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Characterization of *Oryza nivara* introgression lines: A potential prebreeding resource to improve net photosynthetic rate in elite cultivars of rice

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Abstract

Photosynthesis is one of the fundamental processes influencing crop growth and productivity. In order to understand better the basis of variation in net photosynthetic rate (P_N) and yield potential in rice, two backcross populations derived from Swarna × *O. nivara* were studied. Gas exchange and chlorophyll fluorescence measurements were taken at flowering stage and data on yield traits at harvest. P_N was significantly correlated with stomatal conductance, transpiration rate, mesophyll conductance, carboxylation efficiency, yield per plant (YLDP), and dry mass in both populations. Ten introgression lines (ILs) showed higher P_N than their parents. IL 230S showed highest P_N with increased YLDP than the remaining ILs. Single marker analysis showed RM514 and RM48 was positively associated with P_N and YLDP in popA, whereas RM204 and RM122 in popB. The first 4 principal components contributed 92 and 93% to the total genetic variation in each population, respectively.

Additional key words: fluorescence; introgression lines; *Oryza nivara*; photosynthesis; single marker analysis; yield.

Introduction

Rice is vital for more than half of the global population and the second most commercially important cereal crop after wheat. Unpredictable climate changes, degradation of natural resources, and a continuous increase of population threaten global food security. Any increase in rice production can be achieved only through increasing grain yield from the limited land resources available. In order to meet this challenge, rice varieties with a higher yield potential have to be developed to minimise the gap between the yield potential and average farm yields. Among many factors associated with the grain yield, photosynthesis is a fundamental physiological process and a key route to increase crop growth rate and genetic yield potential (Masumoto *et al*. 2004, Long *et al*. 2015).

Improvement in photosynthesis is important for biomass production. This would help to better utilization of solar radiation which can be translated into the grain yield (Long *et al*. 2015). Around 90% of grain dry matter is formed from the products of photosynthesis after heading, particularly from flag leaves (Xie *et al*. 2011). Further, allocation of photosynthates depends on source–sink relationships, which in turn are determined by many morpho-physiological traits (Niinemets 2015). However, flag leaf is an important photosynthetic assimilation organ in rice, especially, during the reproductive stage. It plays a greater role in a grain yield increase by contributing about 41–43% of grain mass (Al-Tahir 2014). In addition, larger flag leaf area during the grain-filling stage also contributes largely to the grain yield by increasing the leaf chlorophyll (Chl) content (Kumari *et al*. 2011, Al-Tahir 2014).

There is a significant positive association between leaf structural traits and physiological traits (Giuliani *et al*. 2013). In general, leaf thickness has a positive association with photosynthesis because of a high Chl content in thicker leaves (Rahman *et al*. 2013) that directly affects the crop biomass (Shen 1980, Chen *et al*. 1995) and grain

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Abbreviations: *Car* – carotenoids; CE – carboxylation efficiency (= P_N/C_i); C_i – intercellular CO_2 concentration; C_c – chloroplast CO_2 concentration; Chl – chlorophyll; TChl – total chlorophyll; DM – dry mass; *E* – transpiration rate; ETR – electron transport rate; F_v/F_m – maximum photochemical efficiency of PSII; F_v'/F_m' – maximum quantum yield of PSII after light adaptation; g_m – mesophyll conductance; g_s – stomatal conductance; HI – harvest index; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; R_D – dark respiration; WUE – water-use efficiency (= P_N/E); WUE_i – intrinsic water-use efficiency (= P_N/g_s); Φ_{CO2} – quantum yield of carboxylation rate; Φ_{PSII} – effective quantum yield of PSII photochemistry. YLDP – yield per plant.

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yield (Xu and Shen 1994). Unlike stomata, mesophyll is also an important decisive factor in rice photosynthesis as 90% of chloroplasts are located there. It affects the CO_2 diffusion (g_m) from intercellular air space to carboxylation site and is also influenced by the constitutive properties of leaf anatomy (Giuliani *et al.* 2013). However, the rate of photosynthesis has been limited by Rubisco carboxylation capacity, which is dependent on the CO_2 concentration in chloroplast stroma (C_c) (Gu *et al.* 2012, Giuliani *et al.* 2013). Since rice is a C_3 plant, the entire photosynthesis process takes place in mesophyll cells. Therefore, it was expected that the grain yield would be improved by exploitation of morphological and physiological characteristics which are related to gas exchange.

The genetic variability has become very low in modern cultivars due to intense selection for crop yield while ignoring other associated traits, such as photosynthetic rate per unit leaf area (Richards 2000). It is well-known that introgressions from wild species helps improve qualitative traits, such as disease and pest resistance (Kumar *et al.* 2013, Sarao *et al.* 2016) as well as quantitative traits, such as the grain yield (Gaikwad *et al.* 2014, Arbelaez *et al.* 2015, Ma *et al.* 2016, Eizenga *et al.* 2016, 2017, Bessho-Uehara *et al.* 2017, Bhatia *et al.* 2017, Haritha *et al.* 2018), and abiotic stress tolerance to salinity (Quan *et al.* 2018), drought (Kaur *et al.* 2017), and heat (Prasanth et al. 2017) in cultivars. *O. rufipogon* and *O. nivara* and their derived lines showed a high Chl content, P_N, stomatal conductance (g_s), transpiration rate (E), carboxylation efficiency (CE), and water-use efficiency (WUE) (Zhao *et al.* 2008, 2010; Kiran *et al.* 2013, Kondamudi *et al.* 2016, Haritha *et al.* 2017, Hamaoka *et al.* 2017). Total dry mass was improved in BC_2 lines derived from *O. sativa* × *O. rufipogon* (Masumoto *et al.* 2004, Haritha *et al.* 2017), whereas, CE was improved in BC_2F_2 lines derived from new plant type (NPT) rice line IR65598-110-2 × *O. longistaminata* (Ding *et al.* 2014) compared to parents. Therefore, wild species could be utilized as a potential source for further improvement in leaf structural and physiological traits which ultimately improve the resource-use efficiency in modern cultivars.

The objective of this study was to investigate the variation in leaf photosynthetic efficiency and related traits in back-cross introgression lines (BC_2F_6–BILs) derived from the wild rice species *O. nivara* and a lowland cultivated *indica* rice variety Swarna. Further, we wanted to examine whether photosynthetic efficiency of Swarna can be improved by the introgressions from *O. nivara* and to provide an insight into how the variation in photosynthetic efficiency is related to improvements in biomass production and grain yield. In addition, we aimed to investigate the marker-trait association for leaf gas exchange and yield traits using SSR markers to determine any common associated loci which influence the photosynthetic efficiency and grain yield.

Materials and methods

Plant material: The experiment was conducted in green house at Indian Institute of Rice Research (IIRR), Hyderabad (17° 32′ N, 78° 40′ E) during dry season 2013 (November 2013 – June 2014) under well-watered conditions. A total of 52 BILs were selected based on their grain yield from two BC_2F_6 populations developed using a common recurrent parent Swarna and two wild accessions of *O. nivara* as donor parents. A population A consisted of 27 BILs derived from Swarna × *O. nivara* IRGC81848, and the population B consisted of 25 BILs derived from Swarna × *O. nivara* IRGC81832. Hereafter, the population A and population B are referred as popA and popB, respectively. Ten-days-old seedlings were transferred to clay pots (26 × 30 cm, volume of 15 L) at a density of three plants per pot filled with loam soil. Each introgression line (IL) was grown with a recommended fertilizer dose in three replicated pots.

The weather parameters recorded during the crop growth period were shown in Fig. 1S (*supplement*). The mean maximum temperature recorded during the crop growth period was 32.6°C, while the mean minimum temperature was 15.9°C and average relative humidity was 79.5%. The mean sunshine duration was 8.3 h per day and mean solar radiation levels was 18.3 W m^{-2}. The temperature and RH were recorded using a datalogger (model *DT-172*, *CEM Instruments*, India) installed inside the greenhouse, whereas data on solar radiation and duration of sunshine were obtained from the automatic weather station (*Campbell Scientific*, USA) installed at IIRR farm.

