A Biochemical Approach to Evaluate Heat Tolerant Rice

การใช้เทคนิคชีวเคมีในการประเมินข้าวทนร้อน

Tarinee Timabud (ธาริณี ทิมาบุตร)* Dr.Paweena Pongdontri (ดร.ปวีณา พงษ์ดนตรี)**

ABSTRACT

This study employed biochemical techniques to determine heat tolerant rice. Enzymatic activities of reactive oxygen species (ROS) scarvenging enzymes and their transcripts of heat tolerant standard rice seedlings, N22, and heat susceptible rice seedlings, IR64, were compared in given heat treatment in a climate chamber. By using those techniques, it suggested that KDML105 tended to be heat tolerant rice.

บทคัดย่อ

งานวิจัยนี้ใช้เทคนิคทางด้านชีวเคมีในการประเมินการทนร้อนของข้าว จากการเปรียบเทียบกิจกรรมและทรานสคริปต์ของเอนไซม์ที่ใช้ในการกำจัดออกซิเจนพลังงานสูงในต้นกล้าข้าวสายพันธุ์ทนร้อนและไม่ทนร้อนมาตรฐานเมื่อให้อยู่ในสภาวะร้อนในตู้ควบคุมสภาพภูมิอากาศ จากการใช้เทคนิคนี้ประเมินข้าวขาวดอกมะลิ 105 พบว่ามีแนวโน้มเป็นข้าวทนร้อน

Key Words: Biochemical technique, Oxidative stress, ROS-scavenging enzyme, Heat stress

คำสำคัญ: เทคนิคทางด้านชีวเคมี สภาพออกซิเดชันสูง เอนไซม์ที่ใช้ในการกำจัด ROS ความเครียดเนื่องจากความร้อน

** Student, Doctor of Philosophy Program in Biochemistry, Department of Biochemistry, Faculty of Science, Khon Kaen University*

*** Assistant Professor, Department of Biochemistry, Faculty of Science, Khon Kaen University*

Introduction

Heat tolerant rice cultivars are demanded for future food security. To date, evaluation of heat tolerant or susceptible rice cultivars takes at least 3 months before reaching reproductive stage in order to apply hot environment and identify individuals yield at the fourth month. Greater yield gains upon heat treatment would be considered as heat tolerant rice (Wahid et al., 2007, Jagadish et al., 2007, Cao et al., 2009). Although, this technique is reliable, it is time consuming. Non-photosensitive rice could be evaluated 3 times a year, however, photosentive rice, such as Khao Dok Mali 105 (KDML105) that grows once a year, are not possible to do so.

Based on general defense mechanism, plants generate reactive oxygen species (ROS) during abiotic or biotic stresses (Aple and Hirt, 2004; Gill and Tuteja, 2010). Under stress conditions, plants would struggle to survive in the presence of uncontrollable ROS content. Tolerant plants usually scavenge the ROS quicker than the susceptible ones (Demiral and Turkan, 2005, Wang et al., 2010, Bonnecarrere et al., 2011). Less ROS, such as H_2O_2, are less detected when being stressed and lower activities of ROS scavenging enzymes are also measured. Oxidative stress responses to heat stress is also different between heat susceptible and heat tolerance in mustard (Dat et al., 1998), cotton (Gür et al., 2010), Kentucky bluegrass (He, 2010) and cucumber (Ara et al., 2013). Lower accumulation of ROS and lipid peroxidation are detected in heat tolerant plants.

This paper presents an alternative approach using biochemical technique to see oxidative stress responses patterns under heat stress of standard heat tolerant and susceptible rice at vegetative stage. Comparison of the oxidative stress response patterns showed that KDML105 tended to be heat tolerant rice.

Materials and methods

Plant materials

Seeds of N22 and IR64 rice cultivars were kindly provided by Dr. Changrong Ye, International Rice Research Institute (IRRI), Phillippines. Khao Dawk Mali 105 seeds were kindly given by Dr. Jirawat Sanitchon, Faculty of Agriculture Khon Kaen University, Thailand. 21-day-old seedlings were prepared in a seedling nursery where average day/night temperature was 36/26 ^{o}C with average light intensity during a day of 76,982 LUX and a 12-hr photoperiod before moving to a climate chamber.

Heat Stress Treatment

The rice seedlings were transferred to a climate chamber and left to be acclimated for 2 days before heat treated to 40^{o}C for 8 hours during the day on day 3. Temperature, relative humidity, light intensity and plant collection points are shown in Fig. 1. Leaf samples were kept at -20 ^{o}C until analyzed.

