RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2015.41.246

ScienceAsia 41 (2015): **246–250**

N-acetylcysteine and Zn regulate corn yield

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Received 17 Sep 2014
Accepted 26 Aug 2015

ABSTRACT: Glutathione (GSH), a non-protein molecule containing thiol groups affects plant growth and development. In this study, the effects of GSH on Zn-induced corn production were evaluated. Different Zn concentrations (0, 0.2, 1.5, and 3.0 ppm of Zn), with or without 100 µm of *N*-acetylcysteine (NAC), were arranged as a completely randomized design with 5 replications. Results show that neither NAC nor Zn affects plant height or leaf numbers. The treatment with NAC did not increase Zn-induced relative water content in leaves while enhancing Zn-induced photosynthesis rate (P_n) and photosynthetically active radiation. Zn-induced chlorophyll (Chl) contents and Chl fluorescence (F_m) were increased by the NAC treatment. In addition, corn showed an improve yield and cob length in NAC-treated plants in the presence of Zn. Taken together, this study suggests that NAC might improve some physiological functions to enhance Zn-induced corn production.

KEYWORDS: glutathione, photosynthesis, chlorophyll content, chlorophyll fluorescence, *Zea mays*

INTRODUCTION

Corn (*Zea mays*), a world leading cereal grain along with rice and wheat, is used as a food source for both humans and animals. Nutrients are one of the factors that limit its growth, yield, and quality. Both macronutrients and micronutrients are essential for the corn growth. Macronutrients are required in a larger quantity than micronutrients. Zinc is one of the micronutrients that affect corn production. Deficiency[1] or excess[2] of Zn might affect plant growth and development, and cause the development of chlorosis, small leaves, and spikelet sterility[3]. Zinc is one of the most abundant trace elements involved in many physiological process. Zn also functions as a cofactor of over 300 enzymes and proteins involved in cell division, nucleic acid metabolism, and protein synthesis[4]. Zinc enhances the growth of cabbage and increases its chlorophyll content[5].

N-acetylcysteine (NAC) is a glutathione (GSH) precursor that enhances GSH content in cells[6]. GSH acts as an antioxidant preventing cell damage caused by the free radicals and peroxides, and functions on guard cells of *Arabidopsis*[7]. In plants, GSH is crucial for biotic and abiotic stress management, cellular defence, and sulphur metabolism[7]. GSH also functions as antioxidant and direct electron donor to peroxide in biochemical reaction catalysed by glutathione peroxidase. These features make GSH a multifunctional metabolite in plants. GSH and Zn are activator of the dipeptidyltranspeptidase that initiates the formation of the heavy metal binding peptide phytochelatin to increase Zn availability in plants[8]. Zinc stabilizes and protects biomembranes against oxidative and peroxidative damage[9]. Zn therefore functions to modulate free radicals and minimize their related damage effects by enhancing antioxidant system of plants[10].

To date, no data were found on the effects of GSH on Zn-induced corn production. The focus of this study was therefore to examine the function of NAC on Zn-induced corn production. The study shows that GSH might influence Zn-induced corn production.

MATERIALS AND METHODS

Plant materials and experimental design

Plant material used in this study was a hybrid corn variety of L41. Two seeds were planted onto seedbed in each hole with a spacing of 25 cm × 75 cm. Eight treatments with five replicates were arranged according to a completely randomized design. Four Zn concentrations (0, 0.2, 1.5, and 3.0 ppm of Zn) were applied, with or without NAC (0 and 100 µM), which increased GSH content in the different cells of leaf[11].

Plant height and leaf number

The numbers of leaf were counted according to Jahan et al[11]. The plant height was measured from the soil surface to the longest leaf emerged from the whorl by straightening the plant to its fullest length, and measured with a measuring ruler alongside.

Yield and yield parameters

Length and weight of cob were determined after the final harvest. The length of corn cob was measured with a measuring scale and weighed for each treatment.

Relative water content

Weight of fresh leaf (FW) was recorded just after collection from the plants and turgid weight (TW) of leaf was taken at full turgidity. The leaves were then dried at 80 °C for 24 h in an oven followed by recording of leaf dry weight (DW). Relative water content was determined as (FW−DW)/(TW−DW), as previously described[12,13].

