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Abstract: N-acetylcysteine (NAC) biosynthesized reduced glutathione (GSH), which maintains redox homeostasis in plants under normal and stressful conditions. To justify the effects of NAC on rice production, we measured yield parameters, chlorophyll (Chl) content, minimum Chl fluorescence (Fo), maximum Chl fluorescence (Fm), quantum yield (Fv/Fm), net photosynthesis rate (Pn), photosynthetically active radiation (PAR), and relative water content (RWC). Four treatments, N1G0 (nitrogen (N) with no NAC), N1G1 (N with NAC), N0G0 (no N and no NAC), and N0G1 (no N but with NAC), were arranged in a completely randomized design with five replications. Nitrogen significantly increased yield and yield parameters of rice plants. Moreover, NAC treatment increased panicle numbers, filled grains per panicle, and yield of rice plants. Nitrogen significantly increased Chl content, Chl fluorescence parameters (Fm, Fv/Fm ratio), Pn rate, and PAR in leaves of the rice plants. Furthermore, NAC treatment induced Chl content, Chl fluorescence (Fm, Fv/Fm ratio), Pn, and PAR in leaves of the rice plants regardless of N treatment. NAC significantly increased RWC in leaves of N-untreated rice plants. In conclusion, this study suggests that NAC may enhance rice yield through modulating physiological functions of rice plants.

Key words: Chlorophyll content, glutathione, N-acetyl-cysteine, *Oryza sativa*, photosynthesis, relative water content

1. Introduction

Rice is the most important staple food in Asia, and approximately 90% of world rice is produced and consumed in Asia (IRRI, 2002). In Malaysia, the current self-sufficiency level for rice is 73%. Rice is imported from neighboring countries such as Thailand, Vietnam, and India to fulfill demand (MARDI, 2002). Therefore, there is pressure to increase rice production in Malaysia.

Nitrogen is one of the major macronutrients and has a vast effect on rice production (De Datta and Buresh, 1989). Deficiency and toxicity of nitrogen in the soil significantly affected growth and development of rice plants. Nitrogen affects meristematic tissues, metabolically active cells and major constituents of proteins, nucleic acids, vitamins, and hormones (Swain and Jagtap, 2010). On the other hand, N-acetyl-cysteine (NAC) is an acetylated cysteine residue that has an optimal thiol redox state that protects cells from oxidative stress. It maintains the biosynthesis of reduced glutathione in cells (Sen, 2001; Jahan et al., 2014). NAC increases glutathione content and maintains the cellular

redox state in cells (Schafer and Buettner, 2001), improves performance suggestive of oxidative stress (Reid et al., 1994), and promotes a positive redox balance within the cell (Medved et al., 2003). Therefore, NAC is an effective scavenger of free radicals as well as a major contributor to the maintainance of cellular glutathione status in cells (Kerksick and Willoughby, 2005).

Glutathione functions in sulfur metabolism, regulation of growth and development, cell defense, redox signaling, and regulation of gene expression (May et al., 1998); however, it is not limited to the antioxidant activity of GSH in plants (Jahan et al., 2011, 2012b; Khokon et al., 2011; Okuma et al., 2011). High doses of nitrogen are toxic to the plant cells (Pilbeam and Kirkby, 1992) and may increase osmotic stress and oxidative damage and induce reactive oxygen species in cells. Glutathione may play a fundamental role in reducing oxidative stress (Foyer and Noctor, 2003; Okuma et al., 2011). Therefore, GSH could have an important role in modifying metabolic functions associated with GSH-regulated genes in plants. The

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content of GSH fluctuates during plant development and in response to a wide array of stimuli (Sánchez-Fernández et al., 1997).

To date, no information was found on the effects of NAC on yield, yield parameters, or physiological parameters in rice plants. However, research on the function of GSH in plant signaling was found (Jahan et al., 2008, 2011; Khokon et al., 2011). This study was conceived to find the effects of NAC on rice production in relation to nitrogen application. We proposed that NAC may increase rice yield through varying plant physiological functions that might enhance metabolic functions. These functions remain to be identified.

