

Growth and physiological performance responses to drought stress under non-flooded rice cultivation with straw mulching

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ABSTRACT

A field experiment was performed to investigate the growth performance and the growth stage-dependent changes in activities of antioxidative enzymes and concentration of malondialdehyde (MDA) in leaves of rice subjected to treatment with (NF-M) or without straw mulching (NF-WM) under non-flooded conditions compared with continuously flooded treatment (CF). Compared with the NF-WM treatment, mulch application significantly increased the flag leaf area per plant before heading, tillers number and plant height at the early period of tillering stage. There was no significant difference between the yield of the NF-WM and CF treatment. However, the yield of NF-WM treatment was significantly lower than CF and NF-M treatments. Significantly higher activities of peroxidase (POD) and catalase (CAT) but lower concentration of superoxide dismutase (SOD) and malondialdehyde (MDA) were observed in straw mulching treatment than in treatment without mulching at elongation, heading and grain filling stages. The change tendency of antioxidant enzyme activity and MDA level was in line both with soil moisture status and rice yields of different treatments.

Keywords: non-flooded rice; straw mulching; drought stress; antioxidant enzymes; yield

Rice (*Oryza sativa* L.) production is challenged by the increasing shortage of water resource in the world and by the limitation of water resource in the seasonal drought areas. Conventional flooded rice cultivation in Asia provides more than 75% of the world's rice supply for half the earth's main staple food (Cabangon et al. 2002). However, rice production consumes about 30% of all freshwater used worldwide. In Asia, flood-irrigated rice consumes more than 45% of total freshwater used (Barker et al. 1999). By 2025, 15 out of 75 million hectare of Asia's flood-irrigated rice crop will experience water shortage (Tuong and Bouman 2003). Alternatives to the conventional flooded rice cultivation were developed worldwide to reduce water consumption and produce more rice with less water.

Non-flooded rice cultivation has been proven to be an effective measure to reduce water consumption. In this paper, non-flooded rice refers to rice grown in non-flooded condition with supplemental irrigation when rainfall is insufficient, in which rice is grown like an upland crop. Non-flooded rice cultivation may decrease water demand and promise substantial water savings by minimizing seepage and percolation and greatly reducing evaporation (Bouman et al. 2002, Huang et al. 2003).

However, compared with continuously flooded rice system, rice cultivation under non-flooded conditions leads to less stable productivity and lower grain yield (De Datta et al. 1973). In Philippines, rapid yield decline under continuous upland rice cropping was documented (Peng et al. 2006). The disadvantages arising from this can be overcome

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to a certain extent by an alternative way of growing lowland rice using rice straw mulch under non-flooded conditions (Huang et al. 2003, Qin et al. 2006). Crop residue mulch has the potential to retain soil moisture (Enrique et al. 2002) and maintain the non-flooded rice production (Huang et al. 2003, Qin et al. 2006). Root development and proliferation depend on soil moisture (Gajri and Prihar 1985) and grain yield under mulches is higher due to longer rooting and higher moisture content in the upper soil layers (Bonfil et al. 1999). It is obvious that water-saving production systems (mulch ground cover) will not entirely replace traditional paddy fields, but in water-scarce environments or under conditions when concurring water demand of non-agricultural users forbids extensive water consumption in agriculture, water-saving ground cover rice production systems may be a promising alternative that greatly reduces seepage and evaporation (Tao et al. 2006).

