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Glutathione Functions on Physiological Characters of Corn Plants to Enhance Mn-induced Corn Production

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ABSTRACT

A non-protein thiol, glutathione (GSH), presents abundantly in plant and affects the growth and development of the plants. In this study, the effects of N-acetyl cysteine (NAC), a precursor of GSH, on manganese (Mn)-induced corn production was evaluated. Different Mn concentrations (0.2, 1.5 and 3.0 ppm of Mn), with or without 100 µM of NAC, were arranged as completely randomised design with 5 replicates. Results show that both NAC and Mn affected plant height and leaf numbers. Treatment of NAC increased Mn-induced relative water content (RWC), photosynthesis (Pn) and photosynthetically active radiation (PAR) in leaves of corn plants. In the Mn-treated plants, chlorophyll (Chl) content, Chl fluorescence (Fm) and quantum yield (Fv/FM) were found significantly higher than the Mn-untreated plants. In addition, corn plants showed improved yield and cob length in NAC-treated plants in the presence of Mn. Thus, this study suggests that NAC might improve some physiological functions of plants to enhance Mn-induced corn production, with 1.5 ppm of Mn showed the best results.

Keywords: Manganese, relative water content, photosynthesis, chlorophyll content and chlorophyll fluorescence

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INTRODUCTION

Corn (*Zea mays* L.) is the world's leading cereal grain used as a food source for both humans and animals. Previous studies stated that the application of micronutrient fertilisers to micronutrient-deficient soils improved the yield and crop quality for cereals, corn, beans, forages and oil seeds (Malakouti, 2007). Meanwhile, the micronutrients' deficiency reduces plants' performance and profitability (Fisher, 2008) and therefore, micronutrients play an important role in controlling plant growth although plants need a proper balance and low quantity to optimise yield.

Manganese (Mn), which is one of the plant's micronutrients, is involved in plant's metabolic functions such as respiration, photosynthesis, amino acid synthesis and hormone activation through Indole Acetic Acid (IAA)-oxidases (Lido *et al.,* 2004; Ducic & Polle, 2005). It is important that Mn works as a co-factor for most antioxidant enzymes (Cakmak *et al.,* 1999). Excessive Mn concentration in plant tissue affects enzyme activity, absorption, translocation and oxidative stress (Ducic & Polle, 2005; Lei *et al.,* 2007).

On the other hand, glutathione (GSH) is known as the antioxidant that prevents the damage of cell caused by free radicals and peroxides. In plants, GSH is crucial for biotic and abiotic stress management, cellular defence and sulphur metabolism (Noctor *et al.,* 2002). Glutathione functions as antioxidant, stomatal aperture regulator and also direct electron donor to peroxide in reaction that is catalysed by glutathione peroxidase (Murphy & Zarini, 2002; Jahan *et al.,* 2011). These features make glutathione as a multifunctional component in plant (Jahan *et al.,* 2008, 2014a). A recent study stated that NAC improves physiological function and yield of rice plants (Nozulaidi *et al.,* 2015) and seed production in *Arabidopsis* plants (Jahan *et al.,* 2014a).

Mn can change plant growth, chlorophyll content and antioxidant activity, whereby an excess of Mn can reduce root and shoot elongation in *V. faba* plant (Fecht-Christoffers *et al.,* 2003), which are related to photosynthesis production (Henriques, 2003). Rahmati *et al.* (2004) reported that superoxide dismutase and catalase activity increased in Mn-treated cells. In addition, excess of Mn increased reactive oxygen species like superoxide radical, hydrogen peroxide and hydroxyl radical (Demirevska-Kepova *et al.,* 2004; Boojar & Goodarzi, 2008). However, the synthesis of non-enzymatic antioxidants like glutathione (GSH) can protect the plant cell from the harmful effect of ROS (Arora *et al.,* 2002).

N-acetyl cysteine (NAC) improves GSH content in the cells of leaf (Jahan *et al.,* 2014a). To date, no research has been conducted on the effects of NAC on Mn-induced corn production. Therefore, the focus of this study was to justify whether NAC induced physiological parameters of corn plants to enhance Mn-induced corn production. We showed that GSH increased Mn-induced corn production.

