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EFFECTS OF DROUGHT STRESS ON PHOTOSYNTHESIS AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY OF TWO BIOMASS ENERGY PLANTS (*Jatrophacurcas* L. and *Verniciafordii* H.)

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ABSTRACT

Drought is considered the main environmental factor limiting plant growth in karst habitats. For this study, seedlings of *Jatrophacurcas* L. and *Verniciafordii* H. were submitted to a 10-day drought stress period at 5 different intensities. Photosynthesis, biomass, carbonic anhydrase (CA), and glucose-6-phosphate dehydrogenase (G6PDH) activities were determined. It was discovered that photosynthesis of the *V. fordii* was more sensitive to drought stress, and the water-use efficiency (WUE) of the *V. fordii* responded earlier in order to adapt to the induced drought stress. However, the *J. curcas* accumulated more biomass with better photosynthetic tolerance under drought stress. As the drought stress increased, the G6PDH activity of the *V. fordii* was activated at 20% or 30% PEG level, the higher G6PDH activity of the *V. fordii* maintained the redox equilibrium of the cells and produced NADPH for the formation of unsaturated fatty acids. As a result, the degree of unsaturation of fatty acids in the *V. fordii* was affected, and its cetane number and iodine value changed; thus, the fuel properties of the biodiesel were affected. Therefore, the photosynthesis and G6PDH activity of a plant should be taken into consideration when selecting appropriate biomass energy plants in karst habitats.

Key words: biofuel, carbonic anhydrase, chlorophyll fluorescence, glucose-6-phosphate dehydrogenase, photosynthesis

INTRODUCTION

Rapidly increasing energy demands have become a serious challenge both in developed and developing countries. The exploitation of biomass energy that is renewable and sustainable are effective solutions to this problem (Pandey *et al.*, 2012; Zhuang *et al.*, 2011). Energy plants are playing an increasingly essential role in the production of biodiesel. *Jatrophacurcas* L. and *Verniciafordii* H., belonging to the *Euphorbiaceae* family, are biomass energy plant species grown mainly in southwestern China (Park *et al.*, 2008; Zhuang *et al.*, 2011), and are also well-suited for cultivating in the marginal lands (Pandey *et al.*, 2012). The development of biomass on marginal lands in southwest China, especially in Guizhou and Sichuan Provinces, has been investigated and proved to be feasible (Wang *et al.*, 2014). Plants in karst regions commonly suffer from water stress during the rainy seasons, but the plants of southwest China are subjected to water stress throughout the year (Liu *et al.*, 2012). The fragile ecological environment leads to low crop production, which means the peasants in this karst area are very poor. However, the inhabitants of this area have the potential to benefit economically by growing energy plants that are adapted to karst conditions. Since drought stress in southwestern China affects the growth of *J. curcas* and *V. fordii*, oil production decreases. Therefore, researching these two biomass energy plants and their adaptability to drought stress is hugely important.

It is well known that one of the primary physiological consequences of drought is photosynthesis inhibition (Bauerle *et al.*, 2003). The photosynthetic activity of plants can be indicated by their chlorophyll *a* fluorescence (ChlF) parameters (Panda *et al.*, 2008), and can also be used as an indicator to measure the response of plants to environmental stress (Guo *et al.*, 2005). Photosynthesis of energy plants is the fundamental system required for the production of potential biomass energy. Advances in optimizing carbon fixation, carbon assimilation, carbon metabolism, and energy transfer are essential, as the efficiencies of these processes are the principal determinants of productivity (Work *et al.*, 2012). While caloric value is the primary energy stored in an energy plant (Lin and Cao, 2008). In order to evaluate the production of biomass energy in energy plants, photosynthesis, caloric value, and biomass production are used as the key research fields.

Influenced by drought stress, stomatal closure and reduced mesophyll conductance are the main cause of reduced CO_2 diffusion from the atmosphere to the site of carboxylation in leaves (Grassi and Magnani, 2005).

