

RESPONSE OF BIOMASS AND PHOTOSYNTHESIS IN NON-HEADING CHINESE CABBAGE TO EXCESS COPPER

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ABSTRACT

Non-heading Chinese cabbage is one of the main vegetables in Asia, especially in China. To analyze the tolerance of non-heading Chinese cabbage to copper (Cu) stress, 10 μ M, 100 μ M and 1mM copper were used to treat two cultivars of 'Wutacai' as well as 'Erqing' and then photosynthesis, chlorophyll fluorescence parameters, biomass and chlorophyll content were investigated. In our study, 1mM copper significantly inhibited the photosynthesis and plant growth of both two cultivars. While at 100 μ M copper, the growth inhibition was just found in 'Erqing', not in 'Wutacai'. The inhibition of copper on the photosynthesis was not only by stomatal factors but also by non-stomatal factors. Copper stress caused damage of photosystem resulting in reduction of PSII efficiency while non-photochemical-quenching parameter NPQ increased. Besides, at low concentration with 10 μ M, copper promoted the photosynthesis and plant growth. Therefore, the copper tolerance of non-heading Chinese cabbage was dependent on the genotype, and the plant growth inhibition under copper stress was due to the damage of photosystem. Our results will provide a theoretical basis for a further study on non-heading Chinese cabbage tolerance to copper and will be helpful for the production of non-heading Chinese cabbage in heavy metals contaminated areas.

Key words: Copper stress, biomass, photosynthesis, chlorophyll fluorescence parameters, non-heading Chinese cabbage (*Brassica campestris* ssp. *chinensis* Makino).

INTRODUCTION

Copper is an essential micronutrient for plant growth and development. Excess copper, however, will arouse significant toxicity by interfering with the protein function and enzyme activity (Marschner, 1988), affecting sites of the electron transport chain and chloroplast lipid biosynthesis, inhibiting plant growth, and even resulting in plant death (Ouzounidou *et al.*, 1994; Monni *et al.*, 2000).

Photosynthesis is the base of plants growth, synthesizing almost 95% dry weight for plant. Growth inhibition of plants under abiotic stresses produces a wide range of toxic effects on physiological and biochemical processes of plants and photosynthesis is the most sensitive process (Ahmad *et al.*, 2011). Copper stress causing reduction in PSII efficiency is associated with the thylakoid membranes of chloroplasts (Pätsikkä *et al.*, 2002), PSII activity (Pätsikkä *et al.*, 1998), and the quantum yield of PSII electron transport (Vinit-Dunand *et al.*, 2002). In *Paspalum distichum* and *Cynodon dactylon*, even 4 μ M copper drastically inhibited the root length (Shu *et al.*, 2002). Stomatal conductance, leaf transpiration rate, net CO₂ assimilation and biomass, however, were not affected until the treatment with 19.7 μ M of copper in the nutrient solution (Zancheta *et al.*, 2011). While, in wheat, compared to the control, dry

matter yield increased up to 35.2% with application of 8mg kg⁻¹ copper (Cu) in soil (Arshad *et al.*, 2011). It seems for various crops, the effect of copper on the photosynthesis and growth is different.

In this paper, we studied the accumulation and tolerance of copper in two non-heading Chinese cabbage cultivars. The effect on biomass, photosynthesis parameters (P_n , g_s , C_i , T_r) and chlorophyll a fluorescence parameters (F_0 , F_v/F_m , qP, PS_{II} , NPQ) were analyzed, aiming to deal with the following questions: 1) The different copper tolerance of the two non-heading Chinese cabbage cultivars. 2) The response of photosystem under different copper stress and the influence on plant growth, which will be helpful for mechanism understanding of non-heading Chinese cabbage tolerance to copper.

MATERIALS AND METHODS

Plant material and treatment: Seeds of two cultivars ('Erqing' and 'Wutacai') of non-heading Chinese cabbage (*Brassica campestris* ssp. *chinensis* Makino) were treated following the method described by Li *et al.* (2009) in the greenhouse of Nanjing Agricultural University in October. After emergence of the third leaf, the seedlings were transplanted into plastic vessels containing 20 L of 1/2 Hoagland full nutrient solution

(Guo, 2003). Plants at 6-leaf stage were treated with copper at four levels of CuSO_4 including $0.32\mu\text{M}$ (as control), $10\mu\text{M}$, $100\mu\text{M}$ and $1000\mu\text{M}$, respectively. The youngest leaves fully expanded were used for analysis. At the 9th day, photosynthesis and chlorophyll fluorescence parameters of leaves were determined in triplicate at 9:00-11:30 a.m., after that plant biomass were determined and leaves were sampled and preserved at -70°C for the determination of other indicators.

