Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2021, 28(2): 178−190

Research Paper

Growth and Photosynthesis Responses of a Super Dwarf Rice Genotype to Shade and Nitrogen Supply

Marc SCHMIERER[1], Oliver KNOPF[2], Folkard ASCH[1]

([1]*Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Stuttgart 70599, Germany;* [2]*Institute for Bio- and Geo-Sciences, IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, Jülich 52428, Germany*)

Abstract: Specific aspects of plant cultivation require tests under fully controlled environmental conditions with restricted energy supply, such as orbit-based space laboratories and low-light conditions. For these growing conditions, super dwarf plants have been developed as model crops, and a gibberellin-deficient Super Dwarf Rice genotype was proposed as a model crop for space flight plant experiments. We tested this genotype in a climate chamber experiment under different illumination and nitrogen supply levels to assess its suitability under scenarios with limited resource availability. A 25% reduction in illumination led to a 75% reduction in yield, mainly due to a 60% reduction in formed tillers and 20% reduction in grain weight, and a 80% reduction in illumination caused total yield loss. Leaf area under reduced illumination was significantly lower, and only marginal changes in the dimensions of leaves were observed. Plant photosynthesis was not significantly different between control and 75% illumination. This was explained by a higher photochemical efficiency under lower light conditions and a reduced mesophyll resistance. Therefore, we concluded that this genotype is well-suited for plant experiments under space and light-limited conditions since it kept its small stature and showed no shade avoidance mechanisms, such as leaf elongation, which would complicate experiments under low-light conditions. Nitrogen concentrations of 2.8 and 1.4 mmol/L led to no differences in plant growth. We concluded that a nitrogen concentration of 1.4 mmol/L is sufficient for this genotype under the light intensities.

Key words: super dwarf rice; photosynthetic limitation analysis; shade; nitrogen

In the life sciences, ‘model’ organisms are used to represent kingdoms, phylla, classes or families, and are often chosen for their ease of handling, non-pathogenicity, or the size of their genome. They play an important role in understanding basic biological concepts. Many major breakthroughs in biology have been driven by research on only a few representative species, such as *Escherichia coli* or *Arabidopsis thaliana* (Russo, 2003; van Norman and Benfey, 2009). However, in crop science, scientists require check varieties for each crop to show generalizable responses to biotic and abiotic factors. In rice, IR64, an economically successful variety developed in 1985 by the International Rice Research Institute (IRRI), has been used as a check variety in a large number of experiments globally (Mackill and Khush, 2018).

Recent technological innovations have increased the focus on cultivating crops in fully controlled environments (Bugbee, 1992; Germer et al, 2011; Pinstrup-Andersen, 2018). Such systems can be of interest for plant cultivation tests in off-the-shelf climate chambers, the recently promoted vertical farms, or even for space-based experiments on plants, such as those already being conducted on the International Space Station. A major constraint for all of these systems is a limited growing area and energy supply.

Received: 13 January 2020; **Accepted:** 9 May 2020

Corresponding author: Marc SCHMIERER (Marc.schmierer@uni-hohenheim.de)

Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/10.1016/j.rsci.2021.01.007

Therefore, experiments consist of either only a few plants, or plants that are not grown through their full growth cycles (Zabel et al, 2016). Bugbee (1999) suggested to identify or breed new genotypes that particularly suit for spaceflight experiments, such as plants with an extremely small stature, known as 'super dwarfs'. These super dwarf crops have potential for cultivation in space-limited systems, as they allow for a larger number of plants to be included in one experiment. Scientifically, this would allow for more treatment factors and an increased statistical power from the increase in the number of replicates. Another useful application would be single-plant cuvettes for measuring gas exchange, an area which has already yielded significant insight into crop physiology (Livingston et al, 1994; Kölling et al, 2015; Sun et al, 2016).

