm and 663 nm.

The Determination of Oxidative Damage and Reactive Oxygen Species Accumulation

The oxidative damage was assayed according to malondialdehyde (MDA) content in the leaves, determined according to Cakmak and Harst (1991). Briefly, 0.2 g fresh leaf homogenized in 5 mL of 0.1% trichloroacetic acid (TCA), centrifuged at 4°C, 13000 rpm for 5 min. The supernatant was used for assay by the reaction of 1 mL of supernatant and 4 mL of 0.5% thiobarbituric acid that dissolved in 20% TCA. The mixture was incubated at 90°C for 30 min and the absorbance was measured at 532 nm and 600 nm. The MDA content was determined by the molar extinction of 155 m*M* cm^{-1}.

The reactive oxygen species (ROS) were estimated by the level of hydrogen peroxide (H_2O_2) in the leaves by using Christou *et al.* (2014) method. The H_2O_2 content was determined based on the standard calibration curve using the same solution without additional samples.

Sample of Extraction and Ascorbate Peroxidase Activity Assay

The extraction was carried out using 0.5 g leaf samples that ground in 0.1 *M* phosphate buffer containing 0.5 m*M* EDTA, then centrifuged at 4°C for 15 min at 10.000 rpm. The APX activity was assayed in 3 mL total reaction volume containing 50 µL of enzyme extract, 50 m*M* phosphate buffer (pH 7.0), 30% H_2O_2, and 5 m*M* ascorbic acid (Nakano and Asada, 1981). The activity was calculated by molar extinction 2.8 mM^{-1} cm^{-1} based on the oxidation of ascorbate at 290 nm for 2 min.

Total RNA Isolation and cDNA Synthesis

The RNA was isolated from 0.1 g leaf samples, following the protocols from total RNA plant isolation kit (NucleoSpin® RNA Plant, Machenery-Nagel). Total elution was 40 µL and the concentration of RNA was measured at 260 nm and 280 nm using nanodrop spectrophotometer (GE Nanovue Plus). For the cDNA synthesis, 900 ng RNA was dissolved in 20 µL RT-Premix (Bioneer Hyper Script RT) and used as template for polymerase chain reaction. Polymerase chain reaction (PCR) condition was conducted at 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and 72°C for 7 min as final extension. The primer used for PCR was *APX2* with the sequence were CCATGGTGAAGAAGAGTTACCCGGAAGT (forward) and TCTGAGATTACTCCTTGTCAGCAAACCCGA (reverse). The primer used for reference gene was *β-tubulin*, ATCGATTCCGTTCTCGATGT (forward) and ATCCAGTTCCTCCTCCCAAC (reverse). PCR product was visualized in 1.5% agarose gel. To evaluate the different of the expression level for each sample, the thickness of DNA band was quantified, using Image J software (Java 1.6.0_20).

Statistical Analysis

The experimental results was subjected to identify the significant difference by using analysis of variance significant difference was continued using Duncan multiple range test at $P \leq 0.05$ using SPSS statistic software (version 17.0).

Results

Effect of Drought Stress on Morphological Characters

Drought stress decreased several morphological characters of Melinjo seedlings (Table 1). Drought stress induced by 5 to 15% PEG significantly decreased the fresh weight, leaf number, leaf area, and shoot diameter, but not significantly effected on the shoot length. The fresh weight was statistically different between control and drought stress treatment, it was 28.4% below the control. For the leaf area and number of leaf, they were decreased up to 38.09 and 36.05% below the control, which found at 15% PEG. The increasing concentration of PEG was statistically different affected on the leaf area. While the shoot diameter was decreased in the 10 to 15% PEG treatment, it was 6.9% below the control. Drought stress induced with 15% PEG caused the lowest value of fresh weight, leaf area, number of leaf, and shoot diameter. The increasing concentration of 5 to 15% PEG was not statistically different for fresh weight and number of leaf. The morphological differences among each treatment were showed at Fig. 1. There were revealed that drought stress induced by PEG inhibited the growth of Melinjo seedlings (Fig. 1a) and the leaf area which also followed with the yellowing leaves (Fig. 1b). The yellowing leaves indicated the lack of chlorophyll that called chlorosis.