Methods

Photosynthesis: Photosynthesis parameters were monitored with a portable photosynthesis system (Li-6400, USA). Net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) of leaves were measured using the youngest leaves fully expanded on a fine morning. Maintain the leaf temperature at 25°C , the relative humidity at 60-70%, CO_2 concentration at $400\mu\text{L L}^{-1}$, and light intensity at $1000\mu\text{mol m}^{-2}\text{ s}^{-1}$ photosynthetic photo flux density. We measured photosynthesis repeating once for each leaf and five leaves per vessel and in triplicate under each treatment (Zhang *et al.*, 2009).

Chlorophyll fluorescence: Chlorophyll fluorescence was measured on at least 3 leaves each treatment, using a portable fluorometer (PAM2100, Walz, Germany), according to the method described by Zhang *et al.* (2009). Minimum fluorescence (F_0), Maximum fluorescence of dark-adapted leaf (F_m), the steady-state fluorescence (F_s), the minimal fluorescence level in the light-adapted state (F_0') and the maximum fluorescence level in the light-adapted state (F_m') were determined. With the use of fluorescence parameters in both dark and light-adapted leaves, the following calculations were made using symbolic formulas designated as (1) the maximum quantum efficiency of PSII - $F_v/F_m = (F_m - F_0) / F_m$, (2) the coefficient of photochemical quenching-qP = $(F_m - F_s) / (F_m' - F_0')$, (3) non-photochemical quenching of the singlet excited state of chlorophyll a- $\text{NPQ} = F_m / F_m' - 1$, (4) the actual efficiency of PSII- $\text{PSII} = F_v' / F_m' \times \text{qP}$.

Chlorophyll content determination: The fully expanded leaves were cut into pieces, extracted with 10 mL mixed liquid A (ethanol: acetone: water = 4.5:4.5:1,v/v/v) in darkness until the leaf debris were completely into white. Liquid A (as the control) and the immersion were respectively measured at 645 and 663nm in spectrophotometer (Zhang, 1992).

Determination of copper content: Copper content of leaves was determined by inductively coupled plasma-atomic emission spectrometer type PerKin Elmer Optima 2100DV (SPECTRAA 220FS) as described by Li *et al.* (2009).

Statistical analysis: The experiment was conducted at

least in three replicates. Statistical analysis was carried out for all the measured parameters by one-way ANOVA-test using SAS software (SAS institute, Cary, NC). Differences were considered significant at $P < 0.05$ (Gai, 2000).

RESULTS AND DISCUSSION

Effect of copper stress on plant growth and copper contents in non-heading Chinese cabbage: Copper is an essential micronutrient for the growth and development of plants. However, it is also a transition metal element leading to environmental pollution and abiotic stress (Caspi *et al.*, 1999). Suitable concentration of copper favored plant development while excess copper inhibited the plant growth, declined fresh and dry weight. Compared with the control ($0.32\mu\text{M}$ copper), 1mM copper treatment significantly decreased fresh and dry weight in both cultivars (Table 1). However, at $100\mu\text{M}$ copper, the inhibition was just found in 'Erqing', not in 'Wutacai'. While for $10\mu\text{M}$ copper, it showed even better plant growth in both two cultivars (Table 1). In our previous study, the plant growth was inhibited in $10\mu\text{M}$ copper when the plant was 2-3 leaves (Li *et al.*, 2009). In this study, plants were at the six-leaf stage. It implies that copper tolerance is due to not only plant genotype but also plant size. Such similar result had been reported in runner bean by Maksymiec *et al.* (1996).

Plants respond to copper toxicity in two different ways including exclusion mechanism and tolerance mechanism. Exclusion mechanism in the plant roots was that it reduced the absorption and transportation to the shoot to lower the heavy metal content. In our results, more than 90% copper accumulating in root (Fig 1) implied that exclusion mechanism provided the primary protection from copper in non-heading Chinese cabbage, as in other plants including *Rumex dentatus* (Liu *et al.*, 2004) and *Oryza sativa* (Thounaojam *et al.*, 2012).