Following the idea of Bugbee (1999), Frantz et al (2004) identified an extremely small growing rice genotype, line N71, from the Konoshita Collection, USA, with a short development cycle, a high harvest index and a full seed set, in contrast to formerly identified super dwarf rice genotypes. The extremely short stature of this genotype is caused by a dysfunction in the synthesis of gibberellin (GA), a plant hormone playing a key role in the reproductive and vegetative developments. GA is a key hormone promoting cell division and elongation, and GA-deficient plants usually show stunted growth and short leaves that can also be wider and thicker than those with normal GA synthesis in maize (*Zea mays*) (de Souza and MacAdam, 2001) and rice (Matsukura et al, 1998). Leaves of GA-deficient plants are often darker in color, probably due to an accumulation process of pigments in response to reduced leaf area (Thomas and Sun, 2004). The identified genotype ('Super Dwarf Rice') grows to a maximum height of around 0.2 m, rendering it a promising candidate as a model crop for rice-based studies conducted in fully-controlled environments, with several benefits over *A. thaliana*, such as larger grain size.

To our knowledge, no research on this Super Dwarf Rice genotype has been published since its introduction by Frantz et al (2004) and there is a lack of data at the plant level of the response of Super Dwarf Rice to limiting environmental conditions, such as light and nitrogen supply and their effect on leaf anatomical structures and photosynthesis. Consequently, it remains unclear whether research on Super Dwarf Rice is transferable to other rice genotypes, especially due to the absent synthesis of GA.

Pigments, mainly chlorophylls and carotenoids, are the key molecules for light harvesting and funneling of excitation energy during photosynthesis. Adjusting their concentrations is one of the first acclimation processes in leaves after changes in the light environment. Weak shading, for example, is shown to increase chlorophyll content in winter wheat and rice, while stronger shade causes a reduction in pigment content (Li H W et al, 2010; Wang et al, 2015). Increasing pigment concentration per unit leaf area allows plants to harvest light energy more efficiently. This is due to not only higher light absorption on a leaf level, but also more efficient light harvesting by the antenna complexes. Excitation energy is more efficiently funneled to the reaction centers and then onward to the electron transport chain, reflected by lower values of light and dark adapted PSII fluorescence (Wang et al, 2015). Typically, shading increases leaf thickness in rice and other species (Terashima et al, 2006; Martins et al, 2014; Wang et al, 2015), but contrary observations are found in winter wheat (Li H W et al, 2010).

Controlled environments are often characterized by low-light conditions, which attribute to the fact that illuminants emit a high thermal load, making it more complicated to maintain a stable temperature and humidity. Further, energy supply can be a critical factor. For example, in all plant cultivation experiments conducted in orbit-based research facilities, light intensities provided inside the growing modules range from very low to medium [50 to 720 μmol/(m^2·s)] (Zabel et al, 2016). For field crops adapted to environmental conditions in the tropics and subtropics, such as rice, these light intensities are uncommonly low. Hence, the focus of this study was on growth and photosynthesis responses of Super Dwarf Rice to different illumination regimes. Also, as light-mediated responses often interact with nitrogen supply, varying nitrogen concentrations in the nutrient solution and their effects on photosynthesis, yield components, and finally yield were investigated.

RESULTS

Yield components

Yield components and their contributions are shown in Table 1. Light intensity had a significant effect on all yield components. However, significant differences between full illumination and 75% illumination were found in No. of tillers per plant and grain yield per plant. Accordingly, all yield components were significantly affected by light intensity. Under 20% illumination, No. of tillers per plant was reduced by 72% and 86% in 2.8 and 1.4 mmol/L N-supply,

Table 1. Grain yield and its components of Super Dwarf Rice N71 grown under different treatments.

