

## PLANT HYDRAULIC CONDUCTIVITY DETERMINES PHOTOSYNTHESIS IN RICE UNDER PEG-INDUCED DROUGHT STRESS

GUANGLONG ZHU<sup>1,2,3\*</sup>, LIFENG GU<sup>1</sup>, YU SHI<sup>2</sup>, HUIZE CHEN<sup>4</sup>, YUQIAN LIU<sup>2</sup>, FAGUANG LU<sup>3</sup>, ZHEN REN<sup>1,2</sup>, YUE WANG<sup>2</sup>, HAITONG LU<sup>3</sup>, ADNAN TABASSUM<sup>1,2\*</sup> AND GUI SHENG ZHOU<sup>1,2,3\*</sup>

<sup>1</sup>Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, Jiangsu, China

<sup>2</sup>Jiangsu Key Laboratory of Crop Genetics and Physiology/ Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College of Yangzhou University, Yangzhou, China

<sup>3</sup>Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou, China

<sup>4</sup>College of Life Science, Shanxi Normal University, Linfen, Shanxi, China

\*Corresponding author's email: zhuguang2007@163.com; matabassum@yzu.edu.cn, gszhou@yzu.edu.cn

### Abstract

Photosynthesis ( $A$ ) plays a key role in maintaining plant carbon balance, but it is sensitive to drought. Both  $A$  and plant hydraulic conductivity ( $K_{\text{plant}}$ ) decrease under water deficit. It is not clearly whether the declined  $K_{\text{plant}}$  is more related to root or leaf, whether the decreased  $A$  is related to  $K_{\text{plant}}$  and/or leaf hydraulic conductivity ( $K_{\text{leaf}}$ ) and diffusive alone or both diffusive and metabolic impairments decreased  $A$ . Two drought-tolerant (DW) contrasting rice genotypes were used to explore the relationship of  $A$ ,  $K_{\text{plant}}$  and  $K_{\text{leaf}}$  under PEG induced drought stress (PEG-DS). The results showed that photosynthesis related parameters of  $A$ , stomatal conductance ( $g_s$ ), transpiration rate ( $Tr$ ), maximum Rubisco carboxylation rate ( $V_{\text{cmax}}$ ), maximum electron transport rate ( $J_{\text{max}}$ ), carboxylation efficiency (CE),  $K_{\text{leaf}}$ ,  $K_{\text{plant}}$  and xylem sap flow rate (XSFR) were all decreased significantly under PEG-DS. These decreases were more severe in DW-sensitive genotype IR64 than DW-tolerant genotype Hanyou-3. However, both intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and  $\text{CO}_2$  concentration inside chloroplasts ( $C_c$ ) were prominently increased in IR64 rather than in Hanyou-3 under PEG-DS. In addition, both  $g_s$  and  $g_m$  (mesophyll conductance to  $\text{CO}_2$ ) were strongly positively correlated with  $A$  ( $R^2=0.98$  &  $0.71$ ). Photosynthesis of both genotypes were increased with increasing  $C_i$  under each treatment. Furthermore,  $A$  and  $g_s$  were significantly correlated with  $K_{\text{plant}}$  ( $R^2=0.94$  &  $0.96$ ) but not with  $K_{\text{leaf}}$ , and  $K_{\text{plant}}$  was not related to  $K_{\text{leaf}}$ .  $K_{\text{plant}}$  rather than  $K_{\text{leaf}}$  determines photosynthesis in rice under drought conditions, which was mainly attributed to  $K_{\text{plant}}$  decreases the stomatal conductance and ultimately lead to decrease in photosynthesis.

**Key words:** Drought stress, Gas exchange, Stomatal conductance, Transpiration rate, Water transport.

### Introduction

Rice is the most staple food for globe population and about 700 million Asians consume it to meet their caloric requirements (He *et al.*, 2013). Rice production consumes a large amount of fresh water (Kar *et al.*, 2017). In Asia, about half of total fresh water resources is used for rice production (Barker *et al.*, 1999). However, the proportion of fresh water for agriculture sector decreases due to the competition between agricultural and non-agricultural consumption (Wang *et al.*, 2010). On the other hand, climatic change and global warming increase plant water requirement. This inverse relationship between fresh water demand and supply gives rise to drought stress (Hu *et al.*, 2015). Drought is major constraint to crop production, and is hazardous for plant survival, establishment, growth, and yield formation worldwide (Fernandez *et al.*, 2006; Gilani *et al.*, 2020).

Photosynthesis ( $A$ ) is the primary physiological process to maintain plant carbon balance, but it is extremely sensitive to water deficit. The research on the constraining factor to photosynthesis under drought is a permanently heated research area (Wang *et al.*, 2018). Decreased  $\text{CO}_2$  diffusion from atmosphere to plant carboxylation sites is considered the major limitation of photosynthesis under drought stress (De Magalhães Erismann *et al.*, 2008; Peeva & Cornic, 2009). As stomatal limitations cannot fully explain the

photosynthetic reductions under drought stress (Zhang *et al.*, 2018), reduction in  $g_m$  (mesophyll conductance to  $\text{CO}_2$ ) and metabolic capacity are thought to be other reasons for declined  $A$  under drought (Flexas *et al.*, 2007, 2008). Moreover, the response of  $g_m$  to environmental changes is as quick as the  $g_s$  (stomatal conductance) (Flexas *et al.*, 2007, 2008). Therefore, at present photosynthesis response to drought stress is divided into two distinguishing phases, including diffusive and metabolic impairments (Zhou *et al.*, 2007).

There is still a controversy about the diffusive and metabolic control of  $A$ . As it was suggested that  $A$  was limited by  $\text{CO}_2$  diffusion under short moderate drought condition, and metabolic impairment occurred only in severe drought condition (Flexas *et al.*, 2009). The water deficit event however, develops gradually from weeks to months under natural conditions, and some accumulative effects occur during this period (Flexas *et al.*, 2006).

Another determinant to photosynthesis is water transport. Plant water transport capacity is expressed as the plant hydraulic conductivity ( $K_{\text{plant}}$ ) (Tyree & Zimmermann, 2002), which is controlled by leaf hydraulic conductivity, stem hydraulic conductivity and root hydraulic conductivity (Martre *et al.*, 2002). Among of them, roots contribute 20% to 35% (Javot & Maurel, 2002) and leaves contribute 25% to the plant hydraulic resistances (Sack & Holbrook, 2006). There are three parallel pathways for water transport in roots: apoplastic, symplastic and trans-

cellular pathways (Miyamoto *et al.*, 2001). Both casparian band and suberin lamellae limit the water transport capacity of apoplastic pathway. It was demonstrated that the formation of aerenchyma could also restrain water uptake through apoplastic pathway (Yang *et al.*, 2012). Symplastic and trans-cellular pathways are difficult to be distinguished, in that case both of them are together called cell-to-cell pathway. Water channel (aquaporin) activity is the main factor which determines water transport capacity in cell-to-cell pathway (Flexas *et al.*, 2008). A large number of studies were conducted to explore the mechanism of root hydraulic decline under drought stress, and the credible evidences were the thicker apoplastic barriers enhancing aerenchyma formation and deactivated aquaporins (Boursiac *et al.*, 2008). In addition, leaf vulnerability can lead to leaf hydraulic conductivity ( $K_{\text{leaf}}$ ) decline under drought (Blackman *et al.*, 2014).

