Tolerance to low radiation during grain filling stage in rice

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***Abstract*—**Low radiation is a determining factor in the development and growth of rice crop and causes yield losses in many regions of the world. We evaluate the diversity of response of rice varieties to a reduction of radiation during grain filling stage yield and yield components were measured in field experiments (two consecutive years). Shade during grain filling significantly reduced grain yield, spikelet fertility and 1000-grain weight in both experiments (dry and wet season). Phenotypic plasticity (GxE) was only observed in both experiments for spikelet filling and 1000-grain weight, suggesting differential genotypic response for these traits under low radiation. Two tolerant genotypes VANDANA and NORUNKAN and three susceptible genotypes SWARNA, ZALCHA and RAY NABJA were selected from the field experiments and studied under controlled growth chamber conditions for low radiation tolerance during the vegetative stage. Shade increased the maximum photosynthesis (A_{max}) for VANDANA, suggesting a shade adapted behavior maximizing carbon gain under low radiation. However; NORUNKAN showed high Amax and the lowest light compensation point (LCP) and respiration under control and shade conditions suggesting a double strategy to maintain carbon (maximize gain and reduce carbon loss under low radiation conditions). This study demonstrates that targeting photosynthetic traits to maximize carbon gain and reduce carbon loss under low radiation conditions is a strategy that should be explored to increase rice tolerance to low radiation conditions during grain filling stage.

Keywords— shade, grain filling, low radiation tolerance.

I. INTRODUCTION

Recent studies on climate variability impact on rice grain yields showed that low radiation is an important yield limiting factor. Low radiation constrained yield in some rice growing regions with about 40 to 50% yield loss in rice grown in India and south east countries [1], [2], China in the regions of Yunnan and Guizhou provinces [3] and Colombia in Latin America [4].

Low radiation significantly reduce yields mainly when low irradiance arrives during the reproductive and maturity stages [5]. In this context, the lack of optimal windows for farmers to sow either due to climatic constrains [6] or management conditions increases the probability that rice crops encountered low radiation conditions during the crop cycle [4]. Grain filling is a key stage depending on light availability, because grain matter increase after heading depends on the availability of assimilates, defined by Carbon (C) assimilation during the grain filling period and assimilate reserves stored in stems [7].

Plants can exhibit a capacity to adjust their morphology and physiology to a particular set of light conditions by acclimation or phenotypic plasticity in order to increase light harvesting and use efficiency. In crops, plants respond to low light modifying leaf morphology and physiology [8]. In rice a reduction of 50% of radiation during grain filling stage decreased source activity (ie.net photosynthetic rate, saturation irradiance and maximum electron transport) reducing spikelet filling and yield [9], [5]. Sink activity can also be limited by low radiation; causing a deleterious impact on grain size, grain number, and even on grain formation processes as observed for sunflower [10].

Plants possess the ability to adjust to different light conditions, differing in their acclimation capacity to shade [11]. However, in crop plants shade acclimation processes that include avoidance (reach more light; increasing plant height) can cause deleterious effects on grain yield (increase lodging, reduction of allocation of resources to reproductive structures for example) [12]; leading to a reduction in grain yield. In this context, breeding has acted to attenuate some but not all shade avoidance responses within modern crop varieties [12]. Thus, it is probable that in breeding programs there is not enough variability for selection in morphological traits to increase tolerance to shade conditions. In fact, for cereal grasses, as wheat [13] observed that the greatest differences were found in the parameters of the light response curve rather than for morphological parameters as specific leaf area or grain yield.

Most of the studies working on plants tolerance to shade suggest that shade tolerant species achieve superior performance in shade conditions by minimizing carbon losses in low light rather than by enhancing maximum potential carbon gain [14]. As high photosynthesis and growth rates require a high concentration of photosynthetic enzymes that are bound to have large maintenance costs, advanced performance of shade-tolerant species in low light has been explained by their lower dark respiration rates, which results in a lower light compensation point [15], [16].

Actually, dark respiration was the strongest determinant of whole-plant light requirements in tropical trees saplings, and it was considered a reliable and simple estimate of shade tolerance [17]. For rice, [18] showed that rice tolerant lines have better accumulation of dry matter under shade conditions, high light harvesting and use [9], contributing to high light use efficiency and grain yields. Suggesting that tolerant rice plants will enhance maximum potential carbon gain (reach high photosynthetic rates under low radiation) rather than minimize CO_2 losses (with low LCP and dark respiration rates). The objective of this study was to (i) See if there is phenotypic plasticity in a larger diversity panel for morphological and grain yield related traits (GxE) in response to low radiation and (ii) understand the carbon gain/loss behavior in tolerant genotypes.

