Sains Malaysiana 45(7)(2016): 1035–1040

Antioxidative Responses of *Cocos nucifera* against Infestation by the Red Palm Weevil (RPW), *Rhynchophorus ferrugineus*, a New Invasive Coconut Pest in Malaysia

(Tindak Balas Antioksida oleh *Cocos nucifera* terhadap Serangan Kumbang Merah Palma (RPW), *Rhynchophorus ferrugineus*, Serangga Perosak Invasif Baharu Pokok Kelapa di Malaysia)

NORHAYATI, Y.*, WAHIZATUL AFZAN, A., SITI NOOR JANNAH, S. & NURUL WAHIDAH, M.R.

ABSTRACT

*Interaction between the Red Palm Weevil (RPW) and coconuts will stimulate the plants' early response by producing the reactive oxygen species (ROS) which causes lipid peroxidation and membrane damage of the host plants. Thus, a multiple defense lines, including both scavenging enzymes and molecular antioxidants have been evolved to promptly inactivate these radicals. This study investigated the responses of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), α-tocopherol, ascorbic acid and carotenoids contents of three coconut cultivars which were PANDAN, MAWA and MATAG against the RPW infestation. The infested PANDAN and MAWA exhibited higher CAT, POD and APX specific activities of 2.60 ± 0.11, 5.0 ± 0.72 and 1.58 ± 0.20 units/mg protein for PANDAN whereas 1.13 ± 0.04, 39.38 ± 2.29 and 1.15 ± 0.16 units/mg, protein for MAWA) compared with controls. However, MATAG cultivar showed no significant difference (*p<0.05*) in the production of both enzymes except for POD specific activities. RPW infestations only managed to trigger the α-tocopherol concentrations in the MAWA cultivar compared with the others. Infested MAWA and MATAG increased the ascorbic acid concentrations, however, a contrast results was observed in infested PANDAN. All coconut cultivars exhibited higher carotenoids content (2771.20 ± 263.90, 3043.20 ± 526.35 and 921.20 ± 281.10 µg/g.fwt of PANDAN, MAWA and MATAG, respectively) compared with their respective controls (455.20 ± 135.10, 1408.40 ± 103.02 and 248.80 ± 110.39 µg/g. fwt). The above results indicated that the oxidative stress induced by the RPW infestation would stimulate the activities of enzymes and molecular antioxidants studied especially in infested MAWA. Thus, it may be suggested that MAWA cultivar was more tolerance towards RPW infestation compared to MATAG and PANDAN. It is hoped that this finding will provide clues on how plant respond toward stress during infestation, thus further action can be activated as soon as possible to control the spread of* R. ferrugineus.

Keywords: Antioxidants; coconut; enzymatic antioxidants; non-enzymatic; oxidative stress; red palm weevil

ABSTRAK

*Interaksi antara Kumbang Merah Palma (RPW) dan pokok kelapa akan mencetuskan tindak balas awal tumbuhan dengan menghasilkan spesies oksigen reaktif (ROS) yang menyebabkan peroksidasi lipid dan kerosakan kepada membran pokok perumah. Sistem pertahanan tumbuhan antaranya enzim dan juga antioksidan telah bertindak balas untuk menyahaktifkan radikal ini. Kajian ini bertujuan mengkaji tindakbalas katalase, (CAT), peroksida (POD), askorbat peroksida (APX), α-tokoferol, asid askorbik dan kandungan karotenoid tiga kultivar kelapa (PANDAN, MAWA dan MATAG) terhadap serangan RPW. Pokok PANDAN dan MAWA yang diserang menunjukkan aktiviti spesifik enzim CAT, APX dan POD yang lebih tinggi iaitu 2.60 ± 0.11, 5.0 ± 0.72 dan 1.58 ± 0.20 unit/mg protein untuk PANDAN manakala 1.13 ± 0.04, 39.38 ± 2.29 dan 1.15 ± 0.16 unit/mg protein untuk MAWA berbanding dengan kawalan. Walau bagaimanapun, tiada perbezaan bererti (*p<0.05*) dalam penghasilan kedua-dua jenis enzim kecuali untuk aktiviti spesifik POD dalam kultivar MATAG. Serangan KPM hanya boleh mencetuskan kepekatan α-tokoferol di dalam kultivar MAWA berbanding dengan kultivar lain. Kandungan asid askorbik dalam kultivar MAWA dan MATAG yang diserang meningkat, walau bagaimanapun, keputusan yang sebaliknya didapati untuk kultivar PANDAN. Semua kultivar pokok kelapa yang telah diserang juga mengandungi kepekatan karotenoid yang lebih tinggi (2771.20 ± 263.90, 3043.20 ± 526.35 dan 921.20 ± 281.10 µg/g.fwt, masing-masing untuk PANDAN, MAWA dan MATAG) berbanding dengan kawalan (455.20 ± 135.10, 1408.40 ± 103.02 dan 248.80 ± 110.39 µg/g.fwt). Keputusan ini menunjukkan tegasan oksidatif yang terhasil daripada serangan KPM mencetuskan aktiviti enzim dan kandungan molekul antioksidan yang dikaji terutamanya dalam kultivar MAWA. Oleh itu, adalah dicadangkan bahawa kultivar MAWA adalah lebih rintang terhadap serangan RPW berbanding dengan kultivar MATAG dan PANDAN. Adalah diharapkan agar hasil kajian ini boleh memberikan gambaran bagaimana tindak balas tumbuhan terhadap serangan RPW, supaya tindakan seterusnya boleh diaktifkan sebaik-baik sahaja serangan berlaku untuk mengawal penyebaran* R. ferrugineus.

