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Gas exchange, carbon balance and stomatal traits in wild and cultivated rice (*Oryza sativa* L.) genotypes

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Abstract Carbon balancing within the plant species is an important feature for climatic adaptability. Photosynthesis and respiration traits are directly linked with carbon balance. These features were studied in 20 wild rice accessions *Oryza* spp., and cultivars. Wide variation was observed within the wild rice accessions for photosynthetic oxygen evolution or photosynthetic rate (A), dark (R_d), and light induced respiration (LIR) rates, as well as stomatal density and number. The mean rate of A varied from 10.49 μmol O_2 m^{-2} s^{-1} in cultivated species and 13.09 μmol O_2 m^{-2} s^{-1} in wild spp., The mean R_d is 2.09 μmol O_2 m^{-2} s^{-1} and 2.31 μmol O_2 m^{-2} s^{-1} in cultivated and wild spp., respectively. Light induced Respiration (LIR) was found to be almost twice in wild rice spp., (16.75 μmol O_2 m^{-2} s^{-1}) compared to cultivated *Oryza* spp., Among the various parameters, this study reveals LIR and A as the key factors for positive carbon balance. Stomatal contribution towards carbon balance appears to be more dependent on abaxial surface where several number of stomata are situated. Correlation analysis indicates that R_d and LIR increase with the increase in A. In this study, *O. nivara* (CR 100100, CR 100097), *O. rufipogon* (IR 103404) and *O. glumaepatula* (IR104387) were identified as potential donors which could be used in rice breeding program. Co-ordination between gas exchange and patchiness in stomatal behaviour appears to be important for carbon balance and environmental adaptation of wild rice accessions, therefore, survival under harsh environment.

Keywords Wild rice accessions · Photosynthesis · Dark respiration · Light induced respiration · Stomata

Introduction

Advent of modelling systems enhanced the predictions of global climate change in terms of carbon dioxide and other gaseous atmosphere. Such studies mostly concentrated on the elevated CO_2, methane and nitrous oxide emissions either due to relative ease to determine the concentration of these gases with the help of modern technological innovations or dependence of global food security on the primary process of photosynthesis. Various evolutionary responses in plants were mainly due to the changes in photosynthesis (Franks and Beerling 2009; Gerhart and Ward 2010) like C_4 and CAM pathways resulted through reorganization of leaf anatomy and metabolism to overcome the inhibitory effect of low atmospheric CO_2 (Sage 2001). These gases are exchanged through stomata and perhaps the changes in atmospheric temperature, are regulated through stomata (Schulze and Hall 1982). From the nearest past, crops are frequently exposed to a plethora of unfavourable or adverse environmental conditions which in turn adversely affects the crop yields (Rodríguez et al. 2005; Acquaah 2007). Rice, being one of the major cereal food crops of Asia, may become susceptible to future climate change wherein water scarcity in combination with increased temperature may result in poor crop productivity and yield. In India, it is cultivated in about 150 million hectares, producing 132 billion metric tons, which covers about 26 % of the global rice production (Sudharshan et al.

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2013). Climatically rice may be considered as the most adaptable cereal, as its cultivation extends from banks of Amur River at 53° North latitude to Central Argentina at 40° South latitude (Subrahmanyam et al. 2011). It is also grown above 2000 m above sea level in the mountains of Himalayas and also in the hot deserts of Egypt (http://www.fao.org/climatechange). Rice yield improvement was accomplished through developing semi-dwarf varieties and heterotic hybrids in the past half-a century. Modification of primary photosynthetic pathway, the process of biomass production under changing climate is one of the requirements for sustaining global food production (Zhu et al. 2008; Kajala et al. 2011). Biomass production is mainly a net product of the difference between photosynthetic carbon assimilation (Makino 2011) and respiratory carbon loss. High temperature responses and photorespiration in a crop, like rice further complicates the understanding on physiological responses under changing climate situation which is yet to be fully understood. For instance, it is known that, for every 1.0 °C increase in temperature two fold increase in respiratory activity results in lower biomass production due to burning of the reserve carbohydrates (Millow et al. 2011) and is associated with mitochondrial enzyme activity (Leakay et al. 2009). Assessing photosynthetic mechanisms in wild rice spp., of diverse genetic bases (Yeo et al. 1994; Kiran et al. 2013) and diversity in the growing environment of the cultivated rice crop (Zhao et al. 2010) might provide insights of various adaptation mechanisms that were lost during breeding for developing cultivable varieties which is of importance to the plant breeders. Conversely, Ziska et al. (2014) had shown that inclusion of wild rice lines may bring variation and assist in the selections for superior physiological traits. The ability of modern rice (*Oryza sativa* L.) to be cultivated in hot climatic regimes may be limited by its narrow gene pool as domesticated rice has only about 10–20 % of the genetic diversity found in wild progenitors (Zhu et al. 2007). However, Second (1985) and Oka (1988) opined that the diversity in the cultivated varieties is insufficient and the differences are little between the cultivated and wild varieties.

