N-acetylcysteine increased nitrogen-induced rice yield

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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 fluorescences (Fo), maximum Chl fluorescences (Fm), quantum yield (Fv/Fm), net photosynthesis rate (Pn), photosynthetically active radiation (PAR) and relative water content (RWC). Four treatments, namely, 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 as 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) and Pn in leaves of the rice plant regardless of N treatments. NAC significantly increased RWC in leaves of N-untreated rice plant. In conclusion, this study suggests that NAC might enhance rice yield through modulating physiological functions of rice plants.

Key Words; *Oryza sativa*, *N*-Acetyl-Cysteine, glutathione, relative water content, chlorophyll content, photosynthesis

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, rice is imported from neighbouring countries, especially from Vietnam and Thailand with the amount at about RM501 million per year to fulfill the country's demand (ASEAN Food Security Information System 2002). Therefore, increasing rice production in Malaysia is in pressure.

Nitrogen is one of major macronutrients has a vast effect on rice production (De Datta and Buresh 1989). Nitrogen affects meristematic tissues, the metabolically active cells and major constituents of proteins, nucleic acids, vitamins and hormones (Swain and Jagtap 2010). On the other hand, Glutathione (GSH) functions on sulfur metabolism, regulation of growth and development, cell defense, redox signaling, and regulation of gene expression (May et al. 1998) but not only limited to the antioxidant activity of GSH in plants (Jahan et al. 2011; Okuma et al. 2011). N-acetyl-cysteine (NAC) is an acetylated cysteine residue that has an optimal thiol redox state which protects cell 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 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 in maintaining the cellular glutathione status in cells (Kerksick and Willougbby 2005).

There is no sufficient research on the effect on NAC on plants. As per our experiement using NAC to *Arabidopsis* and rice plants we found no bad effect on leaf but increase gree pigment of leaves (Jahan et al. 2014) and reduced plant-pathogen infection (Muranaka et al. 2013). In addition, no information was found on the effects of NAC on yield and yield parameters and physiological parameters of rice plants. In consideration this current fact, this study was considered to find the effects of NAC on rice production in relation to the nitrogen application. We presented that NAC might increase rice yield through varying plant physiological functions which might enhance metabolic functions remain to be identified.

Methods

Experimental setup

Experiment was conducted at Gong Badak campus, Universiti Sultan Zainal Abidin, Malaysia. Three rice plants were grown in a pot measuring 30 cm x 25 cm x 35 cm (approx. 20 L). All pot were filled with a soil that was silty clay in texture with 9% sand, 42% silt and 49% clay, soil pH of 5.9, and organic matter of

4.02%. There were four treatments, namely, N1G0 (nitrogen but with no NAC), N1G1 (nitrogen with 100 µM of NAC), N0G0 (no nitrogen and no NAC) and N0G1 (no nitrogen but with 100 µM of NAC), were presented as completely randomized design (CRD) with five replications. Local rice variety of MR 219 was used in this experiment. A 100 µM of NAC solution was prepared using 95% ethanol as surfactant and applied as a foliar spray on leaves of rice plants at 15 days interval. NAC solution was applied until the surfaces of leaves were completely wet. Fertilizers were applied including N at 110 kg/ha (three splits) according to the previous studies (Jahan et al. 2013; Sarwar and Khanif 2005). Proper agronomic procedures were applied according to previous studies (Sarwar et al. 2004).

Determination of plant parameters
Yield and yield parameters were measured as previously described (Sarwar et al., 2004). 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 (Chelah et al., 2011). 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. Five replicates were employed. Net photosynthesis rate was measured using a CI-340 portable photosynthesis meter (CID Biosciences, Inc.). The relative water content (RWC) was determined according to the previous method (Chelah et al. 2011)

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

Results

Yield and yield parameters of rice plants
Nitrogen increased yield and yield parameters of rice plants compared than that of 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 but no impact on unfilled panicles in both of N-treated and -untreated plants (Fig. 1b and c). In addition, the treatment of NAC significantly increased rice yield compared than NAC-untreated plants which was observed in both of with or without N-treated plants (Figure 1d). Fresh and dry weight of rice plants were 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.