Protein extraction

Fresh leaves (2 g) were ground to a fine powder with liquid nitrogen in pre-chilled mortar and pestle and then extracted by 8 mL of 100 mM potassium phosphate buffer, pH 7.0, containing 1% polyvinylpyrrolidone (PVP). After centrifugation at 5,000 g for 1 h at 4^{o}C, the supernatant was collected and keep on ice before measuring enzyme activity, hydrogen peroxide. The protein content was quantified by the Bradford assay (Bradford, 1976).

Determination of H_2O_2, MDA contents and chlorophyll fluorescence

H_2O_2 content in leaf extracts was measured by using modified method of Góth (1991) and expressed as µmole/g fresh weight. MDA content was determined according to Dimeral and Turkan, 2005. Chlorophyll fluorescence (F_v/F_m ratio) was detected by Handy-PEA Hansatech Instrument (USA).

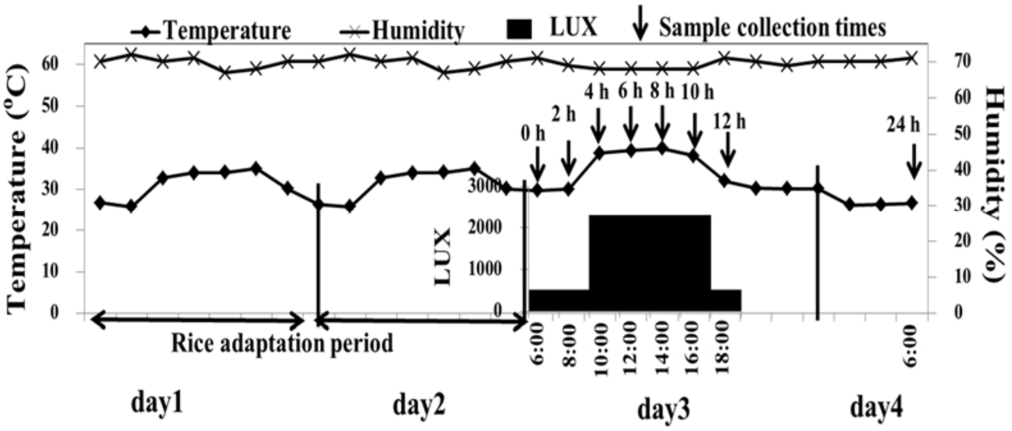

Fig. 1 Heat treatment condition

Assays of catalase and peroxidase activities

Catalase activity was determined according to modified method of Góth (1991) to measure rate of decrease of H_2O_2 content (µmole min^{-1} μg^{-1} protein). Peroxidase activity was determined according to modified method of Chance and Maehly (1955) using guaiacol as the electron acceptor. Unit of the enzyme was expressed as mOD min^{-1} μg^{-1} protein.

Results and Discussion

N22 and IR64 have been known as standard heat tolerant and susceptible cultivars, respectively, by their individual yields obtained after heat treatment at their reproductive stage as performed by International Rice Research Institute (IRRI). These cultivars were non-photosentitive rice, which were able to perform many times a year in order to gain reproducible data. However, in case of photosensitive rice cultivars, which reproductive stage could reach only once a year, it has been not easy to evaluate their heat tolerant traits by using the same technique.

Gathering information of plant response to biotic and abiotic stresses, plants produce reactive oxygen species (ROS) as signaling compounds, such as H_2O_2 (Apel and Hirt, 2004; Gill and Tuteja,2010, Demidchik, 2010). However, excess of ROS accumulation causes oxidative damage to several biomolecules, for example lipid bilayer, protein, genetic materials and etc., which struggles plant survival despite appropriate defense mechanism. A number of reports show that under biotic and abiotic stresses, tolerant plants are able to maintain harmless level of ROS more than susceptible ones.

To avoid determination of heat tolerant rice at reproductive stage, this work attempted to compare oxidative stress response to heat stress of the N22 and IR64 rice seedlings. Several patterns of oxidative stress response under heat treatment of both cultivars a

corresponded to other reported heat stressed plants. By using this approach, a photosensitive rice, Khao Dok Mali 105 (KDML105) could be evaluated as heat tolerant rice during its vegetative stage as will be described.

