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Zinc and α-Ketoglutaric Acid Modulates Plant Growth, Gas Exchange Attributes, Chlorophyll Fluorescence and Zn Content in Rice

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Abstract

The CO_2 increment under climate change leads to adverse growth and quality such as decrease in Zn content of crops. The organic carbon nutrition such as α-ketoglutaric acid (A) is an important carbon source for crops contributing to better crop growth and Zn content in plant. Zn fertilization enhanced its absorption and assimilation resulting in better crop growth. However, the effect of α-ketoglutaric acid and Zn in regulating of its content in rice plant, on growth, photosynthetic attributes and chlorophyll fluorescence are still limited. A pot experiment was conducted by using two aromatic rice cultivars, Meixiangzhan 2 and Yuxiangyouzhan. The rice plants at 3 leaf stage were subjected to two Zn levels (Zn-; 0 $mg{\cdot}kg^{-1}$ and Zn+;10 $mg{\cdot}kg^{-1}$) and two α-ketoglutaric acid levels (A-; 0 $mg{\cdot}L^{-1}$ and A+; 50 $mg{\cdot}L^{-1}$). The results demonstrated strong Zn and α-ketoglutaric acid interactive effect on the plant Zn content and root dry weight. The highest Zn content in plant for Meixiangzhan 2 (19.59 $\mu g{\cdot}g^{-1}$) and Yuxiangyouzhan (25.75 $\mu g{\cdot}g^{-1}$) was observed under Zn-A+ treatment, followed by Zn+A-, Zn+A+ and Zn-A- treatments. The highest root dry weight was found under Zn+A+. Moreover, Zn and α-ketoglutaric acid application and the iteration of Zn and α-ketoglutaric acid strongly affected the chlorophyll fluorescence particularly at 1d, and regulated the photosynthetic parameters. The relationships between the Zn content in plant and the investigated parameters have been also accessed, with Zn content increased, plant height, photosynthetic attributes and chlorophyll fluorescence were improved. The Zn and α-ketoglutaric acid could regulate the Zn content in rice plant which was associated with the modulation of the plant growth, photosynthetic attributes and chlorophyll fluorescence.

Keywords: Zn α-ketoglutaric acid; Plant weight; Photosynthetic attributes; Chlorophyll fluorescence index

Introduction

Rice is one of the important food crops and the increasing population globally raises demand for more rice production (Tilman *et al.* 2011; Valin *et al.* 2014; Searchinger *et al.* 2018). The climate change have great impact on rice yield (Xiong *et al.* 2014; Lv *et al.* 2018) and adverse environmental condition result in decrease of crop yields or grain quality (Lieffering *et al.* 2004; Peng *et al.* 2004; Farooq *et al.* 2009; Högy and Fangmeier 2009; Högy *et al.* 2009; Erbs *et al.* 2010; Samuel *et al.* 2014). Feasible strategies to address the negative influences of climate change on rice yield and quality are desired.

Global warming and CO_2 increment under climate change are two important aspects in affecting crop yield and quality (Pittelkow *et al.* 2014; Samuel *et al.* 2014). The increase of atmospheric CO_2 concentration would result in a significant decline in the content of iron, zinc and crude protein in wheat, rice and soybean (Samuel *et al.* 2014). The increase of CO_2 will also affect the temperature, which influence the physiological and gas exchange attributes of rice (Li *et al.* 2019a). Lieffering *et al.* (2004) indicted that the CO_2-induced increases in yield will affect the nitrogen supply. Shimono *et al.* (2009) suggested that the strong growth enhancement before heading is associated to grain yield under elevated CO_2. Eventually, inconsistent results of grain yield and quality response to the increment of CO_2, a key carbon source of photosynthetic were detected and limitations to CO_2 in improving crop yield and quality is obvious (Ziska and Bunce 2007; Taub *et al.* 2008; Mcgrath and Lobell 2013; Bloom *et al.* 2014; Myers *et al.* 2014; Usui *et al.* 2014 2015). Therefore, Liao *et al.* (2013 2014)

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indicated the organic carbon nutrition application to increased yield and quality of crop. Moreover, Gui *et al.* (2015) suggested that organic carbon nutrition and α-ketoglutaric acid increased dry matter accumulation in rice. Additionally, with application of organic carbon nutrition (α-ketoglutaric acid) the Zn and Fe content in water spinaches were increased (Gui *et al.* 2016). The α-ketoglutaric acid is an important dicarboxylic acid and an intermediate in the tricarboxylic acid cycle (TCC) and amino acid metabolism. It is of particular industrial interest due to its broad application scope, such as building block chemical for synthesis of heterocycles, dietary supplement, component of infusion solutions, and wound healing compounds (Chernyavskaya *et al.* 2000; Stottmeister *et al.* 2005; Huang *et al.* 2006). The α-ketoglutaric acid can be used for the production of an agent that protects humans or animals from oxidative stress by increasing the antioxidant capacity (Otto *et al.* 2011). Thus, approaches such as using the organic carbon nutrition to supply carbon source with less negative effects for crops plant seems viable.

