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Specific Difference in Photorespiration Activity in C4 Subtype Plants and Its Relationship with Drought Tolerance of Leaf Photosynthesis

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Carbon dioxide gas exchange rate (CER), stomatal conductance (Gs), and photosystem II quantum yield (Φ_e) were measured with three C4 subtype plants, corn (*Zea mays* L., NADP–ME type), common millet (*Panicum miliaceum* L., NAD–ME type), guineagrass (*Panicum maximum* Jacq., PEP–CK type) and a C3 type plant, mung bean (*Vigna radiata* L.) grown in droughts. The specific difference in drought tolerance in photosynthesis was discussed in relation to their photorespiratory activity. The C4 plants were superior to mung bean in both photosynthetic sustainability in droughts and photosynthetic recovery under re-watering after droughts. CER and Φ_e of corn showed a large reduction with a lenient decrease in leaf water potential (Ψ_L) compared to those of the other two subtypes. In both guineagrass and common millet Φ_e was sustained at a relatively higher level when their CER was decreased in droughts. A similar specific feature to this was found in leaves measured in the air with low CO₂ concentrations. This may suggest that there is an inter-subtype difference in photorespiration activity. Having a higher photorespiration activity is regarded as a cause for guineagrass and common millet to have superiority in drought tolerance. The inter-subtype difference in photorespiration activity was also able to be estimated from Φ_e of film-sealed leaves.

INTRODUCTION

Today the global warming and its resultant climatic fluctuations are widely predicted. Water is one of the important climatic factors regulating the photosynthesis and production of crops, and the yield reductions of crops due to drought stresses have been frequently reported recently. As a counter measure against this, it is required to release cultivars having a high tolerance under drought stresses and also establish feasible water saving agricultural systems.

Crops included in the C4 type are known to have a high tolerance against drought conditions. The C4 type plants are divided into three sub-types such as NADP–ME, NAD–ME and PEP–CK based on the kind of decarboxylation enzymes and the morphological feature in vascular bundle sheath cells. To compare the specific difference in drought-tolerance among C4 subtypes is interesting in understanding the relationship between photosynthetic metabolism and drought tolerance. Though the difference in photosynthetic activity has not always been significantly determined among the

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sub-types, there are some reports describing that the inter-subtype difference was found in the photosynthetic response in plants grown under drought conditions (Xu *et al.*, 1996; Nagasuga *et al.*, 2002).

It may be predicted that a more distinctive feature is shown in photosynthetic parameters in each C4 subtype plant, if they are exposed to drought conditions. With this prediction our experiment was designed and carried out using four materials such as corn (*Zea mays* L., NADP-ME type), common millet (*Panicum miliaceum* L., NAD-ME subtype), guineagrass (*Panicum maximum* Jacq., PEP-CK subtype) and mung bean (*Vigna radiata* L., C3 type). We tried here to clarify the specific and inter-subtype features in photosynthetic and photorespiratory responses on the basis of gas exchange rate and chlorophyll fluorescence intensity measured in drought-stressed plants, and discussed the relationship between photorespiratory activity and drought-tolerance in C4 subtypes. Furthermore, as an easy method to find the potential activity of photorespiration in C4 plants we employed the leaf-sealing method (Haimeirong *et al.*, 2002) and discussed its usefulness for checking the drought tolerance of C4 subtypes.

MATERIALS AND METHODS

Experimental materials and growth conditions

As experimental materials used are four species: three C4 subtype plants such as corn (*Zea mays* L., NADP-ME type), common millet (*Panicum miliaceum* L., NAD-ME type) and guineagrass (*Panicum maximum* Jacq., PEP-CK type), and a C3 type plant, mung bean (*Vigna radiata* L.). The plants were grown in 8 L pots containing sandy soil in the experimental farm of Kyushu University (33° 35' N, 130° 23' E) during June to August in 2002. As the basal fertilization, a chemical compound fertilizer (N₂:P₂O₅:K₂O = 16:16:16 in weight percentage) was used 5 g per pot.