Previous work showed that non-flooded rice cultivation reduced water consumption by almost 50–70% compared with the continuously flooded system (Huang et al. 2003, Qin et al. 2006). However, there were only limited reports on mechanisms involved in water use efficiency and water stress physiology in particular in non-flooded rice cultivation with mulching (Liang et al. 2003). Although much work was done on the relationships between antioxidative enzymes and drought tolerance in plants, most of such work was undertaken within a shorter experimental duration (from hours to days) under laboratory conditions (Liang et al. 2003). Liang et al. (2003) studied the antioxidative defenses and water deficit-induced oxidative damage in rice growing on non-flooded paddy soils with plastic- and paper-mulching. However, the study of Liang et al. (2003) did not involve the ground mulching type of rice straw. Furthermore, straw mulching is more sustainable and acceptable than the plastic or paper mulching. Hence, in this study we performed field trials subjected to non-flooded cultivation to investigate the yield and growing performance, especially the growth-stage-dependent changes in antioxidative enzyme activity of rice subjected to with or without mulching under non-flooded conditions compared with continuously flooded system. For better understanding the mechanisms of the adaptive responses of rice to water deficit stress, the aim of this study is to examine the effects of straw mulching on rice growing performance and oxidative damages in water-stressed rice grown under non-flooded paddies.

MATERIALS AND METHODS

Soil and climate

The field experiment was conducted at the Yujiang county, Jiangxi province, China (N28°15', E116°55') in late rice season (July–October) of 2004. The research area is a representative of a typical subtropical moist climate with a mean annual temperature of about 17.7°C; a maximum daily temperature of around 40°C in summer; an average of 262 frost-free days and a rainfall of 1750 mm, about 50% of which falls from March to early July. The uneven distribution of rainfall causes strong seasonal droughts in summer and/or autumn.

The soil was developed from alluvial deposits and used for rice cropping for more than 50 years. The soil is anthropogenic soil and the soil horizons include A-P-W1-W2. The initial ploughed soil (0–20 cm) characteristics of the experimental field, which were measured by using routine methods (Lu 1999), are given in Table 1.

Experimental design and water management

The crop system was early rice followed by late rice. The rainfall for the early rice was plentiful and water management trial was performed in the late rice. The early rice was managed under the controlled flooded cultivation, always keeping a standing water depth in the field until two weeks before harvest. In late rice seasons, conventional flooded cultivation (CF) treatments and

Table 1. Initial soil characteristics of the field experiment conducted at Yujiang County

Parameter	Mean	SD
pH	5.2	0.1
Organic C (g/kg)	14.79	1.4
Total N (g/kg)	1.54	0.1
Available N (mg/kg)	95.1	3.2
Available P (mg/kg)	16.1	0.6
Available K (mg/kg)	74.2	2.2
Total S (g/kg)	0.26	0.01
EC (μS/cm)	54	1.5
CEC _{eff} (cmolc/kg)	4.46	0.12
Base saturation (%)	96.5	2.1
Clay (%)	14.2	1.4
Silt (%)	54.9	1.75
Sand (%)	30.9	1.5

non-flooded cultivation treatments (NF), which included non-flooded cultivation with straw mulching (NF-M) and without straw mulching (NF-WM) treatments, were carried out in fields. Rice cultivar Zhongxuan 10 was used in this experiment. All the treatments were arranged with a randomized design. Each treatment was triplicate in the field. Plot size was 60 m² (6 m × 10 m).

In late seasons, rice straw was applied at 5000 kg/hm² in dry weight after transplanting. Seedlings grown in a nursery for a month were then transplanted on 26th of July at a density of 18 hills/m² and two or three plants per hill. Fertilizers of urea, superphosphate and potassium chloride were applied at the rates of 112.5 kg N, 39.3 kg P and 62.2 kg K/ha as basal fertilizer after transplanting and thoroughly incorporated in the soils by hand. Additional urea was spread over the surface soil at a rate of 45 and 67.5 kg N/ha at the tillering and heading periods, respectively. Irrigation water was pumped from an adjacent well and induced through irrigation channel to the experimental plots. The irrigation system and the device to measure the amount of irrigation water were described before (Qin et al. 2006).

Measurement and data collection

Soil moisture. Volumetric soil water content was measured using frequency domain reflectometer probes (MP-406), which were embedded to the depth of 10 cm in the soil. Three probes were used as replicates in each plot.