It is reported that the water transport system regulates the hydraulic conductance from soil to leaves, so plant growth depends upon the capacity of water transport system under water deficit conditions (Virginia *et al.*, 2016). Moreover, linear relationships between  $K_{\text{plant}}$  and water transpiration rate, and  $g_s$ , were observed in higher plants (Saliendra *et al.*, 1995; Hubbard *et al.*, 2001). However, little knowledge about whether the decline  $K_{\text{plant}}$  under drought is more related to root or leaf, whether the decreased  $A$  is related to  $K_{\text{plant}}$  and/or  $K_{\text{leaf}}$ , and diffusive alone or diffusive and metabolic both decreased the photosynthesis. In the present study, two rice genotypes with contrasting responses to drought stress were grown under three levels of PEG induced drought stress (PEG-DS). Gas exchange and hydraulic conductivity were measured to investigate (i) effect of PEG-DS on photosynthesis and hydraulic conductivity in rice; (ii) whether the  $K_{\text{leaf}}$  or  $K_{\text{plant}}$  was related to photosynthetic gas exchange parameters, (iii) whether the diffusive alone or both diffusive and metabolic impairment decreased the photosynthesis.

## Materials and Methods

**Plant material and seedling establishment:** Two rice genotypes with contrasting responses to drought stress (DW) were selected from earlier study were used. Hanyou-3 is DW-tolerant genotype, and IR64 is a DW-intolerant. A controlled study was conducted under natural environmental conditions during August-October.

Seeds of two genotypes were surface sterilized with 10%  $\text{H}_2\text{O}_2$  for 1.5 h and washed with distilled water to remove residual effect of  $\text{H}_2\text{O}_2$ . The sterilized seeds were germinated in petri dishes with moist filter paper and fostered in incubator (maintained temperature 25°C, average photon flux density ranged from 600 to 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , day-night time 12/12 h), after the radical emergence reached 2 cm, the seedlings were transferred to seedling tray and placed in paddy field. The 1/8<sup>th</sup> strength of Hoagland solution was applied on 5-days old seedlings in order to avoid nutrient deficiency.

Fifteen-days old seedlings were transplanted in buckets (50 cm height  $\times$  40 cm diameter), and cultivated with 10.5 L Hoagland solution in each bucket. The components of the full strength nutrient solution were

macronutrients ( $\text{mg L}^{-1}$ ) 40 N as  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{Ca}(\text{NO}_3)_2$ , 40 K as  $\text{K}_2\text{SO}_4$  and  $\text{KH}_2\text{PO}_4$ , 10 P as  $\text{KH}_2\text{PO}_4$ , 40 Mg as  $\text{MgSO}_4$ ; micronutrients ( $\text{mg L}^{-1}$ ) 2.0 Fe as Fe-EDTA, 0.05 Mo as  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ , 0.5 Mn as  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ , 0.2 B as  $\text{H}_3\text{BO}_3$ , 0.01 Cu as  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ , 0.01 Zn as  $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ , 2.8 Si as  $\text{Na}_2\text{SiO}_3\cdot 9\text{H}_2\text{O}$ .<sup>32</sup> Dicyandiamide was mixed into the nutrient solution as a nitrification inhibitor. Solution was changed every 5 d and PH value maintained to  $5.50 \pm 0.05$  by adding 0.1 mol  $\text{L}^{-1}$  HCl or NaOH every day. All buckets were arranged in an open greenhouse with shelter, the greenhouse was covered with during rain.

In this experiment, 4 treatments with 3 replications were arranged; each bucket had 4 seedlings of each genotype and 6 buckets for each replication. The treatments were well (1) watered condition (WWC, control), (2) 5% PEG-DS, (3) 10% PEG-DS and (4) 15% PEG-DS. Treatments were continued at 40 days seedlings age. The different concentration of PEG-DS was confected by PEG6000 (w/v).

**Measurements:** Gas exchange measurements: At 55-day seedling age, all buckets were transferred in growth chamber. All data was recorded inside chamber to avoid the outdoor environmental fluctuations. In growth chamber, the PPFD (photosynthetic photon flux density) was controlled to 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  with T5 fluorescent lamps and halogen incandescent lamps. Three fans built in the roof of the growth chamber to control temperature, and air temperature was set to 30/25°C day/night at 11 h photoperiod. Relative humidity was controlled to 65% in the growth chamber.

Plants were acclimated for 2 h before the start of measurements in growth chamber. Fully developed upper leaves were selected to record gas exchange data during 8:00 to 16:00 by Li-Cor 6400XT portable photosynthesis system (LI-COR 6400XT, NE, USA). PPFD in the leaf chamber maintained at 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $\text{CO}_2$  concentration was about  $400 \pm 10 \mu\text{mol mol}^{-1}$  with a  $\text{CO}_2$  mixture.

Photosynthesis, stomatal conductance, photosynthetic  $\text{CO}_2$  response curves ( $A/C_i$  response curves,  $C_i$  means intercellular  $\text{CO}_2$  concentration) and chlorophyll fluorescence were measured simultaneously. Firstly, leaves were placed in leaf chamber with PPFD of 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $\text{CO}_2$  concentration about  $400 \pm 10 \mu\text{mol mol}^{-1}$  with a  $\text{CO}_2$  mixture. Secondly, after recording the data under 400  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentration at steady status, atmospheric  $\text{CO}_2$  ( $C_a$ ) was controlled through a series of 400, 200, 150, 100, 50, 400, 600, 800, 1000, 1200 and 1500  $\mu\text{mol mol}^{-1}$ . After attaining a steady state,  $F_s$  (fluorescence),  $F_m$  (maximum fluorescence) using a 0.8 s saturating light pulse (about 8000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and gas exchange parameters were measured. The CE (carboxylation efficiency) was calculated as the initial slope of  $\text{CO}_2$  response curves when  $C_a$  was  $\leq 200 \mu\text{mol mol}^{-1}$  and  $\Phi_{\text{PSII}}$  (photochemical efficiency of photosystem II) was calculated as follows:

$$\Phi_{\text{PSII}} = (F_m' - F_s) / F_m'$$

One day later, light response curves and chlorophyll fluorescence were measured simultaneously under low O<sub>2</sub> condition (<2%). Prior to measurements, leaves were attached to the leaf chamber at a PPFD of 1500 μmol m<sup>-2</sup> s<sup>-1</sup>. After ten minutes, PPFD in the leaf chamber was controlled in the sequence of 800, 600, 400, 200, and 100 μmol photons m<sup>-2</sup> s<sup>-1</sup>. After that, gas exchange and chlorophyll fluorescence were recorded in a steady state. The product α<sub>leaf</sub> × β was determined as the slope of linear correlation between the quantum efficiency of CO<sub>2</sub> uptake (Φ<sub>CO2</sub>) and Φ<sub>PSII</sub>, Φ<sub>CO2</sub> was calculated as:

$$\Phi_{CO2}=4(A+R_d)/PPFD$$

where R<sub>d</sub> is assumed to be 1 μmol m<sup>-2</sup> s<sup>-1</sup>. The J (electron transport rate) was calculated as follows:

$$J = \Phi_{PSII} \times PPFD \times \alpha_{leaf} \times \beta$$

The variable J method was used to calculate CO<sub>2</sub> concentration inside chloroplast (C<sub>c</sub>) and mesophyll conductance (g<sub>m</sub>). First CO<sub>2</sub> was calculated as follows:

$$C_c = \Gamma^* (j + 8(A + R_d)) / J - 4(A + R_d)$$

where Γ\* is the Rubisco specific factor and represents CO<sub>2</sub> compensation point in the absence of respiration. Γ\* value of 40 μmol mol<sup>-1</sup> typically for *Oryza* plants were taken from the studies of Tabassum *et al.*, (2016a). Then, g<sub>m</sub> was calculated as follows:

$$g_m = A / (C_i - C_c)$$

The V<sub>cm<sub>max</sub></sub> (maximum velocity of RuBP carboxylation by Rubisco) and the J<sub>max</sub> (capacity for ribulose-1, 5-bisphosphate regeneration) were computed using the FvCB model and its modification (Tabassum *et al.*, 2016b).

Measurement of transpiration rate: Leaf transpiration rate at different time intervals was measured under above mentioned conditions. Leaves of each genotypes were excised in water, then placed in test tube in such a way that the base of the leaves were dipped in the distilled water. Immediately, leaves were recorded *E* at each time interval of 2 minutes by Li-COR 6400XT portable infrared gas analyzer (IRGA) (LI-COR, NE, USA), total 15 readings were recorded.

Measurement of plant and leaf hydraulic conductivities: During the gas exchange measurements, newly and fully developed leaves were used to measure the daytime leaf water potential by a WP4C Dewpoint Potentia Meter (Decagon, Pullman, WA, USA). Plant hydraulic conductivity (K<sub>plant</sub>) was calculated as follows (Taylaran *et al.*, 2011):

$$K_{plant} = E / (\Psi_{solution} - \Psi_{leaf})$$

where Ψ<sub>solution</sub> = 0 for WWC while Ψ<sub>solution</sub> for the 5, 10 and 15% PEG-IWDS was -0.05, -0.18 and -0.38 MPa, respectively.

K<sub>leaf</sub> (Leaf hydraulic conductivity) was measured using modified evaporative flux method.<sup>35,36</sup> Leaf was excised in water, and placed in test tube and the base of the leaf was

dipped in the distilled water. Immediately, leaf was attached to record *E* under above described conditions by Li-COR 6400XT portable infrared gas analyzer (IRGA) (LI-COR, NE, USA). K<sub>leaf</sub> was calculated as follows the method of Taylaran *et al.*, (2011):

$$K_{leaf} = E / (0 - \Psi_{leaf})$$

Xylem sap flow rate: A sharp knife was used to de-top the plants about 5 cm above the interface of the shoots and roots at 17:00 pm. The sap exudation was cleaned to avoid contamination, and 1 g dry cotton was placed above the de-topped shoot. Finally, the plastic film was wrapped around it to avoid the evaporation. It was allowed to collect xylem sap in cotton for 12 h then, wet cotton was removed from de-topped shoot and weighed. Xylem sap flow rate was calculated by the difference in cotton weight (Soejima *et al.*, 1992).

### Statistical analysis

Variance (ANOVA) analyses were performed using factors design and the mean values were compared based on the least significant difference (LSD) test at *p*<0.05 between genotypes with Statistics 8.1 (Analytical software). Correlations and regressions were performed using Sigma Plot 12.0 (SPSS Inc., Chicago, IL, USA).

### Results

**Variation of *A*, g<sub>s</sub> and g<sub>m</sub> under PEG-DS:** Gas exchange parameters of *A* and g<sub>s</sub> were significantly decreased with PEG-DS treatments aggravating and the depression was more severe in IR64 than Hanyou-3 (Table 1). However, g<sub>m</sub> was not significantly affected by PEG-DS in these two contrasting genotypes except under 15% PEG-DS in IR64. Compared to CK, *A* was decreased by 12.1%, 13.0% and 29.2% under 5%, 10% and 15% of PEG-DS in Hanyou-3, but 13.5%, 30.6% and 43.5% in IR64, respectively. As for g<sub>s</sub>, it decreased 7.9%, 15.8% and 34.2% in Hanyou-3, and 9.4%, 21.9% and 50.0% in IR64 under each drought treatment. The ANOVA analysis showed that *A*, g<sub>s</sub> and g<sub>m</sub> were significantly affected by treatments (T) and genotypes (G) (*p*<0.05), but not their interaction (T × G) (Table 1). In addition, both g<sub>s</sub> and g<sub>m</sub> were strongly positively correlated with *A* (R<sup>2</sup>=0.98 & 0.71, Fig. 1). Photosynthesis of both Hanyou-3 and IR64 was increased with increasing C<sub>i</sub> under each treatment (Fig. 2).

**Variation of C<sub>i</sub>, C<sub>c</sub> and Tr under PEG-DS:** Treatments, genotypes and their interaction did not significantly affect C<sub>i</sub> and C<sub>c</sub>, but Tr was prominently affected by T and G (Table 2). Both C<sub>i</sub> and C<sub>c</sub> were not significantly varied in Hanyou-3 under each PEG-DS treatment. However, in IR64, they were significantly increased by 3.8% and 25.6% under 5% PEG-DS and by 6.9% and 22.6% 10% PEG-DS, but decreased by 6.8% and 20.8% under 15% PEG-DS, respectively. In addition, Tr in both Hanyou-3 and IR64 significantly decreased with PEG-DS treatment worsened. The decrease was more severe in IR64 than in Hanyou-3. It was decreased by 13.5% under 5% PEG-DS, 16.2% under 10% PEG-DS and 32.0% under 15% PEG-DS treatment in IR64, but decreased by 12.9%, 27.3% and 41.1% in Hanyou-3, respectively (Table 2).