Treatments

Plants were grown for 40 days under adequately watered soil conditions, before a drought stress was imposed to plants by cutting water supply for two days, and photosynthetic responses to the treatment were measured. Directly after the drought treatment, the plants were re-watered, and their recovery responses were observed 24 hours later. The measurement parameters were described below.

Measurements of leaf water potential (Ψ_L) and soil water potential (Ψ_s)

Leaf discs of 6 mm-diameter were cut off from an adjacent area of the leaf part used for CO₂ exchange rate (CER) measurement by an assimilation chamber. Directly after this, each of the leaf discs was placed in the chamber of a psychrometer (C-52, Wescor, USA) and Ψ_L was measured 3 hours later. At the same time, soil was sampled from three parts in a pot by using a sampling cylinder of 17 mm in diameter, and Ψ_s was measured with a psychrometer (WP4, Decagon, USA).

Measurements of CER and chlorophyll fluorescence (CF)

Fully expanded active leaves were used for measurements. CER and CF were simultaneously measured with a sandwich-type assimilation chamber and a fluorescence meter

(PAM2000, Walz, Germany), respectively.

The measured leaf area inside the assimilation chamber was 5.5 cm². A metal-haloid lamp (LS-M180, Sumita-Glass, Japan) was used as a light source and the light intensity supplied to leaf surface was fixed at 504 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Light intensity was measured with a light meter (LI-189, LI-COR, USA). The temperature of leaf was regulated at 30 ± 1 °C.

Carbon dioxide concentrations and water vapor deficits in the air sampled at the inlet and outlet of the assimilation chamber were measured with an infra-red analyzer (LI-6262, LI-COR, USA). The relative humidity in the air pumped into the assimilation chamber was adjusted at 50% using a dew point generator (LI-610, LI-COR, USA). The rate of air flow to the assimilation chamber was 1.00 L min⁻¹.

The concentration ratio of CO₂, O₂ and N₂ in the air was controlled with a gas mixture (GM-3A, KOFLOC, Japan). The concentration of CO₂ was finely controlled in the range from 0 to 367 μLL^{-1} by passing the air through a cylinder stuffed with soda lime as a CO₂ absorbent. The concentration of O₂ was measured with an oxygen meter (LC-750, TORAY, Japan).

During the measurement of CER, CF was periodically monitored by hitting a white light pulse (0.8 sec) of 8000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ to a leaf placed in the assimilation chamber. Photosystem II quantum yield (Φ_e) was calculated by the equation (1) according to the method described by Genty *et al.* (1989) and Bilger *et al.* (1990).

$$\Phi_e = (\text{Fm}' - \text{Fs}) / \text{Fm}' \quad (1)$$

Where Fs is a CF determined at the light intensity of 504 $\mu\text{mol m}^{-2}\text{s}^{-1}$ supplied for photosynthesis, and Fm' is a spike value of CF obtained when a light pulse of 8000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was supplied.

Measurement of CF with a film-sealed leaf

In advance of the measurement of a sealed leaf, CER and Φ_e were determined using a leaf with full opened stomata under the atmospheric CO₂ concentration, a light intensity of 504 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and a leaf temperature of 30 ± 1 °C. The light pulse for CF spike measurement was 0.8 sec of 8000 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Then both surfaces of this leaf were sealed with transparent films to halt the gas exchange between leaf and the atmosphere, and a stable value of Φ_e was determined 10 min after sealing.

RESULTS AND DISCUSSION

The parameter values of Ψ_L , Ψ_s , CER, Φ_e and stomatal conductance (Gs) are compared among the four species, and the effect of drought treatment on these parameters are shown in Table 1. In control pots without drought treatment, Ψ_s ranged from -0.43 to -0.5 MPa, showing no or little difference in soil water condition where the four species had been grown. On the other hand, a significant difference was found between them under drought conditions: Ψ_s in common millet and guinea grass had a relatively high value showing -1.49 MPa and -1.51 MPa, respectively; while the values of corn and mung bean greatly decreased to -2.87 MPa and -3.44 MPa, respectively.

