RESEARCH ARTICLE

Genotypic variation of photosynthetic gas exchange and stomatal traits in some traditional rice (*Oryza sativa* L.) landraces from Koraput, India for crop improvement

Debabrata Panda¹ · Amar Mahakhud¹ · Biswajeet Mohanty¹ · Swati S. Mishra¹ · Jijnasa Barik¹

Received: 18 February 2018 / Revised: 18 March 2018 / Accepted: 25 April 2018 / Published online: 23 May 2018
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Abstract Variations in photosynthetic gas exchange, stomatal traits and photosystem (PS) II activity were investigated in three popular rice (*Oryza sativa* L.) landraces namely Kalajeera, Machakanta and Haladichudi from Koraput, India and compared with high yielding modern varieties (IR 64 and IR 42) to judge the possibility of using them in crop improvement programmes. The leaf CO₂ photosynthetic rate (P_N), stomatal conductance (gs), water use efficiency and carboxylation efficiency were significantly higher in traditional landraces as compared to the high yielding variety. In contrast, the transpiration rate, internal CO₂ concentration, special analysis device (SPAD) index and chlorophyll were higher in high yielding varieties. In addition, the traditional landraces showed better stomatal traits such as stomatal density (SD), stomatal size (SS) and stomatal index. Further, multiple correlations between different gas-exchange characteristics and other physiological traits revealed that the P_N was not dependent on the leaf pigment content or PS II activity. However, it was dependent on stomatal traits like gs, SD and SS. Taken together, the traditional landraces such as Kalajeera, Machakanta and Haladichudi had superior P_N and stomatal efficiency compared to the high yielding variety under prevailing environmental condition. Further research is required to elucidate the genetic diversity of these popular landraces compared to high-yielding ones in relation to photosynthesis efficiency for future crop improvement programmes.

Keywords Gas exchange · Photosynthesis · Stomatal traits · Traditional landraces · Rice

Introduction

In Asia, rice (*Oryza sativa* L.) yield potential is significantly stagnated and need to increase at least by 60% to provide adequate food for increasing population (Sheehy et al. 2007). During recent decades, increased crop production has been mainly achieved through refining agronomic management and breeding improved crop varieties (Scheben et al. 2016). In the past, multiple traits such as resistance to disease and pest, superior grain quality, and abiotic stress tolerance were incorporated to enhance yield potential (Hibberd et al. 2008; Furbank et al. 2009). However, the yield of rice has already reached a plateau despite of all the agronomic and breeding attempts (Furbank et al. 2009). In order to overcome the yield plateau in rice, an increase in leaf photosynthetic rate for the biomass production is an essential option (Zhao et al. 2010; Kondamudi et al. 2016; Scheben et al. 2016). Several researchers have also suggested that modification of primary photosynthetic process under changing climate is one of the requirements for sustaining global rice production (Zhu et al. 2008; Kajala et al. 2011; Kondamudi et al. 2016). Since most of the plant biomass is derived from photosynthetic carbon, enhancing the leaf photosynthesis would eventually increase the grain yield in rice (Long et al. 2006; Zhu et al. 2010; Makino 2011; Gu et al. 2013). For improving the rice yield potential, the mechanisms employed in the past were canopy architecture (Huang et al. 2015), total biomass production (Long et al. 2006) and photosynthetic efficiency (Zhu et al. 2010). Assessment of genotypic variations of photosynthesis in different

✉ Debabrata Panda
dpanda80@gmail.com

¹ Department of Biodiversity and Conservation of Natural Resources, Central University of Orissa, Koraput, Odisha 764 021, India

rice varieties is an important precondition for a successful breeding programme to increase photosynthesis in rice (Haritha et al. 2017). The variations in photosynthetic traits have been extensively studied in wild *Oryza* species and successfully used in different breeding programmes in the past (Zhao et al. 2010; Kiran et al. 2013; Kondamudi et al. 2016; Haritha et al. 2017). Unlike wild rice, relatively few information have been published regarding the photosynthetic efficiency in traditional rice landraces and there is an information gap on photosynthetic variants among traditional and modern rice genotypes.

