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Varietal differences in photosynthetic characters and chlorophyll fluorescence induction kinetics parameters among intergeneric progeny derived from *Oryza* × *Sorghum*, its parents, and hybrid rice

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Abstract: A comparative study on the photosynthetic parameters among intergeneric progenies derived from *Oryza sativa* L. × *Sorghum vulgare* L., its maternal parent Gui 630 and commercial 3-line hybrid rice Shanyou 63 in pot experiment in greenhouse was conducted. The morphological and photosynthetic characters of canopy leaves and chlorophyll fluorescence kinetic parameters including F_v/F_m, F_v/F_0, photochemical quenching coefficient and non-photochemical coefficient of canopy leaves of 3 varieties were measured. The results showed the progeny, Yuanyou 1, derived from an intergeneric cross of rice and sorghum possesses better canopy spatial architecture with thicker, heavier and bigger canopy leaf than its maternal parent Gui 630. Higher photosynthetic rate due to higher chlorophyll content, higher primary energy transformation efficiency, potential of PSII and non-photochemical quenching coefficient (q_E) were also measured in Yuanyou 1. These explain partly why the intergeneric progeny has higher biomass production, and better tolerance to adverse conditions and higher field yields even under stress conditions.

Key words: intergeneric hybrid, photosynthesis, chlorophyll fluorescence kinetics, non-photochemical quenching

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INTRODUCTION

Light energy absorbed by photosynthetic pigments (chlorophyll and carotenoids) in leaf mesophyll cells is mainly used for photosynthesis. However, a small part of the absorbed light is lost in the de-excitation process of excited chlorophyll a molecules as red chlorophyll fluorescence and infrared radiation (heat emission). Since the discovery of "the Kautsky effect", measurement of chlorophyll fluorescence has been developed as one of the most frequently used measuring tools in basic photosynthesis research. Chlorophyll fluorescence induction kinetics and various parameters and ratios derived from these reveal valuable information on the physiological rate of the photosynthetic apparatus in plants. Presently, various chlorophyll fluorescence parameters are applied to determine the photosynthetic capacity of leaves and the function of photosynthetic apparatus (Babani and Lichtenthaler, 1996). Study of the transient changes of fluorescence with time (Kautsky curve) measured on dark-adapted leaves provides very essential information for understanding the relation between light-harvesting, electron transport, thylakoid energetics and the process of CO_2 fixation. Among these parameters, F_0 is the minimal chlorophyll fluorescence intensity of the dark-adapted leaf; F_M is the maximal chlorophyll fluorescence intensity of the dark-adapted leaf; $F_V/F_0 = (F_M - F_0)/F_M$ is the maximal quantum efficiency of photochemistry of PSII; q_P represents photochemical chlorophyll fluorescence quenching coefficients, q_E the non-photochemical chlorophyll fluorescence quenching coefficients. Various variable chlorophyll fluorescence parameters, e.g. the ratios variable to ground chlorophyll fluorescence (F_V/F_0) and variable to maximum chlorophyll fluorescence (F_V/F_M) have been applied to investigate the variations in primary photochemical reactions of the photosynthetic apparatus (PSII). Any factors which affect the efficiency to capture the excitation energy by open PSII reaction centers, also modifies those chlorophyll fluorescence ratio parameters. The amplitudes of the previously defined coefficients of chlorophyll fluorescence quenching, q_P and q_E, reflect not only photochemical (q_P) due to photochemical energy conversion at PSII reaction centers with primary acceptors QA being oxidized, or non-photochemical (q_E) events as implied in the definitions, but also both photochemical and non-photochemical processes of PSII deactivation. If the partial connection between PSII units is taken into consideration, 1-q_P is non-linearly related to the fraction of closed reaction centers and is dependent on the rate constants of all (photochemical as well as non-photochemical) excitation-consuming process in PSII. On the other hand, 1-q_E equals the (normalized) ratio of the rate constant of photo-chemistry to the combined rate constant of all the non-photochemical deactivation processes excluding the rate constant of energy transfer between PSII units (Schreiber et al., 1986; Havaux et al., 1991).

Recording of chlorophyll fluorescence induction kinetics (Kautsky-effect) and the continuous determination of the photochemical and non-photochemical components of fluorescence quenching can be achieved by employing a measuring system which is designed based on a pulse modulation principle. Combined with the application of saturating light pulses, the modulation fluorometer can provide essential information beyond that obtained with conventional chlorophyll fluorometers (Schreiber et al., 1986).

Non-photochemical quenching of F_0, but not F_V, was found to be dependent upon the wavelength of emission, and was greater at 690 nm than at 730 nm. For emission at 730, compared to at 690 nm, about 30% of F_0 were not affected by non-photochemical quenching processes in leaves of C_3 plants; in maize leaves this was found to be about 50%. A substantial proportion of the pigments contributing to F_0 emission at 730 nm are not quenched by light-induced, non-photochemical quenching processes and large differences were found in the pigment matrices contributing to F_0 and F_V emissions at 730 nm, compared to those at 690 nm (Genty et al., 1990).