Rice growth performance and grain yield. Plant height and tillers number were measured on three different days, separately on 10th and 25th of August and 15th of September. 20 plants were randomly selected for measurements each time. Before heading, CI-203 AREA METER (Cid, INC.USA) was used to measure flag leaf area and mean leaf area in each plot (30 plants were selected randomly). The crop was harvested from a net area of 40 m² when the ears were ripe and straw had turned yellow. Grain yields were recorded after threshing of rice.

Extraction and assay of enzymes. The second youngest fully expanded leaf from 20 plants per treatment was taken at tillering, elongation, heading and grain filling stages respectively. One gram of frozen leaf segments (< 2 mm) was homogenized in 5 ml of ice cold 50 mmol/l Na-phosphate buffer (PBS) (pH 7.8) containing 0.2 mmol/l ethylene diamine tetraacetic acid (EDTA) and 2% (w/v) polyvinylpyrrolidone (PVP). The samples were centrifuged at 10 000 × g for 20 min at 4°C. After

centrifugation, the obtained supernatant sample was used for further processing.

SOD (EC 1.15.1.1) activity was assayed by the photochemical method described by Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme, which produced a 50% inhibition of the rate of NBT (*p*-Nitro Blue tetrazolium chloride) reduction at 560 nm. POD (EC 1.11.1.7) activity was measured by monitoring the increase in absorbance at 470 nm. The reaction mixture consisted of 0.05 ml enzyme extract, 2.55 ml 100 mmol/l 7.0 PBS (contained 0.1 mmol/l EDTA), 0.2 ml 1% guaiacol and 0.3 ml 10 mmol/l H₂O₂. One unit of POD activity was defined as an absorbance change of 0.1 units/min at 470 nm [U/min g/(FW)] (Zou 2000). CAT activity (EC 1.11.1.6) was determined by monitoring the disappearance of H₂O₂, which was carried out by measuring the decrease in absorbance at 240 nm. Activity was expressed as units (μmol of H₂O₂ decomposed per min) per mg of protein (Zou 2000). The reaction mixture contained 50 mmol/l potassium phosphate buffer (pH 7.0), 10 mmol/l H₂O₂ and 200 μl of enzyme extract in a 3-ml volume. MDA content was determined by the thiobarbituric acid (TBA) reaction according to the method of Heath and Packer (1968). The reaction mixture contained 2.0 ml enzyme extract and 2.0 ml TBA reagent [0.5% (w/v) TBA dissolved in 10% (w/v) trichloroacetic acid (TCA)]. All the enzymes were expressed as units per mg of protein. Protein was assayed using bovine serum albumin as reference standard material (Bradford 1976).

Statistical analysis. All the data were subjected to statistical analysis and the means were tested by the least significant difference (LSD) and/or the Duncan's Test at 5% level of significance. All the statistical analyses were conducted using the statistical package SPSS 11.5.

RESULTS

Plant height and tillering. In total, the rice crop was the highest in the CF treatment, followed by the M and ZM treatments (Table 2). On the 10th of August, the rice crop height of the CF treatment was significant higher than the non-flooded treatments. It was shown that rice growth was affected greatly by water stress during the early period of tillering. The crop height on the 15th of September was not significantly different among the three treatments. So, the difference can be reduced in the late period of tillering. The tillers

Table 2. Plant height and tillering number of different periods in the cropping season^a

Treatment	Plant height (cm)			Tillering number		
	10 th Aug.	25 th Aug.	15 th Sep.	10 th Aug.	25 th Aug.	15 th Sep.
CF	66.40 ^a	84.80 ^a	95.57 ^a	15.30 ^a	18.00 ^a	18.43 ^a
NF-M	63.80 ^b	82.97 ^{ab}	94.83 ^a	15.57 ^a	17.43 ^a	18.03 ^a
NF-WM	61.24 ^c	78.86 ^b	95.27 ^a	11.43 ^b	14.73 ^b	17.69 ^b

^aWithin a column for each parameter, means followed by different letters are significantly different at 0.05 probability level according to the least significant difference (LSD) test

number varied among the treatments and the variation was similar to the trend of rice crop height. However, there was no significant difference between the CF and NF-M treatments during the three periods. The tillers number both of the CF and NF-M treatments was significantly greater than the NF-WM treatment.