Gas-exchange and leaf fluorescence measurements: These determinants were measured simultaneously on three fully-expanded flag leaves 3 d after anthesis from each replication, using a portable open gas-exchange system (*LI6400XT*, *LI-COR*, Lincoln, NE, USA) with an integrated fluorescence chamber head (*LI 6400-40*, *LI-COR*, USA) which is used as a light source. Respiration rate (R_D) of the flag leaf was measured by covering the leaf chamber with black cloth in the early hours (05:00–06:00 h) and this parameter was used for calculating mesophyll conductance (g_m). Leaf gas-exchange and fluorescence traits were measured between 09:00–12:00 h on clear days with PPFD at 1,000 µmol m^{-2} s^{-1}. During measurements the leaf chamber air temperature was set at 30°C. The gas-exchange traits, such as P_N, g_s, E, and C_i were measured at ambient CO_2 concentration at 400 µmol mol^{-1}. Water-use efficiency (WUE), intrinsic water-use efficiency (WUE_i), and carboxylation efficiency (CE) were calculated based on the ratios of P_N to E, g_s, and C_i values, respectively.

Similarly leaf Chl fluorescence parameters were calculated according to Björkman and Demmig (1987): maximum quantum efficiency of PSII [$F_v/F_m = (F_m - F_0)/F_m$], maximum quantum yield of PSII after light adaptation [$F_v'/F_m' = (F_m' - F_0')/F_m'$], photochemical quenching coefficient [$q_P = (F_m' - F_s')/(F_m' - F_0')$], nonphotochemical quenching coefficient [$q_N = (F_m - F_m')/F_m'$], effective quantum yield of PSII photochemistry [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] (Genty *et al.* 1989) and quantum yield of carboxylation rate (Φ_{CO2}). Finally, the apparent photosynthetic electron transportation rate through PSII (ETR) was calculated

[ETR = Φ_{PSII} × 0.84 × PPFD × 0.5], where PPFD is photosynthetic photon flux density of absorbed light in μmol(photon) m^{-2} s^{-1} considering 0.84 or 84% light intensity and 0.5 is a factor which accounts for the partitioning of energy between PSII and PSI (Maxwell and Johnson 2000).

Estimation of mesophyll conductance in BILs: Mesophyll conductance (g_m) was measured following the 'variable J' method of Harley *et al.* (1992):

$$g_m = P_N/(C_i - \{\Gamma^*[ETR + 8\,(P_N + R_D)]/[ETR - 4\,(P_N + R_D)]\})$$

where P_N is net photosynthetic rate and C_i is internal CO_2 concentration. These were obtained from gas-exchange measurements. R_D is respiration rate which was measured during the morning hours (05:00–06:00 h). Before the measurement, all plants were covered with black cloth and R_D was measured in dark. Γ^* is the CO_2-compensation point and the value is adapted from the report of Sexton *et al.* (2013). Now, C_c is the chloroplast CO_2 concentration calculated from the following equation of Adachi *et al.* (2013): $C_c = C_i - P_N/g_m$

Leaf Chl and carotenoid (Car) contents: The flag leaves used for the leaf gas-exchange and fluorescence measurements were detached from the plants. Briefly, 0.1 g of leaf tissue was inserted in 50-ml volumetric flask, which contained 25 ml of 80% acetone (v/v) (*Merck,* India). In order to avoid a light exposure, the flasks were wrapped with aluminium foil and kept in dark at room temperature for 48 h. An aliquot (1 ml) of extract was used for measurements of absorbance at 663 (Chl *a*), 645 (Chl *b*), and 470 nm (Car) using a spectrophotometer (*Spectrascan UV 2600*, *Toshniwal Instruments,* India). The amounts of Chl and Car were calculated according to the method of Lichtenthaler and Wellburn (1983). The concentration of Chl was expressed as mg g^{-1}(leaf fresh mass, FM).

Phenotyping of yield and other related traits: The BILs and their parents were harvested at a final maturity stage from each replication to determine the yield and related traits, such as plant height (PH), a number of tillers per plant (NT), number of panicles per plant (NP), panicle length (PL), number of primary branches (PB), number of secondary branches (SB), total number of grains per panicle (TNG), spikelet fertility (SPF), panicle mass (PW), thousand-grain mass (TGW), yield per plant (YLDP), dry mass (DM), and harvest index (HI).

Genotyping: Genomic DNA of parents Swarna, *O. nivara*, and 52 BILs was extracted from young leaves using CTAB (cetyl trimethyl ammonium bromide) method (Rogers and Bendich 1988) and screened using 73 SSR markers which were polymorphic between Swarna and *O. nivara*. The PCR amplification was carried out in 10-μl reaction volume containing 50 ng of template DNA, 0.2 μM of each primer (both forward and reverse primers) and *Emerald Amp PCR Master Mix* (*Takara Bio*, USA). The PCR amplification was performed using a programmable thermal cycler (*Applied Biosystems Veriti, Thermo Fischer Scientifics,* California, USA*)* under the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, followed by the final extension at 72°C for 7 min. Following amplification, the products were checked for marker segregation in 3% agarose gel.

Linkage map construction and single marker analysis: Linkage maps were constructed from the genotyping data of 73 polymorphic markers in both populations separately using the *Mapmaker version 3.0* (Lander and Botstein 1989, Lincoln *et al.* 1993) following Kosambi function (Kosambi 1944). Single marker analysis (SMA) was performed to determine the association between marker and trait using *QTL cartographer ver. 2.5* (Wang *et al*. 2011).

Statistical analysis: Significant differences were determined by analysis of variance (*ANOVA*) and means were compared by *Tukey's* HSD test ($p<0.05$) for all traits using an open source software *R* (*R Core Team* 2012) with *Agricolae* package (de Mendiburu 2012). Multiple correlations were performed between gas exchange and yield related traits using *Microsoft Excel 2007*. Principal component analysis (PCA) was performed based on the covariance matrix to identify the percent contribution of each trait to the total genetic variation in the introgression lines of both populations using statistical analysis for agricultural research *STAR ver. 2.0.1* (IRRI 2013).

Results

Comparative analysis of net photosynthetic rate and other gas-exchange traits: Significant differences were found in P_N between the backcross-introgression lines (BILs) compared to their parents, Swarna and *O. nivara*. P_N ranged from 10.4–23.1 and 9.0–21.5 μmol(CO_2) m^{-2} s^{-1} in popA and popB, respectively. There were 20 BILs in popA and 12 BILs in popB showing higher P_N than that of Swarna. Of these, 4 BILs (230S, 75S, 214S, and 33S) from popA and 6 BILs (173K, 3-1K, 24K, 45K, 75K, and 131K) from popB showed higher P_N than their respective donor wild accession. The variation among BILs was significant for g_s in both populations. It ranged from 0.16–0.83 and 0.19–0.65 mol(H_2O) m^{-2} s^{-1} in popA and popB, respectively. In all, 9 BILs showed higher P_N and g_s than that of their parents. Leaf transpiration rate (E) showed significant differences between the BILs. These differences varied from 3.9–11.2 and 4.8–11.1 mmol(H_2O) m^{-2} s^{-1} in popA and popB, respectively (Fig. 1). Three BILs from each population showed higher E than parents. Similarly, a wide variation was observed in C_i which ranged between 228.5–343.5 and 234.1–341.3 μmol(CO_2) mol^{-1} in popA and in popB, respectively.