Effect of copper stress on photosynthesis: Photosynthesis is one of the main factors determining the plant biomass. Under abiotic stress, such as metal (Pätsikkä *et al.*, 2002), salt (St pie and Klobus, 2006) and water stresses (Jeyaramraja *et al.*, 2005), the damage of photosystem leads to decrease of photosystem II (PSII) efficiency and decline of photosynthesis resulting in the inhibition of plant growth (Zhang *et al.*, 2009). In our study, at $100\mu\text{M}$ and 1mM copper treatment, the net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) of leaves in both cultivars were significantly decreased compared to control (Fig. 2). That was accordance with the decrease of plant biomass (Table 1). While at low concentration with $10\mu\text{M}$, copper promoted the plant growth, which could be explained by the increase of net photosynthesis (P_n). Especially for

'Erqing', g_s and T_r were significant increase (Fig. 2). It implied the difference between genotypes. Another reason for increase of plant growth may be that copper is

an essential micronutrient for photosynthetic organisms of plant (Caspi *et al.*, 1999).

Table 1. Effect of 10 μ M, 100 μ M and 1mM copper on the plant biomass of two cultivars for 9 days. Values are mean \pm SD.

Copper treatment	Q		W	
	Fresh weight	dry weight	Fresh weight	dry weight
Control	9.431 \pm 0.690 b	0.500 \pm 0.009 b	7.973 \pm 0.844 b	0.428 \pm 0.042 b
10 μ M	12.867 \pm 1.129 a	0.762 \pm 0.055 a	9.442 \pm 0.203 a	0.671 \pm 0.017 a
100 μ M	8.514 \pm 0.462 bc	0.425 \pm 0.020 c	6.931 \pm 1.062 b	0.406 \pm 0.018 b
1mM	7.577 \pm 0.563 c	0.384 \pm 0.012 c	4.196 \pm 0.615 c	0.279 \pm 0.004 c

Different letters represent significant differences at $p < 0.05$.

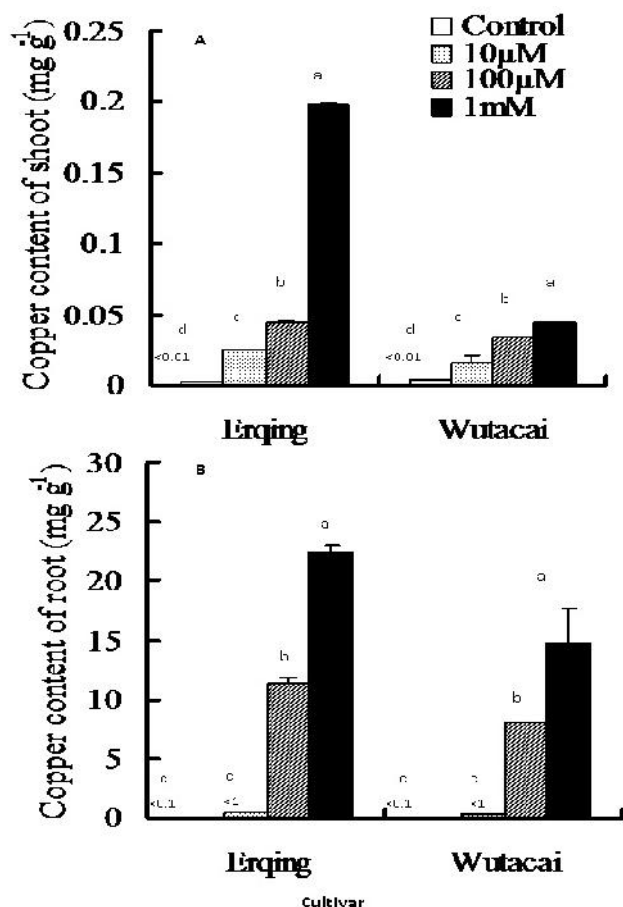


Fig. 1. Copper contents in shoot (A) and root (B) tissues of two cultivars treated by 10 μ M, 100 μ M and 1mM copper. Values are given as mean + SD for each condition. Different letters represent significant differences at $p < 0.05$.

Changes in CO₂ assimilation may be attributable to either stomatal or non-stomatal factors or both. P_n was significantly decreased at both 100 μ M and 1mM copper, accompanied by the decrease of C_i and g_s (Fig. 2). It

suggested that the stomatal factor was the main element influencing CO₂ assimilation. However, when P_n and stomatal closure (T_r and g_s decreased) significantly declined in 1mM copper compared with that at 100 μ M copper, C_i even showed increased (Fig. 2), which implied that non-stomatal limitations to photosynthesis also occurred (Silva *et al.*, 2012).