METHODOLOGY

Plant Material and Experimental Design

In this study, a hybrid corn variety of L41 was undertaken as the test crop. Two seeds were planted onto seedbed in a hole with a spacing size of 25cm X 75cm. Eight treatments with 5 replicates were arranged as completely randomised design. Four Mn concentrations (0, 0.2, 1.5 and 3.0 ppm of

Mn) were applied with or without NAC (0 and 100 μM) as foliar applications in between 10 am to 12 pm.

Soil and Agronomic Practices

Soil was sandy in texture with 87.2% sand, 7.25% silt and 5.50% clay, soil pH of 5.1, total organic matter of 1.02%. Meanwhile, the Sprinkler irrigation method was used to apply water. Manual weeding method was also applied. However, insecticide was not applied.

Determination of Plant Height and Leaf Number

The plant height was measured from the soil surface to the longest leaf emerged from the whorl by using a measuring tape. In addition, leaf numbers were also counted.

Yield and Yield Parameters

Length and weight of cob were determined after harvest. The length of corn cob was measured with a measuring scale and weigh was also recorded for each treatment.

Determination of Relative Water Content

Fresh weight of leaf (FW) was measured just after collection from the plants before taking turgid weight (TW) of the leaf and after obtaining a full turgidity. Then, the leaves were dried at 60°C for 24 h in an oven, followed by measuring leaf dry weight (DW). Relative water content was determined as previously described by Chelah *et al.* (2011) and Jahan *et al.* (2013). Relative Water Content (%) = [(FW-DW) / (TW-DW)] x 100

FW – Sample fresh weight
TW – Sample turgid weight
DW – Sample dry weight

Determination of Chlorophyll Content in Leaves

A chlorophyll determining meter SPAD-502 (Minolta, Japan) was used to acquire a rapid *in-situ* estimation of chlorophyll (Chl) content from the leaf of corn plants (Khairi *et al.,* 2015a, 2015b). Meanwhile, the second uppermost collared-leaf was used to measure the Chl content. Data were taken in between 11 am to 1 pm to avoid wetness effects on the leaf surface (Jahan *et al.* 2014b). A total of five replicates were implemented.

Determination of Chlorophyll Fluorescence Parameters

A portable Chl fluorescence monitoring meter of Junior-PAM (Walz, Germany) was used to quantify chlorophyll fluorescence in the leaves of corn plants (Jahan *et al.,* 2014a; Khairi *et al.,* 2015b). The second uppermost collared-leaf was selected and data were taken in between 11 am to 1 pm. The maximum fluorescence level (Fm) and quantum yields in PS II photochemistry (Fv/Fm) were recorded.

Determination of Net Photosynthesis Rate and Photosysthetically Active Radiation

A CI-340 portable photosynthesis meter (CID Biosciences, Inc.) was used to measure net photosynthesis rate (Pn) according to Munirah *et al.* (2015). A quantum sensor in the measuring cell was attached to determine photosysthetically active radiation (PAR) data together with Pn data. Data taking procedures were according to the manual. Five replicates were implemented.

Statistical Analysis

Data were analysed for the differences in the mean value among the treatments by using ANOVA procedure and LSD and T-test using Minitab-16 and MS Excel software. Differences at $P < 0.05$ are considered as significant.

RESULTS

Effects of the Mn concentrations on plant's height and number of leaves in presence of NAC

The effect of NAC on plant height was found to be significant (Fig.1a). Results indicated that under no NAC, the plant height increased with increasing Mn concentration, whereas under NAC, plant height increased until 0.2 ppm Mn, followed by plateau. However, plant height was greater at low concentrations of Mn when Mn was applied under no NAC and at high concentration of Mn, and there was no influence on plant height both with and without NAC. This result suggests that NAC increases plant height. The results further indicated that leaf number per plant increased with the increasing Mn concentration under no NAC, whereas leaf number increased with the increasing Mn concentration till 1.5 ppm, followed by a decline under NAC. However, leaf number was higher in the NAC-treated plants than the NAC-untreated plants. Taken together, these results suggest that NAC might affect plant's morphological parameters.