The variations observed in WUE and CE were significant in both populations. WUE was the highest in IL 26S [3.06 μmol(CO_2) $mmol^{-1}$(H_2O)] in popA and the lowest one in Swarna [1.44 μmol(CO_2) $mmol^{-1}$(H_2O)]. In popB, it ranged from 1.12 (IL 138K) to 2.62 (IL 45K) μmol(CO_2) $mmol^{-1}$(H_2O). However, CE was maximum in IL 148S

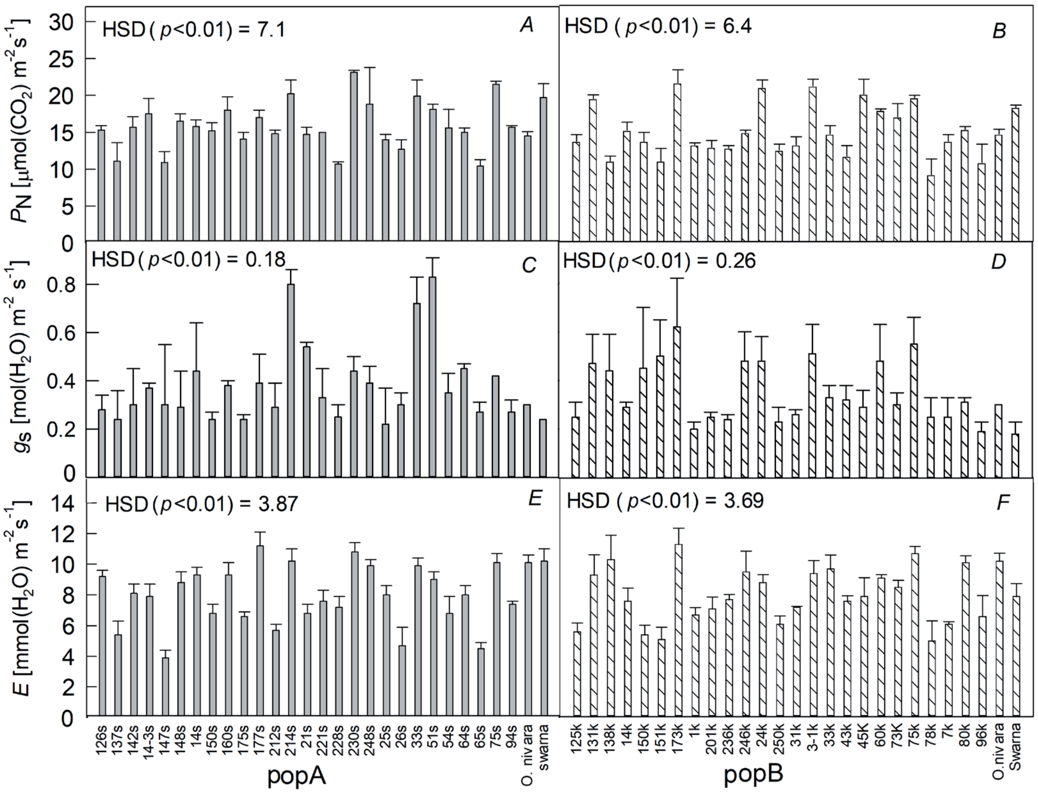

Fig. 1. Variation in net photosynthetic rate (P_N) (*A*,*B*), stomatal conductance (g_s) (*C*,*D*), and transpiration rate (E) (*E*,*F*) in two BC$_2$F$_6$ populations, popA (Swarna × *O. nivara* IRGC818) and popB (Swarna × *O. nivara* IRGC81832). Each bar represent the mean of three replications ± SD.

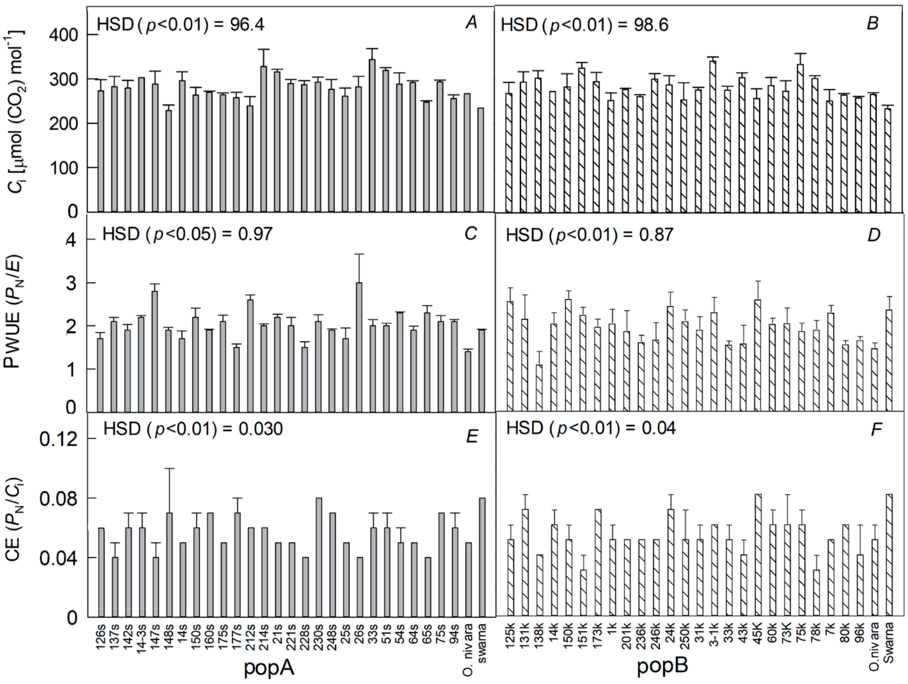

Fig. 2. Variation in intercellular CO$_2$ concentration (C_i) (*A*,*B*), photosynthetic water-use efficiency (WUE) (*C*,*D*), and carboxylation efficiency (CE) (*E*,*F*) in two BC$_2$F$_6$ populations, popA (Swarna × *O. nivara* IRGC818) and popB (Swarna × *O. nivara* IRGC81832). Each bar represent the mean of three replications ± SD.

[0.085 µmol(CO_2) mol^{-1}(air)] and minimum in IL 228S [0.037 µmol(CO_2) mol^{-1}], whereas in popB it ranged from 0.078 (*O. nivara*) to 0.030 (IL 78K) µmol(CO_2) mol^{-1}(air) (Fig. 2).

Chl fluorescence: F_v/F_m was found to be almost identical in both populations and showed no significant variations among the BILs. It ranged between both the parents and was the highest in *O. nivara* (0.77) and the lowest in Swarna (0.68) in both populations. Similarly, F_v'/F_m' showed significant differences ranging from 0.45–0.63 in popA, but differences were not significant in popB. Furthermore, ETR also showed significant variations in both populations. It was the highest in IL 75S (143.8) and IL 60K (135.6), and the lowest in *O. nivara* parents of both populations (Fig. 3). The variation observed for Φ_{PSII} and Φ_{CO2} were significant (Fig. 4). Similarly, q_P and q_N also showed wide variations among the BILs. Swarna showed significantly higher q_P (0.57) in popA compared to than of the BILs. But in popB, IL 125K was the only line which showed higher q_P (0.60) than that of other BILs and parents (Fig. 2S, *supplement*).

Leaf thickness and Chl content: Wide variations were observed for flag leaf thickness in both populations. It ranged from 0.06 (IL 137S) to 0.15 mm (IL 177S) in popA, while in popB it was the highest in IL 24K (0.16 mm) and lowest in IL 201K (0.07 mm). Thickness showed significant association with P_N and YLDP in both populations. Similarly, Car was maximum in IL 221S [1.52 mg g^{-1}(FM)] and 201K [1.49 mg g^{-1}(FM)], respectively (Fig. 5).

The Chl content is an important physiological trait which affects the photosynthetic ability of rice. Significant variations were noted for Chl *a*, Chl *b*, TChl, Chl *a*/*b*, and Car content among BILs of both populations. Chl *a* was the highest in IL 75S [3.75 mg g^{-1}(FM)] and IL 246K [3.63 mg g^{-1}(FM)]. Chl *b* content was maximum in IL 230S [1.38 mg g^{-1}(FM)] in popA and IL 138K [1.25 mg g^{-1}(FM)] in popB. Likewise, TChl was the highest in IL 75S [5.04 mg g^{-1}(FM)] in popA and in IL 246K [4.86 mg g^{-1}(FM)] in popB. The variations for Chl *a/b* ranged from 3.63 (IL 177S) to 2.55 (IL 230S) in popA and 1.97 (IL 96K) to 5.04 (IL 236K) in popB (Table 1S, *supplement*).