Studies on oxygen evolution and consumption during various plant metabolic activities are relatively few. The pathway for enhancing plant biomass includes single leaf photosynthesis (Makino 2011), lower respiration and photorespiration (Peterhansel and Maurino 2011) in conjunction with stomatal features. Identifying the variations in the physiological processes such as photosynthesis, respiration and stomata between the wild and cultivated rice varieties may help in developing rice genotypes with climate resiliance. The relationships of gas exchange parameters and structural traits at leaf interfaces, in wild and cultivated rice was reported in this present communication.

Materials and methods

A total of twenty wild rice accessions (Table 1) belonging to six wild spp., two each of Hybrids (DRRH3 and PA 6444) and varieties (AK-Dhan and Varadhan) and a land race (Moroberekan) were grown as described recently from this laboratory (Kiran et al. 2013).

Oxygen evolution or photosynthesis (*A*), light induced respiration (*LIR*) and dark respiration (R_d) rates

Oxygen evolution rate was measured in fully expanded flag leaves using Oxygraph (Hansatech, Kings Lynn, Norfolk, UK) which has custom built Windows ® software package (Oxygraph Plus). All the measurements were recorded at 25 °C. Each of the leaf sample was stabilized at 25 °C and subjected to a dark period for five minutes in the chamber. Rate of reduction in oxygen level was considered as dark respiration (R_d). After R_d, the samples were illuminated with constant light source (1800 μmol m^{-2} s^{-1}) supplied with instrument and the observed oxygen evolution rates or photosynthesis (*A*) was plotted in real time. Once *A* attained plateau, light source was cut off so as to initiate the oxygen consumption which is termed as "light induced oxygen consumption" or *LIR* as per the instrument manual.

Leaf area and stomatal characteristics

Flag leaf area (LA) was measured using LI-3100C (LI-COR Environmental,USA). Number of stomata per mm^2 on both adaxial surface (ADS) and abaxial surface (ABS), stomatal width and length were measured using microscope (Nikon Model ALPHA PHOT-2 YS2-H, China) as described by Rajagopal et al. (1990). The following parameters were determined using the following formulae.

Single stomatal area (SSA) = Single stomatum width × length
Number of stomata per flag leaf = LA × Stomatal density.
Specific stomatal contribution (SSC)
= Photosynthetic rate (A)/Stomatal density.

Specific stomatal contribution was calculated to evaluate contribution of single stomata to photosynthetic rate.

Statistical analysis

General descriptive statistics, statistical significance of the trait means based on Fisher's LSD test and Pearson Correlation among all observed traits were done using *Statistix 8.1* (Analytical Software Inc. USA) software.

Table 1 Gas exchange and stomata characteristics in cultivated (*Oryza sativa* L.) and wild rice accessions