Ψ_L in control plants of the four species was more than -0.38 MPa. Under drought conditions the parameter values of corn and mung bean were, -1.29 MPa and -1.50 MPa,

respectively, relatively high values among the four species. On the other hand, those of common millet and guinea grass greatly reduced to -2.10 MPa and -2.30 MPa, respectively. Of the three C4 subtypes, corn was characterized by having a lenient depression in Ψ_L under drought conditions.

A large reduction was found in CER of corn under drought condition; on the other hand, the drop of CER by the drought treatment was small in common millet and guinea-grass. The ratio of CER in drought to the control value was in the order of common millet (67.2%), guineagrass (37.2%), corn (27.2%) and mung bean (17.6%). It is well known that a larger depression found in the ratio in C3 plants compared to C4 plants, and the former is inferior in drought tolerance to the latter. In our experimental results, we were interested in the fact that under drought conditions, corn had a less decrease in Ψ_L and had a large depression in CER compared to the other two C4 subtype species.

The response of Gs to droughts was almost similar in tendency to that of CER, which may suggest that CER was chiefly restricted by Gs, or stomatal aperture. A largest depression in Φ_e was detected in corn among the three C4 subtypes. Though showing a large drop in CER as mentioned above, mung bean kept a higher Φ_e under drought conditions. In C3 plants, Φ_e usually holds a high level even when CER has been considerably reduced because chemical energy from the photosystem has been consumed by photorespiratory metabolisms; therefore, a linear relationship is not well established between both parameters. On the other hand, C4 plants with no or little photorespiratory activity lead to have a linear relationship between Φ_e and CER (Krall *et al.*, 1992).

As shown in Table 1, the re-watering after the drought treatment gave different recovering effects on CER, Gs and Φ_e among the four species. The recovery ratios in these parameters were lower in mung bean; for example, the ratio of CER was as low as

Table 1. Soil water potential, leaf water potential, CO₂ exchange rate (CER), quantum yield (Φ_e) and stomatal conductance (Gs) in corn (NADP-ME type), common millet (NAD-ME), guineagrass (PEP-CK type) and mung bean (C3 plant) under well-watered, drought and re-watered conditions.

	Corn (C4 plant, NADP-ME)			Common millet (C4 plant, NAD-ME)		
	well-watered	drought	re-watered	well-watered	drought	re-watered
Soil water potential (MPa)	-0.48	-3.44		-0.45	-1.49	
Leaf water potential (MPa)	-0.35	-1.29		-0.25	-2.10	
CER ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	19.4(100)	1.4(27.2)	16.7(86.1)	19.5(100)	13.1(67.2)	18.8(96.4)
Φ_e	0.52	0.08	0.50	0.50	0.41	0.52
Gs ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	0.21	0.01	0.15	0.16	0.08	0.21

	Guineagrass (C4 plant, PEP-CK)			Mungbean (C3 plant)		
	well-watered	drought	re-watered	well-watered	drought	re-watered
Soil water potential (MPa)	-0.5	-1.51		-0.43	-2.87	
Leaf water potential (MPa)	-0.31	-2.30		-0.38	-1.50	
CER ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	21.5(100)	8.0(37.2)	19.5(90.6)	16.9(100)	2.9(17.6)	8.1(47.9)
Φ_e	0.55	0.29	0.52	0.56	0.35	0.47
Gs ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	0.19	0.10	0.19	0.35	0.03	0.23

47.9%. In both common millet and guineagrass a considerable recovery was found in CER, and also G_s and Φ_e returned up to the pre-treatment levels, though the recovery ratio in these parameters was inferior in corn. When considering the less recovering ability together with the finding that a large drought-induced reduction in CER of this species, corn (NADP-ME) is presumed to be less tolerable to drought stresses compared to the other two C4 subtypes.