Effect of copper stress on chlorophyll fluorescence parameters and chlorophyll content: Chlorophyll fluorescence is considered an effective tool to rapidly detect plant photosynthetic apparatus without damage (Lahive *et al.*, 2012). PSII fluorescence parameters F_0 , F_v/F_m , PS II and qP are called to be photochemical-quenching parameters and NPQ is a non-photochemical-quenching parameter. The increase of the minimum fluorescence (F_0) exhibits the damage of photosystem (Krause and Weis, 1991) and the decrease of the maximum quantum efficiency of PSII (F_v/F_m) demonstrates photo inhibition of plant (Björkman and Demmig, 1987). Value of F_0 significantly increased and the chlorophyll content decreased under 100 μ M and 1mM copper treatment in 'Erqing' and 1mM in 'Wutacai', which exhibited the destruction of photosystem in non-heading Chinese cabbage (Fig. 3-A, Fig. 5). In both cultivars, the maximum quantum efficiency of PSII (F_v/F_m) showed no significant changes under all copper stresses indicating that there may no distinct photo inhibition here (Fig. 3-B).

Value of PS II and qP reflect the actual efficiency of PSII and the proportion of the PSII open reaction center as well as reduction of electron participating in CO₂ fixation, respectively. In our study, PS II and qP showed similar trend that significantly decreased under 100 μ M and 1mM copper in 'Erqing' and 1mM copper in 'Wutacai' compared with 10 μ M (Fig. 4-A, B), demonstrating that the decrease of photosynthesis resulted from the decline of exoteric extent of reaction center that less light was used for photochemical reaction (Hu *et al.*, 2006). It is consistent with spinach that the reduction of photosynthesis can be well associated with

the change of PSII function in excess copper (Shioi *et al.*, 1977). Besides that, excess copper also can prevent the integration of chlorophyll in the photosynthetic membrane, inhibit photosynthesis and result in reduction of biomass (Maksymiec, 1997; Liu *et al.*, 2004). The different tolerance between the two genotypes was also implied here. P_{n} , qP and F_0 changed significantly under 100 μ M indicating the more destruction of PSII in 'Erqing' than 'Wutacai'. NPQ as a non-photochemical-quenching parameter relating to the dark-adapted state, whatever, is associated with a change in the efficiency of

heat dissipation (Li and Ong, 1997). As regards the influence of copper on the non-photochemical processes, the result of NPQ showed completely contrary situation to P_{n} and qP . It significantly increased under 100 μ M and 1mM copper in 'Erqing' as well as 1mM copper in 'Wutacai' (Fig. 4-C). Generally speaking, when chlorophyll molecules absorbed light energy, the increase of NPQ could consume excess light and protect the leaves from light-induced damage (Maxwell and Johnson, 2000).