2. Materials and methods

2.1. Experimental setup
The experiment was conducted at Gong Badak campus, Universiti Sultan Zainal Abidin, Malaysia. Three rice plants were grown in pots measuring 25 cm × 25 cm × 35 cm. All pots were filled with a silty clay soil that was 9% sand, 42% silt, and 49% clay (soil pH, 5.9; organic matter, 4.02%). There were four treatments, N1G0 (nitrogen with no NAC), N1G1 (nitrogen with 100 µM of NAC), N0G0 (no nitrogen and no NAC), and N0G1 (no nitrogen with 100 µM of NAC). Treatments were presented in a completely randomized design (CRD) with five replications. Local rice variety MR 219 was used in this experiment. A 100 µM NAC solution was prepared using 95% ethanol as surfactant and applied as a foliar spray on leaves of rice plants at 15-day intervals. NAC solution was applied until the surfaces of the leaves were completely wet. Fertilizers were applied according to previous studies (Sarwar and Khanif, 2005a, 2005b; Jahan et al., 2013a). Nitrogen (110 kg/ha) as urea in three splits (1/3 as basal + 1/3 at active tillering + 1/3 at late-vegetative stage), P_2O_5 (60 kg/ha) as triple super phosphate (TSP), and K_2O (65 kg/ha) as muriate of potash (MOP) were applied as basal. Rain water collected in a tank was used to irrigate rice plants. Irrigation water was applied through a plastic tube attached to the water tank, and 3 cm of flooding water was maintained. At the ripening stage, standing water was drained to hasten ripening of rice grains. Proper agronomic procedures were applied, according to previous studies (Sarwar et al., 2004; Jahan et al., 2012a, 2013b).

2.2. Determination of yield and yield attributes
Yield and yield parameters were measured as previously described (Sarwar et al., 2004). Plants were harvested 107 days after sowing (DAS), and thereafter the yield parameters were determined. Number of tillers and panicles per pot were recorded. Panicles were detached from straws followed by the separation of grains from panicles. Filled grains were collected from the unfilled grains by salt solution; then they were washed, dried,

weighed, and counted. Grains per panicle and rice yield per pot were recorded.

2.3. Measurement of chlorophyll content in leaves
A SPAD-502 portable chlorophyll meter (Minolta, Japan) was used to acquire a rapid estimation of leaf in situ Chl content at different growing stages (secondary tillering, panicle initiation, and ripening stage). The second uppermost collared-leaf was selected to determine Chl content. Data were taken from 1100 to 1300 hours to avoid the effects of wet conditions on the leaf surfaces (Chelah et al., 2011; Jahan et al., 2013b). Five replicates were implemented.

2.4. Measurements of chlorophyll fluorescence parameters
A portable Junior-PAM Chl fluorescence monitoring meter (Walz, Germany) was used to quantify in situ Chl fluorescence in leaves of rice plants, according to the manual. Data recording times were similar to Chl content data. The second uppermost collared-leaf was selected for taking data between 1100 and 1300. The minimum fluorescence (Fo), maximum fluorescence level (Fm), and quantum yields in PS II photochemistry (Fv/Fm) were recorded. Five replicates were employed.

2.5. Measurement of net photosynthesis rate and photosynthetically active radiation
The net photosynthesis rate was measured using a CI-340 portable photosynthesis meter (CID Biosciences, Inc.). To determine PAR, a quantum sensor in the measuring cell was attached and data were taken together with net Pn data. Data taking procedures were followed according to the manual, and data were taken at three different growing stages (secondary tillering, panicle initiation, and ripening stage). These measurements were taken from 1100 to 1300 on each operational day.

2.6. Measurement of relative water content
Healthy and unblemished leaves were collected from each pot. The fresh weight (FW) of the leaves was measured followed by the turgid weight (TW) after leaves were incubated in distilled water for 24 h to obtain a full turgidity. Dry weight (DW) of the leaves was measured after leaves were dried at 60 °C for 24 h in an oven. RWC data were determined at three different growing stages (primary tillering, secondary tillering, and panicle initiation stages). The relative water content (RWC) was calculated using the following formula (Chelah et al., 2011; Jahan et al., 2013a):

RWC (%) = {(FW – DW)/(TW – DW)} × 100.

2.7. Statistical analysis
Data were analyzed by ANOVA, and differences of means among treatments were determined by LSD and t-test using Minitab-16 and MS Excel software. Differences at $P < 0.05$ were considered significant.