In plant species, the carbon balancing is important for climatic adaptability (Kondamudi et al. 2016). The carbon balance in the leaf is regulated by leaf gas exchange and different stomatal traits such as stomata number, density and size (Hetherington and Woodward 2003). Several studies showed the importance of stomatal density and size and their association with higher photosynthetic rate in different plants including rice (Rebecca et al. 2010; Kondamudi et al. 2016). Apart from the stomatal regulation, there are several plant factors that influence leaf photosynthetic rate, including position of the leaf, sink effects, age and also environmental factors such as light, temperature, nutrition and water availability (Aighewi and Ekanayake 2004). In vivo chlorophyll fluorescence has been used frequently as a rapid and non-invasive tool for studying photosynthetic performance of plants (Kao et al. 2006; Sayed 2003; Strasser et al. 2004). Unlike other plants, limited information on stomatal traits are reported in rice and also its relationship with gas exchange, leaf pigments and PS II activity in traditional rice landraces have not yet been studied.

Koraput districts of Odisha, India, is the home to large number of indigenous rice varieties and considered as the secondary centre of origin of Asian cultivated rice (Mishra et al. 2012; Roy et al. 2016). These indigenous rice landraces are intermediate between wild rice and modern varieties and show many primitive features and are traditionally cultivated and maintained by farmers (Tripathy et al. 2005; Arunachalam et al. 2006; Roy et al. 2016). Although traditional rice landraces are less productive, they offer great promise for ensuring food security and livelihood of poor farming communities due to their tolerance towards a number of biotic and abiotic stresses (Sarkar and Bhattacharjee 2011; Vikram et al. 2016). These diverse landraces have been conserved in different national and base collection centres and their genetic diversity with quality traits were extensively studied (Prashanth et al. 2002; Patra and Dhua 2003; Roy et al. 2016), but genotypic variations of photosynthetic traits in these rice landraces of Koraput have not yet been studied. Since the phenotyping for photosynthetic and stomatal traits is an important prerequisite for breeding programmes. The objective of this

investigation was to identify variations in leaf photosynthetic characteristics and stomatal traits in selected traditional and modern rice genotypes, which may help in rice crop improvement programmes.

Materials and methods

Plant materials

The study was conducted by taking three popular traditional rice landraces, namely Kalajeera, Machakanta and Haladichudi from Koraput, India along with the high yielding modern variety (IR 64 and IR 42) (Table 1). The dry healthy seeds of Kalajeera, Machakanta and Haladichudi were collected from MS Swaminathan Research Foundation (MSSRF), Koraput. These landraces are popular of the region for its fine grain with excellent cooking quality and cultivated by the local population (Arunachalam et al. 2006; Roy et al. 2016). The seeds of high yielding variety such as IR 64 (a high yielding modern variety) and IR 42 (a sister selection of IR 36: IR-156-228-1-2/IR 1733//CR 94-31) (Vikram et al. 2016) were collected from National Rice Research Institute (NRRI), Cuttack, India. The details of genotypes with their yield potential were presented in Table 1.

Growth conditions

The experiment was conducted in the experimental field of Central University of Orissa, Koraput, India (82°44'54"E to 18°46'47"N, 880 m above the mean sea level and average rainfall 1500 mm). Uniform sized seeds were selected and sown directly in earthen pots (45 cm in diameter) containing four kg of farm soil and farmyard manure (3:1). The soil used for the experiment was deep loamy red and lateritic (pH 6.64, organic C 0.15 ppm, total N 0.18 ppm, available P 0.01 ppm, and available K 0.84 ppm). Ten days after germination, the seedlings were thinned and five plants per pot were maintained. Each pot was supplied with 190 mg single super phosphate (P_2O_5) and 50 mg murate of potash (K_2O). N-fertilizer in the form of urea at 1 g per pot was applied thrice after 10, 30 and 50 days of sowing. The experimental design was a randomized block design with three replications in each genotype. Each pot was considered as one replication. Plants were regularly irrigated with tap water and subjected to natural solar radiation, with daily maximum photosynthetic photon flux density, air temperature and relative humidity being about $1460 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$, $33.6 \pm 3^\circ\text{C}$ and 65–70%, respectively throughout the experiment. All the measurements were performed three times after 60 days of sowing (in tillering stage).