Chlorophyll content, Chl fluorescence and FV/FM ratio in leaves of rice plants
Nitrogen significantly increased Chl contents in leaves of rice plants than that in leaves of N-untreated rice plant (Figure 2a). Moreover, NAC significantly increased Chl contents in leaves of rice plants compared to the Chl contents in leaves of NAC-untreated rice plant until plants reached at the panicle initiation stage (Figure 2a). This result suggests that N and NAC both increased Chl content in leaves of rice plants. But Chl content decreased gradually with increasing plant age in leaves of rice plants under N0G0 treatments (Figure 2a). Maximum Chl fluorescences (Fig. 2b) were consistent with Chl content data (Figure 2a). The quantum yield (Fv/Fm ratio; Figure 2c) data supports Chl content data that NAC application increased Fv/Fm ratio in rice plants (Figure 2d). Taken together, these results suggest that NAC might enhance photochemical energy in leaves of rice plants.

Net photosynthesis rate and Relative water content in leaves of rice plants
The net photosynthesis rate and PAR were measured to justify if the 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 ice plants (N1G0) compared than that in leaves of N-untreated plants (N0G0; open round). Figure 3a also shows that the Pn rate increased at the panicle initiation stage then declined thereafter at the ripening stage except in plants grown under N0G0 treatment (Figure 3a, open round). On the other hand, N0G0 treatment narrowed Pn rate gradually with increasing plant age (Figure 3a, open round). Plants showed similar trend results in case of NAC treatment (Figure 3a, closed round and closed square lines). But the Pn rate was significantly higher in leaves of NAC-treated plants than that in leaves of NAC-untreated plants. These results indicate that both applications of N and NAC induced Pn rate in leaves of rice plants.

To explain whether NAC application affects RWC in leaves of the rice plant, RWC was measured in leaves of rice plants at different growing stages. Nitrogen-treated leaves (Figure 3b) accumulated significantly higher RWC than that of leaves of N-untreated plants (Figure 3b). The treatment of NAC did not affect RWC in leaves of N-treated plants (Figure 3b) but affected RWC in N-untreated plants (Figure 3b). In N-untreated plants, NAC treatment significantly increased RWC in leaves compared to NAC-untreated plants (Figure 3b). RWCs in N-untreated plants gradually