3. Results

3.1. Yield and yield parameters of rice plants

To justify whether NAC affects rice production, we measured yield and yield parameters of rice plants after a foliar application of NAC with or without N (urea) (Figure 1). Nitrogen increased yield and yield parameters of rice plants compared to N-untreated plants. Application of NAC did not affect tiller numbers of rice plants regardless of N application (Figure 1a). Nevertheless, NAC increased panicle numbers per plant, total grains per panicle, and filled grains per panicle in both of N-treated and -untreated

plants (Figures 1b and 1c). Furthermore, unfilled grains per panicle decreased in N-treated plants, but no effect was observed on N-untreated plants in the presence of NAC (Figure 1c, closed bars). In addition, treatment with NAC significantly increased rice yield compared to NAC-untreated plants, which was observed in both N-treated and -untreated plants (Figure 1d). Fresh and dry weights of rice plants are presented in Figure 1e. NAC treatment increased fresh (Figure 1e, open bars) and dry weight (Figure 1e, line graph) in N-treated rice plants but not in N-untreated rice plants. These results indicate that NAC
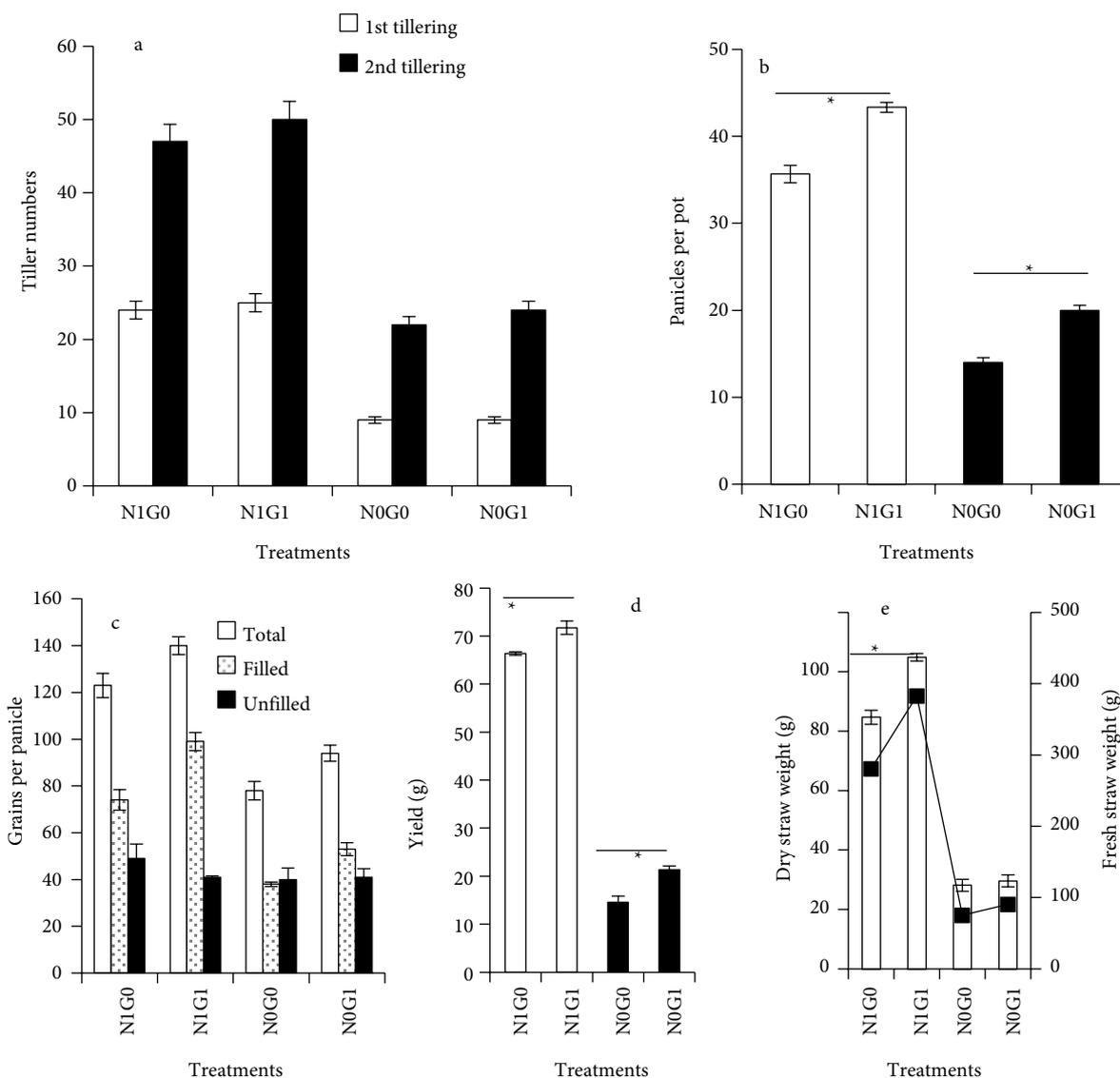