Table 1 Detail of studied genotypes with their special character and yield potential

Sl. no.	Variety	Special character	Yield
1.	Machakanta	Maturity durationc (135–145 days), popular traditional variety, non sticky cooked rice, slender grain with straw colour husk, tall plant type ^{a,b}	25–30 q/ha ^a
2.	Haladichudi	Maturity duration (125–135 days), popular traditional variety, medium slender grain with deep yellow husk color, non-sticky cooked rice with tasty to eat ^{a,b}	18–20 q/ha ^a
3.	Kalajeera	Maturity duration (140–150 days), popular traditional variety, highly aromatic, short bold grain with black colour husk, tall plant type, non-sticky cooked rice and highly palatable ^{a,b}	22–25 q/ha ^a
4.	IR 64	Maturity duration (115 days), hybrid variety with high yield, rainfed lowland areas, semi dwarf, susceptible to abiotic stress ^c	58–61 q/ha ^c
5.	IR42	Maturity duration (130 days), hybrid variety with high yield, intermediate height (105 cm), a sister selection of IR 36: IR-156-228-1-2/IR 1733//CR 94-31 ^c	65–67 q/ha ^c

^aArunachalam et al. (2006), ^bRoy et al. (2016), ^cVikram et al. (2016)

Measurement of leaf gas exchange

The leaf gas exchange parameters such as P_N , E , C_i and g_s were measured between 10 and 12 h on fully matured leaves of each plant using an open system photosynthetic gas analyzer (CI-304, CID, USA) under normal ambient environmental condition. The fully matured 2nd and 3rd leaf from each plant were selected and kept inside the chamber under natural irradiance until stable reading was recorded. The measurements were carried out at $33 \pm 2^\circ\text{C}$, 60–70% Relative humidity, $1024 \pm 38 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic active radiation, $370 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ and 21% O_2 .

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured on the same leaves used for gas exchange measurements in dark- and light-adapted leaves by using a portable chlorophyll fluorometer (JUNIOR-PAM, WALZ, Germany) at mid-day (12:00 h). Different fluorescence parameters like minimal fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence ($F_v = F_m - F_o$) and maximum photochemical efficiency of PS II (F_v/F_m) was measured in 20 min dark-adapted leaves (Maxwell and Johnson 2000). In light adapted leaves at a PPFD of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (for 15 min) steady state fluorescence yield (F_s), maximal fluorescence (F_m') after 0.8 s saturating white light pulse and minimal fluorescence (F_o') were measured when actinic light was turned off. Quenching value due to non-photochemical dissipation of absorbed light energy (NPQ) and the coefficient for photochemical quenching (q_P) was also calculated (Maxwell and Johnson 2000). The electron transport rate (ETR) was measured by the light induction curve with different photosynthetic active radiation (PAR) by the software supplied by the manufacture.

Measurement of leaf pigment (chlorophyll and carotenoid) and SPAD index

After measurement of the gas exchange and chlorophyll fluorescence, the same leaves were pooled and together finely chopped to use for Chl and carotenoid estimation. Fresh leaves of 100 mg were placed in a 25-ml capped-measuring tube containing 10 ml of 80% cold acetone, and kept inside a refrigerator (4°C) for 48 h. The total Chl and carotenoid were measured spectro-photometrically by taking absorbance at 663, 645 and 470 nm. The Chl and carotenoid content were calculated using the equations of Arnon (1949) and Lichtenthaler and Wellburn (1983), respectively. SPAD chlorophyll index was measured on the fully expanded leaf of 5 different plants using a SPAD 502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan), on the intensity of light transmitted 650 nm (Shrestha et al. 2012).

Measurement of leaf area and stomatal traits

Flag leaf area (LA) was measured in each plant by taking the length and breadth of leaf and calculated by the using the formula Leaf area (cm^2) = $0.67 \times \text{length} \times \text{width}$ (Yoshida et al. 1976).

The stomatal traits were measured in the flag leaf of each plant with three leaves in each replication. The abaxial epidermis of the leaf was cleaned by tissue paper, and carefully smeared with nail varnish in the mid-area, then leaf were left for 20 min. The thin film was peeled off from the leaf surface, mounted on a glass slide. Numbers of stomata (s) and epidermal cells (e) for each film strip were counted under a trinocular microscope system with a computer attachment (Olympus model No.MIPS-3 MP CAMERA, Japan). The leaf stomatal density (SD), which was expressed as the number of stomata per unit leaf area,

the number of stomata per flag leaf, stomatal size (SS) and stomatal index (SI) were determined by using following equations (Radoglou and Jarvis 1990)

Number of stomata per flag leaf = LA × Stomatal density.

Stomatal size (SS) = Single stomatum width × length

Stomatal index (SI) = $[s/(e + s)] \times 100$.

Statistical analysis

Differences between various parameters were compared by one-way analysis of variance (ANOVA) using CropStat-7.2 software (International Rice Research Institute, Philippines). The statistical significance of the parameter means were determined by performing the *Fisher's* least significance difference (LSD) test. The standard deviations (SD) and multiple correlation analysis were done by Microsoft Excel 2007. The multivariate analysis including principal component analysis (PCA) and cluster analysis were carried out using PAST-3 (Palaeontological Statistics) software.

