**Supplementary data file 1**

**Survey of photosynthetic traits in background parental lines and photosynthetically diverse plant species**

The trial was carried out in the glass house facilities at the International Rice Research Institute (IRRI), Manila, the Philippines to analyze the photosynthetic properties of diverse plant species belonging to C3, C3-C4, and C4 types to include as control lines in the screen. The parental rice line IR64-21 (C3-type), *Panicum miloides* L. (C3-C4 intermediate type), sorghum (*Sorghum bicolor* (L) Moench*,* C4 type), maize (*Zea mayes*) and *Echinochloa glabrescence* L. (C4 type) were grown in pots (30cmx30cm x60cm) with the sterilized soil medium. These plants were arranged in complete randomized design with four replicates within the glasshouse. When the plants produced their 6th fully expanded leaves, the photosynthesis analyses were made with an infra-red gas analyser, commercial portable photosynthesis system ( LICOR LI-6400 XT, Nebraska, USA). The assimilation against the internal CO2 concentration curves (A/Ci curves) were constructed as per the instructions given by the manufacturer’s manual. Then the curves were analysed with the Microsoft Excel 2007 (Microsoft Corporation, USA). For each of the plant types, the CO2 compensation points ( Г ) were calculated as the x- intercept of a linear regression through the five lowest intercellular CO2 values on a graph of net CO2 assimilation (*A*) versus internal CO2 levels (Ci) (Vogan *et al.,* 2007). The initial slope of *A/Ci* curves were estimated for slopes, as maximum carboxylation efficiency (CE) and overall respiration rate (Ro) estimated by interpolation of liner regression to the Y-axis. Then maximum photosynthetic rate the leaf can attain at saturated light with the provision of 400 µmol mol-1 CO2 levels is referred as ambient level (A400) were also estimated from these A/Ci curves.

A comparison of gas exchange parameters for background parental lines were made. Here, we tested the response of three-types of pathways the C3, C4 and C3-C4 intermediates. Typically, the IR64-21 is the parental rice line for rice deletion mutants follows C3-type, *Panicum miloides* is a C3-C4 intermediate type, whereas sorghum, (*Sorghum bicolor),* maize (*Zea maiz*) and *E.* *glabrescens* strictly follows of C4 type. The reason for testing *E. glabrescens* photosynthesis parameters in the present study was that *E. glabrescens* requires same ecological growth conditions as rice, such as high water-logged conditions coupled with increased growth temperatures ( above 28 oC) and they grow as an invasive C4 weed in rice fields.

**Table 2.** Photosynthetic properties in the 5th leaf of background plant species. The CO2 compensation point (Г) carboxylation efficiencies (CE) and estimated overall respiratory rates (Ro) in IR64 parental lines, *Panicum miloides* , maize, sorghum and *E. glabrescens* are showed. SEM- standard error of mean. Asterisks represent the significance levels when comparing with IR64 plants. \* P<0.05, \*\* P<0.01, n = number of plants tested in the experiment.

E:\Thesis-Post viva voce\Post-viva-Chapter 3 Draft 2\Table 31.tif

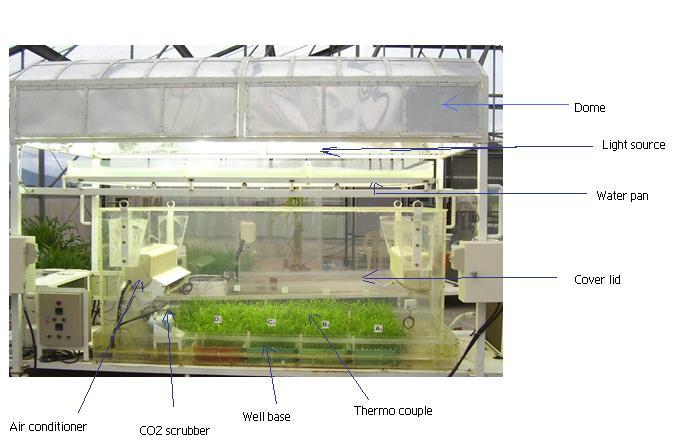
As shown in Table 2, the rice plants possessed significantly increased Г than the C4 and C3-C4 intermediates with an averageГ of 56.2 ±0.48 µmol mol-1. In contrast for *P. miloides* (C3-C4 intermediate) displayed Г value was 24 µmol mol-1, while in typical C4 plants such as maize and sorghum showed Г below 5 µmol mol-1, suggesting that the CO2 compensation point can be used as one of the powerful tool to discriminate plant species. As such, this trait can be used as a criterion for screening of photosynthetically efficient plants.