Treatment		No. of tillers per plant	Percentage of productive tillers (%)	No. of grains per panicle	Percentage of spikelet fertility (%)	Average single grain weight (mg)	Grain yield per plant (g)
N (mmol/L)	Light (L)						
2.8	Full light	24.0 ±0.8 a	66 ±40 a	13.2 ±0.1 a	91 ±2 a	21.0 ±0.3 a	4.002 ±0.323 a
	75% illumination	10.3 ±1.1 b	64 ±11 a	11.3 ±1.3 ab	87 ±0 a	16.4 ±0.4 a	1.051 ±0.155 b
	20% illumination	6.7 ±1.9 b	3 ±2 b	1.3 ±1.1 c	8 ±7 b	4.8 ±3.9 b	0.005 ±0.004 c
1.4	Full light	30.0 ±1.3 a	54 ±3 a	14.2 ±0.9 a	87 ±2 a	20.3 ±0.1 a	3.980 ±0.234 a
	75% illumination	10.3 ±2.1 b	56 ±7 a	12.6 ±1.7 ab	78 ±4 a	15.8 ±0.6 a	0.799 ±0.073 bc
	20% illumination	3.3 ±1.2 b	6 ±5 b	3.7 ±3.0 bc	0 ±0 b	0.0 ±0.0 b	0.000 ±0.000 c
F value	F_L	81.28***	38.91***	19.03***	248.78***	45.71***	185.14***
	F_N	0.37	0.95	0.79	4.40	1.60	0.27
	F_{LN}	3.45	0.53	0.08	0.17	0.70	0.20
Relative decrease compared to control (Full N and full light) (%)							
2.8	75% illumination	-57	-3	-13	-4	-22	-74
	20% illumination	-72	-95	-90	-91	-77	-100
1.4	Full light	25	-18	7	-5	-4	-1
	75% illumination	-57	-15	-5	-14	-25	-80
	20% illumination	-86	-92	-72	-100	-100	-100
Contribution to yield loss (%)							
2.8	75% illumination	58	3	13	4	22	
	20% illumination	17	22	21	21	18	
1.4	Full light	-42	31	-12	8	6	
	75% illumination	49	13	4	12	21	
	20% illumination	19	20	16	22	22	

"-" represents the reduction.

Data are Mean ± SD (n = 3). Different lowercase letters indicate significant differences at $P < 0.05$ according to the Turkey's Honestly Significant Difference analysis. ***, $P < 0.001$.

respectively, percentage of productive tillers and No. of grains per panicle were reduced by 95% and 92%, and 90% and 72%, respectively. Moreover, percentage of spikelet fertility was decreased by 91% and 100%, and average single grain weight was reduced by 77% and 100%. No significant differences of N-supply and no significant interactions between N-supply and light intensity were found. However, plants under full light and 1.4 mmol/L N-supply increased 25% of No. of tillers per plant and 7% of No. of grains per panicle than control plants. This was compensated by losses in the percentage of productive tillers (18%), percentage of spikelet fertility (5%) and average single grain weight (4%), resulting in the grain yield per plant loss of about 1%, where plants under 75% illumination produced 74% and 80% less grain yield under 2.8 and 1.4 mmol/L N-supply, respectively. Furthermore, plants under 20% illumination were sterility.

The analysis of yield component dynamics revealed that No. of tillers per plant was the main factor causing yield reduction under 75% illumination for both N treatments (58% and 49% contribution to yield loss), followed by average single grain weight (22% and 21%, respectively). The contribution of other yield components was less than 13% for both N treatments. Under 20% illumination, the contribution of the different yield components to yield loss was relatively similar in the range of 16% to 22%. Under control light conditions and 1.4 mmol/L N-supply, the higher No. of tillers per plant and the higher No. of grains per panicle accounted for 42% and 12% contribution to yield loss, with percentage of productive tillers accounting for 31%.

Leaf traits and chlorophyll content

In all phenological phases, light intensity significantly affected leaf area, leaf area ratio, average leaf length, special leaf area (SLA) of flag leaves and chlorophyll a content (Table 2). Generally, a lower light intensity led to smaller leaf area accompanied by a higher leaf area ratio. Further, SLA and flag leaf area tended to increase when light intensity increased. For chlorophyll a content, there was a general tendency to decrease with decreasing light intensity.