Figure 1. The effects of NAC on yield and yield parameters. a: NAC-induced tiller numbers at the first tillering (open bars) and 2nd tillering (closed bars). b: NAC-induced panicle numbers per pot in N-treated plants (open bars) and N-untreated plants (closed bars). c: NAC-induced total grains (open bars), filled grains (dotted bars), and unfilled grains per panicle (closed bars). d: NAC-induced yield in N-treated plants (open bars) and N-untreated plants (closed bars). e: NAC-induced fresh weight of plants (open bars) and dry weight of plants (line graph).

treatment may enhance yield and yield parameters of rice plants.

3.2. Chlorophyll content, Chl fluorescence, and Fv/Fm ratio in leaves of rice plants

To determine whether NAC application affects photochemical energy in leaves, we measured Chl contents, Chl fluorescence, and the Fv/Fm ratio in leaves of rice plants (Figure 2). Nitrogen significantly increased Chl contents in leaves of rice plants compared with N-untreated rice plants (Figure 2a). Moreover, NAC significantly increased Chl content in leaves of

rice plants compared to NAC-untreated rice plant until plants reached the panicle initiation stage (Figure 2a). This suggests that N and NAC both increased Chl content in leaves of rice plants; however, Chl content decreased gradually with increasing plant age in leaves of rice plants under N0G0 treatments (Figure 2a). Minimum Chl fluorescence (Figure 2b) and maximum Chl fluorescence (Figure 2c) were consistent with Chl content data (Figure 2a). Quantum yield (Fv/Fm ratio; Figure 2d) data support Chl content data and show that NAC application increased the Fv/Fm ratio in rice plants (Figure 2d). These results
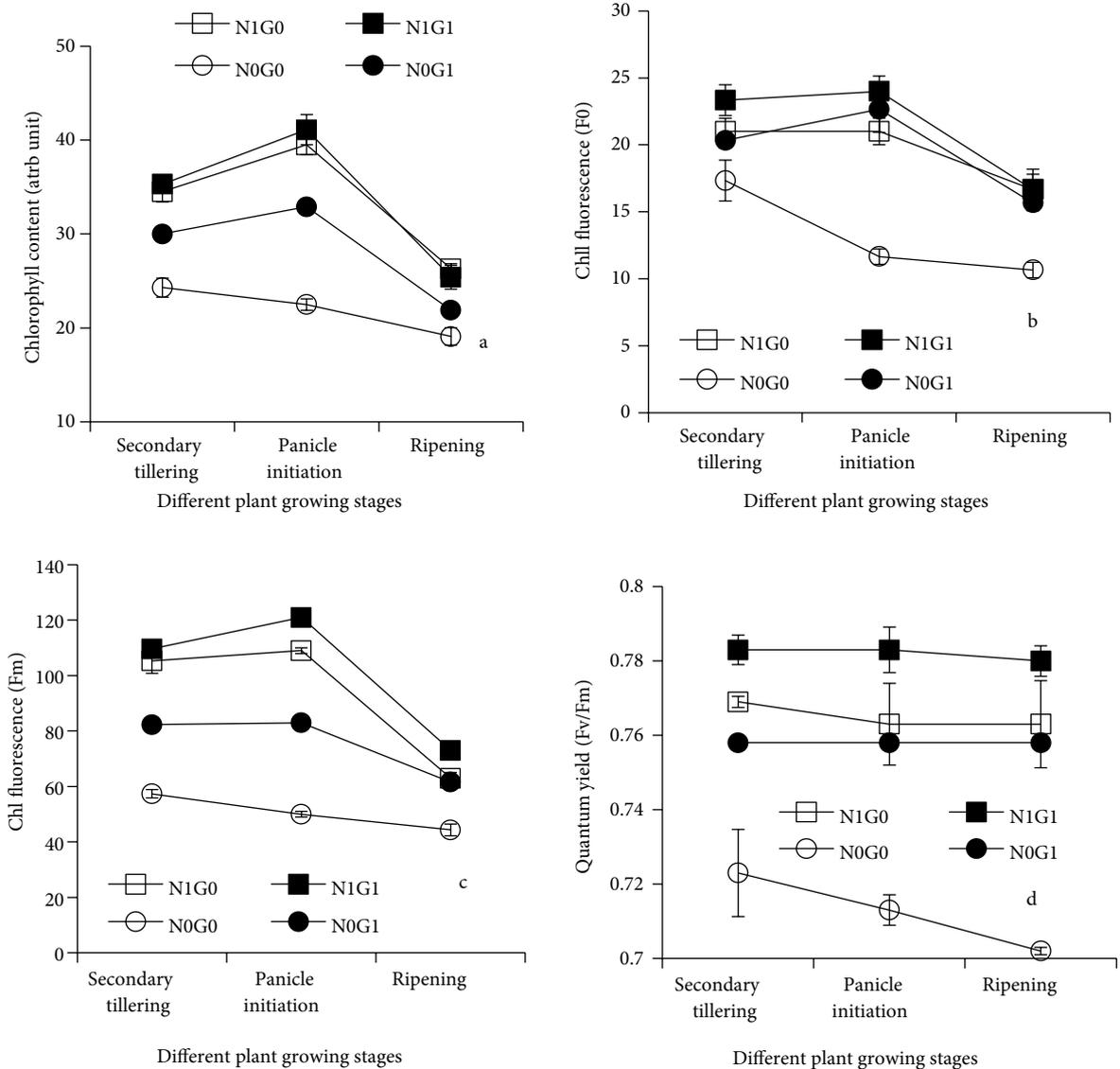