Mesophyll conductance and chloroplast CO_2 concentration: Variable J method of Harley *et al*. (1992) was used to determine the g_m. The differences observed for g_m and C_c were significant in both populations. The g_m ranged from 0.09 in IL 65S to 0. 75 mol m^{-2} s^{-1} in IL 177S of popA and 0.06 in IL 78K to 0.69 mol m^{-2} s^{-1} in IL 173K of popB. Similarly, C_c ranged from 123.5 (IL 65S) to 254.7 µmol mol^{-1} (IL 14-3S) and 108.1 (IL 96K) to 272.2 µmol mol^{-1} (IL 3-1K) in two populations, respectively (Fig. 6).

Comparative analysis of yield and yield-related traits in parents and both populations: We compared component traits of grain yield, as well as total dry mass production in parents and both the populations. The mean and range of all phenotypic traits of parents were given in Table 1. All yield-related traits showed significant variations among BILs of both populations except for HI in popB. Plant height (PH), tiller number (NT), and number of panicles per plant (NP) are important yield determinants during the vegetative growth stage. These traits showed highly significant differences in both populations. PH, NT, NP were the highest in *O. nivara* acc. IRGC81848 (120 cm, 11, and 10, respectively) of popA, whereas in popB, PH was the highest in *O. nivara* acc. IRGC81832 (117 cm), NT and number of productive tillers were the highest in IL 246K (Table 2S, *supplement*).

Panicle length (PL) varied from 18 cm in IL 175S to 26.5 cm in IL 26S, whereas in popB, it ranged from 17 cm in IL 78K to 24 cm in IL 250K. The number of primary branches per panicle (PB) was consistent in both populations, but significant variations were observed for the number of secondary branches per panicle (SB) in popB and popA and it ranged from 12 to 41 (Table 3S), respectively. In popA, maximum number of filled grains (FG) was observed in IL 54S (116), minimum in IL 14-3S (51), and total number of grains per panicle (TNG) was the highest in IL 25S (134) and the lowest in IL 14S (64). However, in popB, IL 75K showed maximum of FG and TNG per panicle. The percentage of spikelet fertility (SPF) was 97% in both populations (Table 4S, *supplement*). Panicle mass (PW) and 1,000-grain mass (TGW) were higher in popB than that in popA. The highest PW was observed in IL 24K (3.2 g) and TGW in IL 45K (23.8 g), whereas in popA, it was observed in IL 26S (3.0 g) and IL 230S (21.7 g), respectively (Fig. 3S, *supplement*).

The carbohydrates produced during the process of photosynthesis are translocated to sink (spikelet) which finally determines the grain yield per plant (YLDP). IL 230S showed significantly higher YLDP (8.7 g per plant), and total dry mass (DM) (21.3 g per plant) in popA. However, in popB, the highest YLDP was in IL 75K (9.2 g per plant) and DM (23.5 g per plant) in IL 173K. Harvest index (HI) was significantly different in popA, ranging from 27.3 (IL 64S) to 45.4% (IL 51S), but it was insignificant in popB (Fig. 4S, *supplement*).

Principal component analysis (PCA) and correlation: PCA was conducted to estimate the percentage contribution of each trait to the total genetic variation. The results showed that first four components accounted 92 and 93% of the total genetic variance in popA and popB, respectively. The first PC accounted about 40 and 43% of total variation and was strongly associated with C_i, C_c, FG, TNG, and ETR in popA, and C_i, C_c, and TNG in popB. The second PC accounted about 32 and 31% of variance and was associated with FG and TNG in popA, but with C_i, ETR, FG, and TNG in popB. The third PC accounted for 12% of total variation and was positively associated with FG, TNG, and C_c in popA, while in popB, it was associated with C_i. The fourth PC accounted for only 6 and 5% of total variation and was associated with C_i, C_c, PH, and TNG in popA, and with ETR, SPF, and HI in popB (Table 5S, *supplement*). The variations among the BILs in both populations based on their Eigen values are shown in Fig. 5S (*supplement)*.

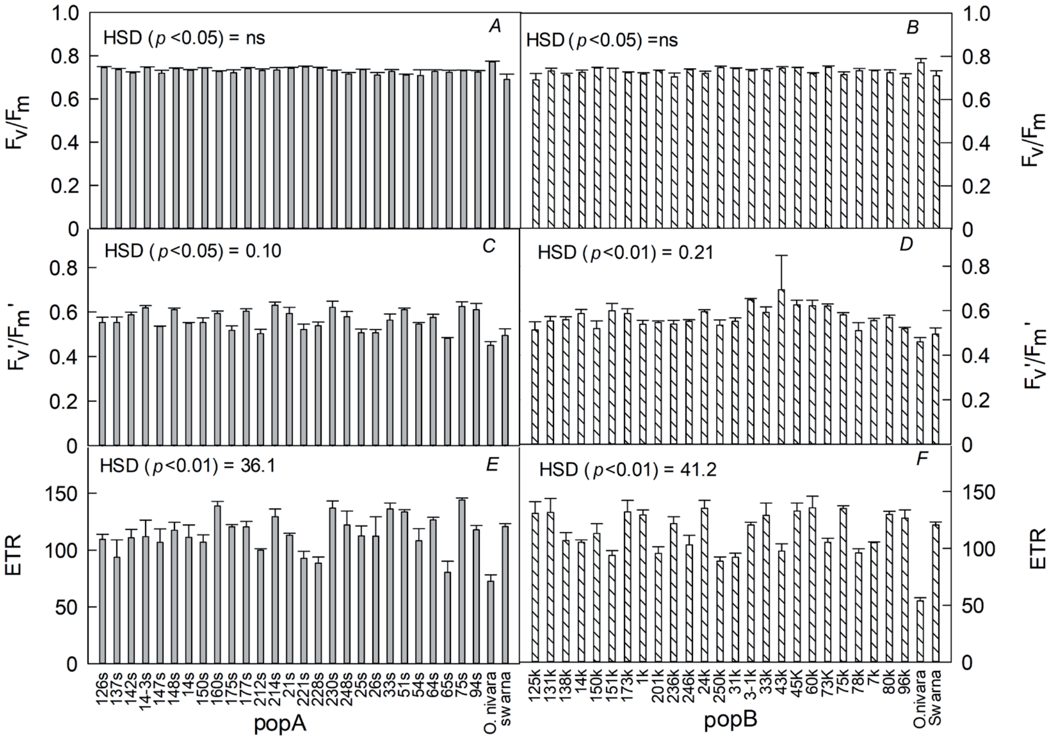

Fig. 3. Variation in important chlorophyll fluorescence traits F_v/F_m (*A*,*B*), F_v'/F_m'(*C*,*D*), and apparent electron transport rate (ETR) (*E*,*F*) in two BC_2F_6 populations popA (Swarna × *O. nivara* IRGC81848) and popB (Swarna × *O. nivara* IRGC81832). Each bar represents the mean of three replications ± SD.

