Influence of strong electric field on MDA and SOD of rice under atmosphere pressure

Jianping Xiong *, Shengyong Hu, Jikai Li, Songqing He, Lixin Feng
Electrostatic Institute, Guangdong University of Petrochemical Technology, Maoming, Guangdong, 525000
E-mail: jianping422@163.com

Abstract. The content of MDA is measured by TBA method in the experiment. The results show that the MDA content of rice seedlings after being radiated in a strong electric field under atmosphere pressure decreases compared to that of those not being radiated while the SOD activity decreases. It indicates that radiated seeds’ resistance against oxidative stress can be greatly enhanced. The mechanism and relation between them are analyzed in this paper.

1. Introduction
An adaption system can be formed in a plant and it is constrained by heredity of which reactive oxygen metabolism plays an important role. It is a primary reaction when the plant is stressed in adversity. The balance of generation and scavenging of intracellular free radicals is destroyed and thus the increase of free radicals causes damage to the cell. MDA is a decomposition product during the membrane lipid peroxidation. The reaction with proteins and nucleic acid modifies its nature to inhibit protein synthesis and it may react with enzyme to make the enzyme lose activity to be a metabolic molecule of error catalysis. Therefore, the content and MDA and permeability change of membrane can reflect the peroxidation degree of membrane lipid and meanwhile are important indexes indicating the damage of plasmalemma. MDA content in lamina of rice seeds in the test is basically decreasing as the SOD of lamina increases. As for rice seeds processed in a strong electric field, enzyme activity of SOD is increased, which indicates that rice seeds’ function after radiation increases against oxidative stress. This is reflected form the test data of MDA content.

2. Measure MDA by TBA method
2.1. Principle
During membrane lipid peroxidation, the generated MDA content can reflect the damage of the plant in adversity. In acid and high temperature conditions, MDA may react with TBA to generate brown 3, 5, 5 trimethyl oxazole 2, 4 diketone of which the maximum absorption wavelength is 532 nm. During the test of MDA in the plant tissue, however, it is disturbed by many kinds of materials, mainly the soluble sugar of which the maximum wavelength of color reaction products with TBA is 450 nm and wavelength of 532 nm can be absorbed too. TBA can be reacted with other materials that may be absorbed at these wavelengths. In order to eliminate influence of the reaction, the absorbance at
wavelength of 600 nm should be measured as the MDA content is being measured, from which the absorbance difference at 532 nm, 600 nm and 450 nm can be used to calculate the MDA content.

2.2. Materials, instrument and reagent

2.2.1. Materials: Rice embryo leaves

2.2.2. Instrument:
Strong electric field radiometer (including high-frequency and high-voltage power supply and the discharge device with dielectric barrier, etc.), high-speed refrigerated centrifuge: TGL-16GR (Shanghai Anting Scientific Instrument Factory), T6 Xinshiji spectrophotometer, micro-syringe, 10 ml test tubes, water bath, electronic balance, mortar and pipettes (5 ml, 2 ml and 1 ml), etc.

![Diagram of chemical reaction](image)

**Figure 1.** The production process of 3, 5, 5 trimethyl oxazole 2, 4 diketone.

2.2.3 Preparation of MDA reagent
(a) 0.05 mol L⁻¹ pH 7.8 sodium phosphate buffer;
(b) 5 % trichloroacetic acid solution: 5 g trichloroacetic acid dissolved in distilled water at a constant volume of 100 ml;
(c) 0.5 % TBA solution: 0.5 g TBA dissolved in 5 % trichloroacetic acid at a constant volume of 100 ml.

2.3 Experimental steps and data

2.3.1. Strong electric field radiation:
(1) Select 6 groups of plump rice seeds in a uniform size, 100 seeds for each group; measure their dry weight and put them in an incubator, one group for reference and the other for test.
(2) Determine the spacing between counter electrodes of strong electric field radiation is 1 cm and apply a field intensity of 100 kV cm⁻¹ for 5, 10, 15, 20 and 25 s.
(3) Add distilled water of same volume into each culture dish and place them in the incubator for soilless cultivation.