II. MATERIALS AND METHODS

A. Field experiments (CIAT – Colombia)

A set of 204 genotypes were characterized in field experiments at CIAT (International Center for Tropical Agriculture), during the dry (experiment 1) and wet season (experiment 2). Given the diversity in the phenology of the genotypes studied and in order to establish the low radiation stress during the grain filling stage for all genotypes a staggered planting was carried out to achieve synchronization at flowering. 142 genotypes synchronized in experiment 1 and 124 genotypes in experiment 2. A ramdomized complete block design with three replicates and two treatments was evaluated each year. When plants reached 50% flowering, shade plant were covered with a black polyethylene mesh (low radiation treatment). Grain yield and yield components were measured for both experiments.

B. *Growth chambers* experiments (CIRAD – France)

Five genotypes (RAY NABJA, SWARNA, VANDANA, ZALCHA and NORUNKAN) with contrasting response to low radiation observed in field experiments were evaluated under controlled conditions at CIRAD, Montpellier, France. Microclima MC1750E growth chambers (Snijders, The Netherlands) were used to simulate low radiation conditions. The experiment was carried out in completely randomized designs with five repetitions. The seedlings were kept in two chambers with the following conditions: temperature: 27/22 °C (day/night), photoperiod: 12/12 hours (day/night), relative humidity: 65/80% (day/night), light intensity: 750 µmol m^{-2} s^{-1}, and CO_2: 400 ppm.

Tolerance to low radiation, with respect to photosynthetic efficiency, was studied during the vegetative stage to avoid an interaction of photosynthetic response with sink activity and size during the reproductive stage.

Therefore, when plants reached seven leaves on the main stem, the light intensity was reduced from 750 µmol m^{-2} s^{-1} to 350 µmol m^{-2} s^{-1} in one growth chamber, leaving the other growth chamber at 750 µmol m^{-2} s^{-1} as a control treatment.

After 25 days under low radiation conditions, photosynthesis light response curves were measured at 11 photosynthetic photon flux density (PPFD) levels (in increasing order of 0, 25, 50, 150, 300, 450, 600, 900, 1200, 1500 and 1750 µmol m^{-2} s^{-1}), by a portable photosynthesis system (LI-6800; LI-COR, Lincoln, NE, USA). The last ligulated leaf of each plant (between leaf number 7 and 10) was measured taking the following parameters: 500 µmol s^{-1}, fan: 10,000 rpm, temperature: 28 °C, relative humidity: 65% and CO_2: 400 ppm.

C. Statistical analysis

Yield and yield components were fitted with a mixed linear model that included the genotype, the treatment and their interaction as a fixed effect and the repetitions as a random effect to obtain adjusted means and analyze the treatment effect. For experiment 3 genotype and treatment were considered as fixed effect. All data was analyzed using R version 3.6.2. The means of each treatment were compared by using the Tukey's test at the 5% significance level by using the agricolae library package of R.

The results of the light curve were adjusted with the Light Response Curve Fitting 1.0 application to obtain the following indicators: maximum photosynthesis (A_{max}), apparent quantum yield (AQY), light compensation point (LCP) and dark respiration rate (R_d).

III. RESULTS

D. Effect of low radiation on yield and yield components

The shade treatment, had a significant effect ($p < 0.01$) and reduced grain yield by 23.28% and 22.47% in experiment 1 and experiment 2 respectively. Only yield components defined during the grain filling stage presented a significant reduction ($p < 0.05$). Spikelet fertility was reduced by 15.43% and 16.57% and 1000-grain weight by 4.30% and 3.69% for experiments 1 and 2 respectively (TABLE I). These suggests that these characteristics were affected by the 50% reduction in radiation, regardless of whether it is in dry or wet season. On the other hand, shade did not have a significant effect in both years on number of panicle per m^2 and number of spikelet per panicle (TABLE I), yield components formed in the development stages prior to flowering. In both experiments, we observed significant phenotypic plasticity (GxT) for spikelet fertility and the 1000-grain weight, suggesting that only for these two traits genotypes responded differently under low radiation.