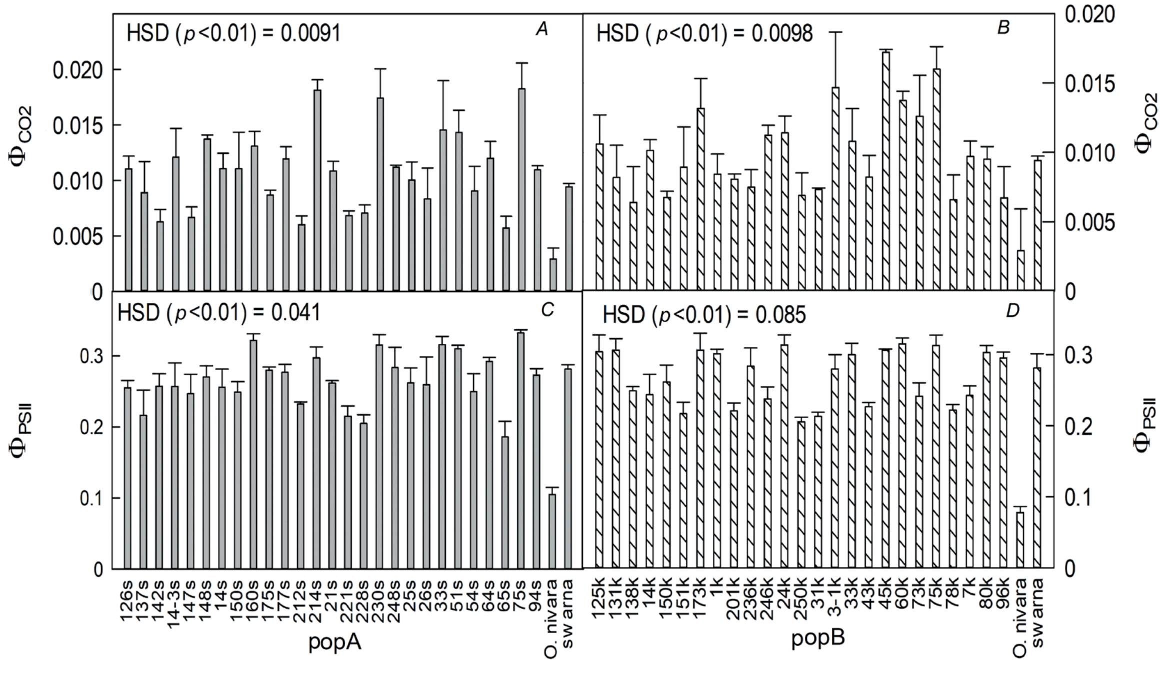

Fig. 4. Variation in important chlorophyll fluorescence traits effective quantum yield of PSII photochemistry (Φ_{PSII}) (*A*,*B*), and quantum yield of carboxylation rate (Φ_{CO2}) (*C*,*D*) in two BC_2F_6 populations popA (Swarna × *O. nivara* IRGC81848) and popB (Swarna × *O. nivara* IRGC81832). Each bar represents the mean of three replications ± SD.

Marker allele constitution of BILs: The percentage of homozygous *O. nivara* allele introgressions was higher in popA than that in popB. It ranged from 0.0 (IL 54S) to 24.7 (IL 14S) with an average introgression of 11.5 in popA. However, in popB, the percentage of homozygous *O. nivara* introgressions ranged from 2.7 (ILs 45K, 75K

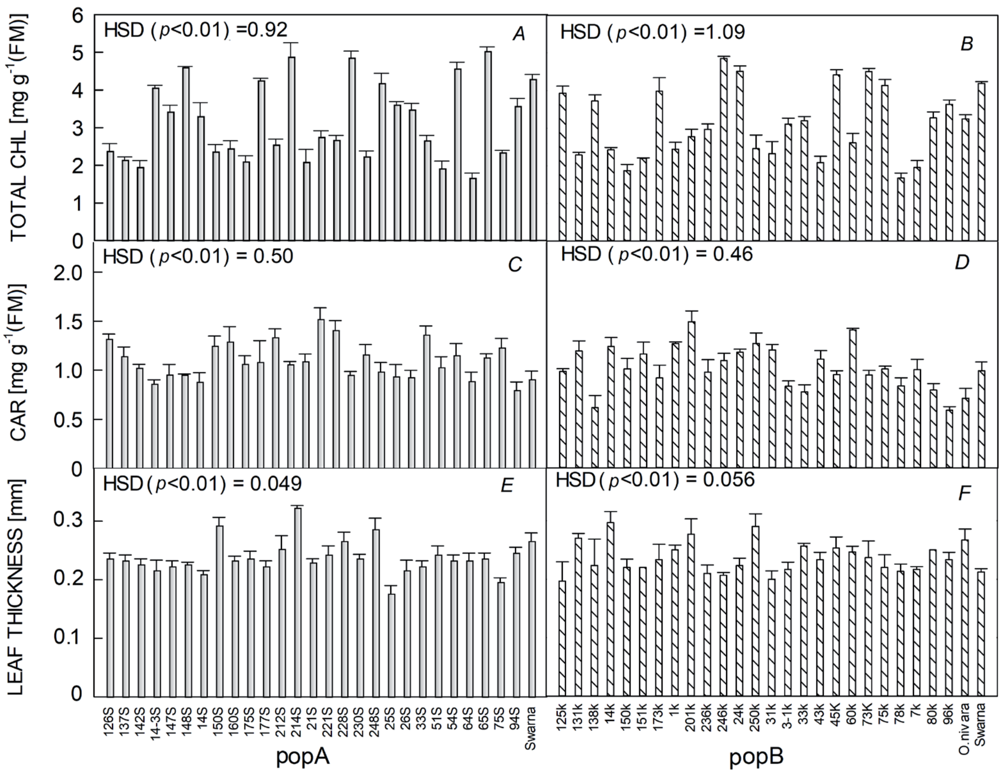

Fig. 5. Variation in total chlorophyll (Chl) (*A*,*B*), carotenoid content (Car) (*C*,*D*) and flag leaf thickness (*E*,*F*) in two BC_2F_6 populations Pop*A* (Swarna × *O. nivara* IRGC81848) and Pop*B* (Swarna × *O. nivara* IRGC81832). Each bar represents the mean of three replications ± SD.

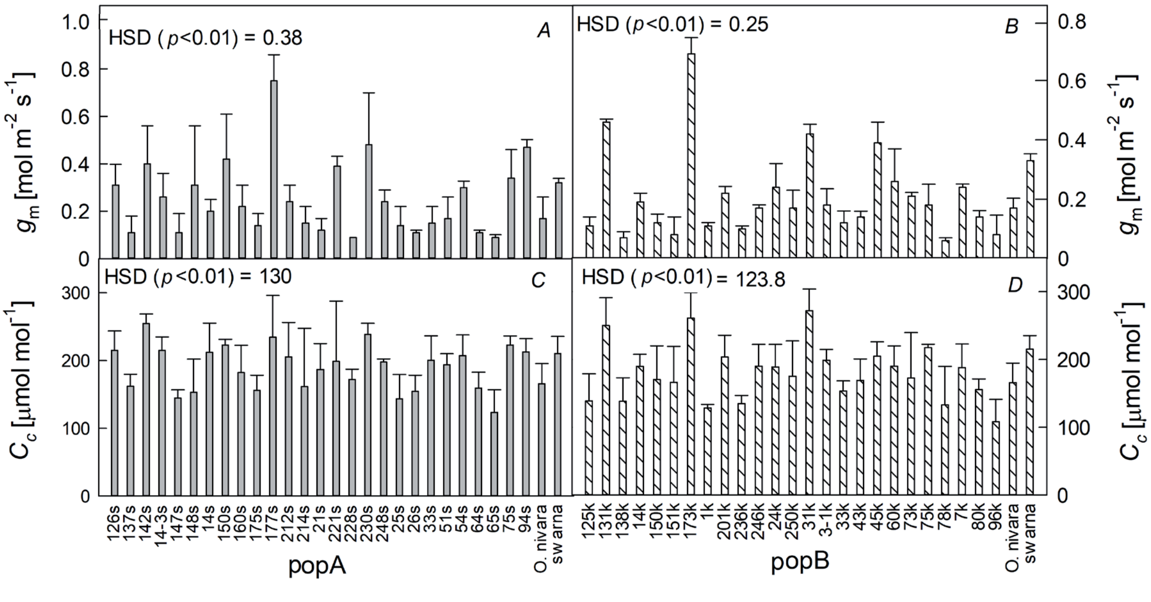

Fig. 6. Variation in mesophyll conductance (g_m) (*A*,*B*) and chloroplast CO_2 concentration (C_c) (*C,D*) in two BC_2F_6 populations popA (Swarna × *O. nivara* IRGC81848) and popB (Swarna × *O. nivara* IRGC81832). Each bar represents the mean of three replications ± SD.

and 201K) to 16.4 (IL 250K) with a mean introgression of 8.7. Likewise, heterozygosity was also higher in popA (6.5%) than that in popB (3.8%).

Marker-trait association: A total of 73 polymorphic SSR markers covering all chromosomes were used to genotype all 52 BILs. Single marker analysis showed that

Table 1. Descriptive statistics for 18 phenotypic traits in parents IRGC81832, IRGC81848 and Swarna (mean ± SE, *n* = 3). PH – plant height; FLL – flag leaf length; FLW – flag leaf width; FLA – flag leaf area; NT – number of tillers per plant; NP – number of panicles per plant; PL – panicle length; PB – primary branches per panicle; SB – secondary branches per panicle; FG – filled grains; YLDP – yield/plant; BM – biomass; DM – total dry mass; HI – harvest index. The means and SDs should have the same numper of decimal places!