Greater levels of carbonic anhydrase (CA, EC 4.2.1.1) activity can enhance the conversion of intracellular bicarbonate to H_2O and CO_2 (Hu *et al.*, 2011). CA is a zinc-containing metallo enzyme that catalyzes the reversible conversion of CO_2 to bicarbonate [$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+$]. It is involved in diverse physiological processes especially the diffusion of inorganic carbon through the cells (Badger and Price, 1994). And glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) is a key enzyme which catalyzes the first reaction in the pathway leading to the production of pentose phosphates

$$[6G6P + 12NADP^+ + 7H_2O \rightarrow 5G6P + 6CO_2 + Pi + 12NADPH + 12H^+],$$

and produces reducing power in the form of NADPH to meet cellular needs for reductive biosynthesis and maintenance of the cellular redox state (Nemoto and Sasakuma, 2000). The synthesis of unsaturated fatty acidswas mainly from pentose phosphate pathway (Gaber*et al.*, 2001).

G6PDH plays an important role in the formation of unsaturated fatty acid. The cetane number (GB/T 20828-2007) and iodine value (EN 14214) meet the standards when the degree of unsaturation of fatty acids is lower than 133.1 (Ramos *et al.*, 2009; Wang *et al.*, 2012). In Indonesia, the degree of unsaturation of fatty acids in *J. curcas* is about 114.2, with a maximum value of 136.1 (Berchmans and Hirata, 2008). In Korea, the degree of unsaturation of fatty acids in *V. fordii* is about 126.8 (Chung, 2010). In southwestern China, the degree of unsaturation of fatty acids in *J. curcas* is 116.9 (Wang *et al.*, 2011), while the degree of unsaturation of fatty acids in *V. fordii* is as high as 184.0 (Xuan *et al.*, 2008). The degree of unsaturation of fatty acids in *V. fordii* is significantly higher in karst areas. The extortionate degree of unsaturation of fatty acids in *V. fordii* causes low cetane numbers and high iodine values, which affect the fuel properties of biodiesel (Ramos *et al.*, 2009; Wang *et al.*, 2012). Therefore, it is necessary to determine the response of G6PDH activities in *J. curcas* or *V. fordii* to a water deficit in karst habitats.

The objective of this study was to determine the photosynthetic characteristics and G6PDH activities in these two biomass energy plant species under drought stress. Photosynthesis, ChlF parameters, CA, and G6PDH activities under PEG6000-induced drought stress were analyzed. and processes of carbon fixation, assimilation, and metabolism in *J. curcas* and *V. fordii* were studied. This research aimed to understand the photosynthetic capacities and variations of G6PDH activities in *J. curcas* and *V. fordii* in drought stress conditions.

MATERIALS AND METHODS

Plant growth and drought stress treatment: Seedlings of *J. curcas* and *V. fordii*were cultivated and treated according to Xing and Wu (2012). Meanwhile, five drought stress levels were applied through incubation in five concentrations of PEG 6000: 0%, 5%, 10%, 20% and 30%. Determination was conducted on Day 10 from the onset of the treatment. Five recent mature leaves from three drought-treated seedlings were measured.

Measurement of net CO_2-assimilation rate: The determination method was same with Xing and Wu (2012).

Measurement of chlorophyll a fluorescence: The determination was conducted according to the method described by Xing and Wu (2012). The photochemical fluorescence quenching (qP) was calculated as (F′m – Fs)/(F′m – F′o).

Biomass: Fresh plant weight was measured with electronic analytical balance (*BSA124S, Sartorius,* Gottingen, Germany). Increment of biomass (P%) was calculated as $100\times(M_t\text{-}M_0)/M_0\%$, where M_0 was fresh plant weight (g) before drought stress and M_t was fresh plant weight (g) on Day 10 from the onset of treatment.

CA activity: It was determined with the electrometrical method of Wilbur and Anderson (Wilbur and Anderson, 1948) with modifications (Xing and Wu, 2012).

G6PDH activity: It was determined with the method described by Brown and Wary (1968).

Statistical analysis: All collected data were analyzed using SPSS software (version 13.0, SPSS Inc). The differences between drought stress levels were assessed using the least significant difference (LSD) post-hoc test at the 5% significance level ($p \leq 0.05$). The data were shown as the means ± standard errors (SE), which were determined using one-sample T test (confidence interval was 95%).

RESULTS

Photosynthesis: In Figures 1A and 1B,except for the *V. fordii* at 10% and 20% PEG levels, the g_s values between the two levels did not change significantly. Compared to the *V. fordii*, the *J. curcas* had higher P_N and g_s values at each PEG level. The P_N value of the *J. curcas* at 5%, 10%, 20%, and 30% PEG levels were 97.5%, 83.6%, 54.0% and 32.0% of that of the control, respectively. While the P_N of the *V. fordii* at 5%, 10%, 20%, and 30% PEG levels were 66.0%, 51.1%, 39.1%, and 22.3% of that at 0 PEG level, respectively.