Patterns of H_2O_2 and MDA contents and chlorophyll fluorescence in N22 and IR64 seedlings during heat treatment

21-day-old seedlings of N22 and IR64 were acclimated in a climate chamber for 2 days (Fig.1) in order to set basal line of oxidative stress (data not shown). To mimic hot climate during day time, heat treatment was performed on day 3 by increasing temperature during 9:00 am – 17:00 pm to 40^{o}C by heat generator and light intensity, which settled at 40^{o}C from 9:30 am onwards. Relative humidity was kept constant to 70% as much as possible. Therefore, this given condition would help analysis of oxidative stress response affected by heat stress only. Leaf samples were collected every 2 hours within 12 hrs and left overnight, finally collected at 24 hr on day 4. During heat treatment, all seedlings were visually wilted; however, N22 seedlings were able to get recovered after temperature declined during the night, but not IR64. Determination of H_2O_2 during the analyzed hours showed that IR64 accumulated H_2O_2 relatively higher than N22 and still much higher than that of N22 at 24 hr of analysis (Fig. 2a). This also corresponded to product of oxidative damage, malondialdehyde (MDA), which lower relative MDA content was detected in N22 than that of IR64 (Fig. 2b). This could also reason why IR64 seedlings were still wilted on day 4. Heat stress could possibly affected thylakoid membrane as lower relative ratio of F_v/F_m of chlorophyll fluorescence detected in IR64 than N22, and again, IR64 was unable to get recovered its photosynthesis system (Fig.1c).

Patterns of catalase and peroxidase activities of N22 and IR64 seedlings during heat treatment

Catalase and peroxidase are H_2O_2 - destroying enzymes as described in following reactions:

$$H_2O_2 + O_2 \xrightarrow{\text{catalase}} 2\,H_2O + \ 1/2\,O_2$$

$$H_2O_2 + e\text{- acceptor} \xrightarrow{\text{peroxidase}} \text{reduced e- acceptor} + 2\,H_2O$$

To determine oxidative stress response of N22 and IR64 seedlings during heat stress, protein extracts from the rice seedlings, collected from each time point (Fig.1), were analyzed for catalase and peroxidase activities (Fig. 3a, b). Relative activities of both enzymes in IR64 seedlings were higher than that of N22. Regard to patterns of H_2O_2 content and H_2O_2 - destroying enzyme activities in both cultivars, this suggested that excess of H_2O_2 in the susceptible rice was not able to remove by higher activity of its destroying enzymes and vice versa. This evidence has been reported as genetically inherited. The susceptible plants tend to overproduce H_2O_2 as well as its destroying enzymes to avoid oxidative damage. In contrast, the tolerant plants tend to keep harmless H_2O_2 level by controlling production of H_2O_2 and levels of their destroying enzymes. However, in this work, their accumulations of H_2O_2, MDA contents and chlorophyll fluorescence, in addition to catalase and peroxidase activities, given in both tolerant and susceptible in cultivars, indicated that patterns of oxidative stress and response upon heat treatment help evaluation of heat tolerant or susceptible rice.

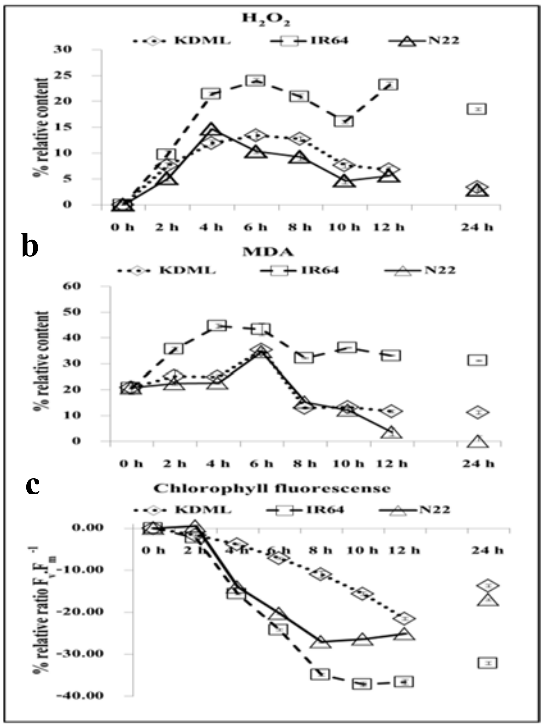

Fig. 2 Percent relative content of H_2O_2 (a), MDA (b), and ratio $F_v.F_m^{-1}$ (c) of KDML-105, IR64 and N22 rice seedling when exposed to high temperature at 40 ^oC during a day.

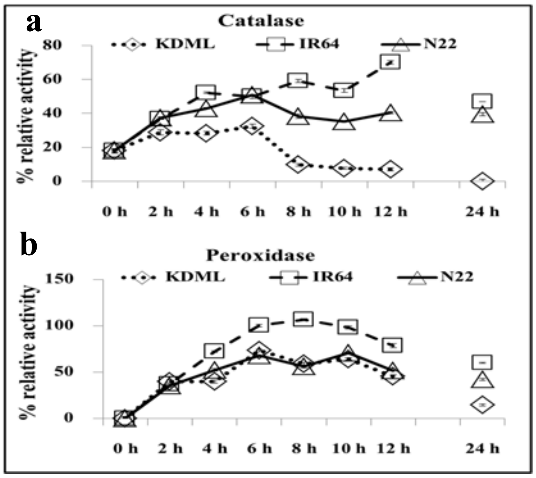

Fig. 3 Percent relative activity of catalase (a), and peroxidase (b) of KDML-105, IR64 and N22 rice seedling when exposed to high temperature at 40 ^oC during a day.