Variable	*Oryza nivara* (IRGC81832)	*Oryza nivara* (IRGC81848)	Swarna
PH	116.97 ± 2.0	119.97 ± 9.39	80.67 ± 1.15
FLL	46.7 ± 4.86	43 ± 9.20	24.53 ± 0.90
FLW	1.4 ± 0.06	1.43 ± 0.12	1.43 ± 0.06
FLA	49.2 ± 6.82	46.43 ± 12.72	26.2 ± 0.10
NT	9 ± 0.58	11 ± 2.65	6.33 ± 0.58
NP	9 ± 0.58	10 ± 3.61	5.33 ± 0.58
PL	23.97 ± 0.33	21.97 ± 1.05	21.73 ± 1.31
PB	7 ± 0	5 ± 1.00	10 ± 1.00
SB	10 ± 0.58	10 ± 1.00	22 ± 1.00
FG	25 ± 1.73	30 ± 2.00	82.33 ± 11.68
TNG	30.67 ± 2.60	40 ± 3.00	112.33 ± 2.52
SPF	81.73 ± 1.44	75.03 ± 0.65	73.17 ± 9.11
PW	0.93 ± 0.03	0.97 ± 0.06	1.83 ± 0.15
TGW	9.5 ± 0.29	9.87 ± 0.78	17.6 ± 0.53
YLDP	1.8 ± 0.47	2.5 ± 0.50	4.4 ± 0.53
BM	16 ± 2.65	13.67 ± 2.31	11 ± 1.00
DM	17.8 ± 2.74	16.17 ± 2.36	13 ± 2.00
HI	10.5 ± 3.02	15.67 ± 3.47	34.47 ± 6.73

47 markers in popA and 48 in popB were significantly associated with more than one trait, indicating pleiotropic effect/linkage (Table 6S, *supplement*). Particularly, in popA, RM514 on chromosome 3 was associated with a maximum of 13 traits and RM85 was associated with 5 traits. There were three such markers on chromosome 2. RM48 was associated with seven traits (P_N, CE, g_m, C_c, Chl *b*, Chl *a/b*, YLDP); RM250 with six traits (P_N, E, CE, F_v'/F_m', q_N, PH) and RM263 with five traits (*E*, WUE, Chl *a/b*, NP, and PB). Another marker, RM209 on chromosome 11, showed significant association with five traits (*E*, WUE, CE, C_c, F_v/F_m, and PH).

Further analysis in popB showed that RM204 on chromosome 6 showed association with maximum of six traits namely P_N, C_i, *E*, g_s, Φ_{CO2}, and q_N. Likewise, RM488 on chromosome 1, RM185 on chromosome 4 were associated with six traits each. Similarly, RM122 on chromosome 5 was associated with five traits, *i.e.*, g_s, C_i, Φ_{CO2}, TNG, and YLDP. There was no common association in both populations.

Multi-trait correlation: Significant correlations were obtained between different physiological and yield-related traits. P_N showed significant positive correlation at 1% level with g_s, *E*, CE, C_c, leaf thickness, Φ_{PSII}, Φ_{CO2}, ETR, YLDP, BM, DM, and HI. On the contrary, it showed a significant negative correlation with q_N in both populations (Fig. 7). However, leaf Chl content showed a positive correlation with P_N at 1 and 5% significance level in popA and popB, respectively.

Discussion

Wild relatives of cultivated rice are an important genetic reservoir which offers novel genes for enhancing crop yield and maintaining future food security. Previous studies on rice have shown that P_N was higher in wild species *O. nivara* 100097 [24.2 µmol(CO$_2$) m^{-2} s^{-1}] and *O. longistaminata* IR105262 [22.9 µmol(CO$_2$) m^{-2} s^{-1}] than that in cultivars and hybrids (Kiran *et al.* 2013, Kondamudi *et al.* 2016). However, Xiong *et al.* (2017) showed that the wild species *O. latifolia* had the highest P_N [35.9 µmol(CO$_2$) m^{-2} s^{-1}] than any other wild and cultivated species of *Oryza*. Nevertheless, Giuliani *et al.* (2013) reported that African cultivated rice *O. glaberrima* showed the highest P_N [27.09 µmol(CO$_2$) m^{-2} s^{-1}], followed by wild species *O. glumaepatula* [25.8 µmol(CO$_2$) m^{-2} s^{-1}]. The BILs derived from wild species *O. rufipogon* and elite cultivar (KMR3) also showed a significant improvement in P_N, grain yield, and dry mass (Haritha *et al.* 2017). In this study, IL 230S from popA showed the highest P_N of 23.1 µmol(CO$_2$) m^{-2} s^{-1} and IL 173K from popB showed 21.5 µmol(CO$_2$) m^{-2} s^{-1}. Considering two populations, a total of 10 BILs showed higher P_N than their parents Swarna and *O. nivara*. Masumoto *et al.* (2004) reported that around 14–15% of BC$_2$ population derived from *O. sativa* × *O. rufipogon* had higher oxygen evolution rates than their parents. P_N increases with increasing g_s (Ono *et al.* 2013). High g_s was reported in wild species *O. longistaminata* IR105262 (Kiran *et al.* 2013). In the present study, g_s was found to be the highest in IL 51S of popA and IL 75K of popB. Notably, as many as 27 BILs

