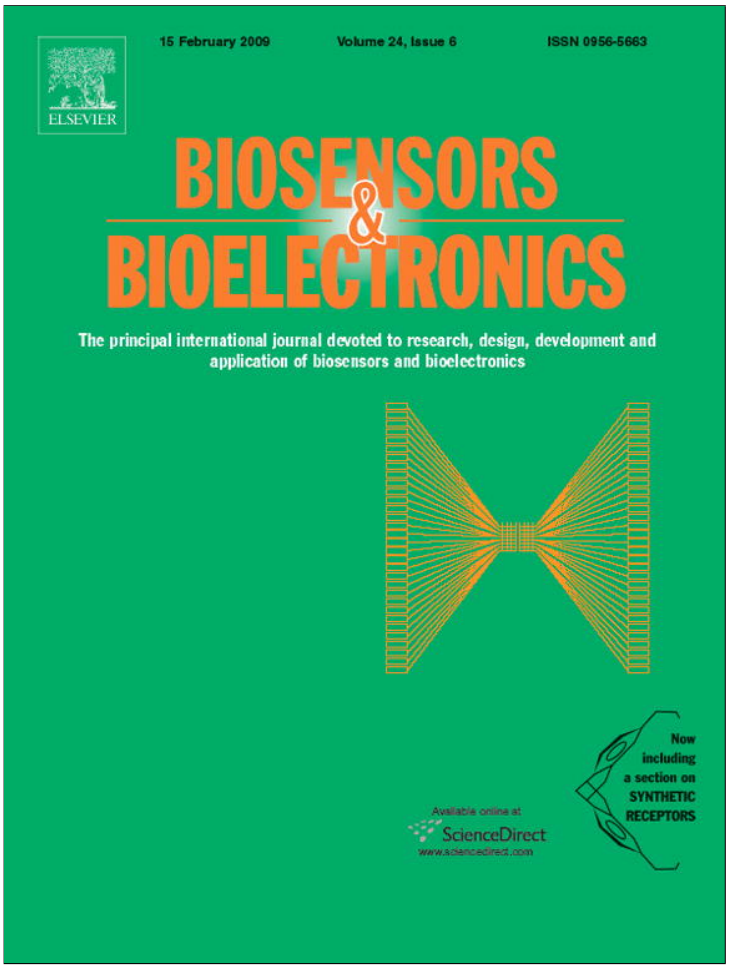

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A non-invasive and rapid seed vigor biosensor based on quantitative measurement of superoxide generated by aleurone cell in intact seeds

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ABSTRACT

Superoxide generated during the early imbibition is an excellent marker for evaluating seed vigor. In this paper, a new principle biosensor for non-invasive detection of seed vigor based on quantitative measurement of superoxide via selective probe 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo [1,2α] pyrazin-3-one (MCLA)-mediated chemiluminescence (CL) was developed. The biosensor, which used a compact single-photon counting module (SPCM) to collect the CL signal, could evaluate seed vigor *in vivo*. Benefiting from the high CL efficiency of MCLA reacting with superoxide and high sensitivity of the SPCM technique, the trace superoxide generated by dry seeds under storage state can be detected to achieve rapid and non-invasive determination of the seed vigor. In comparison with the traditional methods for fast measuring seed vigor based on measurement of physiological and biochemical properties, our proposed technique has significant advantages such as low cost, simplicity, convenient operation and short time consuming. To demonstrate the utility of the system, it was applied to evaluate MCLA-mediated CL of three different plant species wheat (Ze Yu No. 2), maize (Tai Gu No. 1 and 2) and rice (Jing Dao No. 21) seeds with different degrees of aging. The experimental results suggested that there was an excellent positive correlation between the seed vigor assessment from quantitative TTC-test and the detection based on MCLA-mediated CL of superoxide measurement. The new principle of seed vigor measurement is a challenge and breakthrough to conventional method of seed vigor determination and may be a potential technique of the next generation seed vigor detection.

1. Introduction

In order to feed the growing population we need to constantly improve grain unit production on the limited arable land, and high-quality seed provided an important guarantee for enhancing crop unit production. Seed vigor testing, which is the cornerstone of all other seed technologies, tells whether a crop of seeds is worth collecting, whether handling procedures are correct, and how many potential seedlings are available for regeneration. Therefore, the determination of seeds vitality is very important for improving agricultural yield.