Distinguishable variations were also be seen for carboxylation efficiencies among the tested lines. The carboxylation efficiencies of Rubisco enzyme were measured from the slope of A/Ci response curves. The C4 plants showed significantly increased CE than rice plants. However, in P. *miloides*, displayed insignificant differences in CE when compared to the rice plants, although they possessed lower CO2 compensation point. The overall estimated respiratory rates (dark + light) of plants were compared. C4 plants had significantly lower Ro than the wild type rice plants. Similarly, *P.miloides* also possessed lower rates of Ro than the wild type rice counterparts.

**Supplementary data file 2**

**Components of a CO2 chamber**

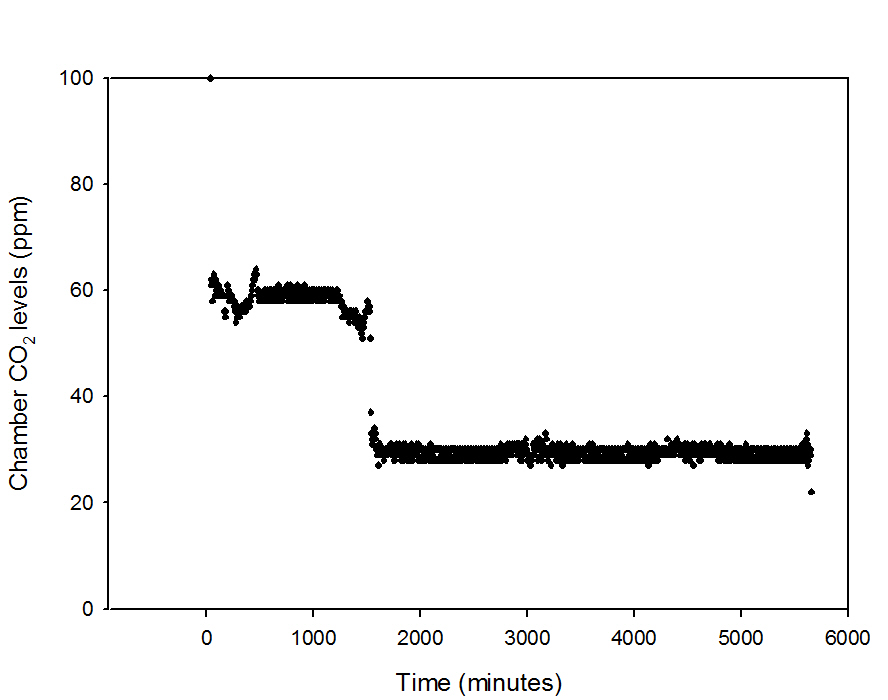
The re-modified screening chambers (1mx1mx1m) at IRRI were designed by John Sheehy *et al*. (IRRI, 2008) after series of discussion with Menz *et al* (1969) for screening of wild rice species at lower CO2 levels (IRRI, unpublished data). The chamber consists of three-primary structures, they are Dome, Cover-lid and well-base (Figure 1). The dome serves as the structure to hold the high-density discharge lamps which are used in day times on cloudy days and throughout the night. The dome also holds a water-pan in order to absorb the heat of the lamps. The cover lid is made of ½” transparent acrylic and serves as air tight unit in the chamber. The well-base is used to keep seedlings and reserves for supply of nutrient solution to the plants (Yoshida 1981).

****

**Figure 1.** Structure ofHigh-throughput CO2 screening chamber. This consists of three major components viz; Dome, cover lid and well base of chamber. Designed and re-modified by John Sheehy *et al.* (2008).

Mechanisms of CO2 control in the chamber

The CO2 levels in the chamber were monitored with 4-major sub units. Viz: WMA-4 analyser a non-dispersive infrared gas analyzer, CO2 control board, CO2 scrubbing unit and the solenoid switch. WMA-4 CO2 analyser (PP Systems, Hitchin, UK) monitors the CO2 levels (ppm) within the chamber and provides electronic signals to the CO2 controller unit, which is fixed in main console. When the CO2 level rises above the set value in the chamber, the control board sends signals to the CO2 scrubber (a suction pump which passes through the air through soda lime). When the CO2 levels goes below the set values in chamber, solenoid valve receives signals from CO2 control board and the solenoid switch allows fresh air to flow inside the chamber. Relative humidity (RH%) and temperatures were monitored with computerised units of the chamber. Generally the RH % was maintained 60% to 65% and the temperature at 28o C, the temperature probes positioned inside the chamber (cover-lid) which were connected to an air-conditioners or air chillier systems.  A constant air flow rate (0.4L/minute) which was maintained with a flow metre through the 4 days of treatment. In terms of CO2 treatment regimes, the 1st day maintained at 60 ppm and the remainder three-days maintained at 30ppm levels.



**Figure 2.** CO2 levels in chambers over the 4-days of treatment. It maintained around 60ppm on the 1st day, then 30ppm for the next 3-days. The CO2 levels were monitored using WMA-CO2 analyzers.