**Table 1. Effect of PEG induced water deficit stress on photosynthesis ( $A$ ), stomatal conductance ( $g_s$ ) and mesophyll conductance ( $g_m$ ) of newly expanded leaves of different rice varieties at vegetative stage. Water deficit stress was simulated by adding 5, 10 and 15% (W/V) PEG6000 to nutrient solution.**

Genotypes	Treatment	$A$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_s$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	$g_m$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )
Hanyou-3	WWC	26.73 $\pm$ 0.39 a	0.38 $\pm$ 0.02 a	0.25 $\pm$ 0.00 a
	PEG-DS5%	23.50 $\pm$ 0.31 a	0.35 $\pm$ 0.02 a	0.25 $\pm$ 0.01 a
	PEG-DS10%	23.26 $\pm$ 1.17 a	0.32 $\pm$ 0.02 ab	0.22 $\pm$ 0.02 a
	PEG-DS15%	18.92 $\pm$ 0.11 b	0.25 $\pm$ 0.01 b	0.22 $\pm$ 0.02 a
IR64	WWC	24.97 $\pm$ 0.24 a	0.32 $\pm$ 0.01 a	0.23 $\pm$ 0.01 a
	PEG-DS5%	21.60 $\pm$ 0.67 b	0.30 $\pm$ 0.01 ab	0.20 $\pm$ 0.01 a
	PEG-DS10%	17.32 $\pm$ 0.13 c	0.25 $\pm$ 0.01 b	0.20 $\pm$ 0.01 a
	PEG-DS15%	14.10 $\pm$ 0.12 d	0.16 $\pm$ 0.00 c	0.12 $\pm$ 0.01 b
T		***	**	*
G		**	*	*
T $\times$ G		ns	ns	ns

WWC=Well watered condition, PEG-DS=PEG induced water deficit stress. Data are presented as Mean $\pm$ SE with 3 replications. ns represents no significant, while \*, \*\* and \*\*\* represent significant at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  levels, respectively. Data followed by the same letters are not significantly different

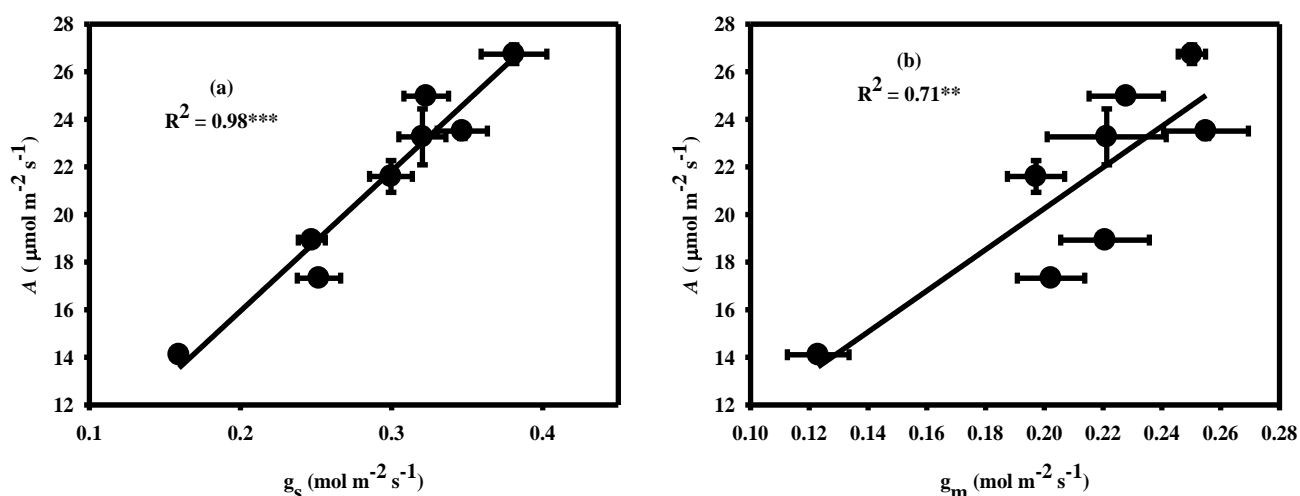


Fig. 1. Relationship of photosynthesis ( $A$ ) with stomatal conductance ( $g_s$ ) and mesophyll conductance ( $g_m$ ). ns represents no significant, while \*, \*\* and \*\*\* represent significant at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  levels, respectively.

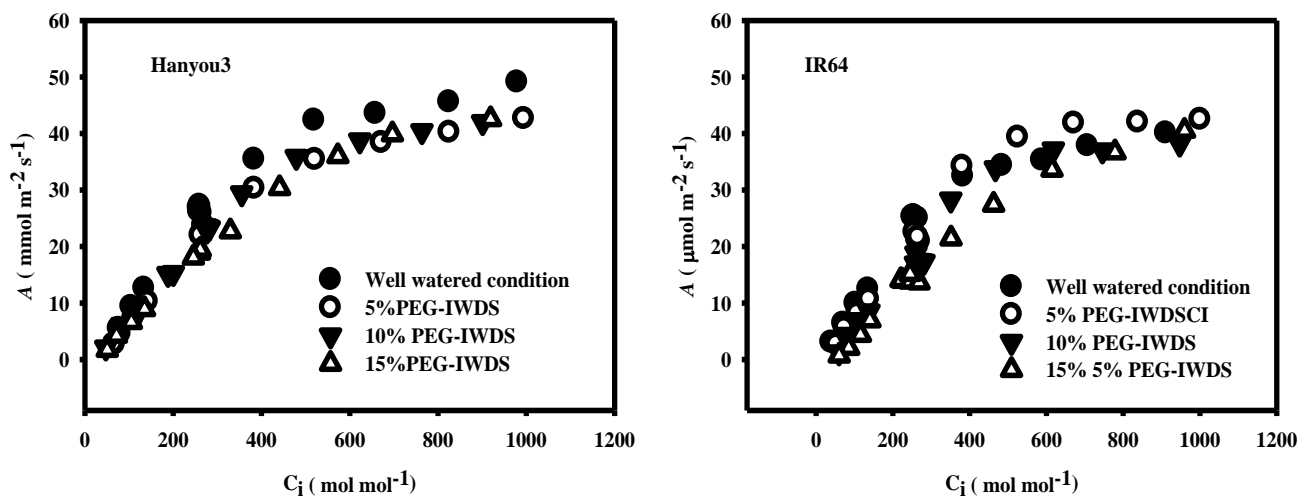


Fig. 2. Photosynthesis ( $A$ ) and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) response curves of Hanyou-3 and IR64 under well-watered condition, 5, 10, and 15% PEG induced water deficit stress levels.