So far two types of methods are commonly used for detection of seed vigor: one is by measuring the seed germination conditions and another is through detecting the physiological and biochemical indicators of seed. The traditional methods, which are based on the germination such as germination rate, seedling length, seedling weight and accelerated aging germination, have several disadvantages such as time consumption and inability to distinguish the dormancy seed. Now, most available methods for measuring seed vigor are based on detecting physiological and biochemical indicators, such as electric conductivity, dehydrogenase activity, ATP content and acid phosphoesterase activity, the above-mentioned assays may involve a longer processing time, destruction of the tested seed, a high cost and specialized skill, and the results are easily affected by environmental factors especially by the containing water in seed (Peters, 2000).

In order to carry out the rapid and non-invasive seed vigor determination, we must investigate the biochemical reactions of seed under store state. Several studies have documented the production of reactive oxygen species (ROS) during seed storage in the dry state (Bucharov and Gantcheff, 1984; Hendry, 1993; McDonald, 1999; Pukacka and Ratajczak, 2005). It has been reported that the production of ROS during seed germination in fact represents an active, beneficial biological reaction that is connected with high germination capacity and vigorous seedling development (Schopfer et al., 2001). This view is supported by the finding that a strong rise in ROS release takes place in the healthy, actively germinating seed and adding extrinsic H_2O_2 can resume

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the seed vigor (Schopfer et al., 2001; Ogawa and Iwabuchi, 2001), namely ROS is related to seed vigor. The active roles of ROS during seed germination have been further demonstrated as follows: (1) H_2O_2 promotes seed germination by the oxidated decomposition of the germination inhibitors present in the pericarp (Ogawa and Iwabuchi, 2001; Oracz et al., 2007). (2) ROS production by germinating seeds represents an active, development control physiological function, protecting the emerging seedling against pathogen attack (Schopfer et al., 2001). (3) ROS can function as cellular second messengers that are likely to modulate many different proteins, leading to a variety of responses (Mori and Schroeder, 2004). However, an enzymatic dismutation step must first take place to convert the free radical $O_2^{\bullet -}$ to the more stable H_2O_2 derivative that is required for a viable long-range cell-to-cell signal or for passing membranes (Allan and Fluhr, 1997). It is possible that there is an endogenous mechanism to generate ROS in dry seed, and this kind of ability gradually declines with the aging of seeds. In our laboratory, the previous work has proposed a new seed vigor detection method based on the measurement of ROS generated during the early imbibition (Chen et al., 2003). Our latest research has suggested that the ROS (most notably superoxide) generated by the catalyzing of NDAPH oxidase (NOX) server as an intrinsic sensor of NADPH, hence, the superoxide can indicate the intensity of NADPH in seed (Liu et al., 2007). It has been reported that the plasma-membrane NOX is involved in ROS generation in rice (Frahry and Schopfer, 2001), maize (Andrés et al., 2007) and wheat (Agarwal et al., 2005; Hao et al., 2006; Yang et al., 2007) and the NOX coding genes in these species have been sequenced. Thus, detecting the superoxide via 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo [1,2α] pyrazin-3-one (MCLA)-mediated chemiluminescence (CL) can be used to determine this endospermic crops seed vigor.

The endosperm of many corps such as rice, maize, wheat and barley grain consists of two differentiated cell types. In the mature ripe grain, an outer layer of living aleurone cells envelopes the dead cells of the starchy endosperm (Olsen et al., 1995; Bethke et al., 2000). The aleurone cells function both as storage tissue and for secretion of hydrolytic enzymes, which upon activation during germination help break down storage tissues (Olsen, 1998). ROS-mediated cell death in cereal aleurone cells accelerates the release of hydrolytic enzymes, thus promoting the seed germination (Bethke and Jones, 2001; Angelika et al., 2002). The ROS is a messenger molecule for seed germination, so the measurement of superoxide generated from aleurone cells can be used to realize this kind of seed vigor.

In this study, a novel fast and portable seed vigor biosensor with new quantitative, sensitive and low cost assay for the assessment of seed vigor was developed based on our previously mentioned principle for measuring seed vigor using MCLA-mediated CL. Our approach utilizes precise measurement of superoxide via the sensitive and selective MCLA-mediated CL during the early imbibition. Furthermore, the single-photon counting technology based on photomultiplier tube (PMT) has been widely used to achieve luminescence detection (Magrisso et al., 2006; Wang et al., 2007). Based on the above principle and technique, a rapid optical measurement system using 89C55 single-chip microcomputer as control center, is designed for seed vigor detection in this paper. It can detect CL using single-photon counting module (SPCM) with high sensitivity and low signal-to-noise ratio basing on ultra-weak luminescence detection technique. Benefitting from the SPCM technique and the high CL efficiency of MCLA reacting with superoxide, the biosensor was high sensitive, and achieved multi-sample detection, simplified operation. It can be used as a feasible measurement system for seed vigor and may have wide application foreground.