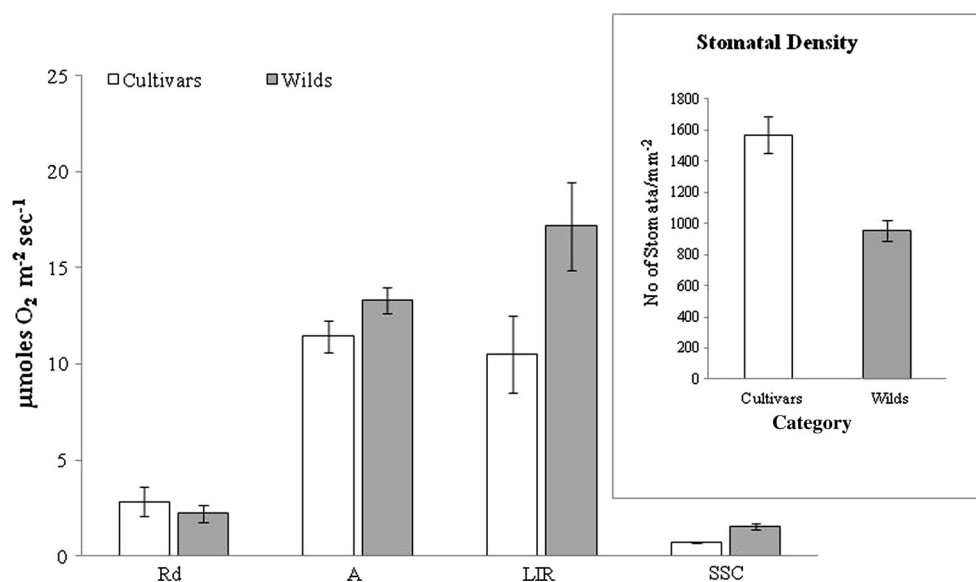| Name of the species with accession number	Country of origin	R_d μmol O$_2$ m^{-2} s^{-1}	A μmol O$_2$ m^{-2} s^{-1}	LIR μmol O$_2$ m^{-2} s^{-1}	A-R_d	A-LIR	SSA μm^{-2}	LA cm^{-2}	Total stomata density (no. mm^{-2})	Total stomatal number (Million LA^{-1})	SSC μmoles O$_2$ m^{-2} s^{-1} (X × 10^{-8})
Oryza sativa (Akshayadhan)	India	2.29c	10.27cd	6.17a	7.99	4.1	444	20.13	1816	3.66	0.58
O. sativa (Varadhan)	India	1.23c	11.84cd	6.23a	10.61	5.61	173	23.2	1766	4.1	0.69
O. sativa (DRR-Dhan-38)	India	1.67bc	9.51cd	9.19a	7.84	0.32	312	15.28	1250	1.91	0.77
O. sativa (PA-6444)	India	3.15bc	10.33cd	14.3a	7.18	-3.97	333	21.9	1449	3.18	0.72
Mean		2.09	10.49	8.97	8.40	1.52	315	20.13	1570	3.21	0.69
Moroberekan	West Africa	5.52abc	14.06bcd	15.57a	8.54	-1.51	514	–	–	–	–
O. barthii (IR 103580)	Chad	1.31abc	12.97bcd	13.33a	11.65	-0.36	411	14.78	765	1.13	1.72
O. barthii (IR 80433)	India	1.33c	12.9bcd	13.21a	11.57	-0.31	416	28.45	782	2.23	1.68
O. eichingeri (IR 100881)	Srilanka	1.54d	9.04c	11.14a	7.5	-2.1	512	20.19	599	1.21	1.53
O. glaberrima (IR 100983)	Nigeria						575	28.25	1366	3.86	0.9
O. glaberrima (IR 102445)	Mali	0.42c	12.29bcd	14.03a	11.87	-1.74	575	43.66	1383	6.04	0.9
O. glaberrima (IR 104033)	Chad	0.42abc	12.3bcd	14.06a	11.89	-1.76	575	11.96	1349	1.61	0.93
O. glaberrima (IR 101800)	Liberia	0.6c	11.51bcd	12.93a	10.92	-1.41	588	12.13	1432	1.74	0.83
O. glaberrima (IR 104020)	Tanzania	0.41abc	12.25bcd	13.99a	11.84	-1.74	575	12.37	1400	1.73	0.89
O. glumaepatula (IR 104387)	Brazil	1.45abc	14.4bc	12.67a	12.96	1.73	483	20.11	683	1.37	2.15
O. longistaminata (IR 104301)	Kenya	1.09c	13.09bcd	13.48a	12	-0.39	522	14.17	1133	1.61	1.18
O. longistaminata (IR 105262)	Gambia	3.7c	22.9a	29.79a	19.2	-6.89	462	30.21	716	2.17	3.26
O. nivara (CR 100008)	India	2.56c	10.52cd	11.79a	7.97	-1.27	352	19.73	966	1.91	1.1
O. nivara (CR 100097)	India	2.76abc	10.92cd	9.94a	8.16	0.98	407	37.15	833	3.1	1.34
O. nivara (CR 100100)	India	2.38c	12.2bcd	8.98a	9.81	3.22	455	11.07	1048	1.16	1.19
O. nivara (IR 104650)	Thailand	2.71ab	9.72cd	14.06a	7.01	-4.34	399	26.17	982	2.57	1.02
O. rufipogon (CR 100018)	India	2.91c	17.2b	17.56a	14.3	-0.36	471	43.76	750	3.28	2.33
O. rufipogon (CR 100267)	India	7.38c	13.94bcd	43.49a	6.56	-29.6	428	21	650	1.37	2.18
O. rufipogon (CR 100309)	India	7.41c	13.88bcd	43.55a	6.47	-29.7	354	33.66	782	2.64	1.81
O. rufipogon (IR 103404)	Philippines	0.83a	13.66bcd	11.53a	12.83	2.13	428	22.58	716	1.62	1.95
O. rufipogon (IR 80774)	Myanmar	1.35c	13bcd	13.25a	11.65	-0.25	446	71.61	733	5.25	1.82
Mean of wilds		2.31	13.09	16.75	10.78	-3.66	472	26.15	953	2.38	1.54
Grand mean		2.26	12.61	15.31	10.34	-2.70	444	24.99	1072	2.54	1.37