Effects of NAC on Mn-induced RWC and Net Photosynthesis Rate

The effect of NAC on RWC was significant (Fig.2a). Results showed that RWC was greater in NAC treated plants than those of NAC-untreated plants. Under no NAC, RWC increased with the increasing Mn concentration, whereas with NAC, the RWC was higher in Mn applied plant

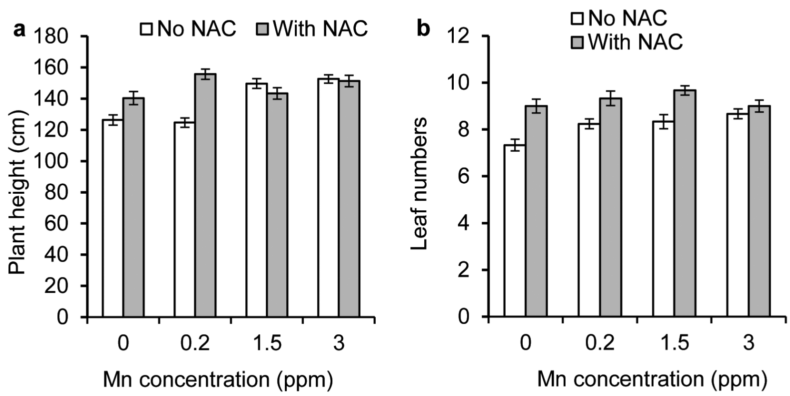

Fig.1: Effects of NAC on Mn-induced plant height (a) and leaf numbers (b) of corn plants.

than the control. In addition, there was no significant difference in RWC amongst the Mn concentrations (Fig.2a; closed bars). This result suggests that NAC might increase RWC in the leaves of corn plants.

Net Pn rate and PAR in the leaves of corn plants were determined under different Mn conditions with or without NAC existence (Fig.2b). Manganese increased Pn rate in the leaves of corn plants than that of Mn-untreated plants (Fig.2b; closed round). In addition, a similar effect of NAC on Pn rate in leaves was observed (Fig.2b; open round). NAC treatment, on the other hand, significantly increased Pn rate, regardless of the Mn treatment. We also measured photosynthetically active radiation which supports Pn data that Pn and PAR might interdependent (Fig.2b; bar graph). This result suggests that NAC enhances Pn rate and PAR in the leaves of corn plants irrespective of the Mn treatment.

Effects of NAC on Mn-induced Chlorophyll Content and Chlorophyll Florescence

The Chl content in the leaves of corn plants was estimated to justify if NAC application affected Mn-induced Chl content in the leaves. Mn-treated plants accumulated higher Chl content than that of the Mn-untreated plants (Fig.3a; open bars). In addition, the Chl contents in the leaves of different Mn-treated plants were similar. In contrast, NAC treatment increased the Chl content in the Mn-untreated plant but not in the Mn-treated plants except 3 ppm of Mn condition (Fig.3a; closed bars), under which the Chl content increased significantly. The chlorophyll fluorescence data (Fig.3b) and quantum yield in photosystem II (Fig.3c) were shown comparable to the chlorophyll content data. This result implies that NAC affects light related reaction in plants which further infers that NAC functions on light-dependent energy production in plants

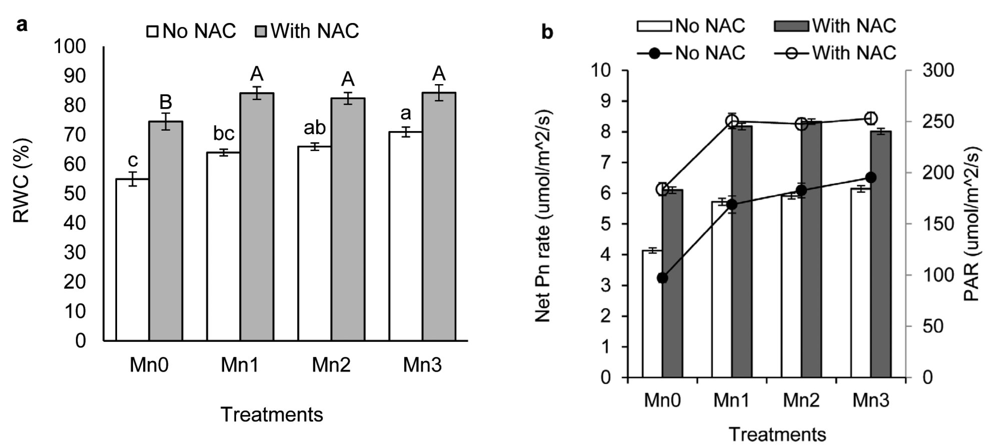

Fig.2: Effects of NAC on different concentrations of Mn-induced RWC (a) and photosynthesis rate (b) in the leaves of corn plants.