Zinc (Zn) deficiency have a negative effect on human health (Wessells and Brown 2012; Black *et al.* 2013) and its malnutrition is attributed to a lack of access to nutritious food and/or the poor Zn content in staple food (Hotz and Brown 2004). Rice is one of the most important cereals, characterized as having low grain Zn content and sensitivity to soil Zn deficiency, particularly those under rice paddy cultivation systems (Wissuwa *et al.* 2008). Zn has important effect on crop yield and quality (Rehman *et al.* 2012; 2018; Ghasal *et al.* 2017; Kumar *et al.* 2017; Li *et al.* 2018) and its application significantly affected plant growth, yield formation, the concentrations and uptakes of Zn in plant (Fageria *et al.* 2011). Thus, sustainable increase in grain quality and with high Zn content in grain in feasible by Zn application (Li *et al.* 2018). It is also important for the biochemical process in plants (Vaughan *et al.* 1982; Cakmak and Marschner 1987; 1988; Obata *et al.* 1999). Additional Zn influences the chlorophyll development (Ramani and Kannan 1985) and its deficiency results in a reduction in the net photosynthetic rate and stomatal conductance (Tavallali 2017). To sum up, Zn supply drive the improvement of Zn content and plant growth even increase crop yield.

It is thus clear that the increase of atmospheric CO_2 concentration could improve the carbon source of plant photosynthesis but lead to decrease in the Zn content and limitation of CO_2 in promoting yield and quality in crops is obvious (Samuel *et al.* 2014). Besides, the supplement of the organic carbon nutrition such as α-ketoglutaric acid, as a carbon source for plant, could improve plant growth and Zn absorption (Gui *et al.* 2016). Moreover, Zn application contributes to better crop growth, yield and quality (Ghasal *et al.* 2017; Kumar *et al.* 2017; Li *et al.* 2018). Therefore, it is hypothesis that, α-ketoglutaric acid and Zn application could improve the rice plant growth and increased the Zn assimilate. However, little study on the effect of Zn and α-ketoglutaric acid on pant growth has been studied. This study was conducted to evaluate the Zn and α-ketoglutaric acid effect on rice plant growth, Zn content in plant and the photosynthesis parameters as well as the chlorophyll fluorescence index; and to access the relationship of the investigated parameters.

Materials and Methods

Experimental description

A pot experiment was carried out at College of Agriculture, South China Agriculture University, Guangzhou, China. The main soil properties of the experiment were as follow: pH 5.1, organic matter content 25.7 $g \cdot kg^{-1}$, available nitrogen 85.51 $mg \cdot kg^{-1}$, available phosphorous 25.11 $mg \cdot kg^{-1}$, available K 153.2 $mg \cdot kg^{-1}$ and available Zn 0.52 $mg \cdot kg^{-1}$.

Experimental design

Before sowing, the seeds of the two commercial aromatic rice cultivars, Meixiangzhan 2 and Yuxiangyouzhan were soaked in water for 24 h at room temperature and germinated in dark chamber at 30°C for next 24 h. To ensure better vegetative growth, NPK fertilizer (15:15:15) was applied at basal stage with 5 $g \cdot kg^{-1}$ soil of fertilizer at 2 days prior to sowing. Both varieties were sown in pots (9 cm height and 15 cm diameter). After thinning, 50 constant plants were kept in each pot for further treatment. At 3 leaf stages, two Zn application levels: 0 $mg \cdot kg^{-1}$ and 10 $mg \cdot kg^{-1}$ (Haldar and Mandal 1981) in the form of $ZnSO_4 \cdot 7H_2O$ to the soil and two levels of α-ketoglutaric acid (0 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$) were sprayed to the plants in once every 24 h and three times in total and each time with 3 mL per pot, the α-ketoglutaric acid solution was mixed with tween-60 (5%) before spraying. The treatments were arranged in randomized complete block design with six replications.

Observations

Plant dry weight and height: At four days after α-ketoglutaric acid treatment, the plant height was measured from 10 seedlings for each replicate. These plants were harvested and washed with distill water, then immediately divided into root and shoot parts, for oven-dried weight at 80°C (Li *et al.* 2019b; Mo *et al.* 2019).

Measurement of plant Zn content: Plant Zn content was measured by using the Atomic Absorption Spectrophotometer (AA6300C, Shimadzu, Japan). Three plant samples were placed in muffle furnace at 500°C and burnt to ashes for the determination of ions (Zn) contents as described by Wu *et al.* (2014).

Photosynthetic parameters

After application of α-ketoglutaric acid, the photosynthesis characters were measured at 1 d, 2 d and 3 d after treatment, with the portable photosynthesis system (LI-6400, LI-COR, U.S.A.) during 9:00 – 11:00 a.m. according to Pan *et al.* (2016). The top fully expanded leaves of three represented seedlings from each three different pots of each cultivar were selected to measure the net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr).