Fig. 1 Means of respiration, Photosynthesis, light induced respiration rates, single stomatal contribution and stomatal density between cultivated and wild rice accessions

Results and discussion

In the present study four cultivated, one land race and 20 wild rice accessions (7 diploid wild spp., with AA genome) were chosen due to the their frequent use in the breeding program under tropical conditions and at IIRR, Hyderabad. The traits of *O. nivara*, *O. rufipogon* and *O. longistaminata* wild spp., include, out crossing ability, biotic tolerance and abiotic tolerance and are incorporated into the breeding program. The other wild spp., are not yet incorporated into the rice breeding program. Thus, a combination of these materials might help us to understand the survival mechanism of wild rices under harsh environments. Differences in the rates of dark respiration (R_d), photosynthesis (A) and light induced respiration (LIR) were studied using oxygraph, based on evolution or consumption of O_2 and the results were compared among wild rice, cultivated rice and hybrids. These results were also correlated with stomatal characteristics. The mean values of three replication for all observed traits and for all 25 genotypes are given in Table 1.

Photosynthetic rate

The range of A in cultivated rice is lower than that of wild rice accessions (Fig. 1). There have been several reports that, high grain yield and biomass production is associated with higher leaf photosynthetic rate and grain filling during late reproductive period (Horie et al. 2003). Molecular marker mapping studies in rice have shown that wild species can contribute genes for improving the complex

traits such as high biomass formation, yield and abiotic stress tolerance, despite their poor phenotype (Placido et al. 2013; Nevo and Chen 2010; Swamy and Sarla 2008) and are therefore source enhancing photosynthetic rates into cultivated rice varieties. Almost 80 % of the wild rice accessions studied in the present investigation had higher A than the mean of cultivars, indicating that A in rice has not yet reached the saturation level and therefore, theoretically a scope to enhance the A is still existing. More recently, increasing air temperature reduced the elevated CO_2 effects in cultivated, wild and weedy rice lines were reported (Ziska et al. 2014). Though carbohydrate is the principle metabolic product of A which could increase biomass (Tausz et al. 2011) under high temperature conditions, due to enhanced respiration rates, the magnitude of assimilation contributed might further decline and therefore anticipating the reduced rice production and productivity under climate change conditions.