Leaves chlorophyll content

A chlorophyll-determining meter SPAD-502 (Minolta, Japan) was used to acquire a rapid in situ estimation of chlorophyll (Chl) content from corn leaf[14,15]. The second uppermost collared-leaf was used to measure the Chl content. Data were taken from 11:00–13:00 to avoid wetness effects on the leaf surface. Five replicates were implemented.

Chlorophyll fluorescence parameters

A portable Chl fluorescence-monitoring meter of Junior-PAM (Walz, Germany) was used to quantify Chl fluorescence in leaves of corn plants[16]. The second uppermost collared-leaf was selected and data were taken in between 11 am until 1 pm. The maximum fluorescence level (F_m) and quantum yields in PS II photochemistry (F_v/F_m) were recorded.

Net photosynthesis rate and photosynthetically active radiation

A CI-340 portable photosynthesis meter (CID Biosciences, Inc.) was used to measure the net photosynthesis rate (P_n). A quantum sensor in the measuring cell was attached to determine PAR data together with P_n data. Data taking procedures were followed according to the equipment manual and previous methods[17,18]. Five replicates were implemented.

Statistical analysis

Data were analysed for differences of mean values among the treatments by ANOVA procedure. LSD and *t*-test by using MINITAB-16 and MS EXCEL were used to determine significant differences among the treatments, and the values of $p < 0.05$ were considered statistically significant.

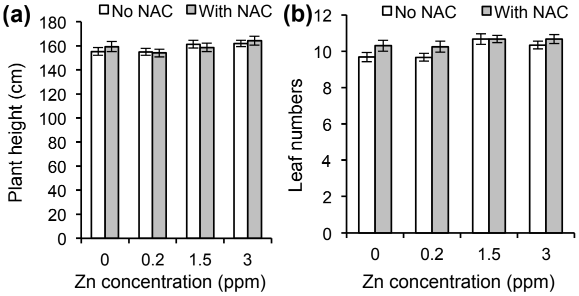

Fig. 1 Effects of NAC on (a) Zn-induced plant height and (b) leaf numbers of corn plants.

RESULTS

Effects of NAC on Zn-induced plant height and leaf number

Zn had no effect on plant height when compared between Zn-treated and Zn-untreated plants (Fig. 1a). Furthermore, NAC did not alter Zn-induced plant height. This result suggests that NAC does not affect Zn-induced plant height. In the presence of Zn, the NAC-untreated and NAC-treated plants showed similar leaf numbers (Fig. 1b). This result also suggests that NAC does not affect Zn-induced plant height.

Effects of NAC on Zn-induced RWC and photosynthesis rate in leaves

To study the effects of NAC on Zn-induced RWC content, RWC in leaves was determined in the presence of Zn and NAC. Zn significantly increased RWC in leaves of corn plants compared to Zn-untreated plants (Fig. 2a). Relative water content increased gradually with increasing Zn concentration and reached a steady level at 1.5 ppm. Nevertheless, the RWC declined significantly thereafter at 3 ppm of Zn compared to those of other Zn treatments. This result indicates that RWC content might depend on the dose of Zn application. In the absence of Zn, NAC significantly increased RWC in leaves of corn plants compared to NAC-untreated plants but, in the presence of Zn, the effects of NAC on RWC were similar to that of Zn-only treatment (Fig. 2a). This result suggests that NAC might increase RWC in Zn-untreated plants but not in Zn-treated corn plants.

Net P_n rate and PAR in leaves of corn plants were determined under different Zn conditions either with or without the presence of NAC (Fig. 2b).

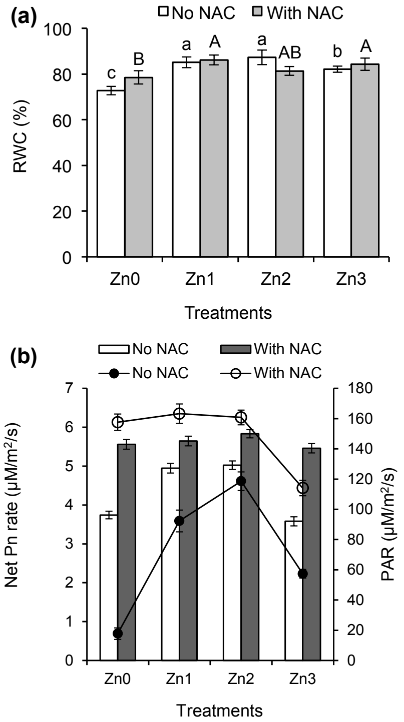

Fig. 2 Effects of NAC on (a) Zn-induced RWC and (b) photosynthesis rate, in leaves of corn plants. In this Fig. and the following, Zn0–Zn3 represent treatments with 0, 0.2, 1.5, and 3.0 ppm of Zn, respectively, and different lower-case or upper-case letters over the bars represent a significant difference at $p \leqslant 0.034$.