Except for at 5% and 10% PEG levels, the Ci/Ca values of the *J. curcas* did not change significantly (Figure 1C). The Ci/Ca values of the *V. fordii* at 10% and 20% PEG levels, which were higher than that at 5% PEG level or control level, were statistically similar. Compared to the *V. fordii*, the *J. curcas* had the higher Ci/Ca values at each PEG level except at the 10% PEG level.

The WUE value of the *J. curcas* increased significantly at 30% PEG level compared to 20% level (Figure 1D). The WUE value of the *V. fordii* at 5% PEG level decreased significantly compared to the control. Although the WUE value of the *V. fordii* increased as the drought stress level increased, the WUE value of the *V. fordii* at 30% PEG level was still lower than at control level. Compared to the *V. fordii*, the *J. curcas* had higher WUE values at each PEG level except for 20% and 30% PEG levels.

Chlorophyll a fluorescence: The Fo value of the *J. curcas* began to increase at 20% PEG level as the drought stress level increased (Figure 2A), while a higher Fo value of the *V. fordii* was associated with a higher drought stress level.

For all species, thevalues of Fv/Fm decreased as the drought stress level increased (Figure 2B). A significant decrease of values of Fv/Fm was observed in the *V. fordii*, especially at 20% and 30% PEG levels. The qP values of the *V. fordii* decreased more significantly as the drought stress level increased compared to the *J. curcas* qP values (Figure 2C).

Increment of biomass: The P% of the *J. curcas* at 5%, 10%, and 20% PEG levels were 21.6%, 21.4%, and 12.0% of that at control level, respectively (Figure 3), and the P% of the *V. fordii* at 5% PEG level was only 8.9% of that at control level.

CA and G6PDH activities: Compared to the *J. curcas*, the *V. fordii* had statistically higher CA activities at each PEG level (Figure 4A). The CA activities of the *J. curcas* at 10% and 20% PEG levels were higher than that at 30% PEG level and lower than that at control level. The CA activities of the *V. fordii* at 10%, 20% and 30% PEG levels were lower than that at the control or 5% PEG levels. And the CA activity of the *V. fordii* at 10% PEG level was lower than that at 20% or 30% PEG level.

The G6PDH activities of the *J. curcas* at 5%, 10%, 20%, and 30% PEG levels were 95.8%, 81.8%, 63.0%, and 52.1% of that at control, respectively (Figure 4B). The G6PDH activities of the *V. fordii* at control, 10%, and 20% PEG levels were lower than that at 5% and 30% PEG levels. The G6PDH activity of the *V. fordii* at 30% PEG level was higher than that at 5% PEG level.

Furthermore, it was observed that the CA and G6PDH activities displayed a good correlation ($Y = 0.001X+0.219$, $R^2 = 0.46$, $n = 30$, $P < 0.0001$) (Figure 5).