Kata kunci: Antioksida; antioksida enzim; bukan-enzim; kelapa; kumbang merah palma; tegasan oksidatif

INTRODUCTION

Coconut palm (*Cocos nucifera* L.) is one of the commercialized plantations in Malaysia. Coconut is the fourth important industrial crop after oil palm, rubber and paddy in terms of total planted area. Statistical data released by Malaysian Department of Agriculture showed that the total area of coconut plantation for the year 2010 was 110,000 ha. The largest area is in Selangor, followed by Johor, Sabah and Sarawak.

Coconut palm belongs to the family of Arecaceae and is widely planted in tropical regions for its versatility as different parts of a coconut tree can produce domestic items, foods and beverages (Chan & Elevitch 2006). The most common commercialized varieties in Malaysia are MAWA (Malayan Dwarf X West Afrcan Tall), MATAG (MYD/MRD X Tagnanan Tall) and Aromatic Dwarf (commonly called Kelapa Pandan).

The coconut plantation is now threatened with the most harmful palm pest, which is *Rhynchophorus ferrugineus* or commonly called the Red Palm Weevil (RPW). In Malaysia, the infestation of RPW was first detected by the Department of Agriculture in 58 localities in seven districts of Terengganu. These infestations brought huge losses to the local agricultural sector, since it caused severe damage to the coconut palm and the occurrence spread very rapidly (DOA 2011).

It was believed that the infestation by RPW could trigger an oxidative stress in coconut plant. Many researchers have reported similar response in cotton leaves during aphid herbivory (Gomez et al. 2004) and in soybean leaves during infestation by two-spotted spider mites (Hildebrand et al. 1986). These events lead to the overproduction of reactive oxygen species (ROS) and free radicals causing serious damage and dysfunction to the structure of organic molecules (Thirupathi et al. 2011). To adapt with this problem, plants were equipped with complex but effective defence mechanisms including enzymatic and non-enzymatic antioxidants. Among the enzymatic antioxidants were peroxidases (POD), glutathione reductases (GR) and catalases (CAT) whereas carotenoid, tocopherol and ascorbic acid were examples of non-enzymatic antioxidants (Mittler 2002). Therefore, the aim of this study was to determine the antioxidative responses of the coconut tree (MAWA, MATAG and PANDAN) against RPW infestation.

MATERIALS AND METHODS

SAMPLES COLLECTION

The cabbage part was selected as it is soft, succulent-structured tissue, which is craved by the RPW larvae. Both healthy and infested coconut cabbages (MAWA, MATAG and PANDAN) were obtained from a highly infested coconut plantation area in Rhu Tapai (5°30'49.88 N, 102°58'36.34 E) Setiu, Terengganu (Figures 1-4). Six replications of each coconut cabbages were put in sealed plastic bag and covered with crushed ices in the ice box during transportation.