**Table 2. Effect of PEG induced water deficit stress on intercellular CO<sub>2</sub> concentration (C<sub>i</sub>), CO<sub>2</sub> concentration inside chloroplast (C<sub>c</sub>) and leaf transpiration rate (Tr) of newly expanded leaves of different rice cultivars at vegetative stage. Water deficit stress was simulated by adding 5, 10 and 15% (W/V) PEG6000 to nutrient solution.**

Genotypes	Treatment	C <sub>i</sub> (μmol mol <sup>-1</sup> )	C <sub>c</sub> (μmol mol <sup>-1</sup> )	Tr (mmol m <sup>-2</sup> s <sup>-1</sup> )
Hanyou-3	WWC	259.65 ± 6.39 a	150.78 ± 7.24 a	6.72 ± 0.29 a
	PEG-DS5%	267.33 ± 4.19 a	171.83 ± 9.02 a	5.81 ± 0.29 ab
	PEG-DS10%	260.64 ± 5.63 a	149.80 ± 9.32 a	5.63 ± 0.25 ab
	PEG-DS15%	256.45 ± 4.87 a	163.84 ± 0.68 a	4.57 ± 0.28 b
IR64	WWC	253.89 ± 5.09 b	142.35 ± 2.66 b	5.57 ± 0.44 a
	PEG-DS5%	263.45 ± 2.58 ab	178.79 ± 15.81a	4.85 ± 0.12 ab
	PEG-DS10%	271.38 ± 5.05 a	174.54 ± 4.65 a	4.05 ± 0.19 bc
	PEG-DS15%	236.67 ± 3.64 c	112.81 ± 7.32c	3.28 ± 0.13 c
T		ns	ns	**
G		ns	ns	*
T × G		ns	ns	ns

WWC=Well watered condition, PEG-DS=PEG induced water deficit stress. Data are presented as Mean±SE with 3 replications. ns represents no significant, while \*, \*\* and \*\*\* represent significant at  $p<0.05$ ,  $p<0.01$  and  $p<0.001$  levels, respectively. Data followed by the same letters are not significantly different

**Table 3. Effect of drought stress on maximum Rubisco carboxylation capacity (V<sub>cmax</sub>), maximum electron transport capacity (J<sub>max</sub>), and carboxylation efficiency (CE) of newly expanded leaves of rice varieties at vegetative stage. Water deficit stress was simulated by adding 5, 10 and 15% (W/V) PEG6000 to nutrient solution.**

Genotypes	Treatment	V <sub>cmax</sub>	J <sub>max</sub>	CE
Hanyou-3	WWC	105 ± 2.71 a	249 ± 9.53 a	0.12 ± 0.003 a
	PEG-DS5%	87 ± 1.95 b	202 ± 5.21 b	0.10 ± 0.004 ab
	PEG-DS10%	85 ± 2.14 bc	201 ± 10.22 b	0.09 ± 0.002 bc
	PEG-DS15%	75 ± 0.33 c	179 ± 6.20 b	0.08 ± 0.005 c
IR64	WWC	102 ± 2.22 a	207 ± 4.99 a	0.12 ± 0.007 a
	PEG-DS5%	81 ± 1.64 b	201 ± 2.99 a	0.09 ± 0.002 b
	PEG-DS10%	69 ± 1.20 bc	171 ± 2.52 b	0.07 ± 0.004 b
	PEG-DS15%	65 ± 3.02 c	161 ± 0.51 b	0.07 ± 0.003 b
T		***	***	***
G		ns	ns	ns
T × G		ns	ns	Ns

WWC=Well watered condition, PEG-DS=PEG induced water deficit stress. Data are presented as Mean±SE with 3 replications. ns represents no significant, while \*, \*\* and \*\*\* represent significant at  $p<0.05$ ,  $p<0.01$  and  $p<0.001$  levels, respectively. Data followed by the same letters are not significantly different

**Variation of V<sub>cmax</sub>, J<sub>max</sub> and CE under PEG-DS:** PEG-DS treatments significantly affected V<sub>cmax</sub>, J<sub>max</sub> and CE, rather than genotypes and their interaction of T × G (Table 3). The V<sub>cmax</sub>, J<sub>max</sub> and CE decreased significantly under PEG-DS treatments, and the decrease were more serious with the PEG-DS treatment aggravating (Table 3). Under PEG-DS treatments, V<sub>cmax</sub> and CE decreased more seriously in IR64 than in Hanyou-3, which declined with 3.5%, 13.3% and 7.7% higher in IR64 under 5%, 10% and 15% PEG-D for V<sub>cmax</sub>, and with 8.3%, 16.7% and 8.4% higher for CE, respectively. On the contrary, J<sub>max</sub> decreased more sharply under PEG-DS treatments in Hanyou-3 than in IR64, which showed 16.0%, 1.9% and 5.9% most decrease in Hanyou-3 under each treatment (Table 3).

**Variation of K<sub>Leaf</sub>, K<sub>Plant</sub> and XSFR under PEG-DS:** Both K<sub>Leaf</sub> and K<sub>Plant</sub> were significantly affected by T and G, were as XSFR was only significantly affected by T. However, Ψ<sub>leaf</sub> was not prominently affected by T, G and T × G (Table 4). In general, K<sub>Leaf</sub>, K<sub>Plant</sub> and XSFR were all significantly decreased with PEG-DS treatments aggravating in both genotypes. From 5% to 15% PEG-DS treatments, K<sub>Leaf</sub>, K<sub>Plant</sub> and XSFR were decreased from 2.6% to 34.9%, 17.6% to 40.4%, and 35.5% to 96.8% in Hanyou-3, but 2.4% to 24.7%, 10.3% to 34.5%, and 23.3% to 95.4% in IR64 compared with CK, respectively. The Ψ<sub>leaf</sub> significant declined under PEG-DS treatments only in Hanyou-3, which decreased by 0.9%, 33.3% and 45.5% under 5%, 10% and 15% PEG-DS treatment, respectively (Table 4).