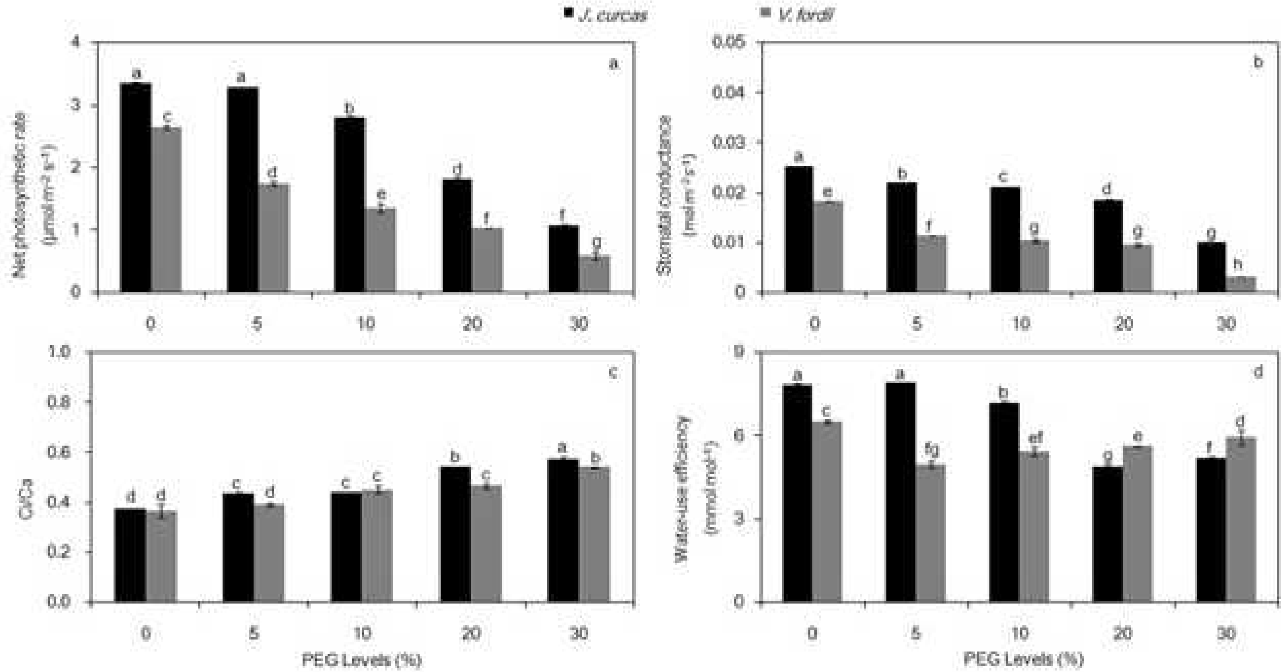

Fig. 1: Effects of drought stress on photosynthetic parameters of the two *Euphorbiaceae* plant species. Mean ± SE (n = 5) followed by different letters in the same parameter indicate significant difference at $P \leq 0.05$, according to one-way ANOVA and t-test