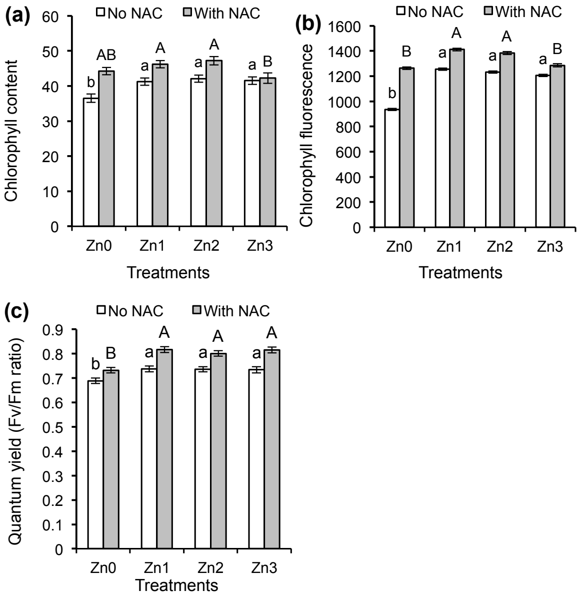

Fig. 3 Effects NAC on (a) Chl content, (b) Chl florescence, and (c) quantum yield, in leaves of Zn-induced corn plants.

Zinc treatment gradually increased P_n rate until it reached a steady level at 1.5 ppm of Zn, then declined thereafter with a Zn concentration of 3.0 ppm. Zinc concentration of 1.5 ppm or less showed better results compared to control or 3.0 ppm of Zn. NAC treatment, on the other hand, significantly increased P_n rate than the Zn-untreated plants. Similar effects of NAC on P_n rate in leaves of plants were observed in case of Zn-treated plants (Fig. 2b). This result suggests that NAC treatment increased the net P_n rate regardless of Zn concentrations. Photosynthetically active radiation was also measured and the result supports the P_n data that P_n and PAR might be interdependent (Fig. 2b). These results suggest that NAC enhances P_n rate and PAR in the leaves of corn plants irrespective of Zn management.

Effects of NAC on Zn-induced chlorophyll content and chlorophyll florescence

Chlorophyll content in leaves was estimated to examine whether NAC application affects Zn-induced Chl content in leaves. Zn-treated plants accumulated higher Chl content than Zn-untreated plants (Fig. 3a). In addition, Chl contents in leaves of Zn-treated plants were similar. These results suggest that Zn application might increase Chl content in leaves of corn plants but different concentrations of Zn might show similar effect on Chl content. In contrast, NAC treatment increased Chl content not only in Zn-untreated plant but also in Zn-treated plants as compared to NAC-untreated plants. The Chl fluorescence data showed similar results to Chl content data where Chl fluorescence increased in leaves of NAC-treated plants than NAC-untreated plants regardless of Zn treatment (Fig. 3b). In addition, quantum yield in photosystem II in leaves of corn plants showed similar data to Chl fluorescence data, which implies that NAC affected light-dependent energy production in plants (Fig. 3c). Taken together, these results support that NAC might increase Zn-induced Chl content in leaves of corn plants, but the mechanism of this phenomenon is still unknown.