ANTIOXIDANT ASSAYS

POD specific activity was assayed according to Agrawal and Patwanadhan (1993). A total of 0.15 g of cabbage was ground up with 1 mL of 100 mM phosphate buffer (pH7.0) in pre-chilled mortar and pestle at 0-4°C. The homogenate then was centrifuged at 10000 rpm (Eppendorf 5840R) at 4°C for 10 min. The reaction mixture consists of 3 mL of solution containing 1 mL 50 mM phosphate buffer (pH7.5), 1 mL 20 mM guaiacol, 1 mL 30 mM H_2O_2 and 100 µL enzyme extract. The changes in absorbance were monitored at 470 nm for 3 min using spectrophotometer (Shimadzu UV-1601). Specific activity for POD was expressed as µmoles of H_2O_2 consumed per minute per mg protein.

CAT specific activity was assayed following the method of Clairborne (1985). A total of 0.15 g cabbage was ground up with 1.0 mL of 50 mM phosphate buffer (pH7.4) and clean sand in prechilled mortar and pestle at 0-4°C. The mixture was centrifuged at 10000 rpm (Eppendorf 5840R) at 4°C for 10 min. The reaction mixture contained 3 mL of reaction buffer (19 mM H_2O_2 in 50 mM phosphate buffer, pH7.0) and 100 µL of enzyme extract was added. The rate of changes in absorbance of the reaction mixture was

FIGURE 1. Healthy coconut palm

FIGURE 2. Crown skirting symptoms appeared on attacked MAWA tree

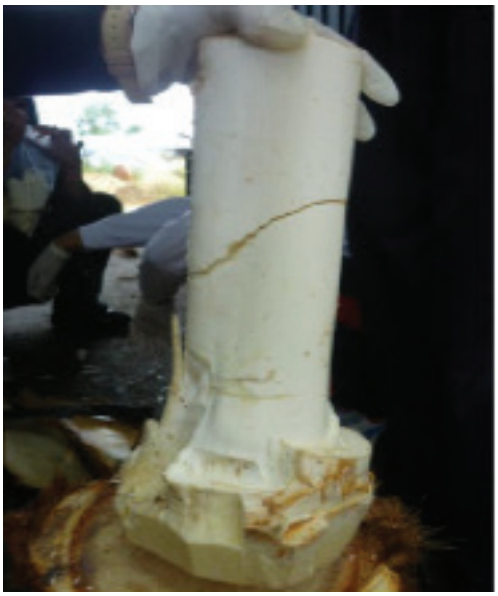

FIGURE 3. Healthy coconut cabbage

FIGURE 4. Coconut cabbage infested by RPW

monitored at 240 nm for 3 min using spectrophotometer (Shimadzu UV-1601). CAT specific activity was expressed in µmoles of H_2O_2 consumed per minutes per mg protein.

APX specific activity was assayed based on Nakano and Asada (1981) and Sairam et al. (1998). Cabbage tissue (0.15 g) was ground up with 1.0 mL of 100 mM phosphate buffer (pH7.0) containing 1 mM ascorbic acid in pre-chilled mortar and pestle at 0-4°C. The homogenate was centrifuge at 10000 rpm (Eppendorf 5840R) at 4°C for 10 min. The reaction mixture consists of 1.5 mL 100 mM phosphate buffer (pH7.0), 0.5 mL 3 mM ascorbic acid, 0.1 mL 3 mM EDTA, 0.4 mL enzyme extract, 0.3 mL distilled water and 0.2 mL 1.5 mM H_2O_2 to start the reaction. The changes in absorbance were monitored at 290 nm for 3 min using spectrophotometer (Shimadzu UV-1601) and the APX specific activity was expressed as µmol ascorbate oxidized per hour per mg protein.

The crude protein concentrations were determined using the method described by Bradford (1976). The Bradford reagent was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 mL 95% ethanol. Concentrated phosphoric acid (100 mL) was then added and the mixture was diluted until 1.0 L with distilled water. The solution was filtered through a filter paper and stored at room temperature in light-proof bottles. Enzyme extract (100 µL) was added to 3 mL of Bradford's reagent and the absorbance was measured at 595 nm after 10 min. The protein concentrations were calculated according to a standard curve prepared with various concentrations (0 to 2 mg/mL) of Bovine Serum Albumin (BSA).