2.3.2 Extraction of MDA
(1) Weight 0.5 g sample and add 2 ml precool 0.05 mol L⁻¹ pH 7.8 phosphate buffers;
(2) Add a little bit quartz sand ground into homogenate in an ice-bathed mortar;
(3) Transfer it in a centrifuge tube of 5 ml scale (including the fluid after the mortar is washed with buffer);
(4) Centrifuge it for 10 min at 4500 revolutions min⁻¹ and exact the supernatant, i.e., MDA extract. See table 1 below for experimental data:
Table 1. MDA extract of experimental groups and reference groups.

|                  | CK group | Experimental group 2 (D2) | Experimental group 3 (D3) | Experimental group 4 (D4) | Experimental group 5 (D5) | Experimental group 6 (D6) |
|------------------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Vt(ml)           | 3.4      | 3.6                       | 3.4                       | 3.7                       | 3.6                       | 3.8                       |
| V1(ml)           | 4.0      | 4.0                       | 4.0                       | 4.0                       | 4.0                       | 4.0                       |
| V2(ml)           | 2.0      | 2.0                       | 2.0                       | 2.0                       | 2.0                       | 2.0                       |

Vt: total volume of sample extract;  
V1: total volume of reaction fluid of sample extract and TBA solution;  
V2: volume of sample extract reacted with TBA; (the same below).

2.3.3. Determination of MDA content
(1) Place 2 ml extract in a graduated test tube;  
(2) Add 3 ml solution of 0.5 % TBA in trichloroacetic acid;  
(3) Heat it for 10 min in a boiling water bath;  
(4) Centrifuge it for 10 min at 4500 revolutions min⁻¹ and exact the supernatant; determine the absorbance for 100 % transmittance with distilled water as the blank at a wavelength of 532 nm and 600 nm.

The experimental data are recorded in table 2 as below:

Table 2. Determination of Absorbance of Each Experimental Group and Reference Group.

|                  | CK group | Experimental group 2 (D2) | Experimental group 3 (D3) | Experimental group 4 (D4) | Experimental group 5 (D5) | Experimental group 6 (D6) |
|------------------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| A532 (abs)       | 0.140    | 0.154                     | 0.109                     | 0.153                     | 0.159                     | 0.160                     |
| k*A532           | 13.860   | 15.246                    | 10.791                    | 15.147                    | 15.741                    | 15.246                    |
| A600             | 0.057    | 0.067                     | 0.034                     | 0.067                     | 0.076                     | 0.076                     |
| k*A600           | 5.643    | 6.633                     | 3.366                     | 6.633                     | 7.029                     | 6.633                     |
| A450 (abs)       | 0.186    | 0.316                     | 0.148                     | 0.316                     | 0.240                     | 0.297                     |
| k*A450           | 18.414   | 31.284                    | 14.652                    | 31.284                    | 21.879                    | 31.284                    |

A532 represents the absorbance at 532 nm and A600 represents the absorbance at 600 nm.

2.3.4. Determination of MDA content
(1) Place 2 ml extract in a graduated test tube and add 3 ml solution of 0.5 % TBA in trichloroacetic acid;  
(2) Heat it for 10 min in a boiling water bath;  
(3) Centrifuge it for 10 min at 4500 revolutions min⁻¹;  
(4) Exact the supernatant and determine the absorbance for 100 % transmittance with distilled water as the blank at a wavelength of 532 nm and 600 nm.

The following is the formula to calculate the MDA content:

\[
C = 6.45(A532 - A600) - 0.56 \times A450
\]  

\[
MDA \text{ content} = \frac{C \times V_1 \times V_2}{1000 \times V_1 \times W}
\]

where 1000 is the coefficient for ml converted into L; W is the mass of fresh samples; A532 represents the absorbance at 532 nm and A600 represents the absorbance at 600 nm; Vt is the total volume of sample extract; V1 is the total volume of reaction fluid of sample extract and TBA solution; V2 is the volume of sample extract reacted with TBA; see table 3 for measured content of each group.
Table 3. MDA content of each experimental group and reference group (umol g⁻¹).

|          | CK group | Experimental group 2 (D2) | Experimental group 3 (D3) | Experimental group 4 (D4) | Experimental group 5 (D5) | Experimental group 6 (D6) |
|----------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| A532     | 0.140    | 0.154                     | 0.109                     | 0.153                     | 0.159                     | 0.160                     |
| A600     | 0.057    | 0.067                     | 0.034                     | 0.067                     | 0.076                     | 0.076                     |
| A450     | 0.186    | 0.316                     | 0.148                     | 0.316                     | 0.240                     | 0.297                     |
| C(umol L⁻¹) | 0.431    | 0.384                     | 0.401                     | 0.378                     | 0.444                     | 0.375                     |
| V(ml)    | 3.400    | 3.600                     | 3.400                     | 3.700                     | 3.600                     | 3.800                     |
| V1(ml)   | 4.000    | 4.000                     | 4.000                     | 4.000                     | 4.000                     | 4.000                     |
| V2(ml)   | 2.000    | 2.000                     | 2.000                     | 2.000                     | 2.000                     | 2.000                     |
| W(g)     | 0.500    | 0.500                     | 0.500                     | 0.500                     | 0.500                     | 0.500                     |
| MDA content (umol g⁻¹) | 0.005864 | 0.005532 | 0.005452 | 0.005591 | 0.005774 | 0.00571 |

2.3.5. Time effect curve

Figure 2. The curve of MDA content of each group

Figure 3. SOD gross activity content curve.