The drought tolerance in crops has been discussed from various physio-ecological and morphological view points related to cultivating managements. When the discussion is limited here to the photosynthesis of a leaf, it may be suggested that if there are some metabolisms consuming chemical energy when stomata are closed under drought condition, the photosynthetic system can be effectively protected against the photo-hazard caused by excessive energy accumulation in leaf. We placed a focus here on photorespiration as an energy consuming metabolism. The existence of photorespiration metabolism in C4 plants was biochemically and morphologically demonstrated by Ueno *et al.* (2001), and the inter-subtypical and inter-specific differences in photorespiration activity were described by Ku *et al.* (1974), Sato *et al.* (2001) and Yoshimura *et al.* (2003). According to these studies, the biochemical and morphological foundations for photorespiration is likely inferior in NADP-ME subtype plants to those of NAD-ME and PEP-CK subtype plants. Furthermore Yoshimura *et al.* (2003) predicted the specific difference in photorespiration activity in plants belonging to the same subtype. If photorespiratory activity is different among the C4 subtypes used here, it may be predicted that the amount of consumption in chemical energy is different among the subtypes and this results in affecting the relationship between CER and Φ_e .

To verify this prediction, the CER- Φ_e regression lines determined based on all the measurements obtained under control and treated plants are compared among the four species in Fig. 1. A linear relationship was found between these two parameters in all the four species. The C3 species showed a high Φ_e , having a lenient slope in the regression line compared to the C4 species. This may be caused by the difference in photorespiratory activity between C3 and C4 plants as mentioned above. Among the three C4 subtypes, a specific difference was found in the inclination of regression lines between CER and Φ_e . At high CER levels like about $20 \mu\text{mol m}^{-2}\text{s}^{-1}$, the inter-subtype difference was not found in Φ_e among the subtypes, but as CER decreased the inter-subtype difference in Φ_e increased. Particularly Φ_e of corn was higher than those of common millet and guineagrass when they were compared at low CER levels. In the case CER decreased by stomatal closure under drought conditions, the inter-cellular CO_2 concentration in leaf is depressed and this may cause the promotion of photorespiration. It may be predicted that the photorespiratory metabolism is more activated in common millet and guineagrass under drought conditions, by which their Φ_e is allowed to hold a higher level. In other words, the photorespiration was hardly enhanced in corn leaf under drought conditions.

As the next step to get an additional verification of the prediction mentioned above, CER and Φ_e were measured in the air with various CO_2 concentrations to observed the relationship between these two parameters. The results are shown in Fig. 2. Φ_e of the C4 subtypes decreased with CER depression caused by a reduction in CO_2 concentration, and the decreasing trend of Φ_e was more conspicuous in corn. The specific feature shown in Fig. 2 was almost similar to the result in Fig. 1. This may suggest that the

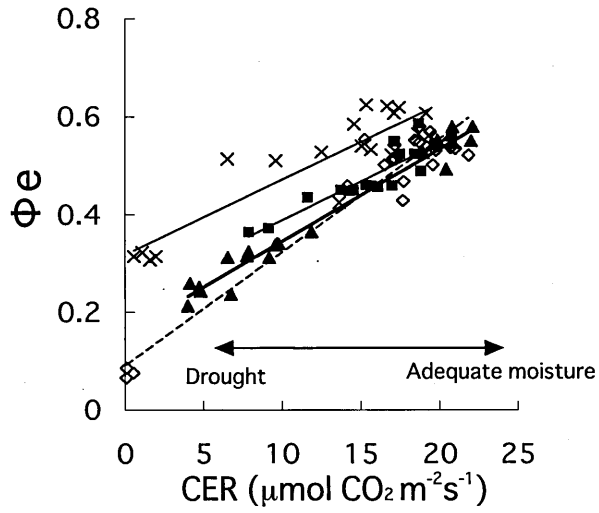


Fig. 1. The relationships between quantum yield (Φ_e) and CO_2 exchange rate (CER) in corn (\diamond , NADP-ME type), common millet (\blacksquare , NAD-ME type), guineagrass (\blacktriangle , PEP-CK type) and mung bean (\times , C3 plant) measured under various soil water conditions.