α-Tocopherol was extracted based on the method by Hodges et al. (1996). Under dim light and over ice, 0.15 g of cabbage tissue was ground up with 1.5 mL acetone and clean sand in a mortar and pestle at 0-4°C. The mixture was extracted with 0.5 mL hexane followed by vortexing for about 30 s. The mixture was then centrifuged at 10000 rpm (Eppendorf 5840R) for 10 min. After the centrifugation, the top layer was removed and the hexane extraction was repeated twice. The assay mixture was prepared as described by Kanno and Yamauchi (1977). A total of 0.5 mL of the hexane-extract was added into 0.4 mL 0.1%(w/v) PDT (3-(2-pyridyl)-5,6-diphenyl-1,2,4 triazine, prepared in ethanol) and 0.4 mL 0.1% (w/v) ferric chloride (prepared in ethanol). The volume was made up to 3.0 mL with absolute ethanol and the mixture was gently swirled and left for 4 min for colour development. Following this, 0.2 mL of 0.2 M orthophosphoric acid was added to the mixture and allowed to stand for 30 min at room temperature before absorbance of the mixture was measured at 554 nm. The blank was prepared in the same manner except that absolute ethanol was used instead of the hexane-extracts. A standard curve was prepared using α-tocopherol (Sigma, type V) at various concentrations (0-1.4 µg/mL). The amounts of α-tocopherol in the samples were calculated based on the standard curve.

Ascorbate was extracted according to the procedure of Jagota and Dani (1982). A total of 0.15 g of cabbage sample was ground with pre-chilled mortar and pestle in 1.0 mL of 10% trichloroacetic acid (TCA) and clean sand under dim light and in ice-cold conditions. The ground sample was then centrifuged (Eppendorf 5840R) at 10000 rpm for 10 min at 4°C. The supernatant obtained (300 µL) was added into 1700 µL distilled water and 200 µL of 10% Folin reagent. The mixture was gently swirled and left on bench under dim light for 10 min. Absorbance of the mixture was then measured at 760 nm. A standard curve was prepared using ascorbic acid at various concentrations (0-60 µg/mL). Ascorbic acid (300 µL) was added into the solution as describe above and the amount of ascorbic acid in the sample was calculated based on the standard curve.

Carotenoid content was analyzed according to the method proposed by Lichtenthaler (1987). Fresh

cabbage tissue (0.15 g) was ground up with 3 mL of 80% (v/v) acetone and clean sand in a mortar and pestle. The homogenate was centrifuged at 10000 rpm for 10 min. Supernatant of the samples were measured spectrophotometrically at 663.2, 646.8 and 470 nm, while 80% acetone was used as a blank.

Statistical analysis was performed using statistical package SPSS window version 16.0. The comparisons between the antioxidants of six cabbages were done using one-way ANOVA analysis (Post Hoc Duncan and Bonferroni) at a significant level of $p<0.05$, while comparison of antioxidant activities between infested and control cabbages of a cultivar were analysed using independent sample t-test at $p<0.05$. Data was expressed as means ± standard error. The differences were considered significant at $p< 0.05$.

Results and Discussion

The level of antioxidative activities obtained in this study may be used as a tool in measuring the tolerance level of every cultivar. Many previous studies has compared the enzymatic antioxidant activities between tolerant and susceptible plants. El-Khallal (2007) suggested that there was significant difference in enzymatic antioxidant performance in resistant leaves of tomato plants after being infested by *Fusarium oxysporum*. Greater increase (more than 0.5 times compared to control) in the activity of antioxidant enzymes especially POD, APX and CAT might be affective in scavenging mechanism to remove hydrogen peroxide (H_2O_2) and superoxide radicals ($O_2^{\cdot-}$) produced in leaves under *F. oxysporum* infection. Other study on comparison of enzymatic antioxidant activities between resistant and susceptible buffalo grasses in response to Chinch Bug feeding also found that there was no stimulation of CAT activity in susceptible plants, while there was an increment of CAT activity in resistant plant (Tiffany et al. 2004).

In this study, both infected PANDAN and MAWA cabbages showed significantly higher POD, CAT and APX specific activities (Table 1). Induced POD, CAT and APX specific activities in infested PANDAN and MAWA were due to the initial attack on the shoot of the tree, enabling the whole cabbages to be severely damage. The infested cabbages might produce huge amount of reactive oxygen species (ROS), acting as signaling molecules to trigger the enzymatic antioxidants defence mechanisms specifically g-POD, CAT and APX. These antioxidants play important role in scavenging the excess ROS and limiting the oxidative damages caused by the RPW.

No changes in the APX and CAT specific activities of healthy and infested MATAG were also observed in this study (Table 1). This might be due to the condition of the infested MATAG tree. According to Abdullah Zawawi (pers. commun), most cases of RPW on MATAG tree start at the trunk base that damaged only this part while the cabbage part is not infested.