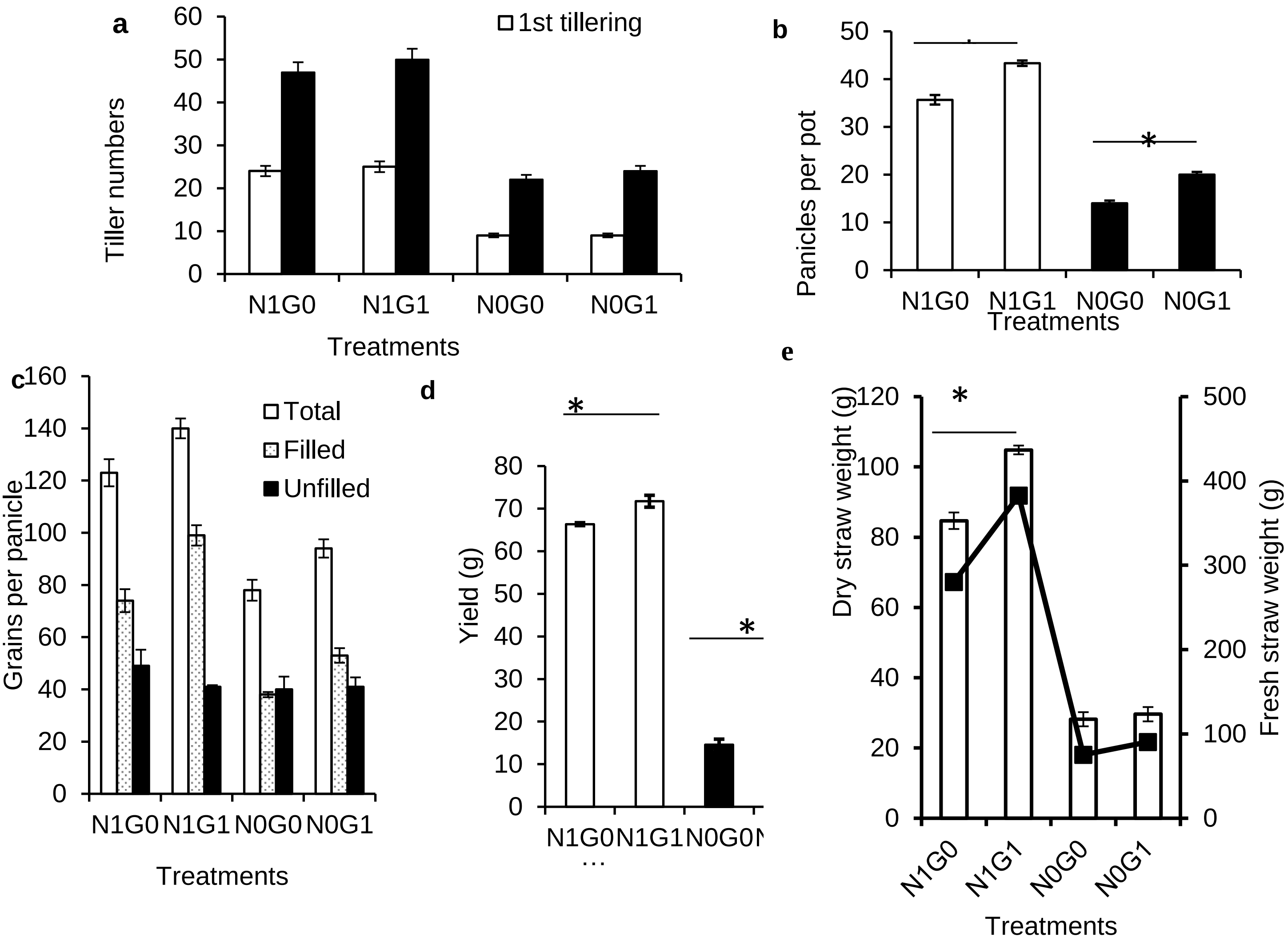

Figure 1. The effects of NAC on yield and yield parameters
a, NAC-induced tiller numbers at the first tillering (open bars) and 2nd tillering (closed bars). b, NAC-induced panicle numbers per pot in N-treated plant (open bars) and N-untreated plants (close bars). c, NAC-induced total grains (open bars)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