Results and discussion

Variation in leaf gas exchange and photosynthetic pigments

Variations in the photosynthetic characteristics of traditional rice landraces were studied by gas-exchange measurements and the results were compared with the modern high-yielding rice varieties. In the present study, wide variations were observed within the studied rice genotypes for leaf gas exchange parameters (Table 2). There were significant differences between traditional and modern high-yielding rice varieties with regard to photosynthetic rate (P_N). The range of P_N in traditional landraces, such as Kalajeera, Haldichudi and Machakanta were significantly ($P < 0.05$) higher than that of modern high yielding rice varieties (IR 64 and IR 42). Similarly, stomatal conductance (g_s) was also higher in traditional landraces, but the transpiration rate (E) was not significantly different among the rice genotypes (Table 2). Further, the water-use efficiency (WUE ; P_N/E) and carboxylation efficiency (CE ; P_N/Ci), varied significantly ($P < 0.05$) among the tested rice genotypes (Table 2). WUE and CE of the traditional landraces, such as Kalajeera, Haldichudi and Machakanta were significantly higher than those of modern high yielding rice varieties. In contrast, the high yielding genotypes, such as IR 64 and IR 42 showed significantly ($P < 0.05$) higher value of internal CO_2 concentration (Ci) compared to other genotypes (Table 2). The leaf photosynthesis is a complex process and is controlled by many

Table 2 Variation in leaf photosynthetic characteristics and leaf pigments in rice genotypes

Variety	P_N ($\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$)	E ($\text{mMol } H_2O \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mMol } H_2O \text{ m}^{-2} \text{ s}^{-1}$)	Ci ($\text{mMol } H_2O \text{ m}^{-2} \text{ s}^{-1}$)	WUE (P_N/E)	CE (P_N/Ci)	SPAD (rel.)	Chlorophyll ($\text{mg g}^{-1} \text{ fwt}$)	Carotenoid ($\text{mg g}^{-1} \text{ fwt}$)
Kalajeera	17.55 ± 1.6^a	3.57 ± 0.18^a	91.7 ± 4.06^a	253 ± 12.5^b	4.91 ± 0.19^a	0.065 ± 0.003^a	32.70 ± 2.38^b	2.84 ± 0.62^b	0.254 ± 0.038^a
Haldichudi	17.37 ± 0.5^a	3.39 ± 0.24^a	88.1 ± 4.25^a	259 ± 6.7^b	5.12 ± 0.10^a	0.063 ± 0.008^a	29.71 ± 2.26^b	2.47 ± 0.55^b	0.249 ± 0.002^a
Machakanta	17.51 ± 1.0^a	3.25 ± 0.57^a	89.2 ± 7.43^a	264 ± 9.5^b	5.38 ± 0.11^a	0.066 ± 0.001^a	29.85 ± 0.35^b	2.66 ± 0.09^b	0.274 ± 0.041^a
IR 64	14.85 ± 0.4^b	3.55 ± 0.07^a	83.1 ± 1.90^b	283 ± 6.9^a	4.18 ± 0.22^b	0.052 ± 0.003^b	36.45 ± 1.34^a	3.87 ± 0.04^a	0.230 ± 0.020^a
IR 42	15.02 ± 1.0^b	3.25 ± 0.07^a	82.4 ± 1.34^b	277 ± 4.7^a	4.62 ± 0.16^b	0.054 ± 0.007^b	38.05 ± 2.73^a	3.94 ± 0.03^a	0.250 ± 0.005^a
Mean	16.28	3.28	86.5	267.6	4.94	0.06	32.5	2.90	0.221
LSD ($P < 0.05$)	1.07	0.40	3.2	16.2	0.31	0.010	3.59	1.06	0.060
CV (%)	8.6	9.8	5.5	6.2	13.1	11.4	14.2	6.3	6.3

Data are the mean of three replications $\pm$ standard deviation

Means followed by a common letter in the same column are not significantly different at the 5% level by *Fisher's* least significance difference (LSD) test