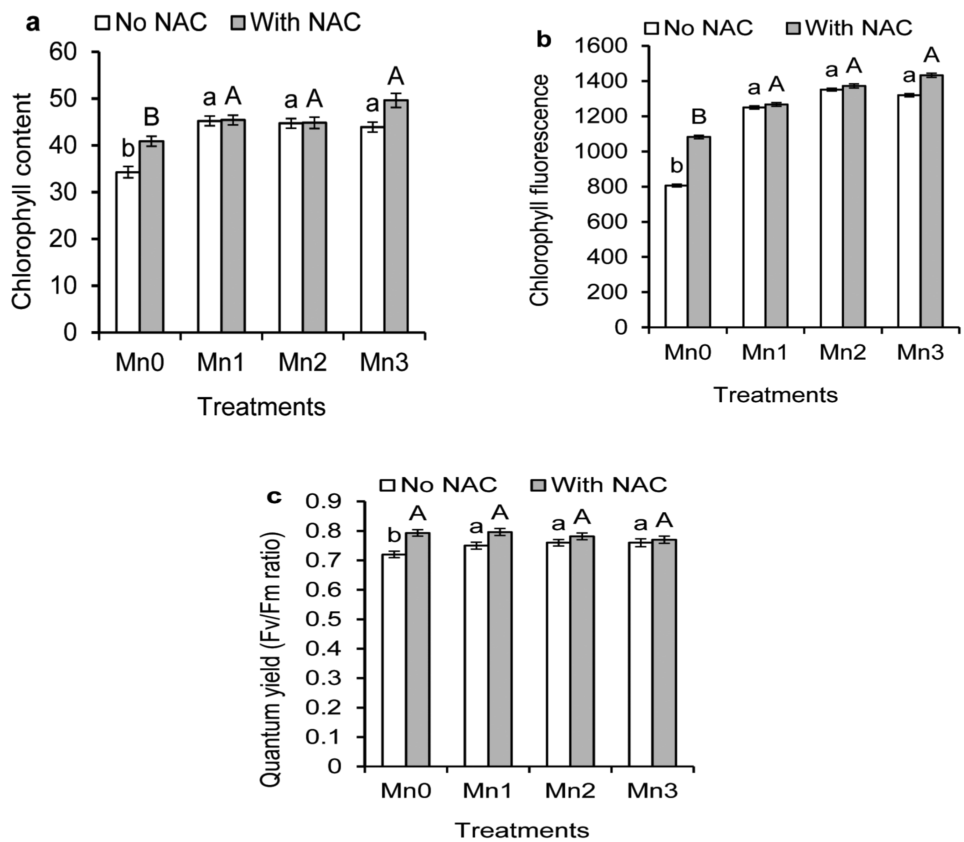

Fig.3:Effects of NAC on Mn-induced Chl content (a), Chl florescence (b), and quantum yield (c) in the leaves of corn plants.

during photosynthesis. Taken together, these results support that NAC might increase Chl content in the leaves of corn plants but the mechanism in this phenomenon is still unknown.

Effects of NAC on Mn-induced Yield of Corn Plants

Results indicated that grain yield was greater in the NAC applied plants than non-NAC applied plant under any doses of Mn (Fig.4a). The results further revealed that under NAC, grain yield increased with the increasing Mn concentration till 1.5 ppm, followed by a decline; under no NAC, however, the grain yield increased with the increasing Mn concentration. In case of the length of corn cob, Mn-treated plants showed longer cob than that of the Mn-untreated plants (Fig.4b; open bars). Additionally, the presence of NAC hastens the size of cob (Fig.4b; closed bars). This result suggests that NAC treatment might increase corn production regardless of Mn application.