Effects of NAC on Zn-induced corn production

The yield and yield parameters were determined based on the weight and length of corn cob (Fig. 4). The yield gradually increased with increasing Zn concentration (Fig. 4a). Although Zn2 (1.5 ppm of Zn) and Zn3 (3.0 ppm of Zn) showed similar performance in terms of yield production, for both treat-

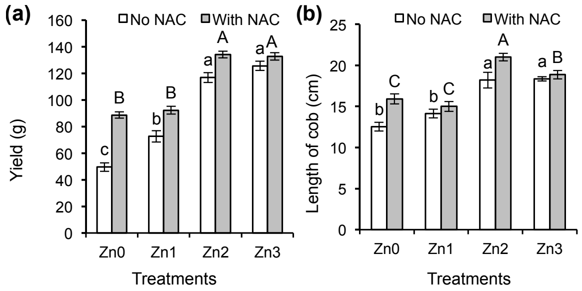

Fig. 4 Effects NAC on (a) Zn-induced yield and (b) cob length of corn plants.

ments the value was significantly higher than for Zn0 (control) and Zn1 (0.2 ppm of Zn) treatments. NAC-treated plants significantly produced higher yield than NAC-untreated plants in the presence or absence of Zn treatment (Fig. 4). This result suggests that regardless of Zn application, NAC treatment might increase corn production. In case of length of corn cob, Zn-treated plants showed larger size than that of Zn-untreated plants (Fig. 4b). In addition, NAC presence in treatment hastened the size of cob.

DISCUSSION

Glutathione has many functions in sulphur metabolism, growth, development, cell defence, and redox signalling. In particular, GSH scavenges reactive oxygen species and detoxifies toxic chemicals[19,20]. Despite the importance of GSH in ROS signalling, few studies have found that GSH contents control plant growth and development[11]. Glutathione level is regulated by ascorbate-GSH cycle (Halliwell and Asada pathway), thus it is expected that alterations of GSH contents may affect plant metabolic function and plant growth. In *Arabidopsis*, a loss-of-function mutation of light harvesting antenna (*ch1-1* mutation) impairs GSH content in cells of leaf and shows depressed growth and development[11]. The current study however reveals that NAC application increases corn yield by modulating Zn-induced physiological parameters.

Zinc and NAC treatments did not affect plant height or leaf numbers (Fig. 1) but it did affect RWC and photosynthetic parameters (Fig. 2), which implies that Zn might modulate physiological parameter while NAC specifically affects photosynthesis but not RWC. Chl content increased in NAC-treated plants (Fig. 3a). Chlorophyll, a green pigment common to all photosynthetic cells, absorbs light for phosphorylation process and transfers electrons from photosystem II into the photosystem I[21]. During the photosynthetic process, light energy is changed to chemical energy using Chl in chloroplast and stored as sugar bond[22]. In this study, we show that F_m values increased in Zn-treated and NAC-treated plants compared to Zn-untreated or NAC-untreated plants, implying that GSH induced by NAC treatment might cause a higher efficiency in energy transfer from PSII to PSI[11] and might control physiological parameters (Fig. 2). Production of rosette leaves was associated with the rate of GSH biosynthesis or the γ-glutamyl cysteine synthetase reaction of GSH synthesis in *Arabidopsis*[22] and light antenna affected GSH content which might regulate the flowering time in *ch1-1* mutant *Arabidopsis* plants[11]. Plant growth and flowering are influenced by nutrient availability, temperature, and light intensity[23]. In addition, the results suggest that the NAC treatment might modulate GSH-regulated plant physiological parameters. In this context, this study showed that NAC controls Chl content and Chl fluorescence in leaves of corn plants (Fig. 3). Glutathione modulates development of *Arabidopsis* plants[24] which is similar to the results by Jahan et al[11] who showed that a deficient GSH content controls leaf development as well as plant growth of *ch1-1* mutant *Arabidopsis* plants. These results agree with our study.

Cellular GSH controls important metabolic functions[6]. Glutathione can enzymatically and non-enzymatically react in cells[25]. GSH deficient in plants might reduce water movement in plants[11] implying that plants might absorb lower amount of water from soil and reduce RWC. This finding supports our study that RWC increased in NAC-treated plants. In addition, Zn application also induces RWC which may be due to functioning of some enzymatic activity. NAC may therefore modulate some physiological function in the presence of Zn application that increases the yield and cob weight (Fig. 4). Taken together, 1.5 ppm of Zn showed the best result with regards to NAC application.

In conclusion, this study implies that NAC might regulate corn yield by enhancing physiological functions and the activity of some enzymes in plants which is to be elucidated in future research. Application of NAC with Zn as a foliar spray would benefit farmers to obtain higher yields.

Acknowledgements: This work was supported by the SEED fund project UniSZA/12/GU(008), Sultan Zainal Abidin University, Terengganu, Malaysia.

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