A. Net photosynthetic rate (P_N); B. Stomatal conductance (g_s); C. Ci/Ca; D. Water-use efficiency (WUE)

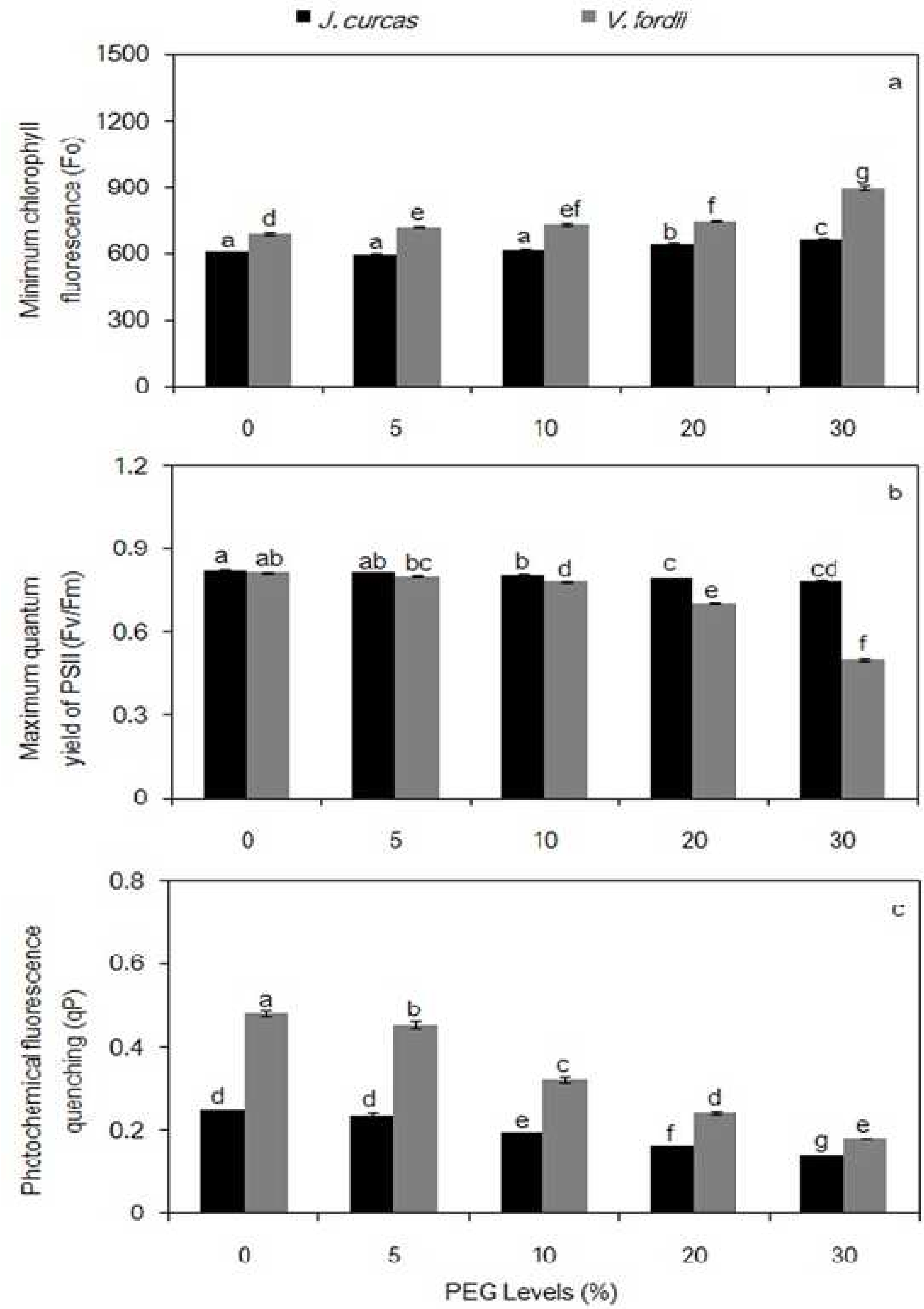

Fig. 2: Effects of drought stress on chlorophyll *a* fluorescence of the two *Euphorbiaceae* plant species. Mean ± SE (*n* = 5) followed by different letters in the same parameter indicate significant difference at $P \leq 0.05$, according to one-way ANOVA and t-test

A. Minimum chlorophyll fluorescence (Fo); B. Maximum quantum yield of PSII (Fv/Fm); C. Photochemical fluorescence quenching (qP)

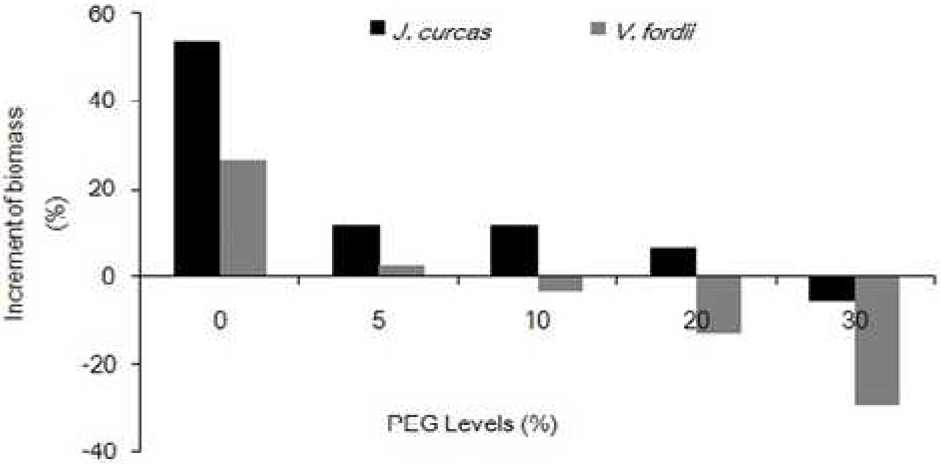

Fig. 3: Effects of drought stress on biomass of the two *Euphorbiaceae* plant species