Light intensity also had significant influence on whole-plant SLA. During heading, lower light intensity generally increased whole-plant SLA, whereas a consistent increase of this parameter during flowering was only measured under 2.8 mmol/L N supply. Under 1.4 mmol/L N supply, an increase of whole-plant SLA was under 75% illumination, but a decrease was observed under 20% illumination. Further, light intensity had a significant effect on average leaf size during ripening and the chlorophyll a/b ratio during flowering, through *post hoc* analysis showed no any differences among the treatment groups. Additionally, light intensity positively affected chlorophyll b content

Table 2. Leaf traits and chlorophyll content of Super Dwarf Rice N71 grown under different treatments.

Developmental stage	Treatment		Leaf area (cm^2)	SLA of whole plant (m^2/kg)	Leaf area ratio (m^2/kg)	Average leaf length (cm)	Average leaf width (cm)	Average leaf size (cm^2)
	N (mmol/L)	Light (L)						
Heading	2.8	Full light	340 ±30 a	276 ±7 b	9.0 ±0.2 b	7.62 ±0.71 b	0.52 ±0.01 a	3.9 ±0.3 a
		75% illumination	219 ±30 bc	308 ±6 ab	11.3 ±0.6 ab	8.04 ±0.32 b	0.54 ±0.04 a	4.4 ±0.5 a
		20% illumination	138 ±18 c	361 ±26 ab	12.6 ±0.4 a	10.20 ±0.16 a	0.49 ±0.01 a	5.0 ±0.2 a
	1.4	Full light	338 ±5 ab	267 ±9 b	8.6 ±0.8 b	7.72 ±0.10 b	0.53 ±0.01 a	4.1 ±0.1 a
		75% illumination	218 ±17 c	295 ±3 ab	11.0 ±0.5 ab	8.42 ±0.14 ab	0.55 ±0.02 a	4.7 ±0.3 a
		20% illumination	132 ±9 c	330 ±13 ab	13.1 ±0.3 a	8.28 ±0.19 ab	0.50 ±0.01 a	4.2 ±0.0 a
	F value	F_L	33.48***	10.52**	19.52***	7.32**	1.91	1.88
		F_N	0.03	1.78	0.01	1.99	0.33	0.26
		F_{LN}	0.01	0.26	0.31	4.53	0.01	1.75
Flowering	2.8	Full light	395 ±29 a	275 ±6 b	5.7 ±0.1 bc	7.60 ±0.30 a	0.59 ±0.02 a	4.5 ±0.3 a
		75% illumination	143 ±28 bc	259 ±6 b	5.0 ±0.8 c	9.08 ±0.09 a	0.59 ±0.02 a	5.3 ±0.2 a
		20% illumination	117 ±31 c	345 ±4 a	10.2 ±0.3 a	8.69 ±0.49 a	0.53 ±0.03 a	4.6 ±0.5 a
	1.4	Full light	277 ±13 ba	260 ±0 b	5.2 ±0.3 c	7.56 ±0.16 a	0.61 ±0.01 a	4.6 ±0.1 a
		75% illumination	140 ±21 c	311 ±19 ab	6.6 ±0.3 bc	8.69 ±0.14 a	0.56 ±0.04 a	4.9 ±0.4 a
		20% illumination	100 ±24 c	279 ±14 b	7.8 ±0.4 ab	8.54 ±0.38 a	0.53 ±0.02 a	4.5 ±0.0 a
	F value	F_L	37.76***	5.62*	27.13***	7.15**	2.37	1.42
		F_N	3.99	1.04	1.14	0.43	0.00	0.26
		F_{LN}	2.45	10.99	7.47	0.11	0.21	0.25
Ripening	2.8	Full light	202 ±6 a	246 ±16 a	2.4 ±0.2 ab	6.77 ±0.21 ab	0.59 ±0.00 a	4.0 ±0.1 a
		75% illumination	53 ±5 b	199 ±17 a	1.8 ±0.2 b	6.36 ±0.56 ab	0.60 ±0.03 a	3.8 ±0.2 a
		20% illumination	73 ±17 b	358 ±60 a	5.7 ±0.8 a	8.83 ±0.43 a	0.58 ±0.02 a	5.1 ±0.3 a
	1.4	Full light	215 ±22 a	235 ±6 a	2.3 ±0.2 ab	5.81 ±0.07 ab	0.56 ±0.01 a	3.3 ±0.1 a
		75% illumination	64 ±5 b	243 ±10 a	2.2 ±0.2 b	5.31 ±0.22 b	0.50 ±0.04 a	2.7 ±0.3 a
		20% illumination	44 ±15 b	211 ±57 a	3.1 ±1.1 ab	7.10 ±1.03 ab	0.57 ±0.04 a	4.1 ±0.9 a
	F value	F_L	56.52***	0.36	6.45*	6.09*	0.35	3.89*
		F_N	0.01	0.29	1.74	5.66*	3.04	4.89*
		F_{LN}	1.04	1.39	2.49	0.22	1.13	0.06