2. Materials and methods

2.1. Theory for measurement of seed vigor using MCLA-mediated CL

In the endosperm seeds, the active aleurone layer cells are located in the outermost layer. In these cells intrinsic NADPH oxidase (NOX) (Liu et al., 2007), and its activity can be activated and amplified rapidly by the influx of Ca^{2+} in the early imbibition. The NOX catalyzes NADPH to generate superoxide anion, which can release outside (Sagi and Fluhr, 2006; Pei et al., 2000) and can be measured by selective CL probe MCLA.

MCLA-mediated CL reflects the NADPH concentration

$$\mathrm{NADPH} + \mathrm{O_2} \xrightarrow{\mathrm{NOX}} \mathrm{NADP^+} + \mathrm{H^+} + \mathrm{O_2^{\bullet -}} \quad \text{(I)}$$

$$\mathrm{MCLA} + \mathrm{O_2^{\bullet -}} \xrightarrow{k_1} \mathrm{MCLA^-} + \mathrm{O_2} + h\nu \quad \text{(CL)} \quad \text{(II)}$$

The equation of kinetics of bisubstrate enzyme-catalyzed reaction (I)

$$\frac{\mathrm{d}[\mathrm{O_2^{\bullet -}}]}{\mathrm{d}t} = \nu = \frac{\nu_{\max}[\mathrm{NADPH}][\mathrm{O_2}]}{K_{\mathrm{m}}^{\mathrm{A}}[\mathrm{O_2}] + K_{\mathrm{m}}^{\mathrm{B}}[\mathrm{NADPH}] + [\mathrm{NADPH}][\mathrm{O_2}]} \quad (1)$$

$\nu_{\max}$ is the maximum rate or maximum velocity; $K_{\mathrm{m}}^{\mathrm{A}}$ is the Michaelis constant for NADPH; $K_{\mathrm{m}}^{\mathrm{B}}$ is the Michaelis constant for O_2. In the measurement condition the intensity of oxygen $[O_2]$ is invariable. The value of $\nu_{\max}$ stands for the activity of NOX, which arrives at culmination after seed mature and declines gradually during the storing. On the assumption that the activity of NOX has not declined, we can consider Eq. (1) as a function that the ratio of superoxide anion production ($\mathrm{d}[\mathrm{O_2^{\bullet -}}]/\mathrm{d}t$) changed with NADPH concentration [NADPH], the intensity of $\mathrm{O_2^{\bullet -}}$ ($[\mathrm{O_2^{\bullet -}}]$) fluctuate with [NADPH], the intensity of MCLA-mediated CL [CL] real-time reflects the change of $[\mathrm{O_2^{\bullet -}}]$. We can conclude that there exists a positive correlation between [CL] and [NADPH]. If NOX activity decreases during the seed conservation, $[\mathrm{O_2^{\bullet -}}]$ will decline. In this case, we can also conclude that there exists a positive correlation between [CL] and [NADPH]. NOX catalyzes NADPH and O_2 to generate superoxide anion, namely, NOX presents in seeds and serves as an intrinsic NADPH sensor. Therefore, the production of superoxide anion can be acted as an indicator of the NADPH concentration in seed, or the composite effects of the change of NOX activity and NADPH concentration, which are directly related to seed vigor (ISTA, 1999).

During the seed germination, the early steps in reserve mobilization are β-oxidation and glycolysis pathway (Angelika et al., 2002), NADPH will produce in these processes. It had been reported that the aleurone layer of cereal grains stores significant amounts of triglycerides that are mobilized shortly after the grain imbibes water (Kristoffer and Allison, 2003). And NADPH concentration is higher in the high vigor seed, namely NADPH is an indicator of seed vigor (Reuzeau and Cavalie, 1995). So, we can investigate the superoxide anion by the MCLA-mediated CL in seed aleurone layer cells instead of NADPH content to assess the seed vigor.

2.2. Materials and reagents

Regular rice seeds (Jing Dao No. 21) was obtained from Guangdong Academy of Agricultural Sciences; maize (Tai Gu No. 1/2) and wheat (Ze Yu No. 2) seeds were obtained from Shanxi Academy of Agricultural Sciences. The rice seeds were harvested in July 2001, 2002, 2004, 2005, and 2006, respectively; the maize and wheat seeds were harvested in 2006. All the samples were taken in separate cloth bags, stored in a desiccator with silica gel and kept in room temperature (15–28 °C). Seeds in all experiments were selected and prepared carefully. MCLA was purchased from

Tokyo Kasei Kogyo Co. Ltd.; MCLA concentrations were based upon $\varepsilon_{430\,nm} = 9.6 \times 10^3$ (mol L^{-1} cm^{-1}). 2,3,5-Triphenyltetrazolium chloride (TTC) and dimethyl sulfoxide (DMSO) were purchased form Sigma–Aldrich, China (Shanghai, China). MCLA was dissolved in DMSO and stored in −20 °C.