Figure 2. The effects of NAC on Chl content, Chl fluorescence, and the Fv/Fm ratio in leaves. a: NAC-treated chlorophyll content in leaves of rice plants at different growing stages. b: NAC-treated chlorophyll fluorescence (Fo) in leaves of rice plants at different growing stages. c: NAC-treated chlorophyll fluorescence (Fm) in leaves of rice plants at different growing stages. d: NAC-treated quantum yields (Fv/Fm ratio) in leaves of rice plants at different growing stages.

suggest that NAC may enhance photochemical energy in the leaves of rice plants.

3.3. Net photosynthesis rate and photosynthetically active radiation in leaves of rice plants

The net photosynthesis rate and PAR were measured to justify whether application of NAC affects Pn rate and PAR in leaves of rice plants. Figure 3a shows that N significantly increased the Pn rate in leaves of rice plants (open square) compared to leaves of N-untreated plants (open circle). Figure 3a also shows that the Pn rate increased with increasing plant age until panicle initiation stage and then declined at the ripening stage, with the exception of plants grown under N0G0 treatment (Figure 3a, open circle). On the other hand, N0G0 treatment narrowed the Pn rate gradually with increasing plant age (Figure 3a,

open circle). Plants showed similar trend results in the case of NAC treatment (Figure 3a, closed circle and closed square lines); however, the Pn rate was significantly higher in leaves of NAC-treated plants than in NAC-untreated plants. These results indicate that both applications of N and NAC induced the Pn rate in leaves of rice plants.

Photosynthetically active radiation was significantly higher in leaves of N-treated plants (Figure 3b, open square) compared to N-untreated plants (Figure 3b, open circle). In addition, PAR increased significantly in leaves of NAC-treated plants compared to NAC-untreated plants (Figure 3b, closed square and closed circle). However, the trends in PAR (Figure 3b) were similar to the Pn rates in leaves of rice plants (Figure 3a). These results indicated that both N and NAC might affect PAR in the leaves of rice plants.