Interestingly, the cabbage of infected MATAG tree exhibited significantly higher POD specific activity compared with the healthy. It can be suggested that MATAG infested cabbage expressed only POD specific activities in scavenging the H_2O_2. The distance between the damaged site and the cabbage was only about six cm, thus excess production of H_2O_2 might be initiated at the cabbage, but still the level was not too high, which enabled only POD to start scavenging H_2O_2.

RPW attack on coconut palm caused severe wounds on plant cabbage that attract microorganisms such as bacteria and fungi depending on the natural opening and wound for invasion (Knogge 1996). This phenomenon might also increase the activities of POD, CAT and APX. Previous study by Zhang et al. (2009) reported that fungal infection on female poplar leaves stimulated the activities of POD, CAT and APX by 1.53, 1.47 and 2.06 times compared with control. These enzymes may act in several ways leading to the protection of plants, such as inhibiting the growth of pathogens inside the cells, accelerating the death of plant cells close to the infection site and alkylating proteins to reduce the bioavailability of such protein (Melo et al. 2006). Powdery mildew infection also caused enhancement in POD and CAT activities in flax leaves through its direct involvement in stopping pathogen development and provide its protection from oxidative damage by rapid removal of H_2O_2 during infection (Ashry & Mohamed 2012).

Highest CAT specific activities were produced by the infested PANDAN. Previous study supported that CAT is one of the enzyme that has the highest turnover number of

TABLE 1. CAT, POD and APX specific activities in the cabbages of healthy and infested coconut cultivars

Enzymatic antioxidants (units/mg protein)		Coconut cultivars		
		PANDAN	MAWA	MATAG
CAT	Infested	2.60 ± 0.11*	1.13 ± 0.04*	0.27 ± 0.10^{ns}
	Control	0.18 ± 0.03	0.57 ± 0.12	0.24 ± 0.10
POD	Infested	5.0 ± 0.72*	39.38 ± 2.29*	10.33 ± 0.42*
	Control	1.56 ± 0.15	9.74 ± 0.87	1.91 ± 0.10
APX	Infested	1.58 ± 0.20*	1.15 ± 0.16*	0.17 ± 0.03^{ns}
	Control	0.92 ± 0.14	0.16 ± 0.05	0.40 ± 0.07

Data are means ± S.E (n=5). *= significantly different and ns = not significantly different between healthy an infested coconut at $p<0.05$

TABLE 2. Amount of α-tocopherol, ascorbic acid and carotenoids in the cabbages of healthy and infested coconut cultivars

Non-enzymatic antioxidants (μg/g.fwt)		Coconut cultivars		
		PANDAN	MAWA	MATAG
α-Tocopherol	Infested	1.43 ± 0.04^{ns}	2.85 ± 0.31*	1.02 ± 0.18^{ns}
	Control	1.36 ± 0.10	0.85 ± 0.16	1.22 ± 0.06
Ascorbic acid	Infested	3.37 ± 1.37	15.71 ± 3.00*	22.49 ± 8.08*
	Control	22.43 ± 6.69*	4.66 ± 2.04	5.94 ± 1.09
Carotenoids	Infested	2771.20 ± 263.90*	3043.20 ± 526.35*	921.20 ± 281.10*
	Control	455.20 ± 135.10	1408.40 ± 103.02	248.80 ± 110.39

Data are means ± S.E (n=5). *= significantly different and ns = not significantly different between healthy and infested coconut at p<0.05

molecules. One molecule of CAT is able to convert 40 000 molecules of H_2O_2 into water and oxygen (Mittler 2002). However from the present study, the specific activity of CAT was lower (2.60 ± 0.11 units/mg protein) than POD (5.0 ± 0.72 units/mg protein). The low activity of CAT could be due to a very low concentration of H_2O_2. CAT has been proven to be inefficient in converting low concentrations of H_2O_2 (Zamocky et al. 2002). Both cabbages of infested PANDAN and MAWA tree exhibited about the same level of APX specific activities. APX was said to be one of the major ROS scavenger in plants. Lower APX specific activities obtained may results from low concentration of its substrate, ascorbate as indicated by Matthew (2002). APX is extremely sensitive to the ascorbate concentration, since it uses ascorbate as the electron donor to reduce H_2O_2 to H_2O (Thirupathi et al. 2011).