In both cultivated rice varieties and wild rice accessions, differences were found with respect to photosynthesis. The mean A in wild rice accessions was 13.09 whereas in cultivated spp., it was lower (10.49). However, at both species level as well as at accession level, photosynthetic rates varied significantly (Fig. 2). For instance, *O. longistaminata* had superior A rates 18.33 that is mainly due to the differences in A observed in the two accessions that are available for the current study, i.e. accessions IR 104387 and IR 105262 with photosynthetic rates 13.09 and 22.9 respectively. *O. rufipogon* and *O. glumaepatula* did not differ (14.50) while in *O. eichingeri* the A was minimum (9.17). Most of the wild rice accessions exhibited superior

Fig. 2 Means of respiration, Photosynthesis and light induced respiration rates in *O. sativa* sps. (Varieties) and different wild rice accessions
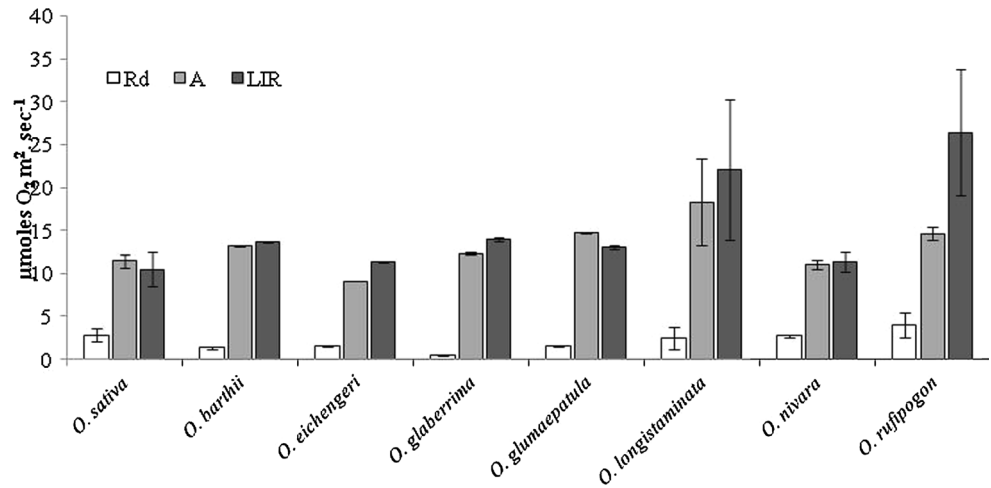
A over the cultivars mean. The *A* of cultivated varieties may be further improved by using some of these accessions as donors in breeding programm. Generally in tropical rice growing areas the effect of increase in global temperature reaches as early as 9.00 hours at which time optimum photosynthesis occurs. Maximum *A* was observed in wild accessions compared to the cultivars at early hours, followed by plateau and a gradual depletion of O_2 evolution. Maximum photosynthesis completed in the first quarter of the photoperiod and the accessions having higher *A* with less photosynthetic duration will be more useful for efficient utilization of light energy. Therefore, the higher *A* and lesser duration in completing the photosynthesis in wild rice accessions could be an important feature in carbon balance and helps to survive under adverse situations.

Respiration and light induced respiration rates

Significant genetic variations were observed at individual genotype level with respect to R_d and *LIR*. The mean R_d rates measured as consumption of O_2 during dark period was 2.31 and 2.09 in wild and cultivated rice spp., respectively. Apparently, there seems to be no much variation with respect to R_d; however, at species level, similar to *A* there were significant differences among the wild spp., *O. rufipogon* had higher maintenance R_d while *O. glaberrima* had lower (0.50). *O. barthii*, *O. eichingeri* and *O. glumaepatula* had R_d in the range of 1.30–1.50 while two of the wild species i.e. *O. nivara* and *O. longistaminata* had similar R_d as that of cultivated rice. Unlike, *A*, the R_d is fairly uniform in the wild rice accessions except *O. rufipogon*. Among all genotypes dark respiration rate (R_d) was recorded lower in *O. glaberrima* (0.50) and higher in *O. rufipogon* (Fig. 1). The difference between *A* and R_d is also more in wild accessions than cultivars and 14 wild accessions exhibited higher difference than the mean of the