2.3. Sample preparation

2.3.1. Artificial accelerated aging

High temperature, high humidity accelerated aging is a good predictor of grain life and quality, and accelerated aging procedures using high temperatures (38–45 °C) and 100% relative humidity (RH) in maize and wheat seeds are adequate for predicting seed viability (McDonough et al., 2004). According to the above methods the maize and wheat seeds were kept in perforated plastic boxes. These boxes were put in a growth chamber (model E7/2; Conviron, winnipeg, MB, Canada) with 100% RH at 41 °C for different time from 0 to 96 h to get different degrees of accelerated aging seeds.

2.3.2. Quantitative TTC reduction test

The pericarp was separated from the endosperm. The endosperm including aleurone layers were separated from dry crop (rice, maize) grains as described previously (Li et al., 2007). 1 mL endosperm cell suspension (10^6 cells mL^{-1}), 1 mL 0.8% (w/v) TTC and 4 mL 0.5 M Na_2HPO_4–KH_2PO_4 (pH 7.4) were put into a 15 mL centrifuge tube with a screw cap. The tubes were mixed by using a vortex mixer for 30 s and incubated at 37 °C for 2 h, 2 mL acetoacetate was then added. The tubes were shaken using the vortex mixer and then centrifuged at 2150 rpm ($975 \times g$) for 20 min. The supernatant was transferred to a test tube. One more extraction of the retained endosperm cells with 2 mL acetoacetate was done as above (more extraction did not show any red color). Finally, the optical density (OD) of the supernatant was measured at 485 nm using a spectrophotometer (Lambda 35, UV/VIS spectrometer, Perkinelmer, America). The control sample was used to zero the spectrophotometer. The triphenyl formazan (TF) concentration was then obtained from the standard curve.

2.4. Measurement of MCLA-mediated CL

MCLA-mediated CL was measured in rice seeds with different natural aging degrees (harvested in different years), as well as maize and wheat seeds with different artificial aging degrees (kept in high temperature and high humidity for different time). Before the measurement of CL, the seeds of equal number were weighed, put in a quartz cuvette and kept in sample ponds of the dark box, the appropriate amount of MCLA solution (the final concentration is 1 μM) was injected equably into the cuvette. After incubation for 10 min in darkness the measurement began. The whole data acquisition time of each experiment was about 5–10 min. The intensity of CL was normalized to cps/g dry weight (cps/g dw). All operations were performed at 37 °C and in darkness. The results of measurements presented in the text were the average CL intensity of five replicates.

2.5. Biosensor system

2.5.1. Concept of operation

Two different operation modes (remote and local control) were optional. In the remote control, the operation of the main instrument was carried out through a PC; while in the local mode, the operation was accomplished on the front panel of the instrument. At first, parameters including MCLA concentration, the position of the sample, the experimental duration and temperature were set. The intact seeds and MCLA mixture solution were in the sample pools; the measurement was based on ultra-weak luminescence detection device (PMT) and the single-photon counting technology and the results were showed on the front panel (local control mode) and the PC display (remote control mode) in terms of MCLA-mediated CL. There were 16 sample pools fixed on the sample tray droved by rotary stepper motor in accordance with the requirements of changing the sample. So, the efficiency was achieved though measuring several samples in one time and avoiding the repeated operation during the sample switching. The measurement process of CL signal was divided into two steps: background survey and meterage of mix signal including auto-oxidation CL and background. CL irradiation dynamics curve was obtained by subtracting background from mix signal. In the biosensor, since the CL signal was stable during several hours, the CL intensity was chosen during the early measuring time as required. Finally, the seed vigor was determined by comparison with the relative average CL intensity.