3. Measure SOD by NBT method

3.1. Preparation of SOD reagent
1. 130 mmol L⁻¹ methionine (Met) solution: 1.9399 g Met dissolved in phosphate buffer at a constant volume of 100 ml;
2. 750 μmol L⁻¹ NBT solution: 0.06133 g NBT dissolved in phosphate buffer at a constant volume of 100 ml;
3. 100 μmol L⁻¹ EDTA-Na₂ solution: 0.03721 g LEDTA-Na₂ dissolved in phosphate buffer at a constant volume of 1000 ml;
4. 20 μmol L⁻¹ riboflavin: 0.00753 g riboflavin at a constant volume of 1000 ml, kept in darkness.

3.2. Collection of SOD extract
1. Take 0.5 g rice leaves in the pre-cool mortar;
2. Add 1 ml pre-cool phosphate buffer ground into homogenate;
3. Add buffer to 5 ml;
4. Take 2 ml solution for centrifugation for 20 min at 1000 rpm;
5. Collect the supernatant, i.e., SOD extract.
3.3. Color reaction
(1) Prepare four 5 ml finger-type tubes with good transparency, two for test and two for reference;
(2) Place solution: 1.5 ml phosphate buffer of 0.05 mol L\(^{-1}\), 0.3 ml Met solution of 130 mmol L\(^{-1}\), 0.3 ml NBT solution of 750 μmol L\(^{-1}\), 0.3 ml EDTA-Na\(_2\) solution of 00 μmol L\(^{-1}\), 0.3 ml riboflavin of 20 μmol L\(^{-1}\), 0.05 ml SOD solution of 10 μmol L\(^{-1}\) and 20 μmol L\(^{-1}\);
(3) Replace the SOD solution with buffer in two reference tubes;
(4) Add 0.25 distilled water to a total volume of 3.0 ml, mix it even and place one in darkness; place other tubes under 4000 Lx sunlight for 20 min reaction; after the reaction is completed, determine absorbance at 560 nm for other tubes.

3.4. Transmittance measuring of each group
(1) Replace the SOD solution with buffer in two reference tubes;
(2) Place 3 ml above solution into the finger-type tubes, place one of the in darkness and other three in positions receiving same light intensity;
(3) Use fluorescent tubes of 4000 Lx (15 W can be used) at 25-30 ℃ for 15-20 min illumination until the color changes;
(4) With the unlighted tubes as the blank and tubes without SOD solution as the reference group, place a spectrophotometer for colorimetric determination at a wavelength of 560 nm.

Record the transmittance as indicated in table 4 below:

**Table 4.** Transmittance by colorimetric method for each group at a wavelength of 560 nm.

|                | CK group | Experimental group 2 (D2) | Experimental group 3 (D3) | Experimental group 4 (D4) | Experimental group 5 (D5) | Experimental group 6 (D6) |
|----------------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Ack (abs)      | 0.260    | 0.241                     | 0.340                     | 0.307                     | 0.213                     | 0.274                     |
| K*Ack          | 25.74    | 23.859                    | 33.660                    | 30.393                    | 21.087                    | 27.126                    |
| Ae1 (abs)      | 0.043    | 0.023                     | 0.028                     | 0.036                     | 0.056                     | 0.042                     |
| K*Ae1          | 4.257    | 2.277                     | 2.772                     | 3.564                     | 5.544                     | 4.158                     |
| Ae2 (abs)      | 0.080    | 0.038                     | 0.053                     | 0.044                     | 0.044                     | 0.055                     |
| K*Ae2          | 7.920    | 3.762                     | 5.247                     | 4.356                     | 4.356                     | 5.445                     |
| Ae (abs)       | 0.0615   | 0.0305                    | 0.0405                    | 0.040                     | 0.050                     | 0.0485                    |

where \( A_e = \frac{Ae1 + Ae2}{2} \) is the average value of light absorption of the sample tube, Ack is the light absorption value of the reference tube, and Ae1 and Ae2 are light absorption values, respectively.