TABLE I

ANALYSIS OF VARIANCE, EFFECTS OF GENOTYPE, TREATMENT, AND INTERACTIONS FOR YIELD AND YIELD COMPONENTS DURING EXPERIMENT 1 (142 GENOTYPES) AND 2 (124 GENOTYPES)

Traits	T	Experiment 1					Experiment 2				
		Mean	**Range**	**G**	**T**	**GxT**	**Mean**	**Range**	**G**	**T**	**GxT**
Grain yield (g)	C	815.65	251.40 - 1,342.62	***	**	ns	585.84	169.94 - 1,333.00	***	**	ns
	S	625.75	320.78 - 1,095.28				454.16	113.10 - 1,173.26			
Spikelet fertility (%)	C	83.98	60.41 - 95.69	***	***	***	71.97	42.75 - 95.20	***	**	**
	S	71.02	43.44 - 93.76				60.04	33.09 - 91.73			
1000-grain weight (g)	C	21.62	14.63 - 30.09	***	*	***	21.41	14.57 - 28.17	***	***	**
	S	20.69	13.21 - 30.05				20.62	13.89 - 25.54			
Number of spikelet per panicle (n)	C	174.72	69.92 - 301.57	***	ns	**	139.08	75.20 - 231.37	***	ns	ns
	S	174.45	84.77 - 315.57				138.28	70.27 - 233.10			
Number of panicle per m^2 (n)	C	277.35	144.44 - 468.89	***	ns	ns	303.04	178.57 - 602.38	***	ns	ns
	S	259.07	142.22 - 526.67				296.73	159.52 - 500.00			

G= Genotype; T= Treatment. C= Control; S= Shade. Significance level: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns = not significant.

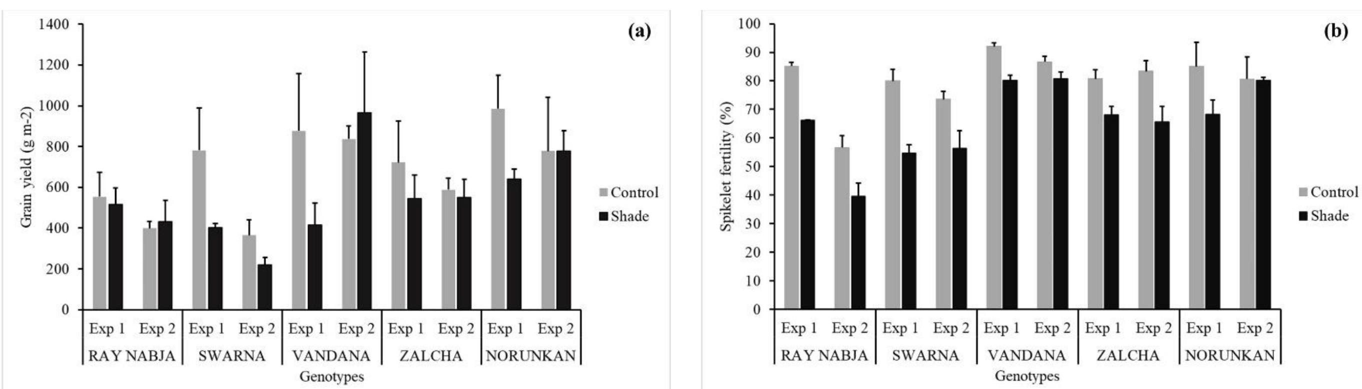

Fig. 1. Grain yield (a) and spikelet fertility (b) of five genotypes (RAY NABJA, SWARNA, VANDANA, ZALCHA and NORUNKAN) during experiment 1 (Exp 1= dry season) and 2 (Exp 2= wet season).

We selected five genotypes for its contrasting response to yield and spikelet fertility under low radiation conditions during grain filling (Fig. 1). VANDANA and NORUNKAN showed the lowest reduction in spikelet fertility for either just the wet season (experiment 2) or for both seasons, while RAY NABJA, SWARNA and ZALCHA showed the highest reductions. This suggest that VANDANA and NORUNKAN are tolerant genotypes and might have different photosynthetic behaviors than RAY NABJA, SWARNA and ZALCHA.

E. Effect of low radiation on photosynthetic parameters

The responses of photosynthetic rate (A) to photosynthetic photon flux density (PPFD) for six rice genotypes are indicated by the differences in the shapes of the light curves in Fig. 2. The light curves showed an increase in A with increasing PPFD for all genotypes in both treatments, reaching a plateau when the photosynthetic pathway was saturated. There were no significant differences in AQY values between rice genotypes under shade or controlled conditions; which indicates that the initial slope of the assimilation (AQY) was similar for the genotypes in both treatments.