Developmental stage	Treatment		Flag leaf area (cm^2)	SLA of flag leaf (m^2/kg)	Chlorophyll a ($\mu g/cm^2$)	Chlorophyll b ($\mu g/cm^2$)	Chlorophyll a/b
	N (mmol/L)	Light (L)					
Heading	2.8	Full light	8.8 ±0.3 a	110 ±1 bc	43.6 ±1.8 a	17.1 ±2.6 a	2.6 ±0.2 a
		75% illumination	11.0 ±1.5 a	127 ±4 ab	42.5 ±1.2 a	18.8 ±1.6 a	2.3 ±0.2 a
		20% illumination	9.0 ±1.2 a	109 ±3 c	37.9 ±2.5 a	13.3 ±1.1 a	2.9 ±0.1 a
	1.4	Full light	8.7 ±0.4 a	108 ±2 c	41.7 ±1.4 a	14.5 ±1.7 a	2.9 ±0.2 a
		75% illumination	9.7 ±0.1 a	130 ±4 a	39.2 ±1.0 a	15.8 ±1.0 a	2.5 ±0.1 a
		20% illumination	10.9 ±0.6 a	125 ±2 abc	33.2 ±2.3 a	11.3 ±0.9 a	2.9 ±0.0 a
	F value	F_L	1.31	14.77***	5.66*	3.82	3.71
		F_N	0.04	3.34	3.32	2.73	1.51
		F_{LN}	1.30	3.52	0.21	0.04	0.16
Flowering	2.8	Full light	8.8 ±0.6 a	119 ±2 a	44.2 ±1.1 ab	17.7 ±1.6 a	2.5 ±0.2 a
		75% illumination	10.6 ±0.5 a	128 ±8 a	34.9 ±4.4 ab	13.2 ±2.6 a	2.7 ±0.2 a
		20% illumination	9.7 ±0.9 a	134 ±4 a	27.1 ±2.0 b	8.3 ±1.1 a	3.4 ±0.3 a
	1.4	Full light	8.4 ±0.6 a	108 ±2 a	45.1 ±3.9 a	18.5 ±2.7 a	2.5 ±0.2 a
		75% illumination	11.5 ±1.1 a	137 ±9 a	32.2 ±3.4 ab	10.1 ±1.5 a	3.2 ±0.2 a
		20% illumination	8.9 ±0.9 a	132 ±4 a	31.1 ±2.4 ab	10.6 ±1.0 a	2.9 ±0.6 a
	F value	F_L	3.46	5.30*	8.96**	7.62**	4.11*
		F_N	0.01	0.05	0.06	0.00	0.02
		F_{LN}	0.44	1.04	0.39	0.72	2.12
Ripening	2.8	Full light	9.9 ±0.6 a	116 ±1 b	43.5 ±0.9 a	16.2 ±0.4 a	2.7 ±0.0 a
		75% illumination	8.1 ±0.4 ab	144 ±4 a	36.6 ±0.3 c	12.9 ±0.6 a	2.9 ±0.2 a
		20% illumination	10.2 ±0.6 a	134 ±4 ab	29.6 ±0.9 d	9.0 ±0.5 b	3.3 ±0.1 a
	1.4	Full light	8.7 ±0.6 ab	118 ±5 b	41.2 ±0.6 ab	15.3 ±0.4 a	2.7 ±0.0 a
		75% illumination	9.2 ±0.4 ab	140 ±7 ab	37.6 ±0.8 bc	13.8 ±0.7 a	2.7 ±0.1 a
		20% illumination	6.9 ±0.1 a	121 ±3 ab	20.6 ±0.9 e	7.8 ±1.1 b	2.7 ±0.3 a
	F value	F_L	1.00	10.36**	182.61***	43.53***	1.44
		F_N	5.79*	1.35	21.07***	0.38	1.76
		F_{LN}	7.65**	1.00	15.16***	1.00	1.02

SLA, Specific leaf area.