2.5.2. System hardware design

The biosensor measured the seed vigor by detecting the luminescence produced by the reaction of superoxide generated during the early imbibition with the CL probe MCLA. The major hardware system block diagram of the biosensor was shown in Fig. 1. The system was mainly composed of the following hardware parts: stepper motor driving, temperature control, sample position identification and change system, single-photon technology modules, data acquisition and processing system, sample ponds and mask cassette et al. According to the emission wavelength of CL probe, SPCM (PMT, MP-962, Perkinelmer, Wiesbaden, Germany) was adopted to receive the CL signal, whose typical value of dark counting was 20–30 cps, detection wavelength range 165–850 nm, which including the MCLA emission peak (463 nm). In order to achieve real-time multi-sample detection, the rotary stepper motor was employed to switch the sample ponds. Proportional-integral-derivative (PID) technology was applied to control the system temperature and the precision reached 0.1 °C. Data acquisition and processing was accomplished by micro control unit (MCU) (AT89c55) in the local control mode and PC in the remote control mode.

2.5.3. Design of stepping motor control system

In our system, the driving circuit of stepper motors included univoltage drive circuit, high and low voltage switching drive circuit, constant current chopped drive circuit and so on. Among them the constant current chopped drive circuit was best performance, in this approach the driving current waveform was greatly improved, so that the basic constant current output, lower system power consumption and high power efficiency were realized (You et al., 2005). The system employed a single-chip sinusoidal subdivision two-phase stepper motor driver application specific integrated circuit (ASIC) (TA8435H; Toshiba, Tokyo, Japan), which could be droved by two-phase stepper motor, with the circuit simple and reliable. The chip also had the following features: wide voltage range (10–40 V), the average output current up to 1.5 A, the peak current up to 2.5 A, employed the pulse-width modulation (PWM) chopper drive mode, with clockwise direction and counterclockwise rotation control functions, as it can be done 1/8 subdivision operation, the vibration and noise generated by two-phase hybrid stepping motor in low-speed operation were overcome. The stepper motor drive control diagram was shown in Fig. 2. Single chip micyocomputer (MCU) controlled the stepper motor driving, meanwhile, adjusted the position of sample pool accurately by infrared signal the launch and testing devices installed beside the tray, the position calibration was executed each rotary lap. These ensured that SPCM was always in the same sample pool position during measurement, avoiding the error induced by the position warp.

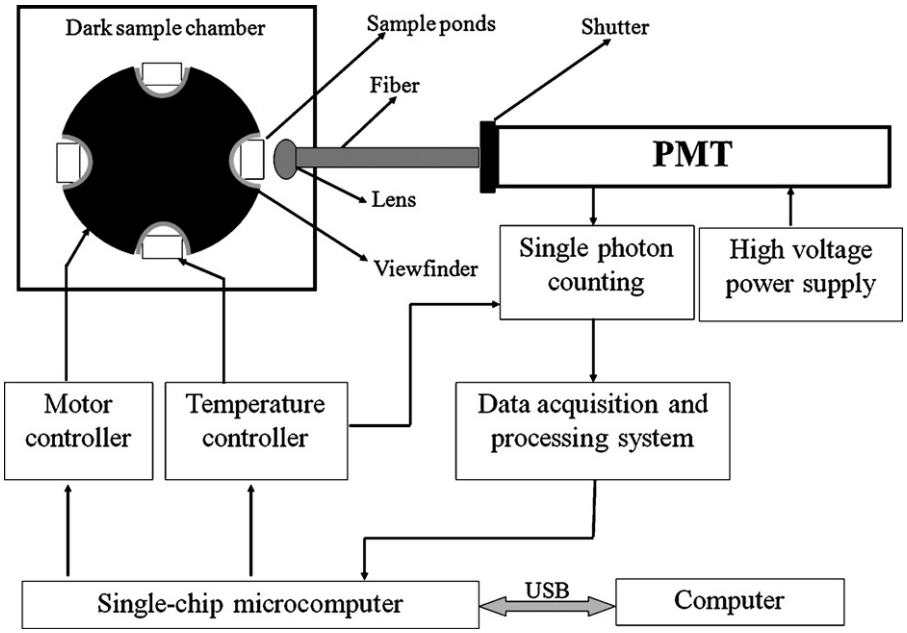

Fig. 1. MCLA-mediated CL-based seed vigor detection biosensor block diagram illustrating major hardware subsystems. Power supplies omitted for sake of clarity.

2.5.4. Dark sample chamber

The structure of dark sample chamber was described in the top left corner of Fig. 1. The dark chamber included periphery light tight dark box and center chamber, which were a rubber cylinder and an adiabatic plastic tray stick together, driven by the rotary stepper motor. There were 16 semi-oval cylinder grooves on the verge of the cylinder. The silver–gilt grooves wall functioned as a viewfinder to collect the light to the lens. These semi-oval cylinder grooves were used to place the sample measuremental cuvette. There was a temperature sensor and temperature adjusting system at the center of the chamber.