**Table 4. Effect of PEG induced water deficit stress on leaf water potential ( $\Psi_{\text{leaf}}$ ), leaf hydraulic conductivity ( $K_{\text{leaf}}$ ), plant hydraulic conductivity ( $K_{\text{plant}}$ ) and xylem sap flow rate (XSFR) of newly expanded leaves of rice cultivars at vegetative stage. Water deficit stress was simulated by adding 5, 10 and 15% (W/V) PEG6000 to nutrient solution.**

Genotypes	Treatment	$\Psi_{\text{leaf}}$ (MPa)	$K_{\text{leaf}}$ ( $\text{mmol.m}^{-2}.\text{s}^{-1} \text{MPa}^{-1}$ )	$K_{\text{plant}}$ ( $\text{mmol.m}^{-2}.\text{s}^{-1} \text{MPa}^{-1}$ )	XSFR ( $\text{gh}^{-1}$ )
Hanyou-3	WWC	$-1.23 \pm 0.05$ a	$5.45 \pm 0.17$ a	$5.45 \pm 0.20$ a	$0.62 \pm 0.02$ a
	PEG-DS5%	$-1.34 \pm 0.10$ ab	$5.31 \pm 0.16$ a	$4.49 \pm 0.19$ ab	$0.40 \pm 0.02$ b
	PEG-DS10%	$-1.64 \pm 0.08$ bc	$4.04 \pm 0.18$ b	$4.07 \pm 0.19$ b	$0.06 \pm 0.00$ c
	PEG-DS15%	$-1.79 \pm 0.01$ c	$3.55 \pm 0.17$ b	$3.25 \pm 0.16$ b	$0.02 \pm 0.00$ c
IR64	WWC	$-1.43 \pm 0.07$ a	$6.15 \pm 0.27$ a	$3.89 \pm 0.25$ a	$0.43 \pm 0.01$ a
	PEG-DS5%	$-1.44 \pm 0.04$ a	$6.30 \pm 0.08$ a	$3.49 \pm 0.07$ ab	$0.33 \pm 0.01$ b
	PEG-DS10%	$-1.54 \pm 0.09$ a	$5.35 \pm 0.35$ ab	$2.97 \pm 0.11$ bc	$0.04 \pm 0.00$ c
	PEG-DS15%	$-1.66 \pm 0.10$ a	$4.63 \pm 0.17$ b	$2.55 \pm 0.08$ c	$0.02 \pm 0.00$ c
T	ns	**	**	***	
G	ns	*	*	ns	
T $\times$ G	ns	ns	ns	Ns	

WWC=Well watered condition, PEG-DS=PEG induced water deficit stress. Data are presented as Means $\pm$ SE with 3 replications. ns represents no significant, while \*, \*\* and \*\*\* represent significant at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  levels, Data followed by the same letters are not significantly different

**Variation of Tr and relationship of  $K_{\text{leaf}}$ ,  $K_{\text{plant}}$  and A under PEG-DS:** After detaching the leaves, transpiration rate of Hanyou-3 increased and reached the maximum value at 16<sup>th</sup> minute. The maximum Tr was  $6.72 \pm 0.21$ ,  $7.13 \pm 0.21$ ,  $6.60 \pm 0.29$  and  $6.34 \pm 0.30$  under CK, 5%, 10% and 15%, respectively (Fig. 3). Similarly, IR64 attained the maximum value on 14<sup>th</sup> minute under both control and 10% PEG-DS treatment, and on 16<sup>th</sup> minute under 5% and 15% PEG-DS treatment. The maximum Tr was  $8.82 \pm 0.39$ ,  $8.46 \pm 0.11$ ,  $8.26 \pm 0.53$  and  $7.70 \pm 0.29$  under CK, 5%, 10% and 15%, respectively. IR64 showed a higher Tr of the detached leaves than Hanyou-3, although the Tr of the attached leaves was lower than in Hanyou-3. Moreover, PEG-DS had only slight effect on Tr in detached leaves but severely decreased in attached leaves. Furthermore, A and  $g_s$  were significantly correlated with  $K_{\text{plant}}$  ( $R^2=0.94$  &  $0.96$ , Fig. 4), but not with  $K_{\text{leaf}}$  (Fig. 4). In addition,  $K_{\text{plant}}$  was not related to  $K_{\text{leaf}}$  (Fig. 5).

## Discussion

The photosynthesis is limited by  $g_s$  under drought as the stomata controls the  $\text{CO}_2$  entry from atmosphere to the intercellular air spaces inside leaves at the cost of conserving water loss (Chaves *et al.*, 2002; Lawlor & Cornic, 2002). As for  $g_s$ , metabolic or biochemical impairment and  $g_m$  depression are the non-stomatal causes of A decrease (Maroco *et al.*, 2002; Santos-Filho *et al.*, 2014). Mesophyll conductance ( $g_m$ ) is considered to be finite due to the difference between  $C_i$  and  $C_c$  (Ubierna *et al.*, 2016). Variation in  $g_m$  is the physiological response to drought, and it limits photosynthesis partially (Warren, 2008). Previous studies show there is a positive correlation photosynthesis and  $g_s$  and  $g_m$  (Galle *et al.*, 2009). Similar result was also found in our study that A significantly positively correlated with  $g_s$  and  $g_m$ , but the correlation coefficient was lower in A versus  $g_m$  than in A versus  $g_s$  (Fig. 1). This suggested that  $g_s$  contributed more than  $g_m$  in the determination of A, and the lower correlation coefficient in A versus  $g_m$ , which was resulted from the unparallel changes of A and  $g_m$  in Hanyou-3 (Table 1).

Photosynthesis was declined under drought stress not only by the diffusive components ( $g_s$  and  $g_m$ ) but also by metabolic/biochemical impairments. Zhou *et al.*, (2007) reported that maximum velocity of RuBP carboxylation by Rubisco  $V_{\text{cmax}}$  and the capacity for ribulose-1,5-bis phosphate regeneration, determined by  $J_{\text{max}}$ , played significant role in decreasing photosynthesis in rice under 30% PEG induced drought stress. Similarly, current study showed significant decrease in  $V_{\text{cmax}}$ ,  $J_{\text{max}}$ , and CE under all PEG-IWDS levels in both varieties (Table 3). Rubisco carboxylation efficiency was reduced due to the inactivation of Rubisco enzyme, while the decrease in  $J_{\text{max}}$  was probably resulted from the deactivation of key regulatory enzymes of the Calvin cycle likesedoheptulose-1,7-bisphosphatase (SBPase) and fructose-1,6-bisphosphatase (Nogués & Baker, 2000; Ölçer *et al.*, 2001).

It was reported that leaf photosynthesis was determined by  $\text{CO}_2$  diffusion under moderate drought condition and metabolic impairment contributed only under severe drought condition (Zhou *et al.*, 2007). An  $A/C_i$  response curve can illustrate whether A is limited by stomatal or non-stomatal processes (Flexas *et al.*, 2006). In the current study, the  $A/C_i$  response curves revealed that photosynthesis under all PEG-IDWDS levels in Hanyou-3 was similar with the WWC treatment, while the photosynthesis of IR64 under all PEG-IWDS was significantly different from WWC (Fig 2). This suggested that the depression of A in Hanyou-3 was probable resulted from stomatal closure, while in IR64 was from the decreased  $g_s$ ,  $g_m$  and/or biochemical capacities.