Total Chlorophyll Content on Melinjo Exposed with Drought Stress

The effect of drought stress on chlorophyll content was showed in Table 2. It revealed that drought stress was significantly decreased the chlorophyll *a*, chlorophyll *b*, and total chlorophyll content. The chlorophyll *a* decreased 22.88, 39.41; and 33.90% for 5, 10 and 15% PEG below the control respectively. The chlorophyll *b* in drought stress treatment decreased up to 50.4% below the control in 15% PEG treatment. While the total chlorophyll decreased up to 42.36% below the control. The increasing concentration of PEG from 5 to 15% did not affect the decrease of chlorophyll content significantly.

Malondialdehyde and Reactive Oxygen Species Level in Response to Drought Stress

Drought stress increased the level of malondialdehyde and reactive oxygen species (Fig. 2). The content of malondialdehyde (MDA) showed the high amount of lipid peroxidation in leaf. There was statistically different among each treatment, the MDA content increased by 29.27, 57.36, and 104.91% for 5, 10, and 15% PEG compared to the control. While ROS accumulation was shown in the level of hydrogen peroxide (H_2O_2) in leaf. It was statistically different, between control and drought stress treatment or among the increasing level of PEG concentration. The highest accumulation of H_2O_2 was found at 15% PEG treatment, it was 116.42% above the control. While the treatment of 5 and 10% PEG was increased the H_2O_2 were 33.27 and 103.69% above the control.

Expression of Ascorbate Peroxidase in Response to Drought Stress

Drought stress induced the expression of ascorbate peroxidase that estimated based on the transcript level and post translation level. The transcript level was determined according to the band area of cDNA as the result of reverse transcription of RNA that isolated from Melinjo leaf for each treatment. While in the post translation level was estimated by the enzyme activity of APX. The expression of ascorbate peroxides in RNA level was estimated based on the band area of the cDNA that visualized in 1.5% agarose gel (Fig. 3**)**. Based on the band area, there was significant difference between the control and PEG treatment 5, 10, and 15% PEG treatment it was 20.98, 40.22, and 92.87% above the control. The band area may estimate the expression level of the gene. This result revealed that drought stress by PEG up to 15% was able to induce the expression level of APX. Tubulin was used for the gene reference to show that the increasing level of APX expression was only caused by PEG.

The activity of APX showed the similar result as its expression on the RNA level. The activity of APX increased in drought stress treatment induced by PEG. The increasing concentration up to 15% PEG was able to induce the activity of APX. There was a significant difference between control and PEG treatment 5 to 15%. The activity of APX was extremely increased in 15% PEG, it was 77.08% above the control (Fig. 4). While the concentration of 5 and 10% PEG might increase the activity 27.08% and 37.5% above the control.

Discussion

Drought stress is one major environmental stress that affected mostly in crop production over the worldwide. The crop plants for instance fruits, vegetables, and grains have an economical value for human being which the production rate is influenced by environments (Basu *et al*., 2016). Drought stress induced by polyethylene glycol caused the reduction of fresh weight, leaf area, shoot diameter, number of leaf, and chlorophyll content of Melinjo seedlings used in this experiment. On the contrary, drought stress caused the increasing level of MDA and H_2O_2 content, also the expression and enzyme activity of APX. Agele (2003) reported that the drought stress reduced the shoot length, shoot diameter, leaf area of sunflower during vegetative stage. Drought stress impact the loss of turgor in leaf (Farooq *et al*., 2010) and the decrease of the fresh biomass production (Farooq *et al*., 2009). The turgor pressure in leaf plays a pivotal role in cell expansion and cell enlargement (Tardieu *et al*., 2011). It can correlate with the reduction of the plant growth. Leaf area due to the leaf growth is important for

Table 1: Effect of PEG on morphological characters in Melinjo seedlings six weeks after treatment

PEG (%)	Shoot length (cm)	Fresh weight (g)	Leaf area (cm^2)	Number of leaf	Shoot diameter (cm)
0	76.5^{a}	79.19^{b}	45.23^{d}	40.00^{b}	0.72^{b}
5	$71.9\ 0^{a}$	58.73^{a}	40.00^{c}	27.25^{a}	0.70^{ab}
10	74.14^{a}	57.65^{a}	31.75^{b}	25.5^{a}	0.67^{a}
15	75.75^{a}	56.70^{a}	28.00^{a}	24.00^{a}	0.67^{a}