2.5.5. Temperature control

The difference between the environmental temperature and the set drove the executant (thermal energy converter (TEC)) to work to stabilize the environmental temperature at the set temperature. TEC can stabilize the temperature through refrigerating one side and heating the other side by changing current direction during the process of working. Proportion integral control was utilized to reduce static state error and improve control precision. In the system, the temperature control range is 15–40 °C and precision can reach 0.1 °C.

3. Results and discussion

3.1. Response of CL intensity to temperature

The relationship between sample temperature and the intensity of CL was first investigated. As shown in Fig. 3, average CL intensity increased with the temperature and reached the maximum at 37 °C then declined. So, this kind of seed vigor determination had a relationship with temperature, in order to achieve maximum efficiency, the proposed measuring temperature was 37 °C.

3.2. Correlations between CL and seed vigor

According to the principle of seed vigor assessment via TTC-test, TTC can be reduced by reductive hydrogen (NADPH and NADH)

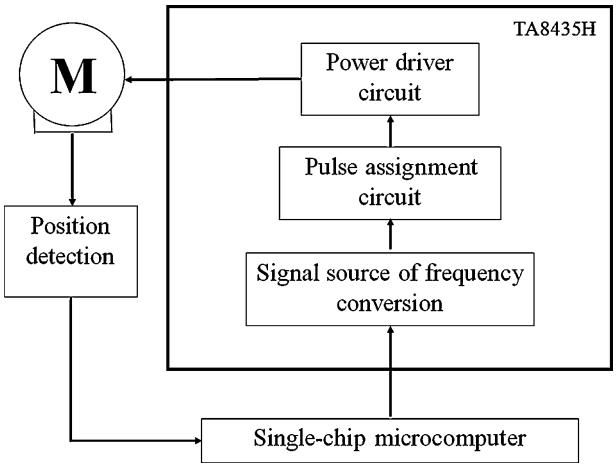

Fig. 2. Schematic of stepper motor drive system.

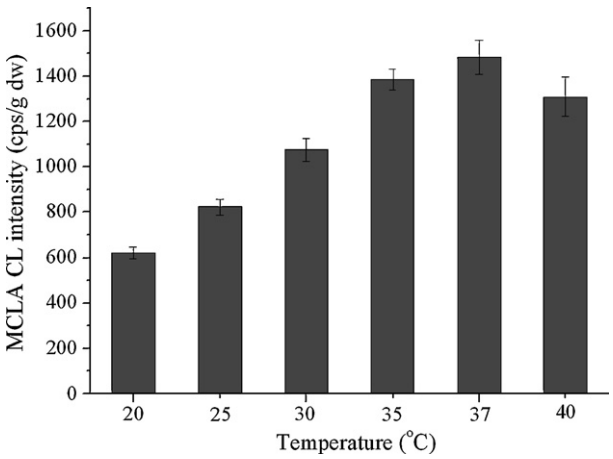

Fig. 3. Effect of the temperature on the MCLA-mediated CL. Concentration of O_2, 138–166 mmHg. Data are the means ± S.E. of five replicates.

generated in seed, to form TF, a red fluorescent compound. The TF concentration was measured by light intensity at the wavelength of 485 nm, and the value of OD_{485} was used to stand for the concentration of reductive hydrogen, which positively correlated with seed germination. We measured the concentration of reductive hydrogen of rice seeds harvested in different years via TTC-test, also examined MCLA-mediated CL induced by superoxide generated from aleurone cells in these seeds. Interestingly, there existed a same trend between MCLA-mediated CL and TF concentration (Fig. 4A). Correlative analysis indicated that this kind of MCLA-mediated CL had an obviously positive correlation to TF concentration (stand for seed vigor quantitatively) (Fig. 4B). So, our methods using MCLA-mediated CL can be used for rapid and non-invasive detection of seed vigor.

3.3. Accelerated aging test

To further demonstrate the ability of the developed biosensor, contrast experiments for measuring seed vigor using the biosensor and quantitative TTC-test were performed for three different plant species maize (Tai Gu No. 1 and 2) and wheat (Ze Yu No. 2) seeds under the temperature of 37 °C and O_2 concentration of 138–166 mmHg. The seed vigor from the biosensor exhibited good accordance with that from the quantitative TTC-test for different plant species (Table 1 and Supplementary figure 1). In addition, the statistical results showed that the measurement time using the biosensor was less than 10 min, while a visible seed vigor value via TTC-test required more than 2 h. Furthermore, the developed biosensor with remote control had the predominance of low cost, simple and convenient operation, as well as original and compact appearance. These merits would make the new biosensor have powerful competition in rapid examination and non-invasive inspection of seed vigor development for precision agriculture. The new principle of seed vigor measurement is a challenge and breakthrough to conventional method of seed vigor detection based on monitoring physiological and biochemical properties, and it may be a potential technique of new generation seed vigor measurement.