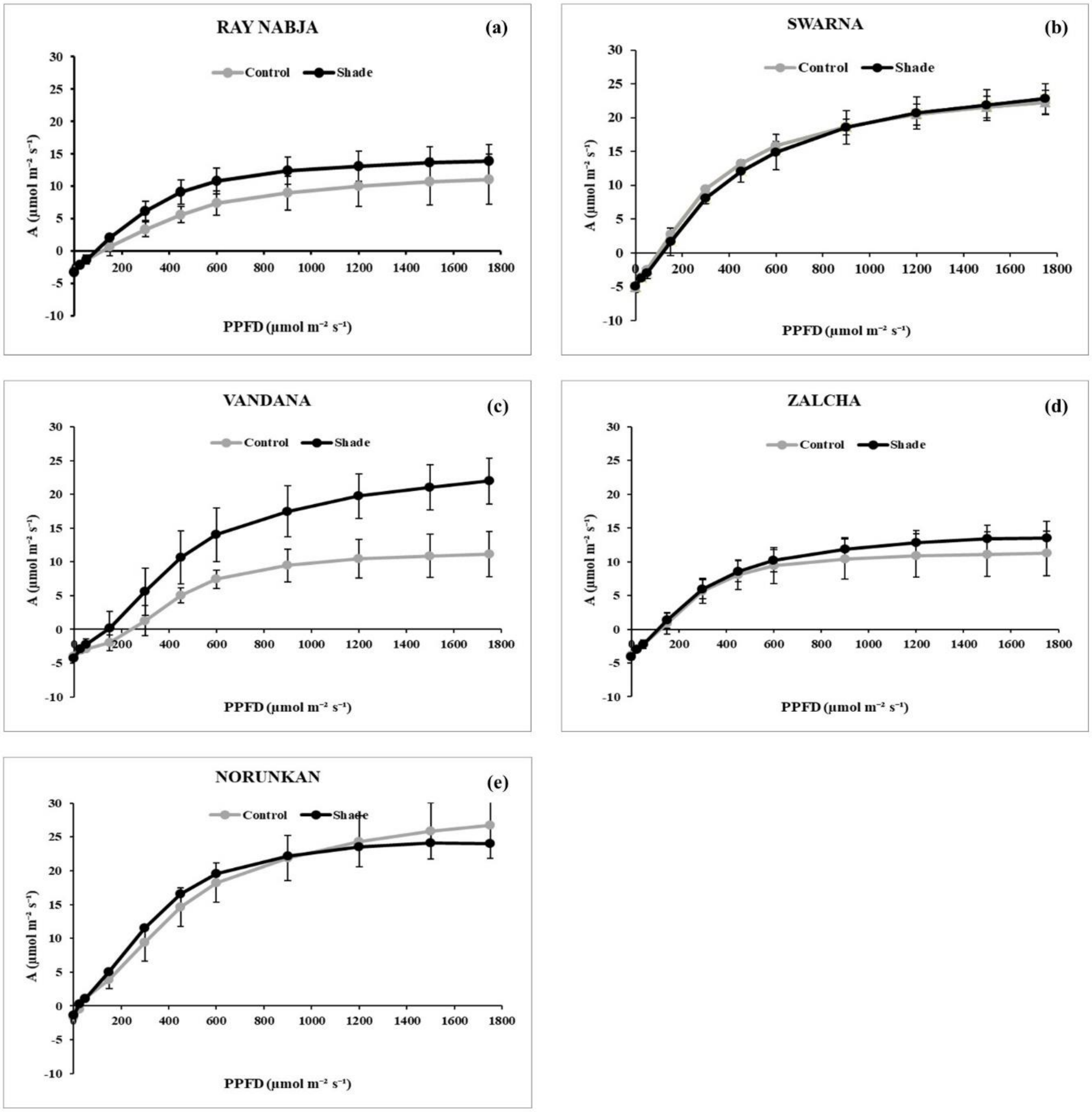

Fig. 2. Fitting light response curves of six rice genotypes in two light intensity treatments. RAY NABJA (a), SWARNA (b), VANDANA (c), ZALCHA (d) and NORUNKAN (e). Bars represent standard deviation (SD) (n = 5).

The shade treatment did not affect the light response curve in terms of carbon assimilation and maximum photosynthesis; except for VANDANA (TABLE I) with higher A_{max} values in shade treatment than in control conditions; this is a typical response of a shade-adapted plant that become intolerant to higher intensities of light.

NORUNKAN and SWARNA showed the highest A_{max}, however only NORUNKAN showed the lowest values for both the light compensation point (LCP) and respiration (R_d) under shade and control conditions (TABLE II). These results suggest that NORUNKAN increases the use of carbon (A_{max}) under shade and maintains its ability to reduce the loss of carbon with low LCP and R_d under low radiation conditions.