Values are means with letter by Duncan's multiple range test at $P \leq 0.05$, the same letters in a column are statistically non-significant

Table 2: Effect of PEG treatment on the Chlorophyll Content in Melinjo seedling

PEG (%)	Total Chlorophyll (mg/g FW)	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)
0	3.82^{b}	2.36^{b}	1.45^{b}
5	2.62^{a}	1.82^{ab}	0.80^{a}
10	2.20^{a}	1.43^{a}	0.76^{a}
15	2.28^{a}	1.56^{a}	0.72^{a}

Values are means with letter by Duncan's multiple range tests at $P \leq 0.05$, the same letters in a column are statistically non-significant

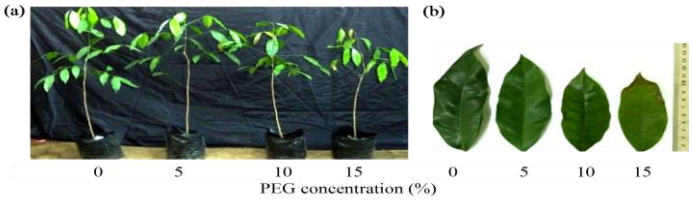

Fig. 1: Morphological character of Melinjo seedlings 6 weeks after treatment. (a) Melinjo seedlings after 6 weeks treatment, (b) Leaves area and leaves color after 6 weeks treatment

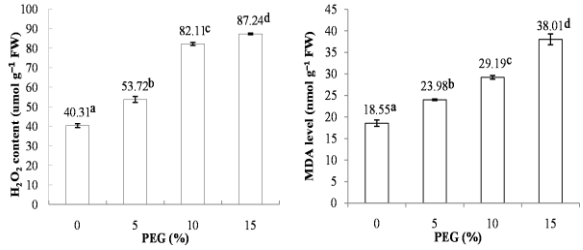

Fig. 2: H_2O_2 content and MDA content of Melinjo seedlings 6 weeks after treatment, 0 (control), 5, 10, and 15 % PEG. The values were followed with standard deviation. Values are means with letter by Duncan's multiple range test at $P \leq 0.05$, the same letter on each graph is statistically non-significant

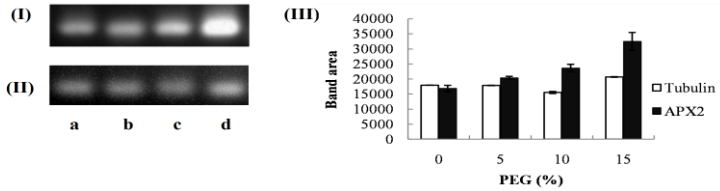

Fig. 3: The cDNA band visualization in 1.5 % agarose gel, using primer of *APX2*(I) and tubulin (II), band thickness quantification using image J software (III). a. 0 % PEG; b. 5 % PEG; c. 10 % PEG; d. 15 % PEG

photosynthesis metabolism. Water deficit may cause reduction in leaf growth in the plant (Farooq *et al.*, 2009). The reduction of leaf area impacts the decrease of the photosynthetic rate in line with the photosynthetic products. The photosynthetic rate was not only affected by the reduction of the leaf area, but also by the loss of the chlorophyll content visualized on the degradation of the green leaf color. Chlorophyll is the green component in the plant cell that crucially acts in the photosynthetic process by absorbing the light used for energy in carbohydrate synthesis

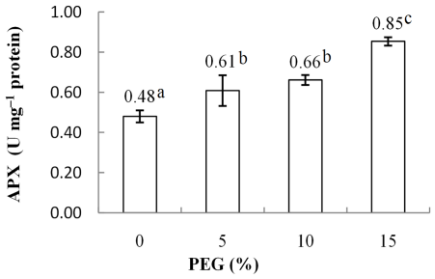

Fig. 4: APX activity in Melinjo seedlings 6 weeks after treatment, 0 (control), 5, 10, and 15% PEG. Values are means with standard deviation and letter by Duncan's multiple range test at $P \leq 0.05$, the same letter on each graph is statistically non-significant

from carbon dioxide and water (Khalegi *et al*., 2012). There are two major parts of the chlorophyll sensitively affected by drought stress they are chlorophyll *a* and chlorophyll *b*. The same result was found at sunflower exposed by drought stress that caused the reduction in the chlorophyll *a*, chlorophyll *b*, and total chlorophyll (Manivannan *et al*., 2007) and chickpea at the vegetative and flowering stages (Mafakheri *et al.,* 2010). The reduced amount of chlorophyll in leaf is related to the accumulation of ROS during drought stress, it leads to lipid peroxidation that followed with the degradation of chlorophyll (Kumar *et al.,* 2011).