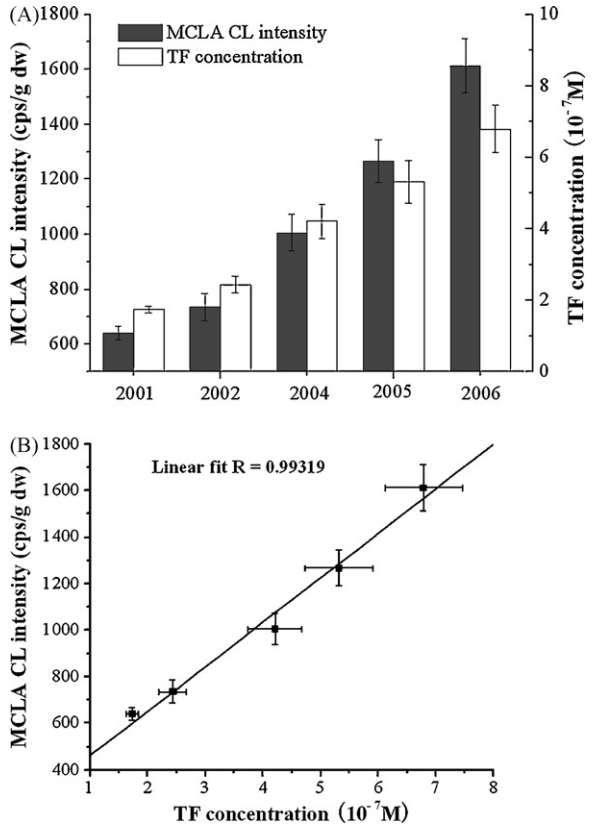

Fig. 4. Correlation between average MCLA-mediated CL intensity and quantitative TTC-test (shown as TF concentration) of rice seeds harvested in different years. Experimental conditions: temperature, 37 °C; concentration of O_2, 138–166 mmHg. Data are the means ± S.E. of five replicates.

Table 1
Linear analysis of relationship between MCLA-mediated CL intensity and TF concentration

Plant species	*R*	S.D.	*N*	*P*
Rice				
Jing Dao No. 21	0.99319	53.99319	5	6.73518E−4
Wheat				
Ze Yu No. 2	0.98241	67.358425	5	0.00279
Maize				
Tai Gu No. 1	0.98485	63.466395	5	0.00223
Tai Gu No. 2	0.98701	55.6285	5	0.00177

R: correlation coefficient. *P*: value-probability (that *R* is zero). *N*: number of data points. S.D.: standard deviation of the fit.

4. Conclusions

In this study, a novel seed vigor biosensor based on quantitative measurement of superoxide *in vivo* was developed. The novel features of the biosensor described here include: (1) the biosensor was based on a new principle-measuring seed vigor using CL from the reaction of MCLA with superoxide generated in seeds, the detection results from this method could be less interfered by the environmental conditions (temperature and RH during storage and the water content of the seed) when compared with conventional method for measuring seed vigor. Because of the high CL efficiency of MCLA reacting with superoxide and high sensitivity of the SPCM detection technique, the new method let us detect the trace superoxide in dry intact seed under storage state. The procedure of our new method is just putting the seeds into the MCLA solution and after incubation for several minutes in darkness then collecting the chemiluminescence, and it only needed 10–20 min to accomplish the measurement. But the conventional method of TTC-test needs a certain time imbibition (usually over 2 h) to activate the seed and to accumulate the red reduzate TF for naked-eye observation, or further using a complicated extraction progress to gather the TF for quantitative analysis via light density determination under spectrophotometer. So, in comparison with the conventional method, the operation of our proposed method for seed vigor determination is less time-consuming, much easier and convenient. The notable advantages of the biosensor are rapid and nondestructive which are significant for the seed vigor determination of large number of seed species and rare species. (2) The only reaction reagent in the proposed method is MCLA, theoretically the cost of each test is less than 0.5 cent, which is very low. The new method has the merits of low cost, simple and convenient operation, and remote control, which would make it have the powerful competition in fast, non-invasive inspection of seed vigor and development and wide application in precision agriculture. (3) The biosensor is portable because of the use of MCU technique. (4) The biosensor has an important application. The main grain crops such as rice, wheat and maize belong to endosperm seeds and have NOX, which is capable of catalyzing superoxide generation. So, these categories of seed vigor can be measured using the new biosensor. In the future, this method can be further extended to other types of NOX containing seeds. (5) The biosensor accomplished wonderful vigor measurement based on the comparison between CL intensity and corresponding TF analy-

sis. In addition, a straightforward combination of CL intensity and reductive hydrogen, which is a good indicator of seed vigor, made the biosensor to be applied accurately and credibly.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.06.040.