Data are Mean ± SD (n = 3). Different lowercase letters indicate significant difference according to the Turkey's Honestly Significant Difference analysis . *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

during flowering and ripening. The significant effects of N-supply were found during ripening on chlorophyll a content, flag leaf area, and average leaf length and size.

Gas exchange

In all phenological phases, $P_{(g)max}$ measured for plants under 75% illumination were higher than those under full and 20% illumination, whereas the lowest $P_{g(max)}$ was exposed under 20% illumination (Table 3). Photosynthesis rates generally decreased towards the end of the generative phase and this decrease was the greatest under 20% illumination. In 1.4 mmol/L N-supply treatment, N-supply significantly increased $P_{g(max)}$ during ripening. $P_{g(max)}$ values under 20% illumination significantly lower than those under 75% illumination, and also significantly lower than those of control plants under 2.8 mmol/L N-supply. Reduced light intensity significantly decreased $P_{g(max)}$ during heading and ripening. During heading, I_{comp} of plants under 20% illumination were more than 60% lower compared to those in full illumination. Under 75% illumination $f_{I(0)}$ under 1.4 mmol/L N-supply was increased compared to 2.8 mmol/L N-supply.

For fully illuminated plants, assimilation values under the respective growing light intensities under 2.8 and 1.4 mmol/L N-supply were 12.8 and 9.8 μmol/(m^2·s) at heading, 9.8 and 8.6 μmol/(m^2·s) at flowering, and 10.4 and 8.6 μmol/(m^2·s) during ripening (Fig. 1). For 75% illuminated plants, the values were 11.1 and 9.6 μmol/(m^2·s) at heading, 10.5 and 9.8 μmol/(m^2·s) at flowering, and 10.3 and 10.0 μmol/(m^2·s) during ripening. For 20% illuminated plants, the values were 3.5 and 3.2 μmol/(m^2·s) at heading, 2.9 and 3.0 μmol/(m^2·s) at flowering, and 3.5 and 2.6 μmol/(m^2·s) during ripening. No statistical differences were found between fully and 75% illuminated plants and between 2.8 and 1.4 mmol/L N-supply during the whole phases. However, plants under 20% illumination always had significant lower assimilation values under the growing light intensitiy (Fig. 1).

F_o/F_m was not significantly affected by any treatment or phenological stages. Values persisted close to 0.8 or higher, giving no indication of damage to photosystem II (Table 3). During ripening, light intensity significantly affected J_{max} with plants under 75% illumination

Table 3. Photosynthetic parameters extracted from light response and A/Ci curves of Super Dwarf Rice N71 grown under different treatments.