Lipid peroxidation formed by the reaction of oxygen by taking the electron from the unsaturated lipid which contains reactive hydrogen atom in the plant cell. It can be detected by MDA level as a secondary product of the oxidation process in lipid, it often used as biomarker of lipid peroxidation. Mirzae *et al.* (2013) noted that the level of MDA was raised along with the higher concentration of PEG in canola (*Brassica napus* L.) SLM046 cultivar. The high amount of MDA content in leaf may show the cellular damage generated by ROS accumulation. The accumulation of ROS was indicated by the H_2O_2 content in leaf. It is most stable among the other ROS compounds and was able to reactively oxidize other molecules (Michelet *et al*., 2013). Based on the result, H_2O_2 content was increased during the increasing level of PEG concentration that induced the higher level of drought stress. The increasing amount of H_2O_2 showed the level of oxidative damage and complex signaling mechanism evolved in response to stress (Gunes *et al*., 2007). When the H_2O_2 is too highly accumulated in cell, it may cause the cellular damage. In the low level, H_2O_2 may play a pivotal role as the intracellular signal that mediated several cellular responses (Sharma *et al.,* 2012). On the other hand, it may trigger the higher activity of the antioxidant enzymes due to its role as a ROS precursor (Mittler, 2002).

The expression of APX was higher than the control, both in the RNA level and the enzyme activity. This suggested that the antioxidant enzyme mechanism was against ROS accumulation produced in drought stress treatment. Drought stress would induce the change of the gene expression in the nucleus by transcription and was continued by translation process (Buchanan *et al*., 2015). Ascorbate peroxidase is an antioxidant enzyme, which acts an important role in drought stress and its recovery system (Sofo *et al*., 2015) and in the reduction of H_2O_2 into H_2O in many cell compartments (Tarchoune *et al.,* 2010). It scavenges potential adverse of H_2O_2 from the chloroplasts and cytosol of the plant cells (Faize *et al.,* 2011). There are many genes which encode the APX as the antioxidant enzyme, one is *APX2*. The result was based on the RNA level shown in Fig. 4 estimating the expression level. While the enzyme activity was used to estimate the post translational level, as the expression product that directly takes a part in H_2O_2 scavenging system. The different expression level of *APX2* in Melinjo seedlings was estimated based on the band thickness using Image J software. The result was based on the band area from Image J software gives the similar trend with the qPCR result, as reported by Antiabong *et al.* (2016) who has analyzed the DNA concentration from *Fusarium necrophorum* using conventional PCR and qPCR and the result showed both have similar trends.

Drought stress induced by polyethylene glycol could trigger the expression of *APX2* in Melinjo seedlings. The concentration of 15% PEG showed the highest expression than the other. The same result was found in the rice, *OSAPX4* gene whichcodes the APX enzyme in rice leaf and root has increased when exposed to a biotic stress, including PEG 6000 (Guan *et al.,* 2010). This result was in correlation with the APX activity. The significant increase and highest activity was found in 15% PEG treatment. The same result was reported by Mirzae *et al.* (2013) in Hyola cultivar 308, that 10% and 15% PEG was significantly induced by the higher level of APX activity in shoots and roots. Enzymes that scavenge ROS must pretend in two roles, in the active and deactivated state. In active state, enzymes were activated by signaling components to keep ROS concentration at safe levels. While in deactivated state, the ROS concentrations reach the crucial level which causes the signaling components and unable to activate the enzymes (Michelet *et al*., 2013) to prevent the cellular damage caused by ROS. The result indicates that the concentration of PEG up to 15% was able to place the APX in the active state to keep ROS at normal levels. Onthe other hand, it may imply that the expression of APX in Melinjo seedlings was induced by drought stress.