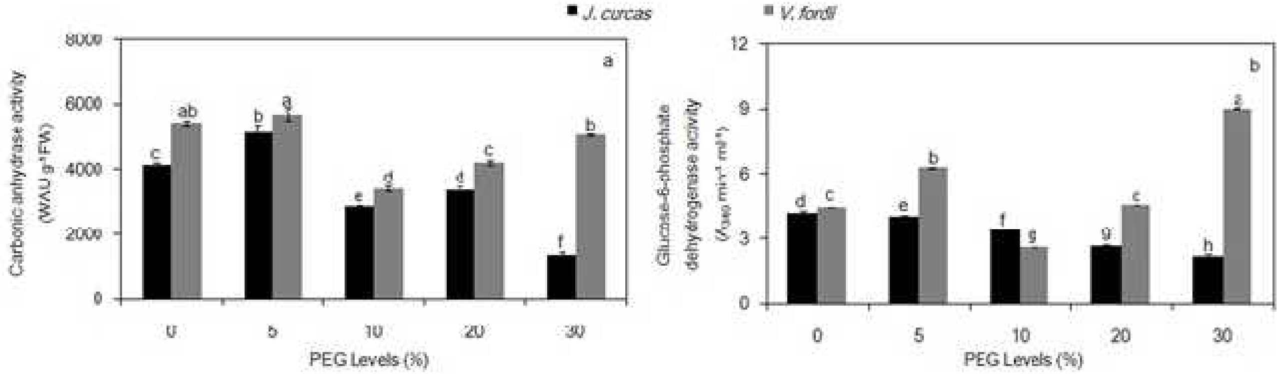

Fig. 4: Effects of drought stress on carbonic anhydrase and glucose-6-phosphate dehydrogenase activities of the two *Euphorbiaceae* plant species. Mean ± SE (*n* = 5) followed by different letters in the same parameter indicate significant difference at $P \leq 0.05$ according to one-way ANOVA and t-test

A. Carbonic anhydrase (CA) activity; B. Glucose-6-phosphate dehydrogenase (G6PDH) activity

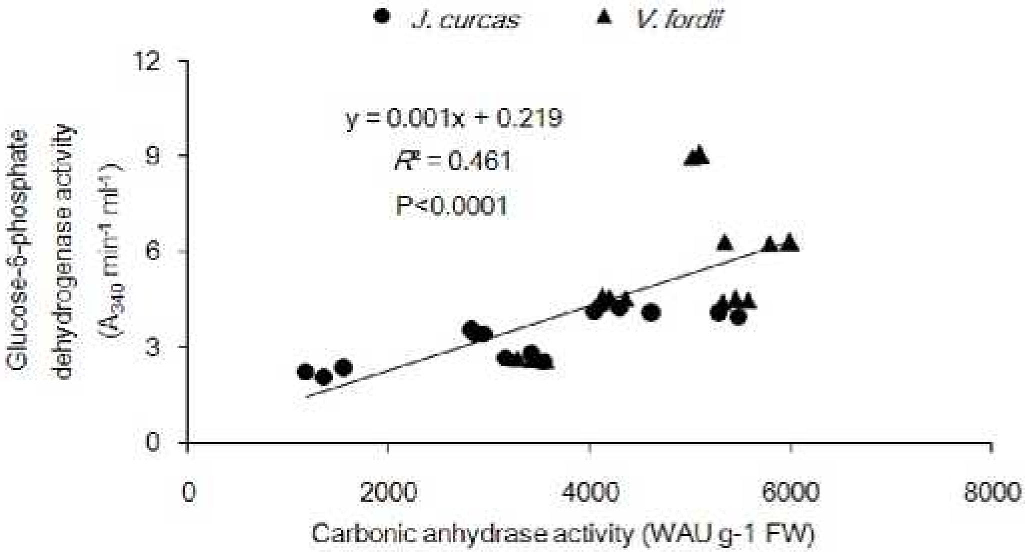

Fig. 5: Relationship between carbonic anhydrase (CA) and glucose-6-phosphate dehydrogenase (G6PDH) activities

DISCUSSION

Photosynthesis and biomass: In this study, the HCO_3^- concentrations in treatment solutions (10 mM) were higher than that in surface runoff water (5 mM) in the karst regions (Yan *et al.*, 2012). Stomatal conductance of the *J. curcas* and *V. fordii* were significantly lower due to high HCO_3^- concentrations. Bie *et al.* (2004) showed similar findings in two butterhead lettuce (*Lactuca sativa* L.) cultivars. The principal path of carbon from the atmosphere to the plant was via diffusion of carbon dioxide through leaf stomata into the leaf intracellular spaces and then through the walls of the mesophyll cells to the sites of fixation (carboxylation) (Mooney, 1972). When the stomatal of the *J. curcas* and *V. fordii* closed, it became harder for atmospheric CO_2 to enter into the intracellular spaces of the leaves. Under drought stress conditions, CA provided CO_2 and water converted from intracellular HCO_3^- for *P. nil* and *L. japonica* in response to low stomatal conductance (Xing and Wu, 2012). CA with higher activity also catalyzed the conversion of intracellular HCO_3^- to CO_2 and provided another carbon source for photosynthesis of the *J. curcas* and *V. fordii* under drought stress, which prevented the P_N of the *J. curcas* from decreasing, and prevented the PSII of the *V. fordii* from damage at 5% PEG level. Stomatal limitations are often considered a short-term response to drought stress, whereas non-stomatal effects are usually considered more important during longer and more severe drought stress conditions (Rouhi *et al.*, 2007). Results presented here clearly demonstrated that the P_N of the *V. fordii* was more sensitive to drought stress than in the *J. curcas*. The P_N of the *J. curcas* and *V. fordii* were inhibited by non-stomatal limitations between PEG levels of 10-30%. In order to be resistant to drought stress, the WUE of the *V. fordii* increased between PEG levels ranging from 5-30%; thus, the *V. fordii* gained higher inorganic carbon based on water loss during the transpiration process.