Leaf area. The effect of rice grown in non-flooded plots on flag leaf area was very great (Figure 1). Compared to the CF treatment, the flag leaf area was decreased significantly in NF-WM treatment. However, there was no significant different for

mean leaf area among the three treatments. In order to alleviate the effect of water stress, leaf area was decreased to reduce transpiration in the non-flooded plots. Sheng et al. (2004) reported that straw mulching can increase leaf area index (LAI) compared with the treatment without mulching under non-flooded rice cultivation system.

Grain yields. There was no significant difference between the yields of the NF-M treatment and the CF treatment (Figure 2). Both of them were significantly higher than the yield of NF-WM treatment. In the non-flooded plots, mulching

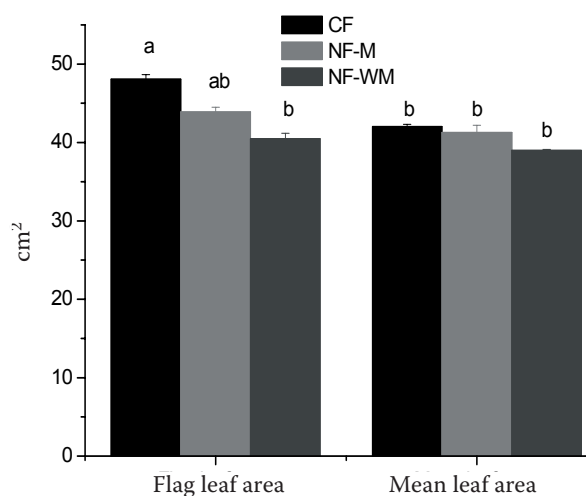


Figure 1. Effect of ground mulching treatment on flag leaf and mean leaf area before heading stage of rice grown under non-flooded and flooded conditions. Data are means of three replicates. The means marked with different letters at the same growth stage are significantly different at $P < 0.05$ by the Duncan's New Multiple Range Test

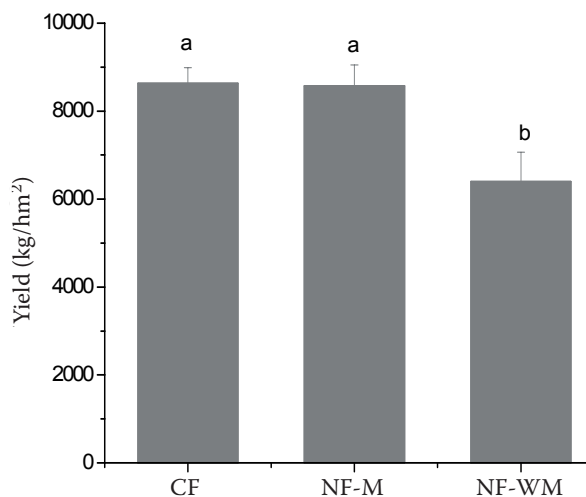


Figure 2. Grain yield of rice grown under non-flooded conditions with or without mulching and under continuously flooded condition

Table 3. Water content (v/v, %) of cultivated soil horizon at different growth stages of rice grown under non-flooded and flooded conditions^a

Treatment	Tillering	Elongation	Heading	Grain filling
CF	47.35 ± 0.12 ^a	47.15 ± 0.11 ^a	46.30 ± 0.21 ^a	46.85 ± 0.15 ^a
NF-M	45.80 ± 0.14 ^{ab}	44.95 ± 0.21 ^b	42.55 ± 0.07 ^b	43.35 ± 0.20 ^{ab}
NF-WM	44.85 ± 0.22 ^b	41.45 ± 0.16 ^b	39.30 ± 0.25 ^c	39.35 ± 0.12 ^b

^aWithin a column for each parameter, means followed by different letters are significantly different at 0.05 probability level according to the least significant difference (LSD) test

with rice straw can maintain high production of rice. Furthermore, in this trial, straw mulching treatment significantly increased soil moisture content at all stages compared to the treatment without mulching (Table 3).