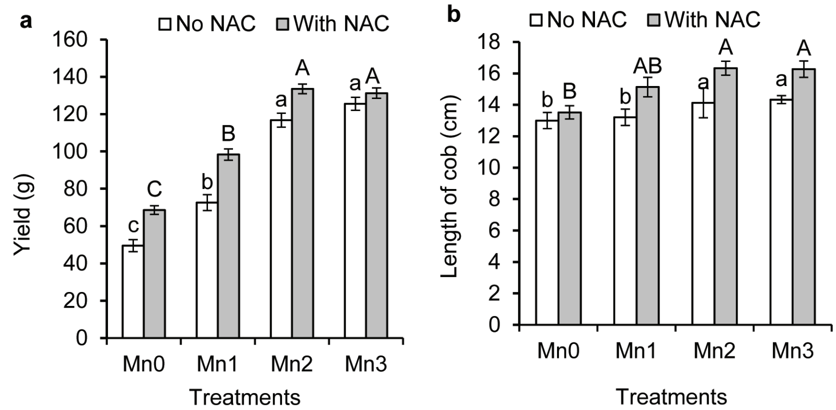

Fig.4: Effects of NAC on Mn-induced yield (a) and cob length (b) of corn plants

DISCUSSION

Glutathione functions on sulphur metabolism, growth, development, cell defense and redox signalling. In specific, GSH scavenges ROS and detoxify toxic chemicals (Noctor & Foyer, 1998; Blum *et al*., 2007). Ascorbate-GSH cycle regulates glutathione level (Halliwell & Asada pathway), and it is likely that alteration of GSH contents may result in adjustment of plant's metabolic function, as well as plant's growth and development (Jahan *et al*., 2008, 2011; Nozulaidi *et al*., 2015). This current study reveals that NAC application increased corn yield through regulation of Mn-induced physiological parameters of corn plants.

Manganese and NAC treatments increased plant height and leaf number (Fig.1), whereby it was observed that plant's height and leaf numbers are regulated by GSH and Mn. A previous study stated that NAC application improved plant physiological parameters of Arabidopsis plants (Jahan *et al*., 2014a) and rice plants (Nozulaidi *et al*., 2015). This may be due to the effects of increasing GSH content in leaves (Jahan *et al*., 2014a) and GSH derivatives synthesised phytochelatins (Rea *et al*., 2004) with micronutrients, including Mn which improves plants' physiological parameters.

In addition, increment of RWC and photosynthetic parameters (Fig.2) implied that Mn might modulate plants' physiological parameters, whereas NAC might enhance Mn-induced enzymatic activity. Chlorophyll, which is a green pigment common to all photosynthetic cells, absorbs light for phosphorylation process and transfers electrons from photosystem II into the photosystem I (Purves *et al*., 1997), suggesting that Chl content increased in the NAC-treated plants (Fig.3a). During the photosynthesis process, light energy uses chloroplastic Chl content and changes it into chemical energy which is stored as sugar bond (Barber, 2006). In this study, Fm values were up in the Mn-treated and

NAC-treated plants than the Mn- and NAC-untreated plants, involving that GSH induced by NAC treatment, might cause energy transfer from PSII to PSI (Jahan *et al*., 2014a) and control physiological parameters (Fig.2). GSH biosynthesis regulates rosette leaves production (Ogawa *et al*., 2004) and flowering time in *ch1-1* Arabidopsis mutants (Jahan *et al*., 2014a). Plant growth and flowering are influenced by nutrient availability, temperature and light intensity (Bernier *et al*., 1993). In addition, these results suggest that NAC treatment might regulate plant physiological parameters, the finding which is supported by Jahan *et al*. (2014a). In this context, this study has shown that NAC controlled the Chl content and Chl fluorescence in the leaves of corn plants (Fig.3). Jiang *et al*. (2010) showed that glutathione modulates the development of Arabidopsis plants, which is similar to the finding of Jahan *et al*. (2014a) that the deficient in GSH content reduced leaf development and plant growth. These results support this study.