cultivars. There is much variation in wild rice respiring duration compared to cultivars. It indicated that the maintenance energy of wild accessions is lower than the cultivars (Fig. 1) and perhaps associated with increase in plant biomass or positive carbon balance. Maintenance respiration has shown highly significant positive correlation with *A* and *LIR*. Millow et al. (2011) and Leakay et al. (2009) opined that the ratio of oxygen consumption to evolution may provide better understanding of plant biomass formation. In general, C_3 crops had known to be having higher light (photo) respiration rates and rice, is no exception to this phenomenon. The difference between *A* and R_d was maximum (19.2 μmol O_2 m^{-2} s^{-1}) recorded in *O. longistaminata (IR 105262)* accession and it was also noticed that this difference in wild accessions was 1.3 times to that of cultivars and hybrids. It indicates the efficient carbon balance system in wild accessions and may be associated with increase in plant biomass. The current results were in harmony with the reports of Yeo et al. (1994) that wild *Oryza* species had higher photosynthesis than cultivated *Oryza sativa*. Earlier, Kiran et al. (2013) reported using photosynthetic measurements in wild accessions, *O. nivara* (CR 100097), *O. rufipogon* (CR 100267), and *O. nivara* (CR 100008) were superior and useful for breeding purpose (Kiran et al. 2013). However, Cook and Evans (1983) reported higher photosynthetic rates in cultivated rice. The genetic differences in photosynthetic rates and dark respiration rates may be attributable to the differences in leaf anatomy. The photosynthetic rates (10.49 and 13.09 μmol O_2 m^{-2} s^{-1}) and dark respiration rates (2.09 and 2.31 μmol O_2 m^{-2} s^{-1}) in rice species recorded in the present study are comparable with earlier reported values for rice spp., (Ohsuni et al. 2007, Davey et al. 2004; Gu et al. 2012). Depletion of O_2 concentration is a result of burst of O_2 evolution and is a combination of both dark as well as light conditions. Once, the plateau of oxygen (*A*) is reached,

light illumination was turned off and immediate subsequent oxygen in the chamber reduced and is termed as *LIR*. This includes dark or maintenance respiration also. *LIR* was higher than the R_d in all the accessions. The mean *LIR* of cultivated rice is about 8.97 while that of the wild rice accessions is almost two times i.e. 16.75. Two of the spp., *O. longistaminata* and *O. rufipogon* had higher *LIR* while in others it was more uniform like R_d (Fig. 2). Variation of *LIR* within the accessions was noticed with respect to *O. nivara* and *O. rufipogon*. The mean *LIR* was found to be more in wild rice accessions.

Two NADPH$_2$ and three ATP molecules are required to fix one CO_2 molecule (Hopkins and Huner 2009). Non-cyclic photophosporylation supplies reducing power (NADPH$_2$) and insufficient ATP for fixing one CO_2. The remaining ATP is met from the cyclic photophosporylation or cellular energy or both. The carbon fixed during photosynthesis can be mobilized to the sink, converted to other biomolecules and may be oxidised and the proportions may vary among entries. During carbon conversion, glucose is partially oxidised and occurrence of electron transport chain is low, hence low oxygen evolution. However, during glucose combustion, electron transport chain is more active and oxygen released will be higher. Generally, in metabolism, synthesis of any molecule occurs when energy status i.e. nucleotide triphosphate ratio (NTP/NMP) of cell is high. The energy released during *LIR* might help the cell in retaining high energy status or it might be utilised for carbon conversion or both. If most of the energy required for glucose synthesis is harvested by photosystems then *LIR* may be less. Hence, it is important to verify the influence of *LIR* on carbon balance. It is presumed that varieties having low R_d as well as *LIR* and high *A* leads to positive carbon balance and indicated that wilds having higher photosynthetic efficiency relative to cultivars. Entries AK-Dhan, PA6444 and all accessions of *O. nivara* (IR-104650) showed similar R_d and *A,* but varied in *LIR*. Among these, *LIR* of AK-Dhan is lower and it is approximately two folds lower than others. *LIR*, R_d and *A* are almost similar in *O. glaberrima* accessions. *A* is more than *LIR* in seven cases only wherein gain in carbon can be expected. Among these 7, three are cultivated varieties i.e. Varadhan, Ak-Dhan and DRR-Dhan-38. The other four wild rice accessions are *O. rufipogon* IR 103404; *O. glumaepatula* IR 104387; *O. nivara* CR 100100 and *O. nivara* CR 100097. In this study, as high as 18 entries showed maintenance respiration lower than the mean (Fig. 1) and *O. nivara* (IR-104650). The present results indicate that, *LIR* might play a pivotal role in the carbon balance and helps in determining the extent of adaptability to the environmental situation.

The boundary wall for the plant atmospheric continuum is stomata and its regulatory mechanism is well known.