SOD activity. In total, SOD activity was higher in non-flooded cultivation than in continuously flooded condition (Figure 3). At tillering stage, no significant difference in SOD activity was noted among the three treatments. At other three stages, SOD activity in NF-WM treatments was highest, followed by the NF-M and CF treatments. At last three stages, SOD activity in NF-WM treatment was significantly higher than in CF treatment. However, there was no significant difference between the CF and NF-M treatments at elongation and heading stages. At grain filling stage, SOD activity in CF treatment was significantly lower than in NF-M and NF-WM treatments, while in NF-M treatment it was also significantly lower than in NF-WM treatment.

POD activity. No significant difference in POD activity was noted among the three treatments

at tillering stage (Figure 4). POD activity in CF and NF-M treatments was significantly higher than in NF-WM treatment at elongation, heading and grain filling stages. At elongation stage, POD activity in CF-treatment was significantly higher than in NF-M treatment. However, there was no significant difference between the CF and NF-M treatments. At heading and grain filling stages, POD activity in NF-M treatment was the highest, followed by CF and NF-WM treatments.

CAT activity. In the trial, no significant difference was noted between CF and NF-M treatments in CAT activity at four growing stages (Figure 5). At tillering stage, there was no significant difference among the three treatments. At elongation and heading stages, CAT activity in CF treatment was highest, followed by NF-M and NF-WM treatments. Differently, CAT activity in NF-M treatment was highest, followed by CF and NF-WM treatments at grain filling stage. CAT activity both in CF and NF-M treatments was significantly higher than in NF-WM treatment at elongation, heading and grain filling stages.

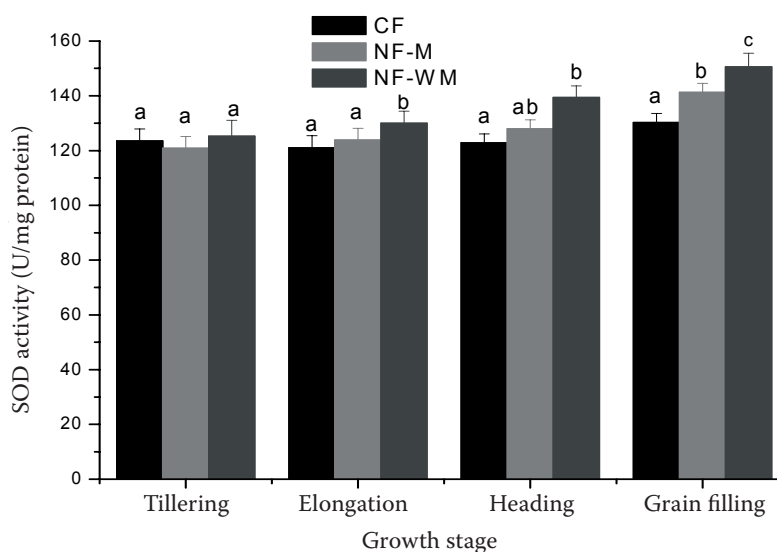


Figure 3. SOD activity of rice growing with or without straw mulching under non-flooded condition compared with continuously flooded system. Data are means of three replicates. The means marked with different letters at the same growth stage are significantly different at $P < 0.05$ by the Duncan's New Multiple Range Test