The intergeneric hybrid Yuanyou 1 showed greater grain yield potential, significantly higher photosynthetic rate and outstanding tolerance to gleying paddy soil, poor irrigation and other adverse conditions than its maternal parent and 2-line hybrid rice and 3-line hybrid rice in the fields production in South China (Li Damo et al., 1993; Tang Jianjun et al., 1995a; 1995b; 1996a; 1996b; 1997a; 1997b; 1997c; 1998a; 1998b). In order to elucidate the causative mechanisms leading to the high yield and photosynthetic capacity of Yuanyou 1, varietal comparison on the characteristics of chlorophyll a fluorescence induction kinetics parameters was conducted with its parents and 3-line hybrid rice and 2-line hybrid rice in pot soil culture experiments under greenhouse conditions. Parameters that related to photosynthesis involving chlorophyll content were analyzed too.

MATERIAL AND METHODS

1. Plants preparation

Materials used in this study were: (1) Yuanyou 1, a stabilized intergeneric hybrid derived from the progeny of *Oryza sativa* L. + *Sorghum vulgare* L., bred by means of Gui 630 self-fertilization supplementarily pollinated by sorghum pollen and induction by remote biotic active pollens; (2) Gui 630, the maternal cultivar of Yuanyou 1, an *indica* type rice variety; (3) 3-line hybrid rice Shanyou 63.

All materials were germinated in growth incubator and sand cultured for 25 days and then transplanted into pot soil with sufficient fertilizers or Yoshida's rice culture solution. Management practices of materials were kept same as those in the field production as previously described (Tang Jianjun, 1998). Considering the impossibility of measuring the parameters of leaves with the same age stage from different cultivars which have different growth duration, sampling and measurement were done at same date instead of the same growth stage. Then all results were evaluated with careful consideration of the leaf physiological age.

2. Pigment determination

The chlorophyll was spectrophotometrically determined. The fresh part of the functional canopy leaves were sampled and chlorophyll was completely extracted in 80% acetone in the dark for 36 hours and the chlorophyll content was quantitatively determined by 721-spectrophotometer and using the re-determined coefficients and equation. The values given are the means of 3 determinations.

3. CO_2 fixation measurement of functional canopy leaves

The photosynthetic rate of functional canopy leaves was measured by LI-6200 photosynthesis system (LI-COR Inc., Nebrasca, USA). The condition for photosynthesis measurement in the leaf chamber was not controlled but automatically detected on real time.

4. Chlorophyll fluorescence kinetics measurement

In vivo chlorophyll a fluorescence was measured at room temperature with pulse amplitude modulation chlorophyll fluorometer PAM 2000 (Walz, Effectrich, Germany) as described (Schreiber et al., 1986). The leaf samples were dark-adapted (at least 2.5 h) before the beginning of experiments. The initial chlorophyll fluorescence intensity of the dark-adapted sample (F_0) was obtained upon excitation with a weak measuring beam. Then a higher intensity was chosen to ensure a reliable signal. An 800 ms saturating pulse was used for determination of the maximal fluorescence intensity (F_M) of the dark-adapted samples. The correct intensity and pulse length was checked via the kinetics of the chlorophyll fluorescence response to the light pulse. Maximum chlorophyll fluorescence intensity during the actinic illumination (F_M') was determined using additional saturating pulses. The first pulse was applied 2s after switching on the actinic PAR and the following ones in 20s intervals. The $1-q_P$ parameter was calculated using the equation $1-q_P = 1-(F_M' - F_t)/(F_M' - F_0)$, where F_t was the chlorophyll fluorescence intensity at the time t of the actinic illumination. The coefficient of non-photochemical quenching (q_E) was calculated from the equation $q_E = (F_M - F_M')/(F_M/F_0)$ (Schreiber et al., 1986).

RESULTS AND DISCUSSION

1. Morphological and photosynthetic characters of functional leaves

Morphological characters related to photosynthesis were comparatively shown in Table 1. The results showed that the intergeneric progeny Yuanyou 1 derived from the germplasm recombination of distant C_4 donor and C_3 parent had significantly larger, heavier and thicker functional canopy than its maternal parent and commercial hybrid rice. Its ideal plant type, especially canopy architecture and foliar characteristics might be considered as one of some reasons for the high photosynthetic efficiency of the Yuanyou 1 population.

Table 1 Morphological characters of canopy at late tillering stage and chlorophyll content in flag leaf in early season

Material	The uppermost leaf area per plant (cm^2)	Leaf dry weight per plant (mg)	Specific leaf weight of functional leaves(mg/cm^2)	Chlorophyll content of flag leaf(FW[a] mg/g)
Yuanyou 1	98.3 ± 7.5	83 ± 0.21	3.136	2.03 (early milky stage)
Gui 630	63.5 ± 5.2	24 ± 0.07	2.330	1.67 (panicle initiation)
Shanyou 63	81.0 ± 1.4	70 ± 0.21	2.373	1.86 (flowering stage)

[a] Fresh Weight

In addition, Yuanyou 1's chlorophyll content also had advantages over other materials despite its development was faster than that of Gui 630 and Shanyou 63. Generally speaking, the chlorophyll content in functional canopy leaves of a rice variety decreases gradually since flowering, i.e. chlorophyll content of canopy leaves at more aged stage is lower than that from younger plant sample. For the same reason, physiological measurement of leaf sampled at lower position is usually lower than that sampled at higher leaf position of the same culm.