The photochemical apparatus of the *V. fordii* was damaged, and more specifically, the number of functional chlorophylls not connected to the reaction centers of the PSII increased at 30% PEG level (Panda *et al.*, 2008). There was more severe damage on the PSII reaction centers of the *V. fordii* compared to the *J. curcas*. The quantum yield of non-cyclic electron transport was found to be directly proportional to the production of the qP and the efficiency of excitation captured by open PSII reaction centers (Genty *et al.*, 1989). Higher qP was always accompanied with higher electron transport activity in PSII. Electron transport activity in the PSII of the *V. fordii* was higher than in the *J. curcas*, but electron transport activity in the PSII of the *V. fordii* was more sensitive to drought stress, especially at 20% and 30% PEG levels. *J. curcas* showed better drought resistance, as it exhibited better photosynthetic tolerance than the *V. fordii*.

Therefore, the *J. curcas* accumulated more biomass with better photosynthetic tolerance and inorganic carbon assimilation ability under drought stress, but the primary energy calorific value that was stored in organisms of the *V. fordii* could be higher than the *J. curcas* in karst areas (Pang *et al.*, 2013).

G6PDH and degree of unsaturation of fatty acid: As drought stress increased, the photosynthetic inorganic carbon assimilation ability of the *J. curcas* was inhibited. Carbohydrate synthesis, which was limited by photosynthetic efficiency, was reduced (Zhu *et al.*, 2013). The G6PDH was a rate-limiting enzyme in the pentose phosphate pathway, and the G6PDH activity of the *J. curcas* was inhibited by increasing intracellular CO_2 concentrations under drought stress. As a result, productive efficiency of unsaturated fatty acid in the *J. curcas* decreased. Inhibited by drought stress, the G6PDH activity of the *V. fordii* decreased at 10% PEG level, but then increased between PEG levels of 10-30%. Thus promoted the formation of unsaturated fatty acid in the *V. fordii*. As a result, the proportion of unsaturated fatty acid and degree of unsaturation in the *V. fordii* increased. Although the calorific value of the *V. fordii* was higher than the *J. curcas*, the higher degree of unsaturation of fatty acid in the *V. fordii* caused low cetane numbers and high iodine values, which could affect the fuel properties of the biodiesel.

Furthermore, higher G6PDH activities produced more CO_2 from the pentose phosphate pathway, and in order to alleviate the damage of excessive intracellular CO_2, the CA activities in the cells were activated to catalyze the conversion of CO_2 to bicarbonate.

Conclusions: Several conclusions were drawn from the above-described study. Photosynthesis of the *V. fordii* was more sensitive to drought stress. The PSII of the *V. fordii* showed more severe damage especially at 20% and 30% PEG levels. The WUE of the *V. fordii* responded earlier than the *J. curcas* in order to better adapt to drought stress. The *J. curcas* accumulated more biomass with better photosynthetic tolerance under drought stress. However, as drought stress increased, the G6PDH activity of the *V. fordii* was activated at 20% and 30% PEG levels. Higher G6PDH activity in the *V. fordii* helped to maintain the redox equilibrium of the cells and produce more NADPH for the formation of unsaturated fatty acid. As a result, the degree of unsaturation of fatty acid in the *V. fordii* was affected, the cetane number and iodine value in the *V. fordii* changed, and the fuel properties of the biodiesel were affected by drought stress. Therefore, the photosynthesis and G6PDH activity of a plant should be taken into consideration when selecting appropriate biomass energy plant species in karst habitats.

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