The plant is one part of Soil-Plant-Atmosphere Continuum, facing two different environments, and subjects to water deficit stress at irregular intervals. Stomatal closure under water deficit stress is mainly caused by decreased leaf turgor pressure and atmospheric vapour pressure deficit (Chaves *et al.*, 2009). As higher  $g_s$  results in a higher photosynthetic rate (Hirasawa *et al.*, 2010), therefore  $g_s$  and boundary layer conductance determine the  $T_r$  (transpiration rate), and  $K_{\text{plant}}$  is the determinant of water potential at that  $T_r$  (Tyree & Zimmermann, 2002). Thus,  $K_{\text{plant}}$  is the regulator of  $g_s$  without desiccating the leaves (Virginia *et al.*, 2016). In the present study, significant and positive relationships between  $K_{\text{plant}}$  and A as well as  $g_s$  (Fig. 4) showed that photosynthesis could be substantially affected by  $K_{\text{plant}}$ .

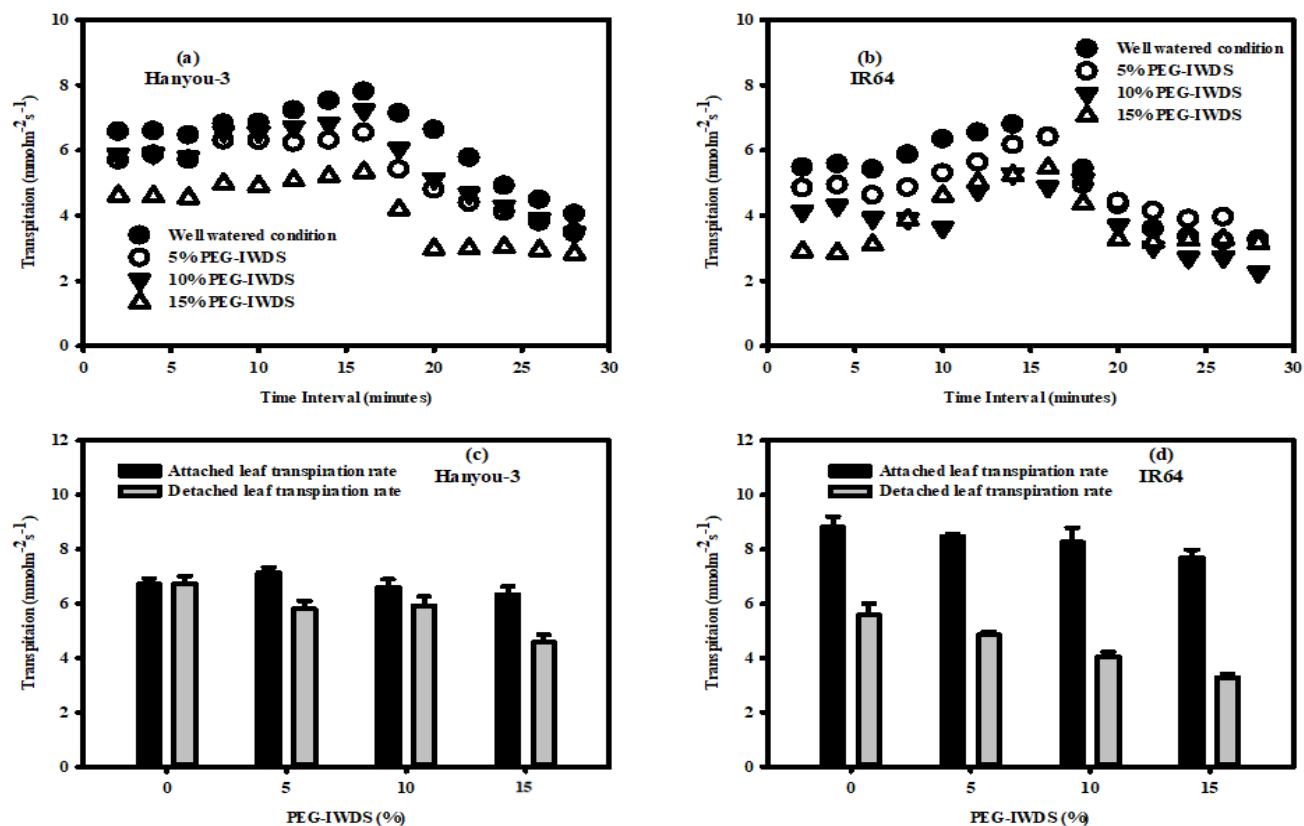


Fig. 3. Transpiration rate at different time intervals of Hanyou3 (a) and IR64 (b) and maximum transpiration rate of Hanyou3 (c) and IR64 (d) of under well-watered condition, 5, 10 and 15% PEG induced water deficit stress (PEG-IWDS) levels.