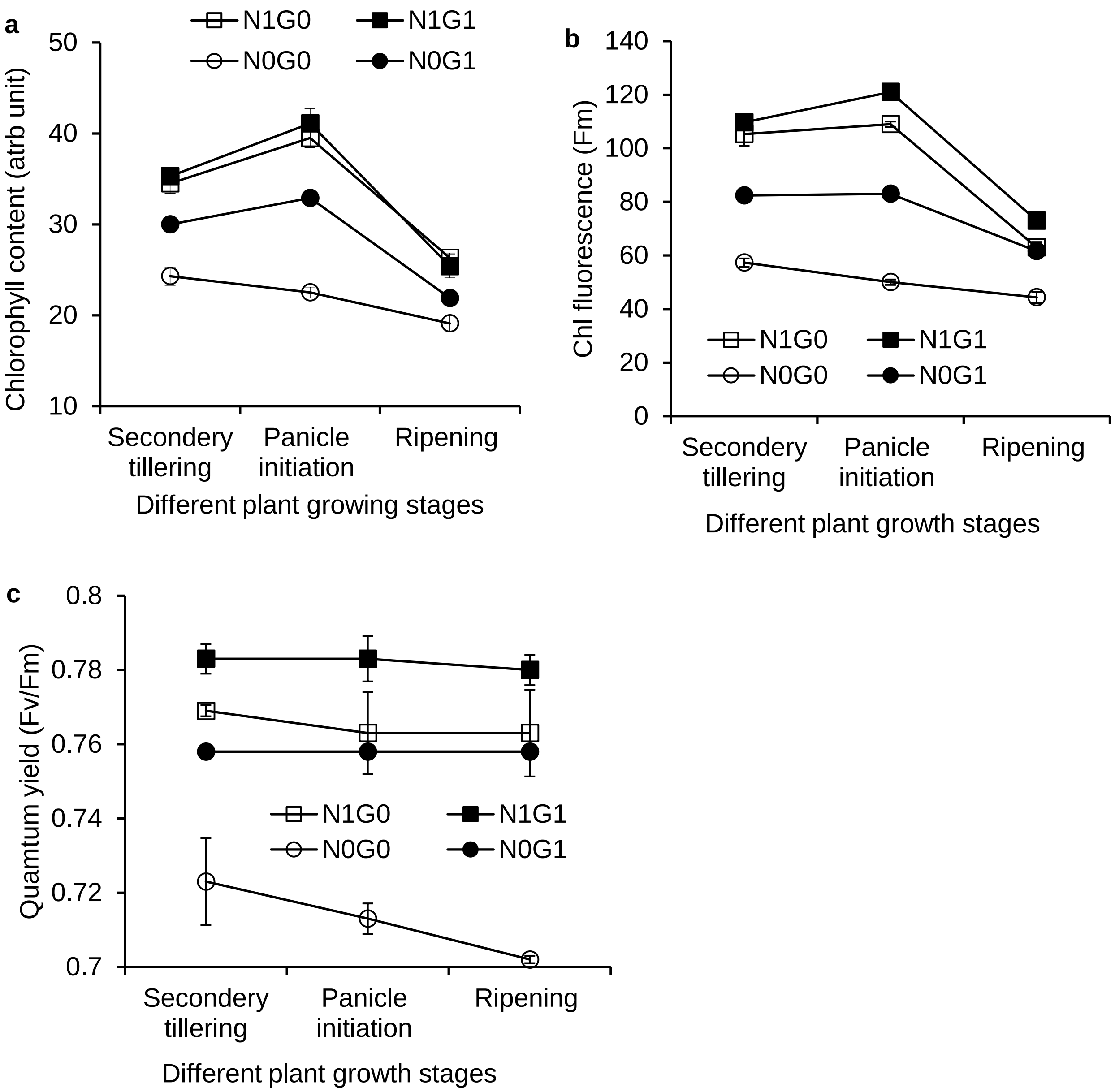

Figure 2. The effects of NAC on Chl content, Chl fluorescence and FV/Fm ratio in leaves.
a, NAC-treated chlorophyll content in leaves of rice plants at different growing stages. b, NAC-treated chlorophyll fluorescence (Fm) in leaves of rice plants at different growing stages. c, NAC-treated quantum yields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

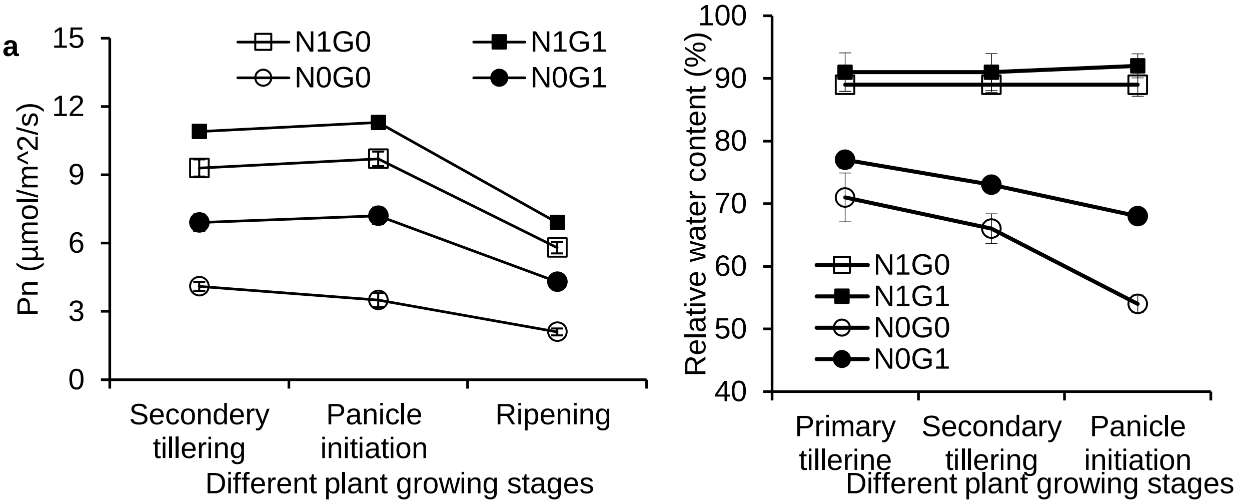

Figure 3. The effects of NAC on the photosynthesis rate and relative water content in leaves of rice plants

decreased with increasing plant age irrespective of NAC treatments (Fig. 3b; open round and closed round). In N-treated plants, RWCs were similar at different plant growing stages. This result suggests that NAC might involve in retaining higher water levels in leaves of rice plants.