Chlorophyll fluorescence index

The Pulse Amplitude Modulation (PAM) fluorometry was conducted using a PAM2500 (Walz) at 1 d, 2 d and 3 d after treatment (Ritchie and Bunthawin 2010; Cuddy *et al.* 2013). The top fully expanded leaves of three represented seedlings from each three different pots of each cultivar were selected for chlorophyll fluorescence measurement. The values of Fo, Fm, Ft, Fm' and Fo' were used to calculate the fluorescence parameters: the maximum photochemical efficiency of photosystem II (Fv/Fm), electron transport rate (ETR), effective photochemical efficiency of photosystem II (Φ(PSII)), regulated heat dissipation quantum yield Y(NPQ), non-regulated heat dissipation quantum yield Y(NO), photochemical quenching (qP) and non-photochemical quenching (NPQ) were calculated.

Statistical analysis

Analysis of variances (ANOVA) was performed by the Linear Model Procedure of Statistix version 8 (Statistix 8, Analystical, Tallahassee, FL, USA). Comparisons of means among different treatments were made according to the least significant difference (LSD) at the 5% probability level. The figures were made by using the SigmaPlot for windows version 10.0 (Systat Software Inc., San Jose, CA, USA).

Results

Analysis of variance of the investigated parameters

Varieties (V) significantly affected Zn content, plant height, shoot and root dry weights, Pn and Tr at 3 d after treatment. Remarkable difference of Zn application was observed for plant height, Pn and Ci at 3 d, gs and Tr at 2 d and 3 d. The α-ketoglutaric acid (A) significantly affected Pn, gs and Ci as well as Tr at 2 d, Ci at 3 d. Significant differences of V×Z was detected in Pn at 3 d, gs and Tr at 2 d, Ci at 1 d. Besides, Ci and Tr at 2 d, Ci at 3 d were affected considerably for V×A. Moreover, Z×A strongly affected the Zn content and root dry weight. And significant effects of V×Z×A were observed for Zn content. Further, dramatically effect of V, Z, V×Z, V×A, Z×A, V×Z×A were observed for the chlorophyll fluorescence parameters at 1d and for less chlorophyll fluorescence at 2 and 3 d (Table 1).

Plant Zn content and height: The Zn+A-, Zn-A+ and Zn+A+ treatment improved Zn content in plant. Compared with Zn-A-, the Zn content in Yuxiangyouzhan in shoot for Zn+A-, Zn-A+, and Zn+A+ significantly increased by 27.93, 41.19 and 7.02%, respectively, but no significant effect was detected for Meixiangzhan 2. Besides, remarkable increased in plant height for Zn-A+ treatment was observed for Yuxiangyouzhan only (Fig. 1).

Shoot and root dry weights: Slightly increase in shoot dry weight was observed. Significant increase in root dry weight was observed for Zn+A+ treatment for Yuxiangyouzhan, but no significant difference in root dry weight for Meixiangzhan 2 (Fig. 2).

Photosynthetic parameters: The highest net photosynthetic rate (Pn) at 1d, was observed in plants subjected to Zn+A+, but there was no significant difference in Pn at 1 d for Meixiangzhan 2. In case of Zn-A+ treatment, remarkable increment of Pn at 2 d was detected for Meixiangzhan 2 and no significant difference in Pn at 3 d (Fig. 3A). For treatments with Zn-A+ and Zn+A+ increase was noted in Pn at 1 d for Yuxiangyouzhan but no significant difference in Pn at 2 d. Apart from the lowest Pn at 3 d found at Zn+A+ for Yuxiangyouzhan with a significant reduction of 15.77%, there was no dramatic difference in other treatments of Yuxiangyouzhan (Fig. 3B). No significant difference in stomatal conductance (gs) at 1 d for Meixiangzhan 2. While lowest gs was obtained at 2 d under Zn+A- for Meixiangzhan 2. Meanwhile, there was significant difference noted in Zn-A- and Zn+A- (Fig. 3C). In plants treated by Zn+A+ notable decrease in gs at 1d was detected for Yuxiangyouzhan. The treatment Zn-A- showed highest gs at 2 d for Yuxiangyouzhan and lowest in plants treated with Zn+A+ at 3 d for Yuxiangyouzhan (Fig. 3D). The intercellular carbon dioxide concentration (Ci) at 1 d value decreased with treatment Zn-A+ and Zn+A+ for Meixiangzhan 2 and Yuxiangyouzhan. The significant decrease in Ci at 2 d value was found under Zn+A+ for both varieties. The Ci at 3 d decreased significantly after application of Zn+A+ for Meixiangzhan 2, Zn-A+ for Yuxiangyouzhan (Fig. 3E and F). Compared with Zn-A- for Meixiangzhan 2, Zn+A-, Zn-A+ and Zn+A+ significantly decreased the transpiration rate (Tr) at 1 d value. There was decrease in Zn+A- at 2 d for Meixiangzhan 2. Meanwhile, Zn-A+ and Zn+A+ decrease dramatically at 2 d for Yuxiangyouzhan. In plants treated with Zn-A+ and Zn+A+ decrement was observed in Tr at 3 d for both varieties (Fig. 3G and H).