The photosynthetic rate of Yuanyou 1 was higher than that of Shanyou 63 and Gui 630 at all leaf positions even though Yuanyou 1 was at the late milky stage while the other two materials were at the early milky stage or even panicle initiation stage when being measured (see Table 2). The higher photosynthetic rate of Yuanyou 1 might be related to its distant C_4 parent. Yuanyou 1 showed a slower decrease of photosynthetic rate downward along the stem than that of other materials. This showed that Yuanyou 1 had ideal foliar architecture compared to that of Shanyou 63 and Gui 630. The chloroplast water photolysis capacity, which is another indicator of the carbon fixation ability of mesophyll, provides additional evidence for the higher photosynthetic potential of Yuanyou 1. Despite the leaf age, the Hill reaction activity of chloroplast from Yuanyou 1's canopy leaves was 65.0% and 19.8% higher than that of Shanyou 63 and Gui 630 respectively. On the other hand, the compensation point of the flag leaf measured by LI6200 with zero net photosynthesis showed Yuanyou 1 had a lower compensation point than that of Shanyou 63 and Gui 630 despite its physiologically more aged flag leaf (see Table 2). The elder leaf, for example, the leaf sample at a lower position of the same culm usually showed a higher carbon dioxide compensation point in this study. All photosynthetic parameters of Yuanyou 1 differed notably from its maternal parent.

2. Comparison of chlorophyll a fluorescence induction kinetics parameters among materials

As mentioned above, analysis of measured results of chlorophyll a fluorescence induction kinetics parameters could reveal the mechanism of the photosynthetic potential of Photo-system II.

Table 2 Varietal comparison of photosynthetic characters of canopy leaves cultivated as late season crop

Material	Photosynthetic rate (CO_2 μmol/m^2·s)			Hill reaction activity of chloroplast from canopy leaves (Fe^{2+} μmol/chl mg·h)	CO_2 compensation point of flag leaf (μmol/L)	Growth stage
	The flag leaf	The 2nd uppermost leaf	The 3rd uppermost leaf			
Yuanyou 1	16.75 ± 4.60 (100)	14.79 (88.30%)	13.26 (79.16%)	6.60	3.2011	Late milky
Gui 630	14.64 ± 3.16 (100)	8.19 (55.94%)	6.45 (44.06%)	5.51	4.1521	Panicle initiation
Shanyou 63	12.81 ± 1.13 (100)	10.26 (80.09%)	9.54 (74.47%)	4.00	3.3484	Early milky

Table 3 F_V/F_0 and F_V/F_M ratio of chlorophyll *a* in the functional leaves at the reproductive stage in early season

Material	F_V/F_0 (potential activity of PSII)	F_V/F_M (primary energy transformation efficiency)	Growth and development stage
Yuanyou 1	4.5208 ± 0.3787	0.8146 ± 0.0005	booting stage
Gui 630	4.2293 ± 0.0831	0.8087 ± 0.0003	panicle initiation phase
Shanyou 63	4.8020 ± 0.5199	0.8156 ± 0.0006	Booting stage

The maximal quantum efficiency (F_V/F_0) of PSII varied slightly among different materials grown in the early season (see Table 3). Yuanyou 1 had an intermediate value of 4.5208. The primary energy transformation efficiency of PSII of Yuanyou 1 and Shanyou 63 did not differ significantly, but the difference between Yuanyou 1 and Gui 630 was significant. The heterosis from wide cross was obvious. But the relationship between the F_V/F_M or F_V/F_0 and photosynthetic rate was not so clear.

To determine the response of PSII to excess solar radiation, materials sampled at 10:00 am when the solar radiation is mild and at 13:30 when the solar density is excessive in the late cultivating season were measured. Results obtained are shown in Table 4.

Table 4 Chlorophyll a fluorescence induction kinetics parameters in the functional leaves at the ripening stage in the late cultivating season

Material	Reducing capacity $(1-q_P)$ at 10:00 in sunny day	q_E measured at 10:00 in sunny day	q_E measured at 13:30 in sunny day	Growth stage
Yuanyou 1	0.3719	0.8845	0.6378	Late dough
Gui 630	0.2826	0.8175	0.5858	Late flowering
Shanyou 63	0.2500	0.7719	0.5260	Yellow maturity

Under sufficient solar condition, the intergeneric hybrid Yuanyou 1 showed higher reducing capacity, which is related to carbon dioxide fixation, than that of its maternal parent and Shanyou 63. q_E reflects the non-photochemical quenching ability of PSII and is related to anti-photo-inhibition under excessive solar radiation. The results obtained suggested that intergeneric hybrid Yuanyou 1 had more adaptive potential than its maternal parent and 3-line hybrid rice to varying solar condition.

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