P_N Photosynthetic rate ($\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$), g_s stomatal conductance ($\text{mMol } H_2O \text{ m}^{-2} \text{ s}^{-1}$), Ci internal CO_2 concentration ($\text{mMol m}^{-2} \text{ s}^{-1}$), WUE water use efficiency (P_N/E), CE carboxylation efficiency (P_N/Ci), chlorophyll ($\text{mg g}^{-1} \text{ fwt}$), carotenoid ($\text{mg g}^{-1} \text{ fwt}$), CV coefficient of variance

genes (Shi et al. 2005). Such photosynthetic variation among the genotypes might be related to their origin and genetics of the varieties. In the present study, leaf P_N recorded in different rice genotypes was lower than the earlier reports (Zhao et al. 2010; Kiran et al. 2013). The lower values of P_N could be due to high temperatures prevailing during measurements. The result are also consistent with the earlier findings of Xie et al. (2011), who reported in the context of climate change that there is a decline in photosynthesis with atmospheric air temperature higher than 35 °C. Results of the present study indicated that traditional landraces showed better photosynthetic efficiency but unable to increase grain yield, because increase in leaf photosynthesis may not necessarily leads to increase in grain yield (Gu et al. 2013). In rice, higher leaf P_N was reported to be correlated with g_s and carboxylation capacity (Gauthami et al. 2014). The WUE can be influenced by both variation in P_N or E and both, which have been reported earlier in rice (Gauthami et al. 2014). There have been several reports that the incorporation of desired natural genetic variation in photosynthesis through breeding programmes, leads to increase in crop photosynthesis (Haritha et al. 2017). The results suggested that the traditional rice landraces are associated with high leaf photosynthetic rate, which can contribute genes for crop improvement despite their poor phenotypes (Placido et al. 2013; Vikram et al. 2016).

Leaf pigments reflect photosynthetic properties of the plants, as they indicate the amount of light-harvesting capacity (Elfeky et al. 2007). The significant differences in Chl and SPAD index were observed among the studied rice genotypes (Table 2). The high yielding varieties (IR 64 and IR 42) showed significantly higher Chl and SPAD index compared to the traditional landraces, but no significant difference in carotenoid content was observed among the genotypes. Earlier studies pointed out that, genotypes maintaining higher leaf Chl during the growth period may produce higher biomass and photosynthetic capacity (Hassan et al. 2009; Haritha et al. 2017). However, in the present study high yielding varieties (IR 64 and IR 42) showing more Chl and SPAD index were unable to increase the leaf P_N because of less g_s , WUE and CE.

Variations in leaf PS II activity and electron transport rate

Leaf Chl fluorescence measurement helps to assess the intrinsic photosynthetic performance of plants. It provides important information of PSII, reflecting actual performance of the plant in prevalent environmental conditions in the field (Strasser et al. 2004; Batra et al. 2016). The leaf PSII activity was studied by measuring different Chl fluorescence parameters such as F_o , F_m , F_v/F_m , $Y(II)$, qP ,

and NPQ, which were widely used in plant physiology studies (Murchie and Lawson 2013). There were no significant differences in values of F_o , F_m , F_v/F_m , $Y(II)$, qP , and NPQ observed among traditional and modern high yielding rice varieties (Table 3).

The intrinsic photosynthetic capacity of the plants is reflected by light-response curves (LCs) (Lüttge et al. 2007). The LC measurements provide valuable information on electron transport rate (ETR), saturating photosynthetically active radiation (PAR), and photosynthetic yield, which can be used to assess the performance of the plants (Batra et al. 2016). In the present study, different ranges of PAR (65–820 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were given to measure the electron transport rate of selected rice genotypes for determining the PAR saturation. It was found that, with increase in PAR range, the ETR also gradually increased. Maximum ETR was observed in traditional rice landraces (Kalajeera and Haldichudi) and they showed better photochemical activity in the prevalent environmental conditions as compared to the other genotypes (Fig. 1).

Variations in leaf area and stomatal traits

There were no significant differences in leaf area among traditional and modern high yielding rice varieties (Table 4). The leaf gas exchange are controlled by different stomatal traits such as stomata number, density and size (Hetherington and Woodward 2003; Buckley 2005). However, very little information on the relationship of gaseous exchange with stomatal behaviour in rice was reported (Buckley 2005). Therefore, an attempt was made in this direction, which led to interesting observations (Table 4; Fig. 2). The stomatal density (SD), their number and stomatal index (SI) showed significant ($P < 0.05$) differences between traditional and modern rice genotypes. The SD, total number of stomata per LA, SS and SI were found to be higher in traditional rice landraces, such as Kalajeera, Haldichudi and Machakanta compared to the high yielding genotypes (Table 4; Fig. 2). Genotypic variations in stomatal density and size have been reported earlier in different rice genotypes (Kondamudi et al. 2016). However, the results were contrast to earlier findings in hybrid rice cultivars producing higher grain yield, which might be due to higher number of large sized stomata causing higher photosynthetic efficacy (Sarwar et al. 2013). The overall effects of stomatal traits are in agreement with earlier reports of active involvement of stomata in photosynthetic enhancement (Goh et al. 1997, 2002). There may not be genetic link between stomatal traits such as density and size and they may not be pleiotropically controlled. These traits can be used as basis for selection at the leaf level towards balance of carbon and water uptake (Rebecca et al. 2010). The results of the present study suggested that