Chlorophyll fluorescence: Compared with Zn-A- treatment, the value of the maximal photochemical efficiency of photosystem II (Fv/Fm) at 1 d significantly increased with Zn+A-, Zn-A+ and Zn+A+ for Meixiangzhan 2 (Fig. 4A). For Yuxiangyouzhan, significant increase in Fv/Fm at 1 d in Zn+A+ was observed (Fig. 4B). There was no significant difference in Fv/Fm at 2 d or 3 d for both Meixiangzhan 2 and Yuxiangyouzhan varieties.

The electron transfer rate (ETR) at 1d was strongly affected by Zn+A-, Zn-A+ and Zn+A+ for Meixiangzhan 2 with an increment of 27.04, 46.59 and 44.89%, respectively. The introduction of Zn-A+ resulted in a significant reduction in ETR for Meixiangzhan 2. There was no difference in ETR

Table 1: Analysis of variance of investigated parameters

Index	V	Z	A	V×Z	V×A	Z×A	V×Z×A
Zn content	28.81*	4.99	0.47	2.52	0.25	53.23**	20.80**
Plant height	210.79**	12.58**	0.27	4.71	0.34	3.48	0.12
Shoot dry weight	114.97**	0.32	0.09	0.09	0.03	0.03	0
Root dry weight	806.1**	2.17	2.71	0.29	0.36	9.31**	0.1
Pn at 1 d	0.69	16.67*	8.66*	0.09	1.08	0.08	3.53
Pn at 2 d	0.06	7.55	16.34**	3.18	0.18	1.73	0.17
Pn at 3 d	179.63**	80.34**	8.86*	29.22**	0.03	4.76	5.16
gs at 1 d	2.29	2.01	3.55	0.06	1.30	1.11	0.21
gs at 2 d	2.29	56.42**	38.42**	63.61**	4.92	1.66	1.19
gs at 3 d	15.82	132.38**	0.14	4.31	0.30	2.88	0
Ci at 1 d	0.01	44.26*	0	18.55*	0.05	0.36	1.15
Ci at 2 d	1.49	13.62	14.24**	0.06	7.24*	0.42	0.05
Ci at 3 d	3.24	70.23*	16.96**	1.32	14.41**	1.25	0.14
Tr at 1 d	1.52	0.95	1.68	0.14	1.20	0.80	0.95
Tr at 2 d	2.68	13.67*	35.23**	36.55**	6.53*	2.69	1.58
Tr at 3 d	56.3*	69.25**	0.01	0.24	0.05	2.20	0.03
Fv/Fm at 1 d	17.76	122384**	168105**	222852**	95443.4**	9392.78**	28561.6**
Fv/Fm at 2 d	0.50	5.5	3.00	3.95	1.19	0.14	0.01
Fv/Fm at 3 d	117.44	8.47	2.31	32*	2.35	0.61	0.03
ETR at 1 d	122551**	1056591**	138233**	56512.8**	108544**	21554**	112313**
ETR at 2 d	0.11	35.24*	3.81	0.15	0.23	1.20	0.46
ETR at 3 d	30.17	1578.91**	0.13	74.71*	2.04	0.64	0.75
Φ(PSII) at 1 d	150508**	1064652**	142733**	57389.4**	108248**	209351**	106573**
Φ(PSII) at 2 d	0.16	33.63*	3.75	0.14	0.21	1.22	0.41
Φ(PSII) at 3 d	29.76	1224.57**	0.12	62.77*	2.22	0.64	0.66
Y(NPQ) at 1 d	32195.30**	128964**	28230.70**	107173**	11290.7**	149318**	1580.39**
Y(NPQ) at 2 d	46460.4**	0.46	2.42	0.03	0.16	0.07	0.49
Y(NPQ) at 3 d	25.82	71.6*	7.35	34.86	4.76	19.59*	1.05
Y(NO) at 1 d	380162**	114661**	881793**	72854.4**	54617.8**	51456.4**	147274**
Y(NO) at 2 d	35.92	1.19	20.53*	0.01	1.28	0.50	0.22
Y(NO) at 3 d	674.77*	7.42	3.62	16.53	0.09	2.89	0.06
qP at 1 d	7.2E+30**	6.6E+31**	5.5E+31**	9.0E+29**	3.7E+31**	5.6E+30**	1.4E+31**
qP at 2 d	0	6.86	8.99	0.02	1.85	3.12	1.06
qP at 3 d	4751.03**	118.75**	0.40	0.62	1.77	0.02	0.14
NPQ at 1 d	21000000**	7124.05**	1636299**	1417281**	57399.8**	311471**	544756**
NPQ at 2 d	1.40	0.05	2.81	1.11	0.07	0.12	0.49
NPQ at 3 d	109.03	3.62	7.03	12.04	0.80	9.89*	1.26
qN at 1 d	8.3E+29**	6.4E+29**	1.9E+31**	1.6E+31**	6.3E+28**	1.2E+31**	1.7E+30**
qN at 2 d	11.93	0.12	5.41	0.07	0.35	0.02	0.51
qN at 3 d	34.11	32.5*	7.63	33.11*	0.59	19.88*	0.93