Conclusion

Drought stress induced by 15% PEG triggered the highest expression of ascorbate peroxidase, both in RNA level and enzyme activity. It can be inferred that APX is an inducible agent that playsan important role in defense mechanism of Melinjo seedlings under drought stress. Onthe other hand, this study was able to be a model way to increase the antioxidant compound in plant.

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References

Agele, S.O., 2003. Sunflower responses to weather variations in rainy and dry, cropping seasons in a tropical rainforest zone. *Int. J. Biotronics*, 32: 17–33

Antiabong, J.F., G. Mafora and S.A. Adakol, 2016. Semi-quantitative digital analysis of polymerase chain reaction electrophoresis gel: potential applications in low-income veterinary laboratories. *Vet. World*, 9: 935–939

Basu, S., R. Venkategowda, K. Anuj and P. Andy, 2016. Plant adaptation to drought stress. *F1000 Res.*, 5: 1554–1564

Buchanan, B.B., G. Wilhelm and L.J. Russel, 2015. *Biochemistry and Molecular Biology of Plants*, 2nd Edition. Wiley Blackwell., New York, America

Cakmak, I. and J. Horst, 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*., 83: 463–468

Caverzan, A., P. Gisele, B.R. Silvia, W.R. Carolina, L. Fernanda and M.P. Marcia, 2012. Plant response to stresses: role of ascorbate peroxidase in the antioxidant protection. *Genet. Mol. Biol*., 35: 1–10

Christou, A., M. George and F. Vasileios, 2014. Systemic mitigation of salt stress by hydrogen peroxide and sodium nitroprusside in strawberry plants cia transcriptional regulation of enzymatic and non-enzymatic antioxidants. *Environ. Exp. Bot*., 107: 46–54

Correa-Aragunde, N., N. Foresi, M. Delladonne and L. Lamattina, 2013. Auxin induces redox regulation of ascorbate peroxidase 1 activity by s-nitrosylation/denitrosylation balance resulting in changes of root growth pattern of *Arabidopsis*. *J. Exp. Bot.*, 64: 3339–3349

Dabrowska,G., K. Alexandra, G. Anna, S.H. Magdalena and S. Edyta, 2007. Characteristics of the plant ascorbate peroxidase family. *Acta. Biol. Cracov. Bot*., 49: 7–17

Dorota, S.K., P. Jaroslaw, S.Z. Danuta, S. Jadwiga and M. Waldemar, 2016. The effect of drought stresas on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars. *Breed. Sci*., 66: 328–331

Faize, M., L. Burgos, L. Faize, A. Piqueras, E. Nicolas, G. Barba-Espin, M.J. Clemente-Moreno, R. Alcobendas, T. Artlip and J.A. Hernandez, 2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. *J. Exp. Bot*., 62: 2599–2613

Farooq, M., A. Wahid, N. Kobayashi, D. Fujta and S.M.A. Basra, 2009. Plant drought stress: effects, mechanisms, and management. *Agron. Sustain. Dev*., 29: 185–212

Farooq, M., A. Wahid, D.J. Lee, S.A. Cheema and T. Aziz, 2010. Drought stress: comparative time course action of the foliar applied glycinebetain, salicylic acid, nitrous oxide, brassinosteroids and spermine in improving drought resistance of rice. *J. Agron. Crop Sci*., 196: 336–345

Hisada, H., M. Asahara, Kato and F. Sakan, 2005. *Antibacterial and Antioxidative Constituents of Melinjo seeds and their Application to Foods*. Science Links Japan, Japan

Guan, Q., X. Dexi and L. Shenkui, 2010. OsAPX4 gene response to several environmental stresses in rice (*Oryza sativa* L.*). Afr. J. Biotechnol*., 9: 5908–5913

Gunes, A., A. Inal, M. Alpaslan, F. Eraslan, E.G. Bagci and N. Cicek, 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition inmaize (*Zea mays* L.) grown under salinity. *J. Plant Physiol*., 164: 728–736

Jiang, Y., W. Erie, L. Shuwei, Y. Xiaoqing and L. Na, 2010. Antioxidative response and candidate gene expression in *Prairie junegrass* under drought stress. *J. Amer. Soc. Hortic. Sci*., 135: 303–309