Table 3 Variation in leaf chlorophyll fluorescence parameters in rice genotypes

Variety	Fo (rel.)	Fm (rel.)	Fv/Fm (ratio)	Y(II) (rel.)	qP (rel.)	NPQ (rel.)
Kalajeera	436 ± 16 ^a	1781 ± 59 ^a	0.755 ± 0.002 ^a	0.68 ± 0.04 ^a	0.87 ± 0.04 ^a	0.56 ± 0.01 ^a
Haldichudi	403 ± 45 ^a	1779 ± 53 ^a	0.777 ± 0.001 ^a	0.65 ± 0.01 ^a	0.90 ± 0.01 ^a	0.42 ± 0.02 ^a
Machakanta	419 ± 24 ^a	1743 ± 28 ^a	0.759 ± 0.001 ^a	0.67 ± 0.03 ^a	0.93 ± 0.04 ^a	0.35 ± 0.02 ^a
IR64	468 ± 14 ^a	1778 ± 70 ^a	0.736 ± 0.018 ^a	0.68 ± 0.02 ^a	0.93 ± 0.02 ^a	0.32 ± 0.01 ^a
IR42	426 ± 38 ^a	1725 ± 38 ^a	0.753 ± 0.028 ^a	0.68 ± 0.01 ^a	0.96 ± 0.03 ^a	0.34 ± 0.00 ^a
Mean	430	1761	0.760	0.62	0.92	0.30
LSD ($P < 0.05$)	65	147	0.047	0.08	0.09	0.098
CV (%)	12.7	9.2	1.2	4.3	9.7	6.0

Data are the mean of three replications ± standard deviation

Means followed by a common letter in the same column are not significantly different at the 5% level by Fisher's least significance difference (LSD) test

Fo minimum fluorescence yield obtained with dark-adapted leaf, Fm maximum Chl fluorescence yield obtained with dark-adapted leaf, Fv/Fm maximal photochemical efficiency of PS II, NPQ non-photochemical quenching, qP photochemical quenching, Y(II) yield of PSII photochemistry, CV coefficient of variance

Fig. 1 Variations in electron transport rate (ETR) in rice genotypes under different photosynthetic active radiation (PAR). Data are the mean of three replications ($n = 3$) and vertical bar represents standard deviation

the traditional rice landraces were more resilient to the prevailing environments and this may be due to stomatal traits combined with efficient gas exchange. Further, it may be an adaptive mechanism of plants to local climatic conditions as has been reported earlier in case of wild rice (Rebecca et al. 2010; Kondamudi et al. 2016).

Correlation

Relationship between leaf photosynthetic parameters and stomatal traits in rice genotypes were studied by multiple correlation analysis (Table 5). The results revealed that the rate of photosynthesis (P_N) was not significantly influenced by leaf pigment contents, PS II photochemical activity and leaf area. A strong positive correlation between P_N with g_s , CE and WUE ($r = 0.60^{**}$, 0.90^{**} and 0.64^{**} respectively, $P < 0.01$) was observed whereas, leaf P_N was negatively

correlated with C_i ($r = -0.50^*$, $P < 0.05$). The results indicated that the observed variations in P_N in different rice genotypes were not based on pigment contents or PS II photochemistry, but related to the leaf CE and WUE as have been reported earlier in rice (Yeo et al. 1994; Kiran et al. 2013; Haritha et al. 2017). A strong correlation between g_s and P_N was also observed in various studies and was reported that stomatal aperture and g_s were strongly correlated with leaf photosynthesis in rice (Hetherington and Woodward 2003; Kusumi et al. 2012). Further, a strong positive correlation between P_N with SD, SLA, SS and SI ($*P < 0.05$) were observed in the present study (Table 5). The findings suggest that shape, size and number of stomata were the major regulating factor for leaf photosynthesis in rice reported in different plant species (Giuliani et al. 2011; Chandra and Das 2000) and also in rice (Kondamudi et al. 2016). The leaf Chl content is an important physiological trait, closely associated with photosynthesis and used to predict yield potential (Teng et al. 2004; Abhilash Joseph et al. 2014). Present study suggested that screening of rice genotypes with high P_N , on the basis of high Chl content, leaf PS II activity or leaf area may not be a useful strategy. However, improvement in stomatal traits is an essential prerequisite for breeding varieties with higher P_N (Ohsumi et al. 2007; Zhao et al. 2010).