* and ** represent significant difference at 0.05 and 0.01 level, respectively

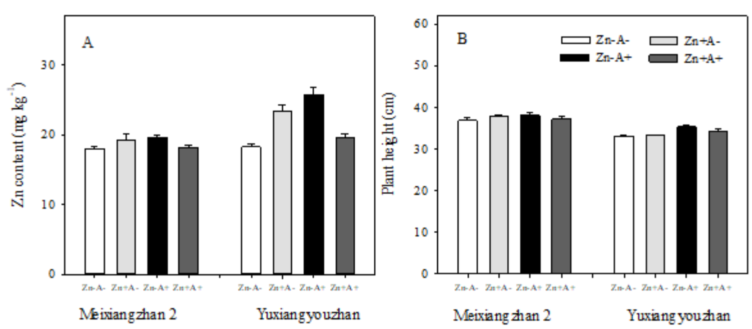

Fig. 1: Effect of zinc and α-ketoglutaric acid on Zn content in rice plant and plant height. Vertical bars are means of the investigated parameters and the capped bars represent SD

at 3 d for Meixiangzhan 2 (Fig. 4C). Nevertheless, for Yuxiangyouzhan, Zn+A-, Zn-A+ and Zn+A+ resulted in a 2.67, 19.75 and 18.43% improvement in ETR at 1 d respectively comparing to Zn-A-. The treatment Zn-A- slightly increased the ETR at 2 d for Yuxiangyouzhan. The highest of ETR at 2 d was detected in Zn-A+ treatment and significantly higher than Zn-A-. It should be noted that for Yuxiangyouzhan, treatment Zn-A+ and Zn+A+ caused a visible increase in ETR at 3 d (Fig. 4D).

A significant increase of the effective photochemical efficiency of photosystem II (Φ(PSII)) in Zn-A+ and Zn+A+ at 1 d for Meixiangzhan 2 was observed when comparing with Zn-A- and up to 46.28% for Zn-A+ and 44.89% for Zn+A+. However, Zn+A- showed significant decrease in

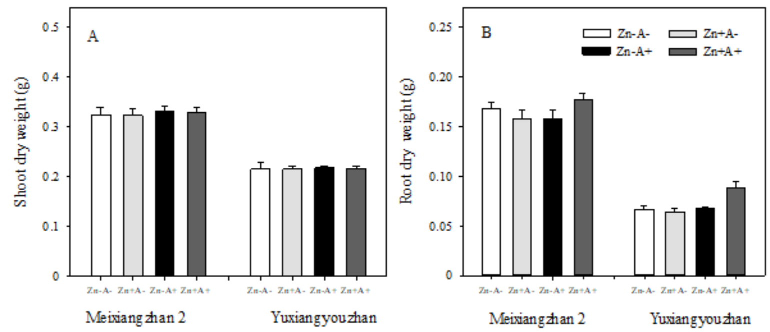

Fig. 2: Effect of zinc and α-ketoglutaric acid on shoot and root dry weight. Vertical bars are means of the investigated parameters and the capped bars represent SD

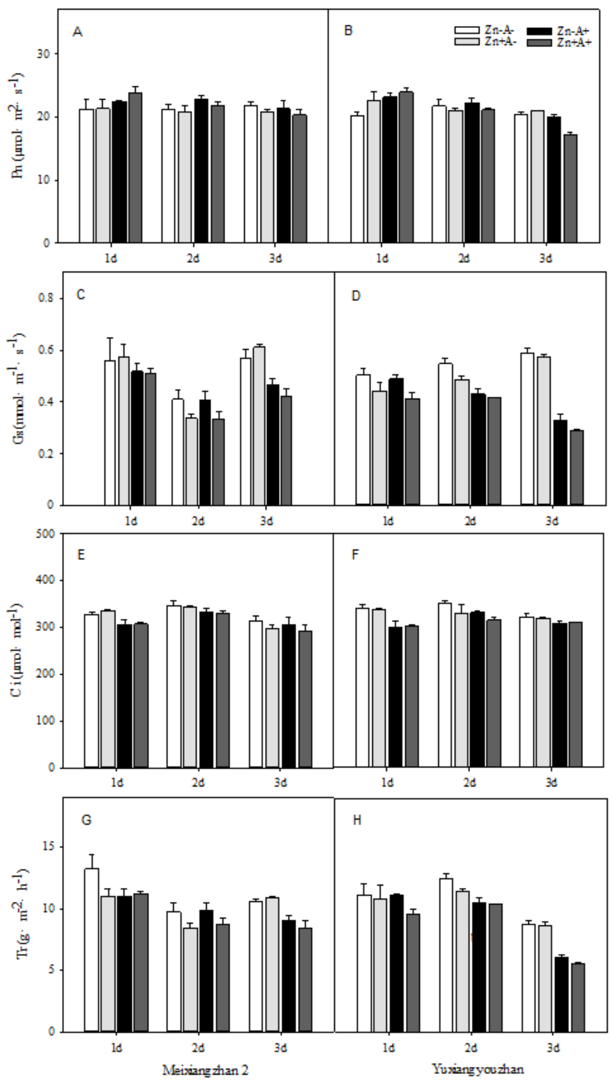

Fig. 3: Effect of zinc and α-ketoglutaric acid on photosynthetic parameters Vertical bars are means of the investigated parameters and the capped bars represent SD