References

Agarwal, S., Sairam, R.K., Srivastava, G.C., Tyagi, A., Meena, R.C., 2005. Plant Sci. 169, 559–570.
Allan, A.C., Fluhr, R., 1997. The Plant Cell 9, 1559–1572.
Andrés, A., Rodríguez, H., Ramiro, L., Dolores, B., Edith, T., 2007. J. Plant Physiol. 164, 223–230.
Angelika, F., Paul, B., Veronica, B., Russell, J., 2002. J. Exp. Bot. 372, 1273–1282.
Bethke, P.C., Jacobsen, J.V., Jones, R.L., 2000. In: Black, M., Bewley, D. (Eds.), Seed Technology and its Biological Basis. Sheffield Academic Press, Sheffield, UK, pp. 184–225.
Bethke, P.C., Jones, R.L., 2001. Plant J. 25, 19–29.
Bucharov, P., Gantcheff, T., 1984. Plant Physiol. 60, 53–56.
Chen, W.L., Xing, D., Wang, J., He, Y.H., 2003. Luminescence 18, 19–24.
Frahry, G., Schopfer, P., 2001. Planta 212, 175–183.
Hao, F.Sh., Wang, X.Ch., Chen, J., 2006. Plant Sci. 170, 151–158.
Hendry, G.A.F., 1993. Seed Sci. Res. 3, 141–153.
International Seed Testing Association, 1999. International Rules for Seed testing. Seed Sci. Technol. 27 (supplement), 33–35.
Kristoffer, P., Allison, R., 2003. Free Rad. Biol. Med. 10, 1261–1270.
Li, L., Blanco, M., Jane, J.L., 2007. Carbohydrate Polym. 67, 630–639.
Liu, X.J., Xing, D., Li, L.L., Zhang, L.R., 2007. Photochem. Photobiol. Sci. 6, 767–774.
McDonald, M.B., 1999. Seed Sci. Technol. 27, 177–237.
McDonough, C.M., Floyd, C.D., Waniska, R.D., Rooney, L.W., 2004. J. Cereal Sci. 39, 351–361.
Magrisso, M., Etzion, O., Pilch, G., Novodvoretz, A., Perez-Avraham, G., Schlaeffer, F., Marks, R., 2006. Biosens. Bioelectron. 21, 1210–1218.
Mori, I.C., Schroeder, J.I., 2004. Plant Physiol. 135, 702–708.
Ogawa, K., Iwabuchi, M., 2001. Plant Cell Physiol. 42, 286–291.
Olsen, O.A., 1998. Plant Cell 10, 485–488.
Olsen, O.A., Brown, R., Kalla, R., Kleczkowski, L.A., Lemmon, B., Linnestad, K., Nielsen, P.S., Potter, R., Shimamoto, K., Thorbjornsen, T., Villand, P., 1995. J Plant Physiol. 145, 587–591.
Oracz, K., Bouteau, H.E.-M., Farrant, J.M., Cooper, K., Belghazi, M., Job, C., Job, D., Corbineau, F., Bailly, C., 2007. Plant J. 50, 452–465.
Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G.J., Grill, E., Schroeder, J.I., 2000. Nature 406, 731–734.
Peters, J. (Ed.), 2000. Association of Official Seed Analysts (AOSA): Tetrazolium Testing Handbook. Contribution No. 29, New Mexico, 302 pp.
Pukacka, S., Ratajczak, E.J., 2005. J. Plant Physiol. 162, 873–885.
Reuzeau, C., Cavalie, G., 1995. New Phytologist 130, 59–66.
Sagi, M., Fluhr, R., 2006. Plant Physiol. 141, 336–340.
Schopfer, P., Plachy, C., Frahry, G., 2001. Plant Physiol. 125, 1591–1602.
Wang, J.S., Xing, D., Zhang, L.R., Jia, L., 2007. Biosens. Bioelectron. 20, 2861–2868.
Yang, Y.L., Xu, S.J., An, L.Z., Chen, N.L., 2007. J. Plant Physiol. 164, 1429–1435.
You, T., Feng, J.M., Lu, M.L., 2005. Electric Switchgear 5, 34–35 (in Chinese).