Among the three enzymatic antioxidants, POD specific activities were significantly higher in the infested MAWA cabbage. An enhancement in POD specific activity in this study suggested that this enzyme serves as intrinsic defense tool to resist H_2O_2 in coconut cabbage. Previous study on POD activity in induced fluoride stress on Mulberry leaves (Kumar et al. 2009) also showed similar results. In addition, wide distribution of PODs in plant tissue, due to their important role in many physiological processes may be the reason for the high POD activities (Thirupathi et al. 2011; Welinder 1992).

The α-tocopherol content in PANDAN and MATAG cultivars were almost similar in their healthy and infected cabbages. The infested MAWA cultivar produced higher (2.85 ± 0.31 μg/g.fwt) α-tocopherol as shown in Table 2. This was due to change of α-tocopherol level, which depends on the magnitude of the infestation and species sensitivity to RPW. Infested PANDAN cultivar exhibited lower ascorbic acid content (3.37 ± 1.37 μg/g.fwt) compared with control (22.43 ± 6.69 μg/g.fwt). However, elevated activities of other antioxidants studied like CAT, g-POD, APX specific activities as well as the carotenoids content proved that PANDAN have an alternative routes to detoxify the ROS, thus converting these ROS to stable molecules. All infested coconut cultivars produced higher carotenoids content compared to the healthy ones, thus indicating the activation of the mechanism to neutralize the potentially harmful singlet oxygen ($^1O_2$).

This study also indicated that antioxidants in the coconut cultivars may act together to reduce ROS level more effectively than single antioxidants, because they can function as synergists. In plant, tocopherols and carotenoids are the major lipid-soluble antioxidants, whereas ascorbate and glutathione are hydrosoluble antioxidants, therefore they have different action sites against ROS. However, a tight cooperation has been reported for these metabolites between their scavenging for oxygen radicals and their protection of cell metabolism (Munne-Bosch 2005; Trebst et al. 2002). Thus, an understanding of the interplay between these antioxidant molecules and the enzymatic ROS scavenging mechanism is crucial for identifying the key components involved in oxidative defense mechanism against RPW infestation.

In normal condition, there is equilibrium between antioxidative defense system and the destruction of ROS. Antioxidative defense system in plants has its own limits of capacity to respond to stress. In many stress situations, the enzymatic antioxidant activities will double, but the response might be moderate if the plant system is geared to destruction (Arora et al. 2002).

CONCLUSION

Enhanced POD, CAT and APX specific activities as well as α-tocopherol, ascorbic acid and carotenoids content in the infested cabbages indicated that these three cultivars have the ability to trigger defense mechanism towards the infestation of RPW. This study shows that all cultivars were tolerance towards RPW infestation especially MAWA. RPW infestation on coconut palm negatively altered normal physiological processes, which could threaten its survival. It is suggested that urgent actions to combat RPW occurrence have to be taken for the sake of the country agricultural stakeholders and farmers to prevent continuous economic losses. In addition, study on the effect of RPW attack on productivity and growth of coconut palm should also be carried out.

ACKNOWLEDGEMENTS

We thank Mr. Zazali Bin Chik and the colleagues from the Department of Agriculture Kuala Terengganu and the

Department of Agriculture Lundang, Kelantan for technical support. This work was funded by the Fundamental Research Grant Scheme, Vot: 59219.

REFERENCES

Agrawal, R. & Patwardhan, M.V. 1993. Production of peroxidase enzyme by callus cultures of *Citrus aurantifolia*. *Journal Science Food Agriculture* 61: 377-378.

Arora, A., Sairam, R.K. & Srivastava, G.C. 2002. Oxidative stress and antioxidative system in plants. *Current Science* 82(10): 1227-1238.

Ashry, N.N. & Mohamed, H.I. 2012. Impact of secondary metabolites and related enzymes in flax resistance and/ or susceptibility to powdery mildew. *African Journal of Biotechnology* 11(5): 1073-1077.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Chan, E. & Elevitch, C.R. 2006. *Cocos nucifera* (coconut). Profiles for Pacific Island agroforestry. http://www.agroforestry.net/tti/Cocos-coconut.pdf. Accessed on 30 January 2014.

Clairborne, A. 1985. Catalase activity. In *Handbook of Method for Oxygen Radical Research*, edited by Greenwald, E.A. Boca Raton: CRC Press. pp: 283-284.

Department of Agriculture (DOA). 2011. Report on current status of attack of Red Palm Weevil, *Rhynchophorus ferrugineus* in Terengganu. Putrajaya: DOA Press.