Cellular GSH have important consequences in the cell through modification of metabolic functions associated with GSH-regulated functions (Noctor *et al*., 2002). Glutathione can enzymatically and non-enzymatically react in cells (Hwang & Lee, 2006). In more specific, GSH deficient in plants reduces water movement in plants (Jahan *et al*., 2014a), which indicates that NAC may enhance water absorption from soil and induce RWC in leaf (Fig.2a). In addition, Mn application also induces RWC, which may be due to some enzyme activities. Therefore, there may be a positive effect that NAC modulates some extent of physiological functions in plants in the presence of Mn application so that the yield and cob weight increased in Mn- and NAC-treated plants (Fig.4). Taken together, 1.5 ppm of Mn showed the best results in regards to NAC application.

In conclusion, NAC might regulate corn yield through enhancing physiological functions and the functional activity of some enzymes in plants which are to be elucidated in the future research. Application of NAC with Mn as a foliar spray would be benefited the farmers by achieving higher yields.

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REFERENCES

Arora, A., Sairam, R. K., & Srivastava, G. C. (2002). Oxidative stress and antioxidative system in plants. *Current Science, 82*, 1227-1238.

Barber, J. (2006). Photosystem II: an enzyme of global significance. *Biochemical Society Transactions, 34,* 619–631.

Bernier, G., Havelange, A., Houssa, C., Petitjean, A., & Lejeune, P. (1993). Physiological signals that induce flowering. *Plant Cell, 5*, 1147–1155.

Blum, R., Beck, A., Korte, A., Stengel, A., Letzel, T., Lendzian, K., & Grill, E. (2007). Function of phytochelatin synthase in catabolism of glutathione-conjugates. *Plant Journal, 49,* 740-749.

Boojar, M. M. A., & Goodarzi, F. (2008). Comparative evaluation of oxidative stress status and manganese availability in plants growing on manganese mine. *Ecotoxicology and Environmental Safety, 71*, 692-699.

Cakmak, I., & Engels, C. (1999). Role of Mineral Nutrients in Photosynthesis and Yield Formation. In Z. Rengel (Ed.), *Mineral Nutrition of Crops* (pp. 141–168). New York,NY, USA: Haworth Press.

Chelah, M. K. B., Nordin, M. N. B., Musliania, M. I., Khanif, Y. M., & Jahan, M. S. (2011). Composting Increases BRIS Soil Health and Sustains Rice Production on BRIS Soil. *Scienceasia, 37*, 291-295.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Holzer, R., & Feller, U. (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. *Environmental and Experimental Botany, 52*, 253-266.

Ducic, T., & Polle, A. (2005). Transport and detoxification of manganese and copper in plants. *Brazilian Journal of Plant Physiology, 17*, 103-112.

Fecht-Christoffers, M.M., Maier, P., & Horst, W.J. (2003). Apoplastic peroxidases and ascorbate are involved in manganese toxicity and tolerance of Vigna unguiculata. *Physiologia Plantarum, 117*, 237-244.

Fisher, G. E. J. (2008). Micronutrients and animal nutrition and the link between the application of micronutrients to crops and animal health. *Turkish Journal of Agriculture and Forestry, 32*, 221-233.

Henriques, F. S. (2003). Gas exchange, chlorophyll fluorescence kinetics and lipid peroxidation of pecan leaves with varying manganese concentrations. *Plant Science, 65,* 239-244.

Hwang, E. S., & Lee, H. J. (2006). Induction of quinine reductase by allylisothiocyanate (AITC) and the N – acetylecysteine conjugate of AITC in Hepalc1c7 mouse hepatoma cells. *Biofactors, 26*, 7-15.

Jahan, M. S., Ogawa, K., Nakamura, Y., Shimoishi, Y., Mori, I. C., & Murata, Y. (2008). Deficient glutathione in guard cells facilitates abscisic acid-induced stomatal closure but does not affect light-induced stomatal opening. *Bioscience Biotechnology Biochemistry, 72*, 2795–2798.

Jahan, M.S., Nakamura, Y., & Murata, Y. (2011). Histochemical Quantification of GSH Contents in Guard Cells of Arabidopsis Thaliana. *Scienceasia, 37,* 291–295.