Stomatal size and their frequency have been used as an indicator of water loss by many workers (Venora and Calcagno 1991). However, information on the connecting link for gaseous exchange from the leaf surfaces in the plants through stomata is lacking. Therefore, an attempt was made in this direction which led to interesting observations (Table 1). The difference in photosynthesis and respiration rates may be attributable to stomatal features and is studied in these limited genetic materials. Although both adaxial and abaxial surface stomata were analyzed, for brevity stomatal density, stomatal number per leaf as there existed a wide variation with reference to the size of the leaf and single stomatal contribution per leaf were computed. There were significant differences between wilds and cultivated rice with regard to specific stomatal contribution and also total leaf surface. More number of stomata was observed in abaxial surface than in adaxial surface. Though, cultivated rice are semi-dwarf and possess lesser leaf area, stomatal size and single stomatum contribution to photosynthesis, they may compensate these drawbacks by comprising more number of stomata and density to contribute for more yields when compared to wild rice species.

Inspite of high significant differences in stomatal length between wild rice species and cultivated rice, there were no considerable difference in single stomatal area (SSA) because of no differences in stomatal width. SSA in wilds was 0.54 times more to that of cultivars and hybrids. Significant ($p < 0.001$) variations were noticed in stomatal density between wild rice and cultivated rice on the basis of total leaf surface area. Significant variation was observed between wild and cultivated rice with respect to leaf area (Table 1). Mean stomatal number (1570) in cultivated rices, AK-Dhan and Varadhan had maximum stomatal density and total leaf surface, to that of in wild rice (953). Similarly, total stomatal number on both leaf surfaces was more in cultivated and hybrid rice when compared to wild rice.

Specific stomatal contribution was calculated to evaluate contribution of single stomata to photosynthesis. Stomatal number and size may be an adaptive mechanism in plants to local climatic conditions and breeding targets. Perhaps, wild species are following the route of environmental adaptation with lesser stomata number associated with large size, operating in patches and in tandem. On the other hand, the cultivated rice are bred for higher yield hence, the gaseous exchange has to be in favour of producing more biomass and yield. Higher stomata number coupled with smaller size might have occured during selection procedure for higher yields. The resultant effect of stomatal density, area and specific stomatal contribution are in agreement with earlier reports of more involvement of stomata in photosynthesis enhancement (Goh et al. 1997, 2002). Thus,

Table 2 Pearson correlation values among photosynthetic rate, dark respiration rate and other stomatal characteristics
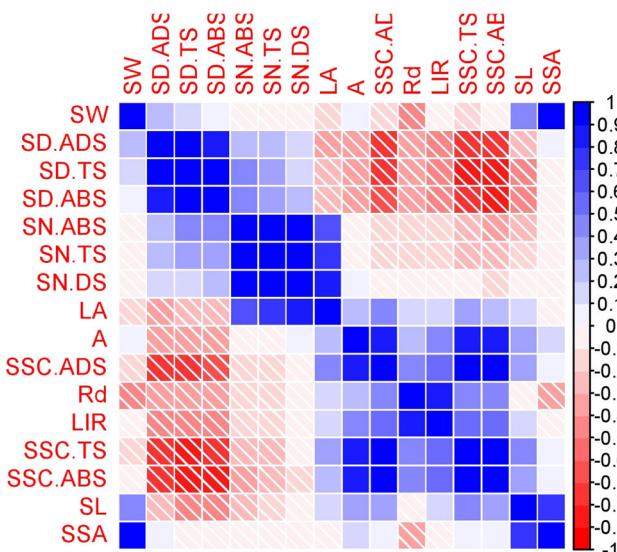
ABS and ADS single stomatal contribution (SSC) abaxial (ABS) and adaxial (ADS) surfaces, *SD.TS* total stomatal density, *LA* leaf area, *SSA* single stomatal area, *SW* stomatal width, *SL* stomatal length, *LIR* light induced respiration rate, *A* photosynthesis rate, *Rd* dark respiration rate

resilience to environment in wild rice spp. may be due to lesser stomata number combined with efficient gas exchange appears to be of paramount significance.