KDML105 tended to be heat tolerant rice

To evaluate KDML105 whether it is heat tolerant or susceptible cultivar, classical method by identifying individual yield after heat treatment during reproductive stage is time consuming because of its photosensitive trait. To date, KDML105 has not been known how it responses to heat. By using 21-day old seedlings treated as performed in N22 and IR64, patterns of H_2O_2, MDA contents and chlorophyll fluorescence (Fig.2a,b, c, respectively), in addition to catalase and peroxidase activities were analyzed (Fig.3a,b, respectively). Similar patterns to N22 suggested that KDML105 rice tended to be heat tolerant rice. This experiment took one month to evaluate and less rice cultivating area was used. However, it is still required classical method to support this conclusion as well as more of standard cultivars should be compared.

Conclusions

Employing plant oxidative stress response to heat stress, under given heat stress condition, heat tolerant rice cultivar, N22, showed different patterns of H_2O_2 and MDA contents, chlorophyll fluorescence catalase and peroxidase activities from that of IR64, standard heat susceptible rice. These patterns were compared to that of KDML105 and suggested that KDML105 was rather heat tolerant rice. This biochemical technique is presented as a new approach to determine heat tolerant rice trait by using its vegetative stage. However, supporting evidences from reproductive stage and more rice standards are still required.

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References

Apel, K. and Hirt, H. (2004) Reactive oxygen species: metabolism , oxidative stress, and signal transduction. Annual Review of Plant Biology. 55: 373-399.

Ara, N., Nakkanong, K., Lv, W., Yang, J., Hu, Z. and Zhang, M. (2013) Antioxidant enzymatic activities and gene expression associated with heat tolerance in the stems and roots of two cucurbit species (“Cucurbita maxima ” and “Cucurbita moschata”) and their interspecific inbred line “Maxchata”. International Journal of Molecular Sciences. 14: 24008-24028.

Bonnecarrere, V., Borsani, O., Diaz, P., Capdevielle, F., Blanco, P. and Monza, J. (2011) Response to photoxidative stress induced by cold in japonica rice is genotype dependent. Plant Science. 180: 726-732.

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

Cao, Y.Y., Duan, H., Yang, L.N., Wang, Z.Q., Liu, L.J. and Yang, J.C. (2009) Effect of high temperature during heading and early filling on grain yield and physiological characteristics in *Indica* rice. *Acta Agromomica Sinica*. 35(3): 512-521.

Chance, B., and Maehly, A. C. (1955) Assay of catalase and peroxidases. Methods Enzymology. 2:764-775.

Dat, J.F., Lopez-Delgado, H., Foyer, C.H., and Scott, I.M. (1998) Parallel change in H2O2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in Mustard seedlings. Plant Physiology. 116: 1351-1357.

Demidchik, V. (2015) Mechanisms of oxidative stress in plants: from classical chemistry. Environmental and Experimental Botany. 109: 212-228.

Demiral, T. and Türkan, I. (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environmental and Experimental Botany. 53: 247–257.

Gill, S.S and Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 48: 909-930.

Góth, L. (1991) A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta. 196:143-152

Gür, A., Demirel, U., Özden, M., Kahraman, A., and Çopur, O. (2010) Diurnal gradual heat stress affects antioxidant enzymes, proline accumulationand some physiological components in cotton (Gossypium hirsutun L.). African Journal ofBiotechnology. 9(7): 1008-1015.

He, Y. (2010) Differential responses to heat stress in activity and isozymes of four antioxidant enzymes for two cultivars of Kentucky bluegrass contrasting in heat tolerance. Journal of America Horti Science. 135(2): 116-124.

Jagadish , S.V.K., Craufurd, P.Q. and Wheeler, T.R. (2007) High temperature stress and spikelet fertility in rice (Oryza sativa L.). Journal of Experimental Botany. 58(7): 1627-1635.

Wahid, A., Gelani, S., Ashraf, M. and Foolad, M.R. (2007) Heat tolerance in plants: An overview. Environmental and Experimental Botany. 61: 199-223.

Wang, H.Z., Zhang, L.H., Ma, J., Li, X.Y., Zhang, R.P and Wang, R.Q. (2010) Effects of water stress on reactive oxygen species generation and protection system in rice during grain-filling stage. Agricultural Sciences in China. 9(5): 633-641.