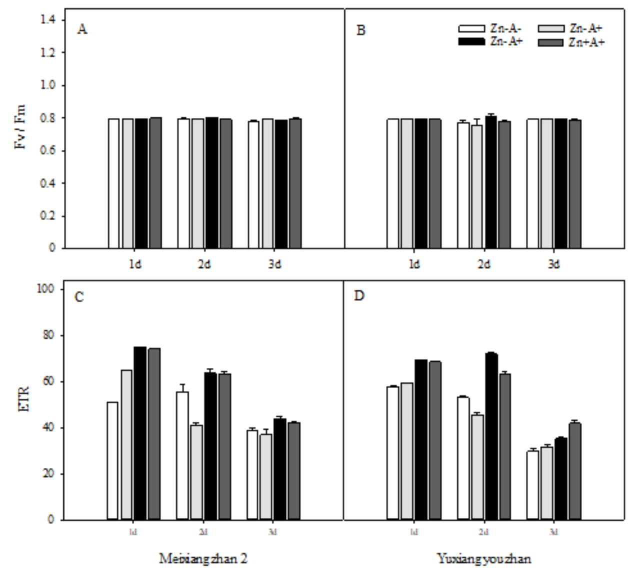

Fig. 4: Effect of zinc and α-ketoglutaric acid on Fv/Fm and ETR in rice leaves

Vertical bars are means of the investigated parameters and the capped bars represent SD

Φ(PSII) and Zn-A-, Zn+A+ showed significant increase at 2 d for Meixiangzhan 2. But there was no remarkable difference of Φ(PSII) at 3 d for Meixiangzhan 2 (Fig. 5A). For Yuxiangyouzhan, comparing to Zn-A-, the increment of Φ(PSII) at 1 d was up to 20% for Zn-A+ and 18.68% for Zn+A+ respectively. There was no significant difference in Φ(PSII) at 2 d under all treatments for Yuxiangyouzhan. Whereas the considerable decrease of Φ(PSII) at 3 d was observed in Zn-A- and Zn+A- for Yuxiangyouzhan (Fig. 5B).

A significant decrease in the regulated heat dissipation quantum yield (Y(NPQ)) at 1 d compared to control group could be observed in Zn-A+ for Meixiangzhan 2 and Yuxiangyouzhan. The higher value of Y(NPQ) at 1 d observed when plants were subjected to treatment Zn-A- for Meixiangzhan 2, and treatment Zn+A+ for Yuxiangyouzhna. There was no notable difference in Y(NPQ) at 2 d and Y(NPQ) at 3 d for both cultivars (Fig. 5C and D).

There was a significant higher value of the non-regulated heat dissipation quantum yield (Y(NO)) at 1 d under Zn-A- for both varieties. For Meixiangzhan 2, no conspicuous effects showed in Y(NO) at 2 d. By contrast,

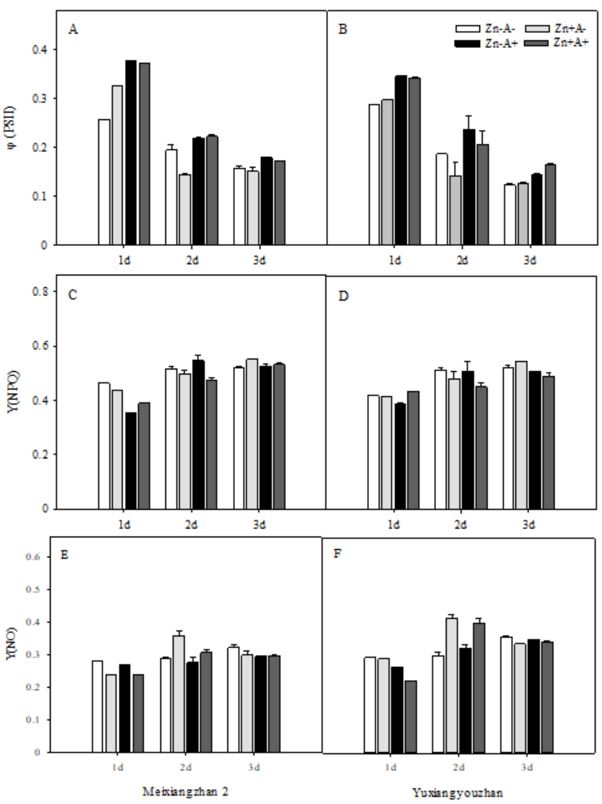

Fig. 5: Effect of zinc and α-ketoglutaric acid on Φ(PSII), Y(NPQ) and Y(NO) in rice leaf