3.5. Measuring SOD gross activity of each group:
With unlighted reference tubes as the blank, measure the light absorption of each tube. As known that SOD activity unit restraining 50 % of NBT photochemical reduction is an enzymatic activity unit, calculate the SOD activity according to the equation below:

\[
SOD \text{ gross activity} = \frac{(A_{ck} - A_e) \times V}{A_{ck} \times 0.5 \times W \times V_t}
\]  

(3)

where the SOD gross activity is in a unit of fresh weight enzyme per gram; Ack is the light absorption value of the reference tube; and Ae is the light absorption value; \( V \) is the total volume of sample solution cm\(^{-3}\); \( V_t \) is the sample consumption cm\(^{-3}\); \( W \) the weight of sample (g).

See table 5 for SOD gross activity of each group:
Table 5. SOD gross activity of each experimental group.

|                | CK group | Experimental group 2 (D2) | Experimental group 3 (D3) | Experimental group 4 (D4) | Experimental group 5 (D5) | Experimental group 6 (D6) |
|----------------|----------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Ack (abs)      | 0.26     | 0.241                     | 0.34                      | 0.317                     | 0.213                     | 0.274                     |
| Ae (abs)       | 0.0615   | 0.0305                    | 0.0405                    | 0.04                      | 0.05                      | 0.0485                    |
| V (ml)         | 3.8      | 3.85                      | 3.95                      | 3.85                      | 3.95                      | 3.95                      |
| Vs (ml)        | 0.05     | 0.05                      | 0.05                      | 0.05                      | 0.05                      | 0.05                      |
| W (g)          | 0.5      | 0.5                       | 0.5                       | 0.5                       | 0.5                       | 0.5                       |
| SOD gross activity | 232.0923 | 269.0207                  | 278.3588                  | 267.8697                  | 241.8216                  | 260.0657                  |

3.6. Time effect curve

Based on table 5, the SOD gross activity time effect curve of each experimental group and reference group is graphed as the right figure.

4. Conclusions

(1) As can be seen in table 1, 2 and 4 and MDA content curve (left), a same electric field of 100 kV cm\(^{-1}\) is used to radiate the rice seedlings and the MDA content in rice seedlings is lower than that of reference, which indicates that time effect occurs for rice seeds in electric field radiation under atmosphere pressure. The MDA content decreases as radiation time extends, for example, group 1, 2 and 3. However, the MDA content, on the contrary, increases as the radiation time extends further, for example, group 4, 5 and 6, while the MDA content is still lower than that of reference group, which indicates that the change of MDA content is not monotonic as the radiation time extends. A threshold value can be found-10s radiation time in group 3. The MDA content is a key index reflecting the level of lipid peroxidation. MDA content in Rice seed after radiation in a strong electric field decreases; lipid peroxidation is weakened; stress resistance is enhanced. These promote the seed germination.

(2) As also indicated in table 4, 5 and the right experimental curve, the SOD gross activity of rice seedlings radiated under atmospheric pressure in a strong electric field is higher than that of reference group and is getting higher as the radiation time extends, for example, group 1, 2 and 3. SOD gross activity of experimental group 3 is the highest (by about 20 %). However, as the radiation time extends to a certain extent, the SOD gross activity decreases on the contrary but is still higher than that of reference group, for example, group 4, 5 and 6. While the activation of SOD eases the lipid peroxidation caused by active oxygen, which protects the membrane and makes it adapt better in the environment and is conducive to seed germination.

(3) After the rice seeds are radiated in a strong electric field, MDA content in rice seedlings decreases and SOD activity increases, which are two aspects of biological effect of a strong electric field, and the effect differs for different radiation time. As shown from research results, for rice seeds after being radiated in a strong electric field, the O\(^2-\) in seedling cells is comparatively lower to ensure the membrane system keeps stable.

(4) The mechanism is that the strong electric field polarizes the ordered arrangement of polar molecules such as cellular protein, carbohydrates and lipids as well as metal ions to cause change of metalloenzyme conformation. While MDA is a product of cell membrane lipid peroxidation and its generation will aggravate the damage of membrane. The decrease of MDA content of the radiated seedlings indicates that the membrane damage is reduced and oxidation resistance enhanced. The SOD, however, is a kind of protective enzymes clearing superoxide anion free radicals O\(^2-\). Commonly, the generation and scavenging of free radicals in a cell is in a dynamic balance and the growth of SOD activity after radiation in a strong electric field is conducive to scavenging oxygen radicals, which eases peroxidation of membrane lipid. It can make the O\(^2-\) in the seedling cells maintain at a low level, thus to ensure stability of membrane system. Therefore, after having been radiated in a strong electric field, seeds’ resistance against oxidative stress can be greatly enhanced. The researches present that...
the SOD activity directly reflects the balance state of generation and scavenging of O2– in a cell and indirectly influences the level of MDA.

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