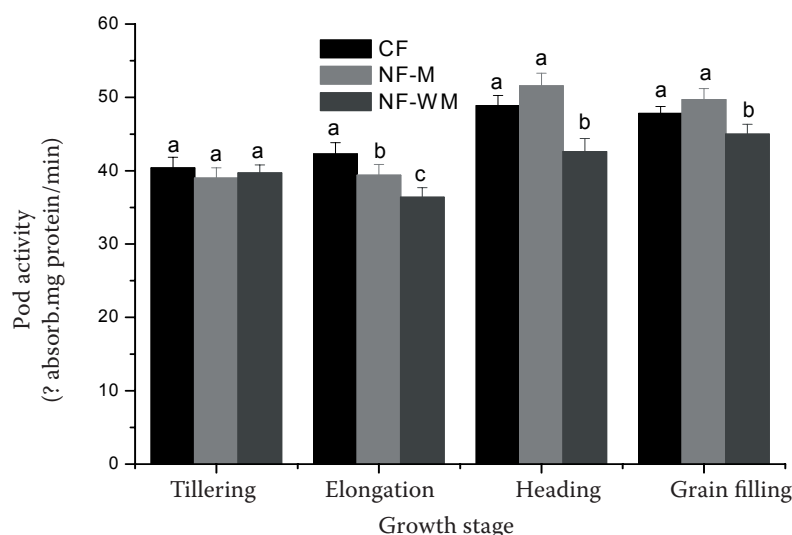


Figure 4. POD activity of rice growing with or without straw mulching under non-flooded condition compared with continuously flooded system. Data are means of three replicates. The means marked with different letters at the same growth stage are significantly different at $P < 0.05$ by the Duncan's New Multiple Range Test

MDA. At four growing stages, MDA concentration in NF-WM treatment was the highest, significantly higher than CF treatment (Figure 6). MDA concentration in NF-WM treatment was significantly higher than in NF-M treatment at elongation, heading and grain filling stages, except at tillering stage. At early stages (tillering and elongation stages), MDA concentrations in all treatments were relatively low compared to the late stages (heading and grain filling stages).

DISCUSSION

Compared to the treatment without mulching, straw mulching can keep soil moisture and reduce the differences between CF and non-flooded treatments in flag leaf area, plant height, tillers

number and grain yield. The soil moisture content in treatment without mulching ranged from only 84.0 to 94.7% of CF treatment, while that of straw mulching treatment from 91.9 to 96.7% of CF treatment, suggesting less severe water deficit stress in straw mulching treatment. Rice growth was deferred greatly in the non-flooded plots. In our previous study, we also found that in non-flooded condition, rice growth time was longer than in the flooded plots (Qin et al. 2006). In non-flooded condition, rice growth was deferred during the tillering and heading stages. Our results coincided with those of Huang et al. (2003) who reported that, in non-flooded condition, the growth time was 5 days longer than under flooded conditions. The reasons for this were as follows: (1) during early periods, rice grew better in flooded plots, and absorbed more nitrogen from soil. So before

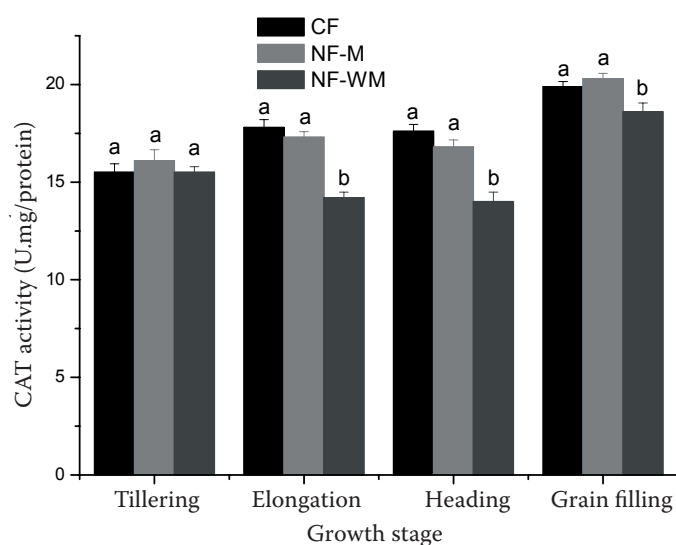


Figure 5. CAT activity of rice growing with or without straw mulching under non-flooded condition compared with continuously flooded system. Data are means of three replicates. The means marked with different letters at the same growth stage are significantly different at $P < 0.05$ by the Duncan's New Multiple Range Test