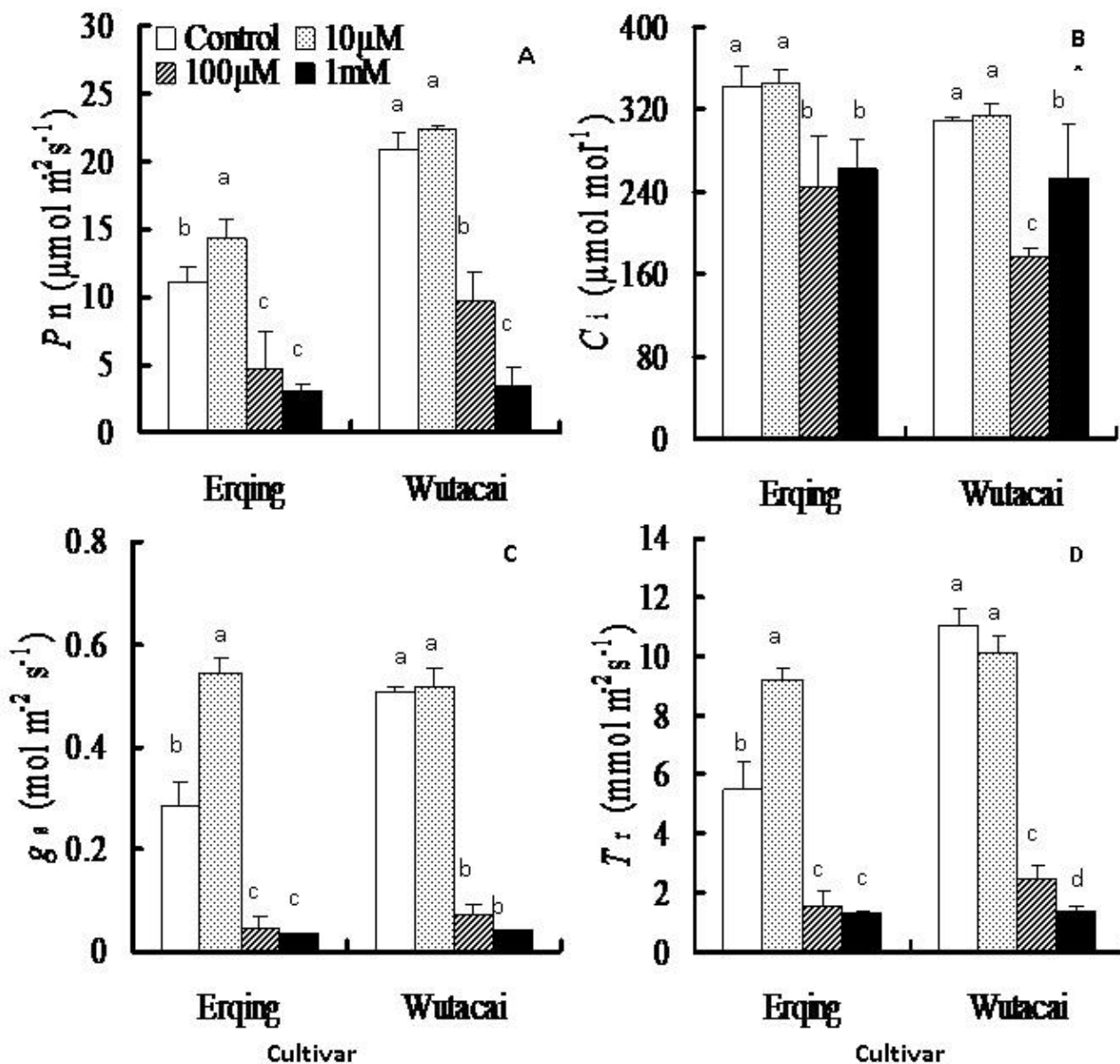


Fig. 2. Effect of 10 μ M, 100 μ M and 1mM copper on net photosynthetic rate (P_n) (A), intercellular CO_2 concentration (C_i) (B), stomatal conductance (g_s) (C) transpiration rate (T_r) (D) of two cultivars. Values are given as mean + SD for each condition. Different letters represent significant differences at $p < 0.05$.

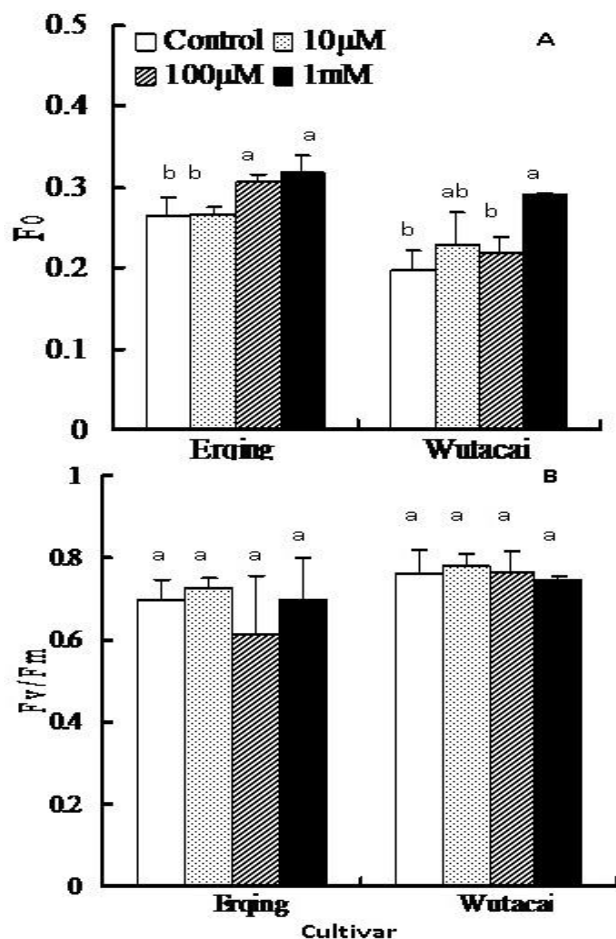


Fig. 3. Effect of 10µM, 100µM and 1mM copper on the chlorophyll a fluorescence parameters minimal fluorescence (F_0) (A) and maximum quantum efficiency (F_v/F_m) (B) of two cultivars. Values are given as mean + SD for each condition. Different letters represent significant differences at $p < 0.05$.