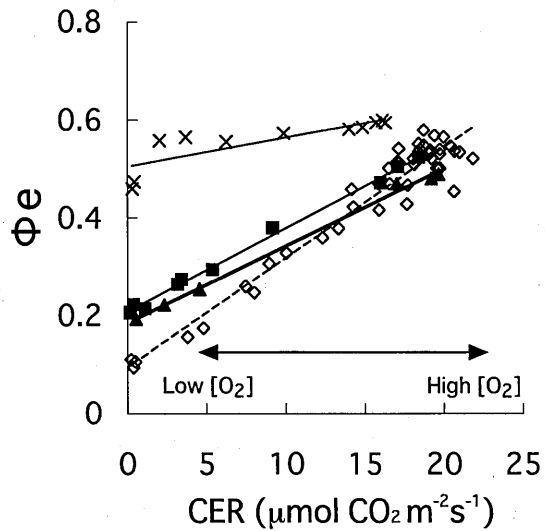


Fig. 2. The relationships between quantum yield (Φ_e) and CO_2 exchange rate (CER) in corn (\diamond , NADP-ME type), common millet (\blacksquare , NAD-ME type), guineagrass (\blacktriangle , PEP-CK type) and mung bean (\times , C3 plant) measured in different O_2 concentrations in the air.

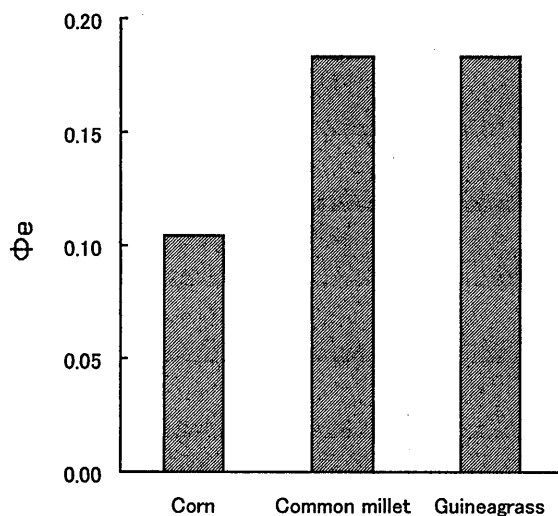


Fig. 3. The quantum yields (Φ_e) measured with film-sealed leaves in corn (NADP-ME type), common millet (NAD-ME type) and guineagrass (PEP-CK type).

inter-subtype differences in Φ_e and photorespiration activity are more definitely shown when the carboxylation activity is more greatly restricted.

Then the leaf-sealing method was employed to restrict carboxylation in a leaf. In this method, both surfaces of a leaf are sealed with transparent films to stop the gas exchange. By monitoring the chlorophyll fluorescence, the electron transporting status inside the sealed leaf is diagnosed. As shown in Fig. 3, there was a significant difference in Φ_e of sealed leaves between the C4 subtypes: the parameter value was high in common millet and guineagrass, and low in corn. This result may correspond to the inter-subtypical and inter-specific differences shown in Fig. 2 and 3. In the atmospheric air, C4 plants have almost no photorespiration, but under the condition where the intercellular CO_2 concentration is reduced by restricting the gas exchange by sealing, the latent photorespiration metabolism is activated and the specific difference in photorespiratory activity may become apparent among C4 plants. The water-water cycle is a different energy-consuming metabolism other than photorespiration (Makino *et al.*, 2002). But in our experiment the light intensity used was $504 \mu\text{mol m}^{-2}\text{s}^{-1}$, then it may be considered that in such a low light intensity the action of this cycle did not significantly affect the consumption of chemical energy.

The level of Φ_e has been often used as one of the parameters indicating the activity of photorespiration. Of the three subtype species used here, corn (NADP-ME) was characterized by having a comparatively low Φ_e when the CER was restricted. This may suggest that corn is a species having no or quite poor foundations for photorespiration, and as the result, this species was readily damaged under drought conditions and showed a less recovery in photosynthesis under the re-watering treatment. The leaf sealing

method is expectantly available to diagnose the photorespiration potential and drought tolerance in C4 subtype species, but further studies using a more variety of materials are necessary to make clear the relationship between photorespiration and drought resistance in leaf photosynthesis.

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