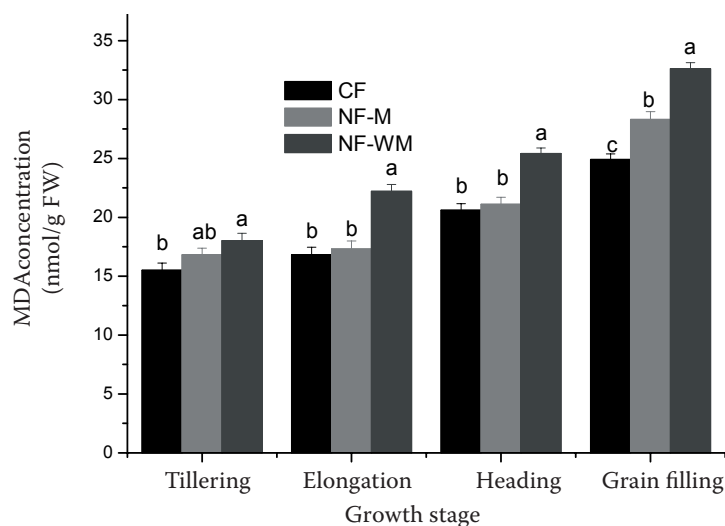


Figure 6. MDA activity of rice growing with or without straw mulching under non-flooded condition compared with continuously flooded system. Data are means of three replicates. The means marked with different letters at the same growth stage are significantly different at $P < 0.05$ by the Duncan's New Multiple Range Test

harvesting, nitrogen content in the non-flooded soil was higher than in the flooded plots. (2) In non-flooded plots, water stress delayed the tillering in early growth period (Table 2), which caused whole growth to be delayed. Mulching with rice straw also increased the flag leaf area and improved the grain yield of rice significantly compared to the NF-WM treatment.

Compared to the treatment without mulching, straw mulching could greatly reduce the effect induced by water deficit stress in non-flooded condition. This was supported by the decreased concentration of MDA, an end product of lipid peroxidation (Figure 6). MDA is one of the main products of lipid peroxidation. Its content reflects the degree of lipid peroxidation. The MDA as lipid peroxidation product can act with many ingredients of plant cells easily and damages enzymes and membrane system severely. In this study, it is proposed that the increased membrane damage induced by elevated active oxygen species could be alleviated by straw mulching via the striking efficiency in the maintenance of soil moisture (Table 3). Higher MDA concentration in NF-WM treatment (Figure 6) at all growing stages was closely related to the decline in activities of POD and CAT (Figures 4 and 5).

In contrast, significantly higher SOD activity (Figure 3) was observed at elongation stage and onward in NF-WM treatment. In this study, SOD activity in NF-WM treatment was significantly higher than in CF treatment at last three stages, which is in line with the findings reported by Kocsy et al. (1991) in wheat and by Liang et al. (2003) in trial with rice under water deficit stress in 1999. On the contrary, the decline in SOD activity under