Jahan, M. S., Nozulaidi, M. B. N., Khairi, M. B. C. L., & Khanif, Y. M. (2013). Effects of water stress on rice production: bioavailability of potassium in soil. *Journal of Stress Physiology and Biochemistry, 9*, 97-107.

Jahan, M. S., Nozulaidi, M. B. N., Moneruzzaman, M. K., Ainun, A., & Husna, N. (2014a). Control of plant growth and water loss by a lack of light-harvesting complexes in photosystem-II in Arabidopsis thaliana ch1-1 mutant. *Acta Physiologia Plantarum, 36*, 1627-1635.

Jahan, M. S., Muslianie, I., & Khandaker, M. M. (2014b). Effects of Soil Amendments on BRIS Soil Health, Crop Physiology and Production. *International Journal of Research and Innovations in Earth Science, 1*, 1-4.

Jiang, H. W., Liu, M. J., Chen, I. C., Huang, C. H., Chao, L. Y., & Hsieh, H. L. (2010). A glutathione S-transferase regulated by light and hormones participates in the modulation of Arabidopsis seedling development. *Plant Physiology, 154*, 1646–1658.

Khairi, M., Nozulaidi, M., Afifah A., & Jahan, M.S. (2015a). Effect of various water regimes on rice production in lowland irrigation. *Australian Journal of Crop Science, 9*,153-59.

Khairi, M., Nozulaidi M., & Jahan, M. S. (2015b). Effects of Different Water Levels on Physiology and Yield of Salinity Rice Variety. *Australian Journal of Basic and Applied Sciences, 9*, 339-345.

Lei, Y., Korpelainen, H., & Li, C. (2007). Physiological and biochemical responses to high Manganese concentrations in two contrasting Populus cathayana populations. *Chemosphere, 68*, 686-694.

Lido, F.C., Barreiro, M., & Ramalho, J. (2004). Manganese accumulation in rice: Implications for photosynthetic functioning. *Journal of Plant Physiology, 161*, 1235-1244.

Malakouti, M. J. (2007). Zinc is a neglected element in the life cycle of plants: A review. Middle East. *Russian Journal of Plant Science and Biotechnology, 1*, 1-12.

Munirah, N., Khairi, M., Nozulaidi, M., Khandaker, M. M., Mat N. & Jahan, M. S. (2015). The Effects of Zinc Application on Physiology and Production of Corn Plants. *Australian Journal of Basic and Applied Sciences, 9*, 339-345.

Murphy, R. C., & Zarini, S. (2002). Glutathione adducts of oxyeicosanoids. *Prostaglandins Other Lipid Mediators, 69*, 471–82.

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology, 49*, 249-279.

Noctor, G., Gomez, L., Decker, H., & Foyer, C. H. (2002). Interaction between biosynthesis, compartmentation and Transport in the Control of Glutathione Homeostasis and Signalling. *Journal of Experimental Botany, 53*, 1283-1304.

Nozulaidi, N., Jahan, M. S., Khairi, M., Khandaker, M. M., Nashriyah, M., & Khanif, Y. M. (2015). N-acetylcysteine increased rice yield. *Turkish Journal of Agriculture and Forestry, 39*, 204-211.

Ogawa, K., Hatano-Iwasaki, A., Yanagida, M., & Iwabuchi, M. (2004). Level of glutathione is regulated by ATP-dependent ligation of glutamate and cysteine through in Arabidopsis thaliana: Mechanism of strong interaction of light intensity with flowering. *Plant Cell Physiology, 45*, 1–8.

Purves, W. K., Orians, G. H., Heller, H. C., & Sadava, D. (1997). *Life-Tire Science of Biology (5th Edition)*. Sinaur Associates, Inc., pp. 165–188.

Rahmati, M., Yazdani, M., & Ghanati, F. (2004). *Effects of excess amount of Mn on activation of certain enzymes of antioxidant system in suspension-cultured tea cells*. The 2nd Congress on Applied Biology (International Approach). 29-30 Sep. 2004, Mashhad, Iran.

Rea, P. A., Vatamaniuk, O. K., & Rigden, D. J. (2004). Weeds, worms, and more. Papain's long-lost cousin, phytochelatin synthase. *Plant Physiology, 136*, 2463–2474.