Conclusion

Nitrogen significantly increased rice yield through increasing chlorophyll parameters, the Pn rate and RWC in levees of rice plants Further research will be needed to justify the effects of NAC application on phytoavailability of other nutrients to sustain rice production. The costing was justified in this study. Furthermore, according to the price of NAC, farmers would be benefitted if NAC could be applied to the plants with micronutrients as a foliar application to reduce detrimental effects of stress conditions as well as to increase NAC-induce rice production.

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References

ASEAN Food Security Information System (2002). The First Technical Meeting Bangkok, Thailand.

Chelah MKB, Nordin MNB, Musliania MI, Khanif YM and Jahan MS (2011). Composting Increases BRIS Soil Health and Sustains Rice Production on BRIS Soil. ScienceAsia 37, 291-295.

De Datta SK and Buresh RJ (1989). Integrated nitrogen management in irrigated rice. Advance Soil Science 10, 43–169.

IRRI (2002). International Rice Research Institute Annual Report for 2002. Los Baños, Philippines

Jahan MS, Khanif YM, Sinniah UR (2013). Effects of low water input on rice yield: Fe and Mn bioavailability in soil. The Pertanika Journal of Tropical Agriculture Sciences 36, 27-34.

Jahan MS, Nakamura Y, Murata Y (2011). Histochemical quantification of GSH Contents in Guard Cells of *Arabidopsis thaliana*. ScienceAsia 37, 281-284.

Jahan MS, Nozulaidi M, Khandaker MM, Afifah A and Husna N (2014) Control of plant growth and water loss by a lack of light-harvesting complexes in photosystem-II in *Arabidopsis thaliana ch1-1* mutant. Acta Physiology Plantarum 36, 1627-1635.

Kerksick C, Willoughby D (2005). The Antioxidant Role of Glutathione and N-Acetyl-Cysteine Supplements and Exercise-Induced Oxidative Stress. Journal of International Sociely of Sports Nutrition 2, 38-44.

May MJ, Vernoux T, Leaver C, Van Montagu M, Inze D (1998). Glutathione homeostasis in plants: implications for environmental sensing and plant development. Journal of Experimental Botany 49, 649-667.

Medved I, Brown M J, Bjorksten AR (2003). N-acetylcysteine infusion alters blood redox status but not time to fatigue during intense exercise in humans. Journal of Applied Physiology 94, 1572-1582.

Muranaka LS, Giorgiano TE, Takita MA et al. (2013). N-acetylcysteine in agriculture, a novel use for an old molecule: focus on controlling the plant-pathogen Xylella fastidiosa. PLoS ONE 8, e72937.

Okuma E, Jahan MS, Munemasa S, Ogawa K, Watanabe-Sugimoto M, Nakamura Y, Shimoishi Y, Mori IC, Murata Y (2011). Negative regulation of abscisic acid-induced stomatal closure by glutathione in Arabidopsis. Journal of Plant Physiology 168 (17), 2048-55.

Reid MB, Stokic DS, Koch SM (1994). N-Acetylcysteine inhibits muscle fatigue in humans. Journal of Clinical Investigation 94, 2468-2474.

Sarwar MJ, Khanif YM (2005). Techniques of Water Saving in Rice Production in Malaysia. Asian Journal of Plant Science 4, 83-84.

Sarwar MJ, Khanif YM, Syed Omar SR, Sinniah UR (2004). The Effect of Different Water Regimes on Yield and Bioavailability of Phosphorus in Rice Production in Malaysia. Malaysian Journal of Soil Science 8, 53-62.

Schafer FQ, Buettner GR (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. F**ree** Radical Biology and Medicine 30, 1191-1212.

Sen CK (2001). Antioxidant and redox regulation of cellular signaling: introduction. Medicine & Science in Sports & Exercise 33, 368-370.

Swain DK, Jagtap SS (2010). Development of spad values of medium-and long duration rice variety for site-specific nitrogen management. Journal of Agronomy 9, 38-44.