In conclusion, excess copper inhibit plant growth, and the inhibition is dependent on not only genotype, but also plant size and even other environment factors. The inhibition of copper on the photosynthesis is not only by stomatal factor but also by non-stomatal factors. At low concentration with 10µM, copper promoted the photosynthesis and plant growth. From this work, although the two cultivars both presented photosynthetic impairment and similar decreases in growth under the highest copper treatment, we found the differences here: 1) copper induced more damages whether in photosystem II (PSII) or biomass in ‘Erqing’ than ‘Wutacai’ (100µM) ; 2) compared with ‘Erqing’, ‘Wutacai’ showed higher capacity to exclude copper and less accumulation in plant.

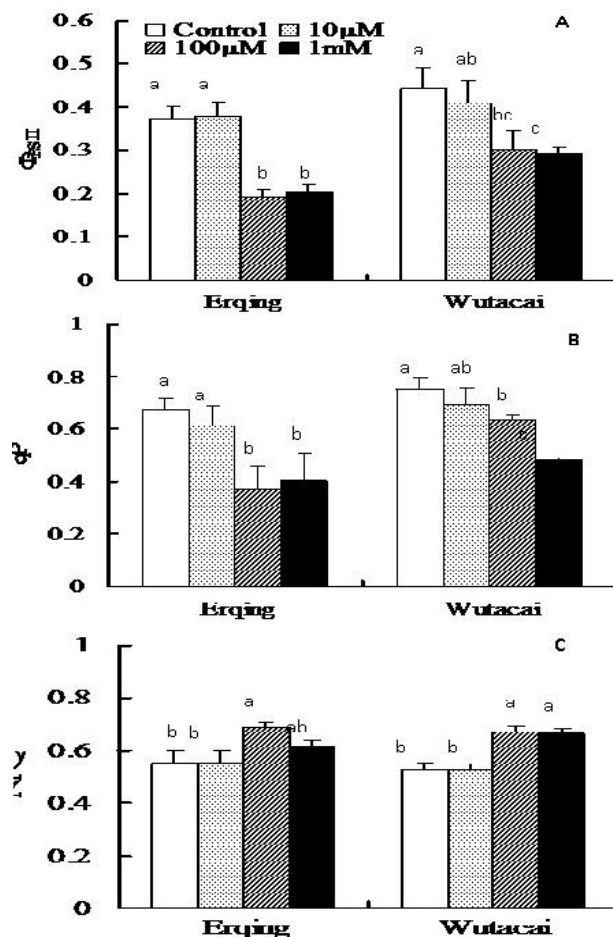


Fig. 4. Effect of 10µM, 100µM and 1mM copper on the chlorophyll a fluorescence parameters the actual efficiency of PSII (ψ_{PSII}) (A), the coefficient of photochemical quenching (q_P) (B), non-photochemical quenching (NPQ) (C) of two cultivars. Values are given as mean + SD for each condition. Different letters represent significant differences at $p < 0.05$.

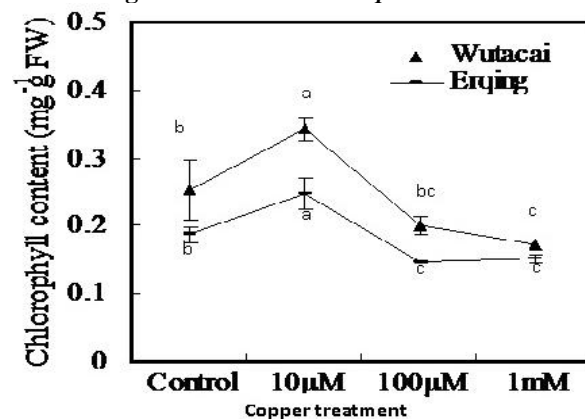


Fig. 5. Effect of 10µM, 100µM and 1mM copper on chlorophyll content of two cultivars. Values are given as mean + SD for each condition. Different letters represent significant differences at $p < 0.05$.

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