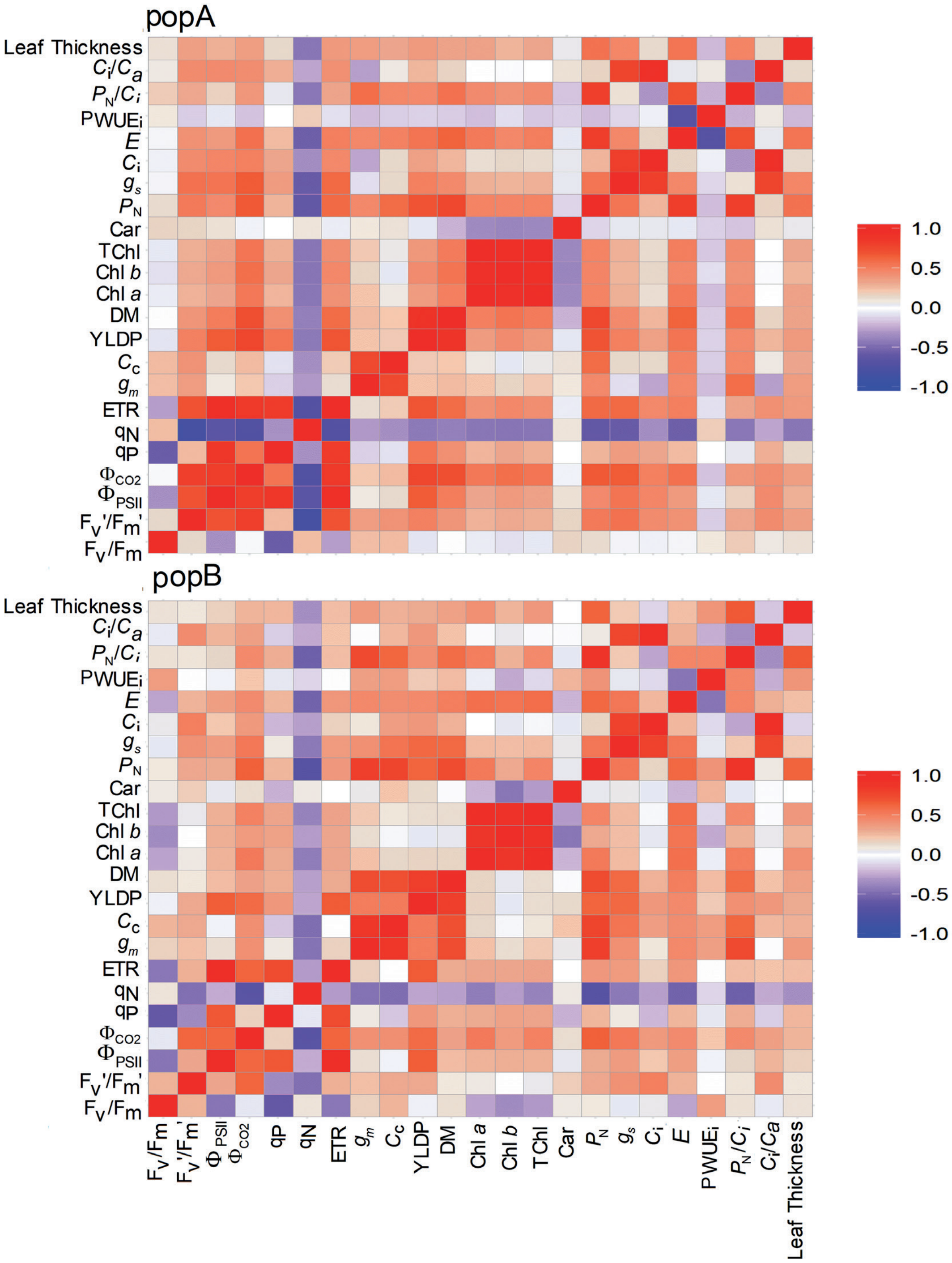

Fig. 7. Heat map of *Pearson's* correlation coefficients between morphological, physiological, and yield related traits in two BC_2F_6 populations of Swarna × *O. nivara*. The squares with red color indicate positive ($p<0.05$) correlation and squares with blue color indicate negative ($p<0.05$) correlations. C_i/C_a – ratio between intercellular CO_2 (C_i) and ambient CO_2 (C_a); P_N/C_i – carboxylation efficiency; WUE_i – photosynthetic water-use efficiency; E – transpiration rate; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; P_N – net photosynthetic rate; Car – carotenoid content; TChl – total chlorophyll content; Chl – chlorophyll; DM – dry mass; YLDP – grain yield per plant; C_c – chloroplast CO_2 concentration; g_m – mesophyll conductance; ETR – electron transport rate; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; Φ_{CO2} – quantum yield of carboxylation rate; Φ_{PSII} – effective quantum yield of PSII photochemistry; F_v/F_m – maximum photochemical efficiency of PSII; F_v'/F_m' – maximum quantum yield of PSII after light adaptation.

showed higher g_s than the parents and 9 of these BILs showed high P_N as well.

Kiran *et al.* (2013) reported that the wild species *O. nivara* 100097 showed maximum *E* [11.1 mmol(H_2O) m^{-2} s^{-1}]. Our previous report showed that P_N was positively correlated with *E* (Haritha *et al.* 2017). However, *E* showed an inverse relation with leaf thickness, but WUE showed positive relation with leaf thickness among the wild and cultivated species of *Oryza* (Giuliani *et al.* 2013). Similarly, the maximum C_i was observed in IL 33S [343.5 µmol(CO_2) mol^{-1}] and IL 3-1K from both populations, respectively. The highest value [280 µmol(CO_2) mol^{-1}] reported previously was in *O. rufipogon* IR103404 (Kiran *et al.* 2013). The derived BILs of Swarna and *O. nivara* showed higher gas-exchange rate than that of KMR3 × *O. rufipogon* BILs in terms of P_N, g_s, C_i, and *E* (Haritha *et al.* 2017). CE showed significant positive association with P_N in both populations. WUE is another important trait for dry matter production. This was determined by P_N and *E*. During the water-deficit conditions, stomatal closure reduces the loss of water from leaf, which leads to an increase in photosynthetic WUE. It was positively associated with leaf stomatal density (Xu *et al.* 2008), photosynthesis, and stomatal conductance (Silva *et al.* 2013). The highest WUE of 4.69 µmol $mmol^{-1}$ in different species of *Oryza* was reported by Giuliani *et al.* (2013). In our previous report, we showed that the IL 106 derived from KMR3 × *O. rufipogon* showed high WUE of 3.43 µmol $mmol^{-1}$. However, in present study, the maximum WUE was 3.06 µmol(CO_2) $mmol^{-1}$(H_2O) in IL 26S, which was higher than the other wild and cultivated species of *Oryza* (2.0 µmol $mmol^{-1}$) (Kiran *et al.* 2013). WUE and WUE_i showed significant variations among the ILs but these were not significantly correlated with the yield-related traits.

On the other hand, g_m and C_c plays a crucial role in the diffusion of CO_2 from sub-stomatal cavities to the site of carboxylation in chloroplast stroma which limits photosynthesis and grain yield in rice (Flexas *et al.* 2008). Our results revealed that 7 BILs of popA showed higher g_m and 9 BILs showed higher C_c than parents and in these 6 BILs (230S, 177S, 94S, 150S, 14-3S, and 75S) were common. However, popB showed slightly lower g_m than that of popA, but 3 BILs (173K, 131K, and 3-1K) showed high g_m and C_c. Giuliani *et al.* (2013) reported high g_m of 0.467 mol m^{-2} s^{-1} in *O. australiensis* and high C_c 0.185 µmol mol^{-1} in *O. glaberrima*. P_N showed a strong positive association with g_m at a significance of 5% in popA and 1% in popB. Similarly, C_c also showed positive association with P_N at 1% level of significance. The positive interrelations among the traits P_N, *E*, g_s, and g_m were also reported by Giuliani *et al.* (2013).

Leaf thickness affects the photosynthetic components per unit leaf area (Murchie *et al.* 2005). This is an important leaf structural trait, which determines the yield capacity of rice. It is positively correlated with P_N largely because of a high Chl content in thicker leaves (Rahman *et al.* 2013). Our results showed significant positive association of leaf thickness with P_N in both populations. Recent study of Guru *et al.* (2017) reported that IL 24K had the highest flag leaf length compared to other lines and hybrids. Takai *et al.* (2010) showed that SPAD and specific leaf area (SLA) play a major role in increasing photosynthesis by increasing the leaf Chl content and leaf thickness. Our results showed the leaf Chl content was positively associated with P_N and it was the highest in IL 75S of popA and in IL 246K of popB. Leaf Chl fluorescence is used to assess the PSII photochemistry, energy absorption, and dissipation of excess energy by PSII (Falqueto *et al.* 2009). Significant positive associations were found between P_N and F_v'/F_m', $\Phi_{PSII,}$ and Φ_{CO2}, as well as negative association with q_N in both populations. Hura *et al.* (2009) showed significant associations between Chl fluorescence traits, yield, and leaf gas-exchange parameters of triticale. However, F_v/F_m showed positive correlation with P_N, g_s, and *E* and negative correlation with C_i in triploid popular hybrid clones of *Populus simonii* and *Populas nigra* (Zhao *et al.* 2015) Maxwell and Johnson (2000) suggested that Φ_{PSII} can provide useful information concerning photosynthetic performance in the field. However, fluorescence measurements alone cannot be used to make comparative measurements of photosynthesis in plants.

P_N showed the significant positive association with YLDP, BM, DM, and HI. As an example, IL 230S, which had high P_N, showed the high yield (8.7 g), high BM (12.6 g), and high DM (21.3 g). Our previous report showed that P_N is associated with DM (Haritha *et al.* 2017). High yield in modern cultivars is associated with biomass (Alvarez *et al.* 2012), 1,000-grain mass (Bhatia *et al.* 2017), number of filled grains per panicle (Bhatia *et al.* 2017), panicle size (Laza *et al.* 2004), and a tiller number per plant (Yeh *et al.* 2015). The component traits of yield in relation to photosynthesis showed significant positive association with NT, PB, and TGW.