Kato, E., Y. Tokunaga and F. Sakan, 2009. Stilbenoids isolated from the seeds of Melinjo (*Gnetum gnemon*. L.) and their biological activity. *J. Agric. Food Chem*., 57: 2544–2549

Khalegi, E., K. Arzani, N. Moallemi and M. Barzegar, 2012. Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b, and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. *World Acad. Sci. Eng. Technol.*, 68: 1154–1157

Kumar, R.R., K. Krishna and G.R. Naik, 2011. Effect of polyethylene glycol induced water stress on physiological and biochemical response in pigeonpea (*Cajanus cajan* L. Millsp*). RRST*, 3: 148–152

Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extract in different solvents. *Biochem. Soc. Trans*., 11: 591–592

Mafakheri, A., A. Siosmardeh, B. Bahramnejad, P.C. Struik and Y. Sohrabi, 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Aust. J. Crop Sci*., 4: 680–585

Manivannan, P., J.C. Abdul, B. Sankar, A. Kishorekumar, R. Somasundaram, G.M.A. Lakshmanan and R. Panneerselvam, 2007. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. *Colloids Surf. B. Bioenterfaces*, 59: 141–149

Manner, H.I. and R.E. Craig, 2006. *Gnetum gnemon* L. (Gnetum). Species Profile for Pacific Island. *Agroforestry*, 1: 1–8

Michelet, L., T. Roach, B.B. Fischer, M. Bedhomme, S.D. Lemaire and A. Krieger-Liszkay, 2013. Down-regulation of catalase activity allows transient accumulation of a hydrogen peroxide signal in *Chlamydomonas reinhardtii*. *Plant Cell Environ*., 36: 1204–1213

Mirzae, M., A. Moieni and F. Ghanati, 2013. Effects of drought stress on lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. *J. Agric. Sci. Tech*., 15: 593–602

Mittler, 2002. Oxidative stress antioxidants and stress tolerance. *Trends Plant Sci*., 7: 405–410

Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiol*., 22: 867–880

Narayanan, K.N., K. Kazuhiro, Y. Yukia, M. Mari, M. Hideki, N. Kazuki, M. George, M. Upender, K.T. Amit and N. Bhagavati, 2011. Antitumor activity of melinjo (*Gnetum gnemon* L.) seed extract in human and murine tumor models in vitro and in a colon-26 tumor-bearing mouse model in vivo. *Cancer Med.*, 4: 1767–1780

Racchi, M.L., 2013. Antioxidant defense in plants with attention to *Prunnus* and *Citrus* Spp. *Antioxidants*, 2: 340–369

Sharma, P., B.J. Ambuj, S.D. Rama and P. Mohammad, 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Botany*, 10: 1–26

Siswoyo, T.A., M. Eka, K.O. Lee and H. Keizo, 2011. Isolation and characteristic of antioxidant protein fraction from Melinjo (*Gnetum gnemon*) seed. *J. Agric. Food Chem*., 59: 5648–5656

Siswoyo, T.A., A. Tri and H. Keizo, 2017. Fermentation-induced changes in antioxidant activities and oxidative DNA damage protection of Melinjo (*Gnetum gnemon*) flour. *J. Food Biochem*., 41: 4-e12382

Sofo, A., A. Scopa, M. Nuzzaci and A. Vitti, 2015. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int. J. Mol. Sci*., 16: 13561–13578

Tarchoune, I., C. Sgherri, R. Izza, M. Lachaal, Z. Ourghi and F. Navari, 2010. Antioxidative response of *Ocimum basilicum* to sodium chloride or sodium sulphate salinization. *Plant Physiol. Biochem*., 48: 772–777

Tardieu, Z., C. Granier and B. Muller, 2011. Water deficit and Growth. Co-ordinating processes without an orchestrator? *Curr. Opin. Plant Biol*., 14: 57–72

Terangpi, R., U. Engtipi and R. Teron, 2013. Utilization of less known plants, *Gnetum gnemon* L. *and Rhynchotechum ellipticum* (Dietr.) A.DC. among the Karbis, Northeast India. *J. Sci. Innovat. Res*., 2: 943–949

Yoshimura, K., Y. Yukinori, I. Takahiro and S. Shigeru, 2000. Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. *Plant Physiol*., 123: 223–233

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