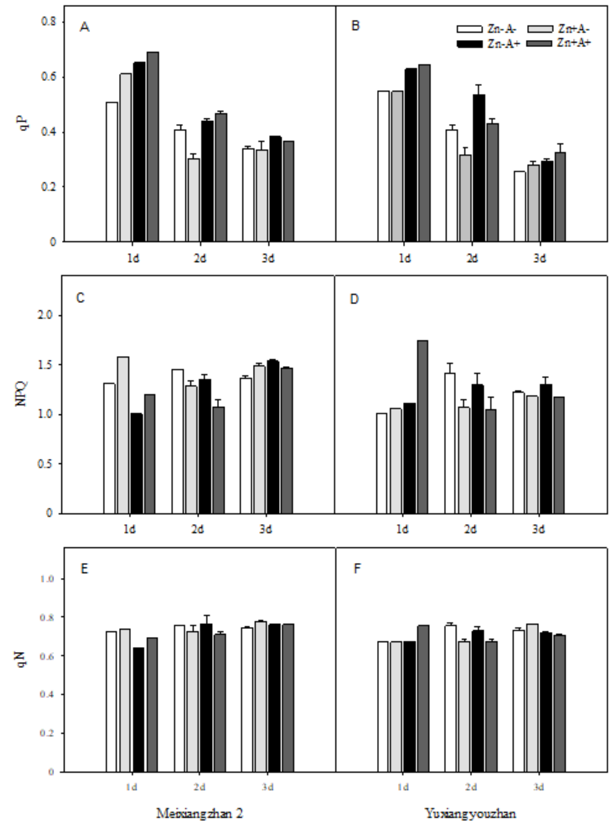

Fig. 6: Effect of zinc and α-ketoglutaric acid on qP, NPQ and qN in rice leaf. Vertical bars are means of the investigated parameters and the capped bars represent SD

Zn-A- significantly decreased Y(NO) at 2 d for Yuxiangyouzhan, others were all increased. Y(NO) at 3 d was not significantly different among treatments or cultivars (Fig. 5E and F).

The treatment Zn-A+ and Zn+A+ resulted in an increment of photochemical quenching (qP) at 1 d and the total of 28.24% and 35.93% for Meixiangzhan respectively (Fig. 6A). A significant improvement was observed in Zn-A+ and Zn+A+ for Yuxiangyouzhan and up to 14.70% and 17.28% respectively (Fig. 6B). The qP at 2 d value of treatment Zn+A- was significantly lower than Zn-A- for both varieties. Besides, remarkable increase was also observed by Zn-A+ compared to Zn-A- for both varieties. But at 3 d, there was no significant difference on value of qP among four treatments for Meixiangzhan 2 and Yuxiangyouzhan (Fig. 6A and B).

For Meixiangzhan 2, the highest value of nonphotochemical quenching (NPQ) at 1 d was observed when plants were subjected to treatment Zn+A-, by contrast, the lowest value of NPQ at 1 d was related to Zn-A+ (Fig. 6C). Furthermore, for Yuxiangyouzhan, a considerable increase was obtained when compared to Zn-A- and up to 72.29% for Zn+A+ (Fig. 6D). There was no significant difference in NPQ at 2 d among these treatments for both varieties. A remarkable decrease was noted in NPQ at 3 d under Zn-A- for Meixiangzhan 2, whereas there was no significant difference among four treatments for Yuxiangyouzhan (Fig. 6C and D).

The value of qN at 1 d significantly increased after application of Zn+A- whereas notable decrease was observed under Zn-A+ for Meixiangzhan 2 (Fig. 6E). The highest value of qN at 1 d was related to plants subjected to Zn+A+ for Yuxiangyouzhan up to 12.64% comparing to Zn+A- (Fig. 6F). There was no significant difference in qN at 2 d and 3 d among four treatments for both varieties respectively (Fig. 6E and F).

Correlation analyses: Zn content was significantly negatively correlated with plant height, root/shoot rate, Tr, ETR, qP and NPQ at 3 d, whereas it positively associated with Pn at 2 d and Fv/Fm at 3 d. While plant height was visibly positively related to root/shoot rate, Fv/Fm at 2 d, Φ(PSII) at 1 d and 3 d, ETR at 1 d and 3 d, qP at 1 d and 3 d, NPQ at 3 d and qN at 3 d but had negative correlation with Cond at 2 d, Ci at 3 d, Tr at 2 d. By contrast, root/shoot rate had positive relation with Φ(PSII) at 3 d, ETR at 3 d, qP at 3 d, NPQ at 3 d, however, it was negatively related to Cond at 2 d, Ci at 3 d, Tr at 2 d.