Correlation

Pearsons Correlation values among all observed traits are given in Table 2. Light induced respiration (*LIR*) was strongly correlated with both photosynthetic rate (*A*) and dark respiration rate (*R*$_d$). There was positive correlation between *A* and *R*$_d$, however, it was not significant. Net photosynthesis was equal to the difference between gross photosynthesis and respiration and the photosynthetic capacity of leaf was closely correlated with nitrogen content, chlorophyll content and respiration rate (Tanaka, 1976). High quantum yield in photosynthesis is coupled with the production of assimilatory power (ATP and NADPH), which is again used in anaerobic pathways and export of these assimilates require energy supplied by respiration. Hence high photosynthetic rates may result in high dark respiration (Barro et al. 1996). Li et al. (2013) also reported similar results in tomato that availability of more carbohydrates and energy were involved in more rate of dark respiration in response to elevated [CO$_2$]. Specific stomatal contribution was strongly correlated with *R*$_d$, *A* and *LIR*. Tsunoda and Fukoshima (1986) reported

correlation between leaf photosynthesis and stomatal density in upland and lowland rice varieties grown on flooded soil. Stomatal length and width were positively correlated with each other and hence also with single stomatal area and specific stomatal contribution. However, stomatal length was negatively correlated with stomatal density and as expected, it was positively correlated with specific stomatal contribution. Ohsuni et al. (2007) also reported similar results of a negative correlation between stomatal density and stomatal length of rice leaf. They also reported that stomatal length is related to specific stomatal conductance which is closely related with leaf photosynthesis, producing higher yield in hybrid cultivars. Hybrid rice cultivars produce higher grain yield, might be due to higher number of large sized stomata causing higher photosynthetic efficacy (Sarwar et al. 2013). Recently, Giuliani et al. (2011) in rice used Pearson Correlation matrix for trait to trait and suggested that large differences are due to trade off in gas exchange parameters and leaf structural features rather than a single individual trait. Numbers of stomata are related with photosynthetic activities and leaves with larger stomata showed higher photosynthesis rate than the leaves with smaller stomata (Chandra and Das 2000). They also reported that broader leaves exhibited larger stomata than narrow leaves. But, the current research obtained contrast results of negative correlation between leaf area and single stomatal area; however, this negative correlation was not significant. Number of stomata per flag leaf was highly correlated with leaf area. Broader stomata facilitate CO$_2$ diffusion into the leaf causing increased stomatal conductance. Though, stomatal density, stomatal number per flag leaf and specific stomatal contribution for photosynthesis were more in abaxial surface, they were highly correlated with that of in adaxial surface. Stomatal conductance of the abaxial surface was higher than that of the adaxial surface at any light intensity in sunflower (Wang et al. 2008). In maize, both stomatal density and area were more on the abaxial surface than adaxial surface and hence more photosynthesis and transpiration rates on abaxial surface (Driscoll et al. 2006).

Conclusion

Genetic variations between wild rice species and cultivated/hybrid rice in photosynthetic rate, dark respiration rate and light induced respiration rates, measured based on O$_2$ evolution or consumption in leaf discs was found to be useful to identify efficient gas exchange occurring at leaf surface of single leaves. Among the various parameters, this study reveals *LIR* and *A* as key factors for positive carbon balance. Stomatal contribution towards carbon balance appears to be more dependent on abaxial surface

where several number of stomata are situated. Correlation analysis indicates that R_d and LIR increase with the increase in A. In this study, *O. nivara* (CR 100100, CR 100097), *O. rufipogon* (IR 103404) and *O. glumaepatula* (IR104387) were identified as potential donors which could be used in rice breeding program. Environmental adaptation in wild rice accessions seems to be associated with stomatal number/leaf, size and stomatal patchiness, leading to their survival under harsh environment.

Author contribution statement Rajesh Kondamudi and Swamy KN: Executed the work, analysed the data, prepared earlier draft of manuscript and maintained potted plants . Venkateswara Rao Y, Vishnu Kiran T and Suman K: rendered technical help, stomatal enumeration work and maintenance of wild rice accessions of this experiment, Sanjeeva Rao D: revised and edited the manuscript. Raghuveer Rao P: contributed to the stomatal work, Subrahmanyam D: Planning, scientific discussions, data analysis, Sarla N: planning of work, manuscript preparation, Ramana Kumari B: Data analysis and Voleti SR*: Conceived, work design and overall supervision.

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