The impact of all physiological and yield component traits to the total genetic variation is determined by PCA. In current study, the percentage of cumulative variance explained by four principal components (PC) was 92% in popA, and 93% in popB indicating there may be a strong correlation among the traits to explain gross diversity. Interestingly, FG and TNG in popA and C_i in popB were common contributing traits for first three PCs. However, considering both populations together, TNG was common to all PCs in both populations. Nachimuthu *et al.* (2014) reported 80.6% of genetic variation explained by the first five PCs, and suggested days to 50% flowering, days to maturity, PH, NT, SPF, PL, and grain length as important for classifying the variation in a set of 192 genotypes of rice. Likewise, Gana *et al.* (2013) also reported 65% of variation explained by five PCs and leaf width, TNG, gall count, and PL showed more contribution to a total genetic variation.

RM514 was significantly associated with as many as 13 traits, P_N, *E*, WUE, WUE_i, CE, F_v'/F_m', Φ_{PSII}, Φ_{CO2}, q_N, ETR, Chl *a*, NP, and TGW. Of these F_v'/F_m' and *E* were strongly associated at significance level of 0.01 and 0.1% with a phenotypic variance of 46.2 and 42.2%, respectively. Likewise, P_N, Φ_{PSII}, Φ_{CO2}, q_N, and ETR showed association at 1% and explained the phenotypic variance of 25.3, 24.9, 25.8, 34.1, and 25.2%, respectively. All other traits were associated at a significance level of 5%. Previous reports showed that RM514 flanks a QTLs linked to panicle length (Wang *et al*. 2012), number of filled grains per panicle (Sellamuthu *et al*. 2015), and grain yield, thousand-grain mass under water-stress conditions (Zou *et al*. 2005). RM85, another marker on chromosome 3, was significantly associated with five traits, P_N, E, F_v'/F_m', Φ_{PSII}, and ETR. It has been reported as flanking marker for QTL *qNSB3.1* for number of secondary branches, *ac3.2* for amylose content (Swamy *et al*. 2011, 2012), and spikelet number per panicle (Xu *et al*. 2004). Thus, these two markers are important to track several traits in marker-assisted selection and breeding.

RM250 is linked to P_N, E, CE, F_v'/F_m', q_N, and PH, and the marker was reported to be related to most of the yield-related traits in BC_2F_2 (Swamy *et al*. 2011). It flanks *nsp2.1* for number of spikelets per plant, *nsp2.1* for number of filled grains per plant, *bm2.1* for vegetative biomass, *yld2.1* for yield per plant, *wup2.1* for water uptake, and *gc2.1* for gel consistency (Swamy *et al*. 2012, 2014), *QPh2* for plant height (Xu *et al*. 2005), *qSBN-2ci* for secondary branches number (Mei *et al*. 2006), and panicle number (Zou *et al*. 2005). RM48 showed epistatic QTL interactions, it was linked to the QTL *tp2* for number of tillers per plant and *gw1c* for grain mass (Xing *et al*. 2002). In our experimnet, it was linked with four physiological traits P_N, C_c, Chl *b*, Ch *a*/*b*, and YLDP. Many studies showed that RM263 is significantly associated with yield and yield-related traits, *hgw2* for heterotic loci linked to 1,000-grain mass (Luo *et al*. 2011), *yld2.1* for yield per plant (Marri *et al*. 2005), and *qHD-2* for heading date (Zou *et al*. 2000). In our study, RM263 was significantly associated with yield-related traits, such as NP, PB, and also to physiological traits *E*, WUE, and Chl *a*/*b* and they were significantly correlated with P_N. This has not been reported previously. Likewise, another marker RM209 was significantly associated with *E* and WUE, whereas C_c, F_v/F_m, and PH were associated at 5% level of significance. Also, RM209 was reported to be associated with *QGy11a* for grain yield per plant (Xu *et al*. 2005), *nt11.1* for number of tillers per plant, *bm11.1* for vegetative biomass, *yldp11.1* for yield per plant (Swamy *et al*. 2011), *mp11.1* for milling percent, *asv11.1* for alkali spreading value, and *gc11.1* for gel consistency (Swamy *et al*. 2012) and protein content (Xu *et al*. 2016).

Furthermore, the analysis of marker trait association in popB showed RM488 on chromosome 1 was associated with F_v/F_m, Chl *b*, Chl *a*/*b*, PL, and PWT. Previous reports showed that RM488 was linked with the QTL *ph1.1* for plant height, *npt1.1* for number of productive tillers per plant, *nsp1.1* for number of spikelets per plant, *nfg1.1* for number of filled grains per plant (Swamy *et al*. 2014), *qNSB1.1* for number of secondary branches (Swamy *et al*. 2011), *mp1.1* for milling percent, *kw1.3* for kernel width, *ver1.1* for volume expansion ratio (Swamy *et al*. 2012), *ph1.3* for plant height, *nt1.3* for number of tillers per plant, *np1.2* for number of panicles per plant, *pl1.2* for panicle length, *nsp1.1* for number of spikelets per plant, *gnp1.1* for number of grains per plant, and *gw1.6* for grain mass (Kaladhar *et al*. 2008). Likewise, RM185 on chromosome 4 was associated with six traits with the significance level at 5%. It was reported to be associated with days to heading (*qDTH4.1*), days to 50% flowering (*qDFF3.2*), days to maturity (*dtm4.1*), kernel width (*kw4.1*), and gel consistency (*gc4.1*) (Swamy *et al*. 2011, 2012, 2014). But in our study, it was highly associated with leaf pigment-related traits, such as Chl *a*, Chl *b*, TChl, Car, and yield-component traits, NT and TGW. RM122 on chromosome 5 is associated with g_s, C_i, Φ_{PSII}, TNG, and YLDP. It was linked to BLB resistance gene *Xa5* in rice genotypes (Sabar *et al*. 2016) and lemma width (Ishikawa *et al*. 2017). RM204 on chromosome 6 was associated with P_N, g_s, C_i, E, Φ_{PSII}, and q_N. Swamy *et al*. (2011) showed that RM204 was linked to QTL *qDTH6.1* for days to heading, *qDFF4.1* for days to 50% flowering, *qSD6.1* for stem diameter. It was linked to *ns6.1* for number of spikelets per panicle, *gp6.1* for number of grains per panicle (Kaladhar *et al*. 2008), and *kw6.1* for kernel width (Swamy *et al*. 2012). Another marker RM162 on chromosome 6 was linked to five traits WUE, WUE_i, Φ_{CO2}, PB, and TGW. Previous reports showed that it was linked to the QTL for *qCC6b* for Chl content (Hu *et al*. 2009), *qNT-6* for number of tillers per plant (Zhou *et al*. 2013), *qPBN-6* for primary branch number (Liu *et al*. 2008), and *qTPH-6* for tallest panicle height (Ma *et al*. 2009).

All the markers, which showed significant association with photosynthetic traits, were previously reported to be linked with yield traits, indicating their pleiotropic effect or strong trait correlation. Interestingly, we did not find any common associated marker for yield or photosynthesis related-traits studied in both populations, indicating marker–trait association varies continuously in different interspecific populations. Though, these two populations were derived from common genetic background of Swarna.

Conclusions: The results showed that wide variations in leaf photosynthetic traits, physiological, yield and yield-related traits among the BILs. P_N was significantly correlated with g_s, E, g_m, C_c, CE, leaf thickness, TChl, F_v'/F_m', Φ_{PSII}, Φ_{CO2}, YLDP, BM, DM, and HI in both populations. Four BILs (IL 230S, IL 75S, IL 214S, and IL 33S) from popA and six BILs (173K, 3-1K, 24K, 45K, 75K, and 131K) from popB showed higher P_N than that of parents. These BILs also showed high g_s, C_i, g_m, C_c, leaf thicknes, TChl, ETR, TGW, YLDP, and DM. The principal component analysis showed the first four PCs explained 92 and 93% of total genetic variation in popA and popB, respectively. These principal components are significantly associated with the traits C_i, C_c, FG, and TNG. Similarly, single marker analysis showed six markers from popA and five markers from popB that were associated with more than five traits indicating pleiotropic effect. These loci are of great importance in improving photosynthetic traits and should be explored in detail for further use in crop improvement. This study helped identify the factors which contribute to photosynthesis and the relation among various gas-exchange and yield-related traits. Further, it shows that introgressions from wild species can help increase several traits. The BILs with high P_N, DM, and grain yield can be used to improve a yield potential in modern cultivars.

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