Discussion

Zinc (Zn) is important for human health (Wessells and

Brown 2012; Black *et al.* 2013) and increased Zn content in staple food is a sustainable approach for Zn supply (Hotz and Brown 2004). Zn is also important for crop growth, yield and quality formation (Wissuwa *et al.* 2008; Ghasal *et al.* 2017; Kumar *et al.* 2017; Li *et al.* 2018). Zn application significantly affected plant growth, yield formation, the concentrations and uptakes of Zn in plant (Tavallali 2017; Li *et al.* 2018). However, difference in Zn application rate, from 4.89 mg·kg^{-1} to 10 mg·kg^{-1}, in the improvement crops, due to the genotypes and soil Zn concentrations differences have been reported (Wissuwa *et al.* 2006; 2008). In this study, due to the low available Zn content in soil, Zn at rate of 10 mg·kg^{-1} was applied to the soil which significantly affected the plant height, some chlorophyll fluorescence indexes and photosynthetic parameters (Table 1). Zn deficiency result in reduction of the net photosynthetic rate and stomatal conductance (Tavallali 2017; Rehman *et al.* 2019; Ullah *et al.* 2019) which affects the plant growth (Pavlíková *et al.* 2014).

The α-ketoglutaric acid is widely used for industrial interest (Chernyavskaya *et al.* 2000; Stottmeister, *et al.* 2005; Huang *et al.* 2006) and can promote plant growth under different nitrogen levels (Gui *et al.* 2016). It has been reported that individual application of Zn and α-ketoglutaric acid can increase the Zn content in plants (Fageria *et al.* 2011; Gui *et al.* 2016). Therefore, Zn and α-ketoglutaric acid affected Zn content various from variety (Table 1 and Fig. 1). Besides, plant growth such as the plant height will decrease under Zn deficiency condition (Wang *et al.* 2009). The plant height in Zn-A+ was remarkably improved for Yuxiangyouxhan (Fig. 1B). And rice absorbed Zn could increase root length and root dry weight (Fageria *et al.* 2016). Crops have the tendency to form more chlorophyll with the addition of Zn (Symeonidis and Karataglis 2008). Thus, remarkably improved root dry weight was observed for Zn+A+ (Fig. 2).

Crops have the tendency to form more chlorophyll with the addition of Zn (Ramani and Kannan 1985). The influences of variety, Zn and α-ketoglutaric acid on photosynthetic parameters and chlorophyll fluorescence were observed (Table 1 and Fig. 3, 4, 5 and 6). Interestingly, the significant impact of Zn, α-ketoglutaric acid and their interactive effect on photosynthetic and chlorophyll fluorescence were notably observed at 1 d after treatment (Table 1). The possible reason for metabolic balance of carbon was regulated by α-ketoglutaric acid and Zn supplied. Further, significant positive relationship between Zn content and plant growth, photosynthetic and chlorophyll fluorescence were detected (Fig. 7). Those results further prove the role of Zn and α-ketoglutaric acid in regulating of plant growth as a consequence of regulating of the photosynthetic and chlorophyll fluorescence.

Conclusion

The Zn×A strong interaction effect the Zn content in plant and root dry weight were observed. Zn-A+ treatment yielded the highest Zn content in plant. The trend of Zn content was as follow: Zn-A+> Zn+A->Zn+A+> Zn-A-. Moreover, the interaction between Zn and α-ketoglutaric acid produced the highest root dry weight. Application of Zn, α-ketoglutaric acid and their interaction strongly affected the chlorophyll fluorescence which regulated the photosynthesis. Zn and α-ketoglutaric acid whose benefit enhanced Zn content in rice plant and was associated with the plant growth, photosynthesis attributes and chlorophyll fluorescence.

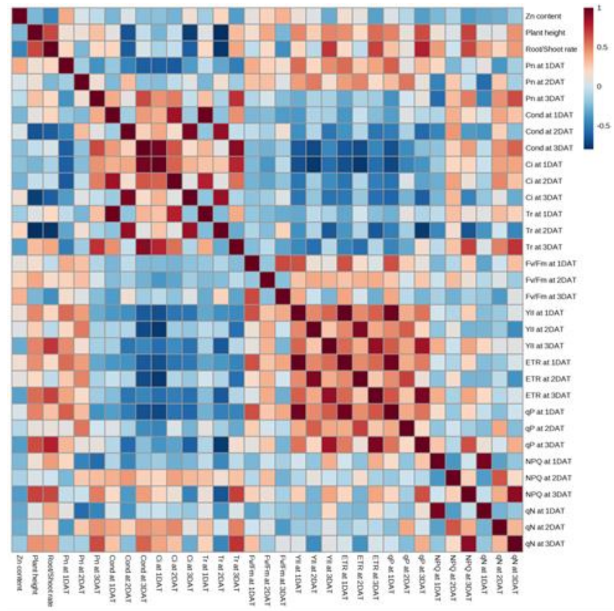

Fig. 7: Correlation analyses of the investigated parameters

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