El-Khallal, S.M. 2007. Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 2-changes in the antioxidant enzymes, phenolic compounds and pathogen related- proteins. *Aust. J. Basic Appl. Sci.* 1(4): 717-732.

Gomez, S.K., Oosterhuis, D.M., Rajguru, S.N. & Johnson, D.R. 2004. Molecular biology and physiology foliar antioxidant enzyme responses in cotton after aphid herbivory. The *Journal of Cotton Science* 8: 99-104.

Hildebrand, D.F., Rodriguez, J.G., Brown, G.C., Luu, K.J. & Volden, C.S. 1986. Peroxidative responses of leaves in two soybean genotypes injured by two spotted spider mites. Acari: Tetranychidae. *Journal of Economic Entomology* 79: 1459-1465.

Hodges, D.M., Andrews, C.J., Johnson, D.A. & Hamilton, R.I. 1996. Antioxidant compound responses to chilling stress in differentially sensitive inbred maize lines. *Physiologia Plantarum* 98: 685-692.

Jagota, S.K. & Dani, H.M. 1982. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. *Analytical Biochemistry* 127: 178-182.

Kanno, C. & Yamauchi, K. 1977. Application of a new iron reagent, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine, to spectrophotometric determination of tocopherols. *Agricultural Biological Chemistry* 41(3): 593-596.

Knogge, W. 1996. Fungal infection of plants. *The Plant Cell* 8: 1711-1722.

Kumar, K.A., Varaprasad, P. & Rao, A.V.B. 2009. Effect of fluoride on catalase, guiacol peroxidase and ascorbate oxidase activities in two verities of Mulberry leaves (*Morus alba* L.). *Research Journal of Earth Sciences* 1(2): 69-73.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology,* edited by Packer, I. & Douce, R. Vol 148. New York: Academic Press. pp. 350-382.

Matthew, C. 2002. A comparison of the amount of the amount of catalase enzyme in different plant aand animal tissue. *Biochemistry* 21: 112-134.

Melo, G.A., Shimizu, M.M. & Mazzafera, P. 2006. Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochemistry* 67: 277-285.

Mittler, R. 2002. Oxidatiev stress antioxidants and stress tolerance. *Trends Plant Science* 7: 405-410.

Munne-Bosh, S. 2005. The role of α-tocopherol in plant stress tolerance. *Journal of Plant Physiology* 162: 743-748.

Nakano, Y. & Asada, K. 1981. Hydrogen peroxide scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* 22: 867-880.

Sairam, R.K., Deshmukh, P.S. & Saxena, D.C. 1998. Roles of antioxidant systems in wheat genotype tolerance to water stress. *Biologia Plantarum* 41: 387-394.

Tiffany, H.M., Sarath, G., Baxendale, F., Novak, D., Bose, S., Ni, X. & Quisenberry, S. 2004. Characterization of oxidative enzyme changes in Buffalograsses challenged by *Blissus occiduus*. *Journal of Economic Entomology* 97(3): 1086-1095.

Thirupathi, K., Jun-Cheol, M., Changsoo, K., Kumariah, M. & Wook, K. 2011. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science* 5(6): 709-725.

Trebst, A., Depka, B. & Hollander-Czytko, H. 2002. A specific role for tocopherol and of chemical singlet oxygen quenchers in the maintenance of photosystem II structure and function in *Chlamydomonas reinhardtii*. *FEBS Letters* 43: 2157-2162.

Welinder, K.G. 1992. Superfamily of plant, fungal and bacterial peroxidases. *Current Opinion Structural Biology* 2: 388-393.

Zamocky, M., Janecek, S. & Koller, F. 2002. Common phylogeny of catalase peroxidase and ascorbate peroxidase. *Gene* 256: 169-182.

Zhang, S., Lu, S., Xu, X., Korpelainen, H. & Li, H. 2009. Changes in antioxidant enzyme activities and isozyme profiles in leaves of male and female *Populus cathayana* infected with *Melampsora larici-populina*. *Tree Physiology* 30: 116-128.

School of Fundamental Science
Faculty of Marine and Environmental Sciences
Universiti Malaysia Terenganu
21030 Kuala Terengganu, Terengganu
Malaysia

*Corresponding author; email: yatiyusuf@umt.edu.my

Received: 2 July 2014
Accepted: 30 January 2016