3.4. Relative water content in leaves of rice plants

To explain whether NAC application affects RWC in rice plant leaves, RWC was measured in leaves at different growing stages. Nitrogen-treated leaves (Figure 4, open square) accumulated significantly higher RWC than leaves of N-untreated plants (Figure 4, closed circle). The NAC treatment did not affect RWC in leaves of N-treated plants (Figure 4, closed square) but affected RWC in N-untreated plants (Figure 4, closed circle). In N-untreated plants, NAC treatment significantly increased RWC in leaves compared to NAC-untreated plants (Figure 4). RWC in N-untreated plants gradually decreased with increasing plant age irrespective of NAC treatments (Figure 4, open circle and closed circle). In N-treated plants, RWC was similar at different plant growing stages. This result suggests that N
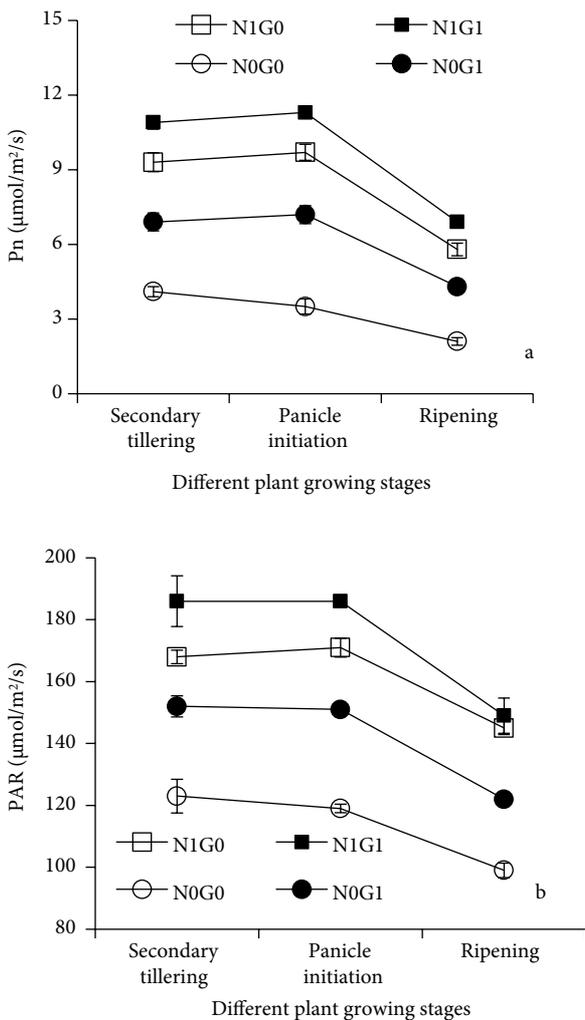

Figure 3. The effects of NAC on the Pn rate and PAR in leaves of rice plants. a: NAC-treated Pn rate in leaves of rice plants at different growing stages. b: NAC-treated PAR in leaves of rice plants at different growing stages.
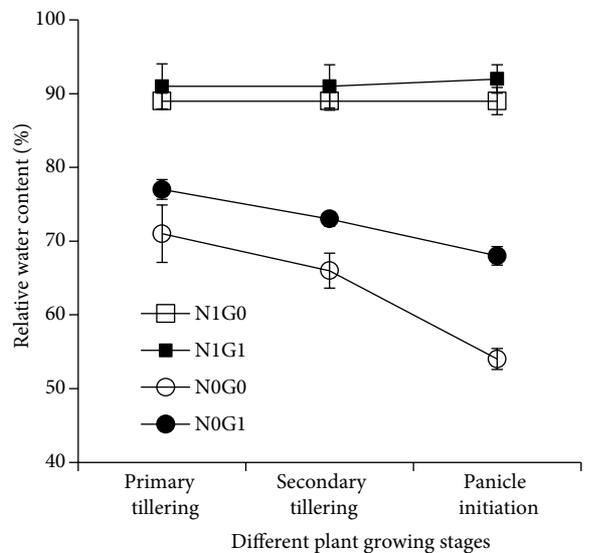

Figure 4. The effects of NAC on relative water content in leaves of rice plants; NAC-treated RWC in leaves of rice plants at different growing stages.

might be involved in retaining higher RWC in leaves in the presence of NAC.

4. Discussion

Accumulation of glutathione in plants changes metabolic functions, regulatory processes (Yan et al., 1995), expression of plant-defense–related genes (Wingsle and Hallgren, 1993; Ball et al., 2004), and plant growth and photochemical reactions including Chl content and Chl fluorescence (Jahan et al., 2014). In contrast, nitrogen had a vast effect on rice plants. In flooded soil, urea hydrolyzed to ammonium form (NH_4^+), which is stable under flood conditions, increasing N availability (Turner and Patrick, 1968); NH_4-N is the main nitrogen source in flooded soil (Turner and Patrick, 1968). This study showed that treatment with N increased the yield and yield parameters of rice plants (Figure 1). In addition, NAC influenced panicle numbers (Figure 1b), filled grains per panicle (Figure 1c, dotted bars), and yields (Figures 1d and 1e) but reduced unfilled grains per panicle (Figure 1c, closed bars). NAC application decreased unfilled grains per panicle but increased filled grains per panicle (Figure 1c), which may influence rice production (Figures 1d and 1e). Therefore, NAC could enhance plant physiological characters to some extent (Jahan et al., 2014). Glutathione increments in plants by NAC treatment influence plant growth and development, while GSH deficiency affects the light-related reaction of plants (Jahan et al., 2008, 2012b, 2014). Nevertheless, whether NAC functions on N effectiveness is still unclear, and more research will be needed to elucidate the mechanism involved in this phenomenon. This study reveals that NAC might affect some physiological functions that could increase the yield of rice plants.