TABLE II

PHOTOSYNTHETIC PARAMETERS FOR THE SIX RICE GENOTYPES DURING EXPERIMENT 3

Genotypes	Treatment	A_{max} (μmom $m^{-2} s^{-2}$)	AQY (μmom $m^{-2} s^{-2}$)	LCP (μmom $m^{-2} s^{-2}$)	R_d (μmom $m^{-2} s^{-2}$)
RAY NABJA	Control	17.022 A b	0.030 A c	121.274 A b	3.032 A d
	Shade	18.350 A c	0.040 A a	90.946 A ab	3.366 A c
SWARNA	Control	30.835 A a	0.064 A c	90.282 A bc	5.390 A d
	Shade	32.642 A a	0.058 A a	105.432 A a	5.483 A a
VANDANA	Control	16.753 B b	0.023 A c	200.927 A a	4.444 A bc
	Shade	28.575 A ab	0.040 A a	131.522 A a	4.511 A b
ZALCHA	Control	15.735 A b	0.037 A bc	119.365 A b	4.073 A c
	Shade	19.473 A c	0.044 A a	108.754 A a	4.267 A b
NORUNKAN	Control	31.457 A a	0.043 A abc	37.690 A c	1.571 A e
	Shade	26.519 A ab	0.046 A a	26.924 B b	1.219 A d

Maximum net photosynthetic rate (A_{max}); Apparent quantum yield (AQY); Light compensation point (LCP); Dark respiration rate R_d). Letters A and B indicate significant differences ($p < 0.05$) between treatments in the same genotype, while a, b, c and d indicate significant differences ($p < 0.05$) between genotypes under the same treatment.

IV. DISCUSSION

V. Low radiation during the grain filling stage reduced yield in dry season and wet season.

Shade significantly reduced grain yield, spikelet fertility and 1000-grain weight in both experiments. These results demonstrate that grain filling and weight were the key factors related to grain yield under shade [19], [20]. In fact, low radiation during grain filling causes a reduction of the net photosynthetic rate and lower accumulation of dry matter significantly reducing the number spikelet fertility and 1000-grain weight [21]. Besides, grain yield, spikelet fertility and 1000-grain weight were similarly affected by shade treatment in the dry and wet season suggesting that the impact of a reduction in radiation in yield is independent of the environment across genotypes.

Tolerant genotypes VANDANA and NORUNKAN originate from India and Sri Lanka respectively, countries in which there are seasonal changes with heavy rainfall and cloudiness during certain times of the year due to the monsoon season. Therefore, the observed tolerance to low radiation may be associated to an adaptation to these cloudy-monsoon conditions.

VI. Effects of low radiation during vegetative stage on photosynthesis parameters.

Photosynthesic-irradiance curves show the efficiency and capacity of plant photosynthesis to respond to different light intensities. Accurate assessment of such relationships is of fundamental importance for understanding the photochemical yield of the process and for studying the responses of plants to environmental changes, such as light stresses [22]. In our study, phenotypic variation was observed in the light curve for the control and stress treatments. The photosynthetic parameters obtained from the light curve showed that VANDANA increased photosynthetic rate under low radiation conditions suggesting greater efficiency in the use of available radiation under low radiation conditions but low adaptation to high radiation conditions.

However, NORUNKAN showed high photosynthetic rates, low R_d, and LCP under both shade and control conditions, suggesting an efficient use of carbon and the capacity to initiate photosynthesis with low levels of radiation. This suggest that rice shade tolerant genotype not only showed low rates of respiration in the dark and therefore lower light compensation points , as observed in other plants [23] [24]; but also maximizes the use of carbon under low and normal radiation conditions.

VII. CONCLUSION

Shade during grain filling significantly reduced by 23% grain yield regardless of whether it was the wet or dry season. This loss of grain yield was related to the reduction of spikelet fertility and 1000-grain weight. The tolerant genotypes NORUNKAN and VANDANA showed higher light use efficiency under low radiation conditions in the growth chamber. However, VANDANA reaches high values of maximum photosynthesis in low radiation, but was sensitive to high radiation conditions. On the contrary, NORUNKAN maximizes carbon gain and reduced carbon loss by reaching high values of maximum photosynthesis and showing low values of LCP and R_d in both shade and control treatments.

Alternatives must be found to carry out hightroughput phenotyping for LCP, Rd and Amax photosynthetic parameters in order to identify plants that maximize carbon gain and reduce carbon loss under low radiation conditions. Besides, assessing spikelet fertility under low radiation conditions seemed a good proxy to identify tolerant genotypes in field experiments.

ACKNOWLEDGEMENTS

The International Center for Tropical Agriculture (CIAT) and program In-silico Multiscale Optimization of Sustainable Agricultural Crops (ÓMICAS)

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