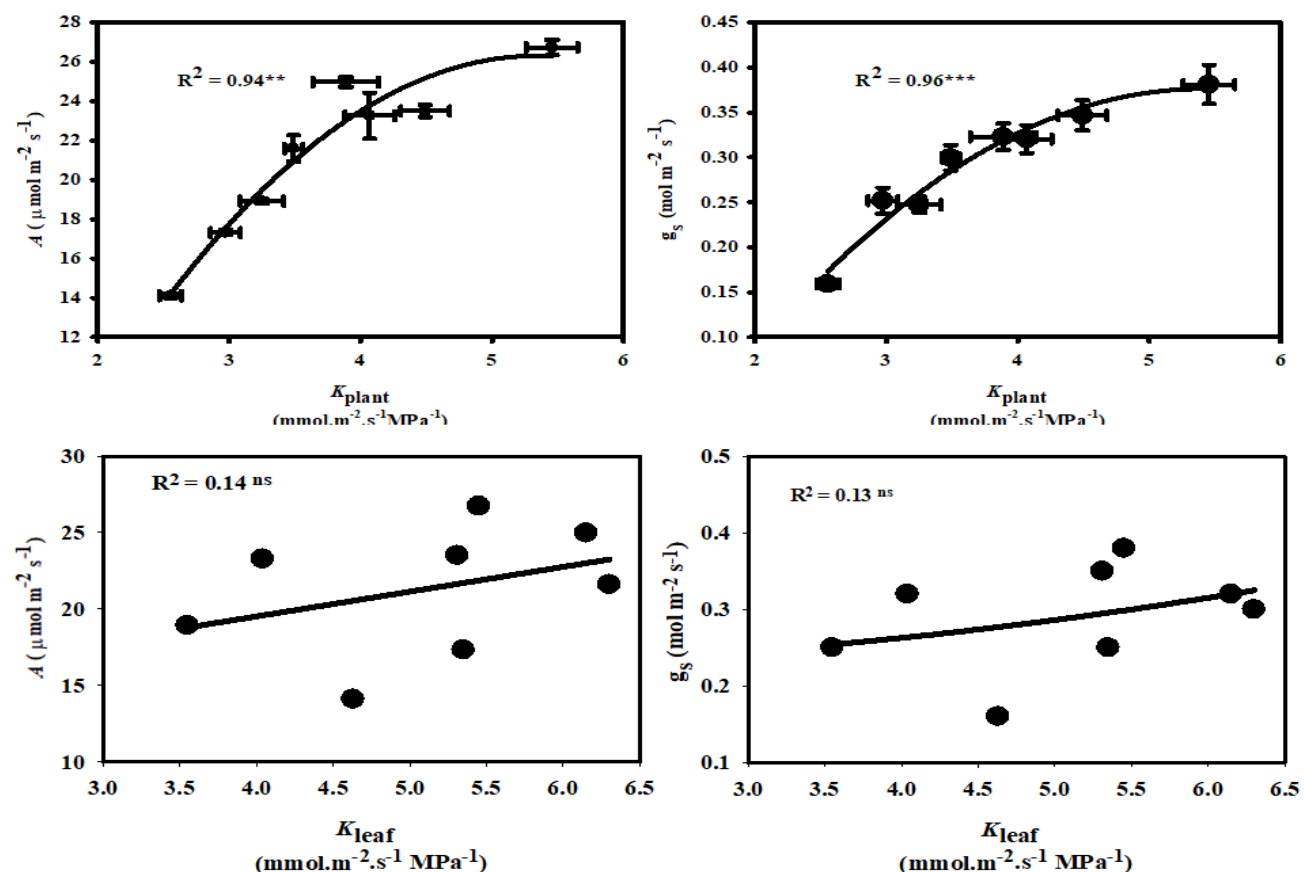


Fig. 4. Relationship between different photosynthetic gas exchange parameters and hydraulic conductivity parameters where, *A*, leaf photosynthesis; *g<sub>s</sub>*, stomatal conductance; *K<sub>plant</sub>*, plant hydraulic conductivity; *K<sub>leaf</sub>*, leaf hydraulic conductivity. ns represents no significant, while \*, \*\* and \*\*\* represent significant at *p*<0.05, *p*<0.01 and *p*<0.001 levels, respectively.

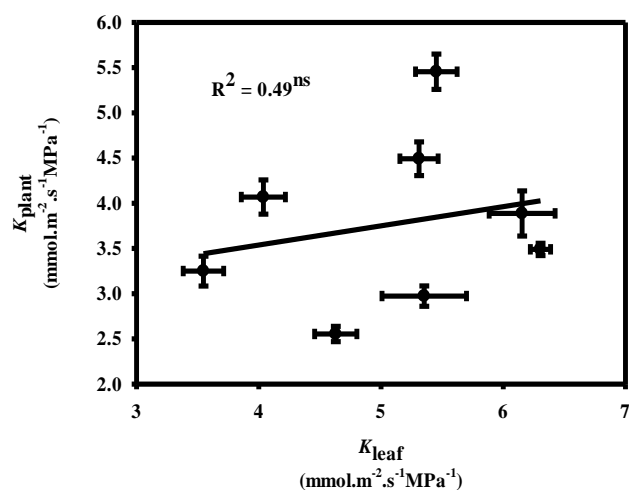


Fig. 5. Relationship between plant ( $K_{plant}$ ) and leaf hydraulic conductivity ( $K_{leaf}$ ) under well-watered condition (WWC), 5, 10 and 15% PEG induced water deficit stress (PEG-IWDS) levels. ns represents no significant, while \*, \*\* and \*\*\* represent significant at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  levels, respectively.

There are two major factors to determine  $K_{plant}$ . Leaf mesophyll cells account up to 30% (Sack *et al.*, 2006), while roots account up to 20% of whole plant resistances to water flow (Javot & Maurel, 2002). It is reported that rice roots under normal water condition have a high hydraulic resistance due to apoplastic barriers in different root tissues (Miyamoto *et al.*, 2001). Moreover, suberization and compaction of endodermis increased under water deficit condition, so changes in root anatomy contribute a major part to the decreased plant hydraulic conductivity (Henry *et al.*, 2012). In the current study, detached leaves were used to measure  $K_{leaf}$ , and found similar values of  $K_{leaf}$  and  $K_{plant}$  in Hanyou-3 under all treatment levels (Table 4), which suggested that leaf resistance was a major part of whole plant hydraulic resistance, and  $K_{leaf}$  was a key constituent of  $K_{plant}$ . This was in accordance with previous studies, which showed leaf resistance can reach 80-98% of whole plant resistance in some plant species (Nardini *et al.*, 2000; Bourne *et al.*, 2017). However,  $K_{leaf}$  was almost doubled in magnitude than the respective  $K_{plant}$  values under all treatment levels in IR64. This suggested that the root resistance in this cultivar also contributed a major part in whole plant resistance (Table 4). Therefore, the contribution of leaf and root resistance to whole plant hydraulic resistance is varietal dependent. The relative higher  $K_{leaf}$  in IR64 than in Hanyou-3 was probably resulted from its higher leaf vein density (Table 1), because leaf vein density was a major determinant of  $K_{leaf}$ .

## Conclusions

Photosynthesis ( $A$ ) was significantly decreased under PEG-DS, which caused by decline in  $g_s$ ,  $Tr$ ,  $V_{cmax}$ ,  $J_{max}$ ,  $CE$ ,  $K_{leaf}$ ,  $K_{plant}$  and  $XSFR$ . These decreases were more severe in DW-sensitive genotype IR64 than DW-tolerant genotype Hanyou-3.  $A$  and  $g_s$  were significantly correlated with  $K_{plant}$  but not with  $K_{leaf}$ , and  $K_{plant}$  was not related to  $K_{leaf}$ .  $K_{plant}$  rather than  $K_{leaf}$  determined photosynthesis in rice under drought conditions, which

was mainly attributed to  $K_{plant}$  decreased the stomatal conductance and ultimately lead to decrease in photosynthesis.

## Acknowledgements

This work was financially supported by the Natural Science Foundation of Jiangsu Province of China (BK20180923), Jiangsu Postdoctoral Sustentation Fund of China (2018K231C), the Natural Science Foundation of Jiangsu Higher Education Institutions of China (17KJB210008), the China National Key Research and Development Program (2017YFD0301205), Postdoctoral Foundation of Jiangsu Province of China (2018K231C), and Science and Technology Innovation Cultivating fund of Yangzhou University (2019CXJ198).

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