Deficient chlorophyll content in leaves inactivates the photosynthesis rate (Evans, 1983). In addition, light-harvesting antennae in photosystem II (PSII) regulate GSH accumulation in leaves of plants (Jahan et al., 2008), and nitrogen controls chlorophyll content in leaves (Tsou, 2004). Several authors stated that there is a positive correlation between nitrogen supply and chlorophyll formation in leaves. This was consistent with our results, which showed that N increased Chl content in the leaves of rice plants (Figure 2a) in the presence of NAC. However, it is not confirmed that N and NAC are interdependent. In addition, NAC increased Chl content in leaves of N-treated plants more than in N-untreated plants. This implies that NAC might function in plant physiological improvement through GSH modulation in the cells of rice plants. Our results confirm that not only N but also NAC increased Chl content in leaves of rice plants (Figure 2a). Chl content might depend on plant age and environmental conditions, and this is why Chl content in leaves at the panicle initiation stage was higher than in the tillering stage and declined

sharply at the ripening stage (Figure 2a). This may be due to the effects of leaf senescence at the ripening stage. Leaf senescence involves catabolism of compounds associated with nutrient relocation to the developing grains (Dai et al., 2011). Therefore, it seems that plant age has an effect on Chl content in plants. Chl fluorescence (Figures 2b and 2c) and quantum yield (Figure 2d) support the Chl content data and indicate that Chl content and Chl fluorescence are correlated and interdependent. Consequently, higher Chl content in leaves indicates higher light-dependent energy production through light reaction in the PSII system, which enhances physiological functions (Jahan et al., 2014). Chlorophyll content depends on the light-harvesting antennae in PSII in which sunlight is used for photosynthesis and energy production (Lodish et al., 2000). In addition, NAC increased chlorophyll content, which suggests that intracellular GSH might have a function in increasing Chl content and light reaction in the leaves of rice plants.

Furthermore, senescing is associated with chlorophyll degradation (Jakhar and Mukherjee, 2014), lipid peroxidation, and membrane alterations (Dai et al., 2011), which together might lead to a decline in the net photosynthesis rate (Figure 3a) and photosynthetically active radiation (Figure 3b) at the ripening stage. Net photosynthesis rate and PAR were consistent with Chl content and suggested that increasing Chl content might be an indicator of increasing Pn and PAR in plants. Therefore, the Pn rate and PAR gradually increased from the tillering stage to the panicle initiation stage but declined sharply at the ripening stage (Figure 3). This suggests that Chl parameters and photosynthetic parameters are correlated and codependent in leaves of rice plants. Increments of Chl contents, Pn rates, and the PAR in leaves through the application of NAC indicate the involvement of GSH in enhancing plant metabolic function and light reaction in PS II. The rice yield increased in the NAC-treated plants.

We found that N and NAC treatment increased relative water content in leaves (Figure 4). This result suggests that NAC may enhance metabolic functions to maintain the flow of water through the plant cell and could regulate nutrient bioavailability for the plants. An earlier study stated that GSH functions in hormone signaling and water loss in plants (Jahan et al., 2014), which might affect relative water content in leaves of rice plants. These results suggest that GSH content in leaves may help the plants cope with N deficiency or improve metabolic functions. As a result, it is suggested that NAC may increase yield and yield parameters of rice plants through aggregating Chl content, Chl fluorescence, the Pn rate, PAR, and relative water content in rice plants.

Nitrogen significantly increased chlorophyll parameters, the Pn rate, PAR, and RWC in leaves of

rice plants and increased rice yield. Application of NAC increased RWC, Chl content, and Chl fluorescence in leaves, which may lead to increases in the Pn rate and PAR through NAC-induced intracellular GSH content in rice plant leaves. Further research will be needed to justify the effects of NAC application on the phytoavailability of other nutrients necessary to sustain rice production. Furthermore, farmers would benefit if NAC could be applied to plants with micronutrients as a foliar application to protect plants from stress conditions while increasing rice yield.

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