Genotypic variations among rice genotypes

To study the genotypic variation among studied rice landraces, the data of different photosynthetic traits were subjected to multivariate analysis including principal component analysis (PCA) and cluster analysis. PCA measures the importance and contribution of each

Table 4 Variation in stomatal traits and leaf area in rice genotypes. Data are the mean of three replications $\pm$ standard deviation

Variety	Leaf area (cm^2)	Stomata density (no. mm^{-2})	Total stomata no. (million LA^{-1})	Stomata size (μm^2)	Stomatal index (SI)
Kalajeera	0.39 ± 0.15^a	347.64 ± 20.8^a	1.27 ± 0.07^a	403.5 ± 13.4^a	7.76 ± 0.36^a
Haldichudi	0.41 ± 0.09^a	318.82 ± 20.8^a	1.10 ± 0.02^b	400.5 ± 19.1^a	6.02 ± 0.20^b
Machakanta	0.30 ± 0.08^a	323.52 ± 21.5^a	1.05 ± 0.08^b	389.5 ± 13.4^a	5.81 ± 0.61^b
IR64	0.59 ± 0.06^a	264.70 ± 11.5^b	0.87 ± 0.06^c	335.5 ± 18.9^b	4.35 ± 0.33^c
IR42	0.39 ± 0.01^a	294.11 ± 21.5^b	0.93 ± 0.02^c	314.0 ± 2.80^b	4.37 ± 0.21^c
Mean	0.42	311	1.04	368.6	5.66
LSD ($P < 0.05$)	0.28	28.15	0.13	33.29	1.01
CV (%)	4.5	8.7	6.0	6.2	7.1

Means followed by a common letter in the same column are not significantly different at the 5% level by Fisher's least significance difference (LSD) test
 LA leaf area (cm^2), SD stomatal density (no. mm^{-2}), SLA total stomata no. (million LA^{-1}), SS stomatal size (μm^2), SI stomatal index, CV coefficient of variance

Fig. 2 Distribution of stomata (black arrows) in abaxial epidermis of flag leaf of different rice genotypes. Genotypes A Kalajeera, B Machakanta, C Haldichudi, D IR 64, E IR 42

component to study the genotypic variation (Sinha and Mishra 2013). The first three axes of the principal component analysis captured 97.9% of the total variations with eigen values more than 1 suggesting the wide genetic variation among the studied landraces. Based on the loading and biplot, it appears that a few traits are the major determinants of phenotypic diversity, among which SI, CE, P_N , SD and SS play a pivotal role. The rest of the characters seem to have a minimal contribution to variability (Fig. 3). A scatter plot between PC1 and PC2 depicted a clear pattern of grouping genotypes in the factor plane (Fig. 3).

Cluster analysis based on the Bray–Curtis paired linkage revealed the percent of similarity in leaf photosynthetic parameters among different rice genotypes are presented in

Table 5 Relationship between leaf photosynthetic parameters and stomatal traits in rice genotypes