stressed conditions was thought to be associated with oxidative damage (Gogorcena et al. 1995). In other words, the SOD activity should be in line with the activity of POD and CAT. For example, it was reported that SOD activity decreased in drought-stressed pea nodules (Gogorcena et al. 1995) and in water deficit stressed rice. In fact, the ability of plants to adapt to environmental stresses does depend not only upon SOD but also on the rice cultivar and the whole antioxidative defense system (Liang et al. 2003). It was reported that POD activity played an important role in drought-stressed plants (Zheng and Han 1997). In the present study, lowest POD activity was observed in the NF-WM treatment and was followed by the NF-M treatment, highest POD activity was noted in CF treatment at all stages (Figure 4). Similar change was noted in CAT with growth stage (Figure 5). This was generally coincident with the results of Liang (Liang et al. 2003). Thus it can be assumed that higher concentration of H_2O_2 formed in the removal of superoxide free radicals by higher SOD in NF-WM treatment could not be eliminated by its lower POD and CAT activities (Figures 4 and 5), thus damaging cell membrane lipids (Figure 6). Higher activities of antioxidative enzymes in rice plants (Figures 3–5) in straw mulching treatment effectively protected the plant tissues from membrane oxidative damage induced by water stress.

Except for the tillering stage, the changes in antioxidative enzymes and MDA with growth stages (Figures 3–6) were in line with the severity of water stress in soils, flag leaf area and rice productivity (Figures 1 and 2), suggesting that antioxidative defenses and lipid peroxidation can well reflect the status of water deficit stress of the field plants at last

three growing stages. At tillering stage, there was no significant difference in antioxidative enzymes and MDA concentration among all the treatments (Figures 3–6). The results were different compared to the report of Liang et al. (2003); although water stress affected the tillers number, the different sampling time for tillers number recording and enzymes assay caused the different results. The earliest date of tillers number recording was on 10th August. However, leaf samples for enzymes assay at tillering stage was sampled on 1st August, only 5 days after transplanting. Before transplanting, soil preparation activities in all the treatments begin with pre-saturation irrigation to saturate the topsoil layer and to create a ponded water layer (about 5 cm) for land soaking. Then, plowing and puddling was applied by hand under saturated soil conditions in different plots (Qin et al. 2006). On 1st August, rice growing was not affected greatly by water stress compared to last three growing stages. Therefore, no significant difference was observed in antioxidative enzymes and MDA concentration among all the treatments as a result of absence of severe water stress at tillering stage.

Rice yield was significantly lower in NF-WM treatment than in NF-M treatment, as well as the antioxidative defenses and oxidative damage between NF-WM and N treatments. The main reason is that rice growth and yield is determined not only by soil water supply but also by many other factors including soil nutrient availability, light intensity, soil temperature etc. The reasons for the beneficial effect of crop residue mulch on non-flooded rice were that: (1) for loss of water by evaporation from a mulched soil, water must change from liquid to vapor at the soil surface. The water vapor must then diffuse through the thickness of mulch, which significantly reduces the rate of water loss when compared with bare soil surface. The mulch reduces the quantity of direct solar radiation reaching the soil surface, thereby reducing the amount of energy available for change of state of water from liquid to water. (2) Mulches also act as insulators to downward conduction of heat into the soil. Mulching treatment can effectively decrease soil temperature in early stages (tillering and elongation stages) and keep soil temperature in late growing stages (heading and grain filling stages). (3) Straw mulching increases the input of organic matter. Use of organic amendments is generally seen as a key issue for soil health and sustainability in intensive rice-based systems, both in terms of maintaining the amount and quality of soil organic matter and in terms of supplying im-

portant micronutrients (Yadav et al. 2000, Timsina and Connor 2001). Retention of crop residues on the soil surface increased organic C and total N in the top 5–15 cm of the soil (Rasmussen and Collins 1991), while straw mulching cultivation resulted in maintained rice yields (Liu et al. 2003, Fan et al. 2002). Rasmussen and Collins (1991) reported that slowly decomposing crop residues on the soil surface increased organic C and total N in the top 5–15 cm of the soil while protecting the surface soil from erosion. In conclusion, straw mulching enhanced antioxidative defenses and reduced water deficit-induced oxidative damage and yield decline in rice grown under non-flooded paddy soils compared to the treatment without straw mulching.

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