Parameters	P _N	g _s	Ci	WUE	CE	SD	SLA	SI	SS	Fv/Fm	qP	NPQ	Chl	CAR
g _s	0.60*													
Ci	– 0.50*	– 0.80**												
WUE	0.64*	0.07**	0.01 ^{ns}											
CE	0.90**	0.81**	– 0.82**	0.40 ^{ns}										
SD	0.67*	0.65*	– 0.75**	0.30 ^{ns}	0.77**									
SLA	0.60*	0.76**	– 0.84**	0.10 ^{ns}	0.76**	0.88**								
SI	0.69*	0.79**	– 0.79**	0.20 ^{ns}	0.81**	0.84**	0.97**							
SS	0.71**	0.71**	– 0.63*	0.30 ^{ns}	0.76**	0.45 ^{ns}	0.70**	0.78**						
Fv/Fm	0.02 ^{ns}	0.39 ^{ns}	– 0.30 ^{ns}	– 0.30 ^{ns}	0.13 ^{ns}	0.34 ^{ns}	0.55*	0.55*	0.23 ^{ns}					
qP	– 0.18 ^{ns}	– 0.55*	0.59*	0.40 ^{ns}	– 0.66*	– 0.56*	– 0.70**	– 0.70**	– 0.68*	– 0.49 ^{ns}				
NPQ	0.35 ^{ns}	0.44 ^{ns}	– 0.55*	– 0.20 ^{ns}	0.46 ^{ns}	0.55*	0.73**	0.73**	0.60*	0.62*	– 0.90*			
Chl	– 0.17 ^{ns}	– 0.50*	0.44 ^{ns}	– 0.70**	– 0.71**	– 0.35 ^{ns}	– 0.45 ^{ns}	– 0.51*	– 0.78**	0.19 ^{ns}	0.32 ^{ns}	– 0.12 ^{ns}		
CAR	0.34 ^{ns}	0.52*	– 0.53*	0.10 ^{ns}	0.48 ^{ns}	0.23 ^{ns}	0.26 ^{ns}	0.29 ^{ns}	0.49 ^{ns}	– 0.44 ^{ns}	– 0.24 ^{ns}	0.07 ^{ns}	– 0.54*	
LA	0.41 ^{ns}	– 0.20 ^{ns}	0.18 ^{ns}	– 0.80**	– 0.47 ^{ns}	– 0.22 ^{ns}	– 0.25 ^{ns}	– 0.29 ^{ns}	– 0.46 ^{ns}	– 0.05*	0.08 ^{ns}	0.03 ^{ns}	0.76**	0.12 ^{ns}

P_N Photosynthetic rate, g_s stomatal conductance, Ci internal CO₂ concentration, WUE water use efficiency (P_N/E), CE carboxylation efficiency (P_N/Ci), SD stomatal density, SLA total stomata no., SI stomatal index, SS stomatal size, Fv/Fm maximum photochemical efficiency of PS II, qP photosynthetic quenching, NPQ non photosynthetic quenching, Chl chlorophyll, CAR carotenoid, LA leaf area, ns non significant

*P < 0.05, **P < 0.01

Fig. 3 Principal component analysis (PCA) of studied rice genotypes showing genotypic relationship in a graphical representation scatter plot on the basis of leaf photosynthetic parameters

Fig. 4 Dendrogram showing the percentage of similarity between rice genotypes constructed using leaf photosynthetic parameters and stomatal traits. Data are the mean of three replications ($n = 3$)

Fig. 4. The Dendrogram showing the similarity forms two major clusters such as Haldichudi, Machakanta and Kalajeera in one cluster with 96.7% similarity and the modern high yielding genotypes IR 64 and IR 42 in a separate cluster having 97.6% similarity. The genotypes under the same cluster are unique as they showed similar leaf

photosynthetic traits. The popular landraces of Koraput, such as Kalajeera, Machakanta and Haladichudi are clearly separated from high yielding genotypes on the basis of leaf photosynthetic efficiency under prevailing environmental condition.

Conclusion

In the present study, significant genotypic variations of leaf photosynthetic traits were observed in the selected rice genotypes. The results indicated that the traditional landraces, such as Machakanta, Haldichudi and Kalajeera showed superior leaf P_N , g_s , water-use and carboxylation efficiency compared to the modern high yielding cultivars (IR 64 and IR 42). The photosynthetic adaptation of these traditional rice landraces seemed to be associated with stomatal density, number, size and stomatal index. Further, the leaf P_N was not significantly influenced by PS II photochemical activity, leaf area and pigment contents, but highly dependent on g_s , WUE and CE. Taken together these traditional landraces are identified as potential donor with superior photosynthetic efficiency, could be useful for improving leaf photosynthesis. Further research aim to elucidate the genetic diversity in relation to photosynthetic traits in these landraces and could be used in rice breeding program.

Acknowledgements The authors are grateful to Dr. S. K. Palita, Head, Department of Biodiversity and Conservation of Natural Resources for providing necessary facilities for the work and valuable suggestions. The Regional Director, MS Swaminathan Research Foundation (MSSRF), Koraput and Director, NRRI, Cuttack are highly acknowledged for providing the rice seeds for the experiment.

Author contributions AM and DP designed the experiments, cultivated the plants. AM, BM, JB and SSM performed the measurement of photosynthetic traits. DP analyzed the data and wrote the paper. All authors read and provided helpful discussions for the manuscript.

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