Developmental stage	Treatment		F_o/F_m	$P_{g(max)}$ [μmol/(m^2·s)]	I_{comp} [μmol/(m^2·s)]	$f_{I(0)}$ [μmol/(m^2·s)]	J_{max} [μmol/(m^2·s)]	$V_{c(max)}$ [μmol/(m^2·s)]
	N (mmol/L)	Light (L)						
Heading	2.8	Full light	0.82 ±0.01 a	19.1 ±0.7 ab	33.6 ±2.6 a	31.8 ±1.2 a	180 ±21 a	138 ±16 a
		75% illumination	0.82 ±0.00 a	19.8 ±2.0 a	24.1 ±3.3 abc	32.8 ±3.2 a	178 ±18 a	142 ±11 a
		20% illumination	0.82 ±0.01 a	14.0 ±0.4 ab	12.4 ±3.3 bc	30.5 ±0.8 a	135 ±10 a	111 ±13 a
	1.4	Full light	0.83 ±0.00 a	15.2 ±1.2 ab	23.0 ±1.7 abc	26.3 ±3.8 a	152 ±2 a	131 ±12 a
		75% illumination	0.83 ±0.01 a	18.1 ±0.7 ab	28.4 ±4.4 ab	29.2 ±1.4 a	156 ±5 a	131 ±8 a
		20% illumination	0.81 ±0.00 a	12.6 ±1.0 b	9.0 ±2.2 c	28.4 ±3.6 a	145 ±5 a	124 ±5 a
	F value	F_L	2.91	8.79**	13.12***	0.20	2.04	1.11
		F_N	0.07	4.31	1.11	1.97	1.07	0.02
		F_{LN}	1.05	0.49	1.96	0.13	0.88	0.45
Flowering	2.8	Full light	0.81 ±0.00 a	14.1 ±0.7 a	32.1 ±1.2 a	25.8 ±1.5 ab	141 ±15 a	124 ±22 a
		75% illumination	0.79 ±0.00 a	14.8 ±0.5 a	18.0 ±3.7 a	42.0 ±5.3 a	133 ±8 a	124 ±10 a
		20% illumination	0.81 ±0.01 a	13.0 ±2.1 a	30.6 ±4.5 a	29.3 ±2.5 ab	170 ±32 a	128 ±12 a
	1.4	Full light	0.81 ±0.01 a	11.7 ±1.5 a	26.9 ±4.5 a	23.8 ±3.6 b	152 ±4 a	127 ±5 a
		75% illumination	0.80 ±0.00 a	14.3 ±1.2 a	18.2 ±3.5 a	34.5 ±1.4 ab	168 ±19 a	161 ±43 a
		20% illumination	0.80 ±0.01 a	9.6 ±1.6 a	19.3 ±2.9 a	32.4 ±0.7 ab	126 ±24 a	110 ±20 a
	F value	F_L	1.6	1.8	3.4	7.0**	0.0	0.4
		F_N	0.0	2.3	2.3	0.6	0.0	0.1
		F_{LN}	1.5	0.4	0.9	1.1	1.4	0.5
Ripening	2.8	Full light	0.81 ±0.01 a	16.7 ±1.8 a	26.5 ±4.1 a	28.8 ±0.6 a	153 ±5 a	122 ±6 a
		75% illumination	0.81 ±0.00 a	16.6 ±0.7 a	28.0 ±6.6 a	36.4 ±3.6 a	170 ±19 a	107 ±5 a
		20% illumination	0.81 ±0.00 a	11.4 ±1.2 ab	10.1 ±2.8 a	34.0 ±1.7 a	121 ±7 a	136 ±30 a
	1.4	Full light	0.79 ±0.01 a	11.6 ±1.5 ab	35.6 ±6.9 a	28.2 ±3.2 a	124 ±16 a	99 ±14 a
		75% illumination	0.81 ±0.01 a	15.8 ±1.4 a	18.6 ±2.8 a	32.4 ±1.3 a	176 ±14 a	136 ±8 a
		20% illumination	0.80 ±0.00 a	7.2 ±0.6 b	20.4 ±2.5 a	31.5 ±3.9 a	114 ±8 a	109 ±7 a
	F value	F_L	2.6	10.3**	3.9	1.8	6.6*	0.3
		F_N	3.8	6.8*	0.5	0.8	0.6	0.3
		F_{LN}	1.5	1.1	1.9	0.1	0.6	1.5

F_o, Minimal fluorescence; F_m, Maximal fluorescence; $P_{g(max)}$, Maximum gross photosynthesis; I_{comp}, Compensation irradiance; $f_{I(0)}$, Quantum yield of photosynthesis at zero irradiance; J_{max}, Maximum electron transport capacity; $V_{c(max)}$, Maximum carboxylation capacity.

Data are Mean ± SD (n = 3). Different lowercase letters indicate significant differences at $P < 0.05$ according to the Turkey's Honestly Significant Difference analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

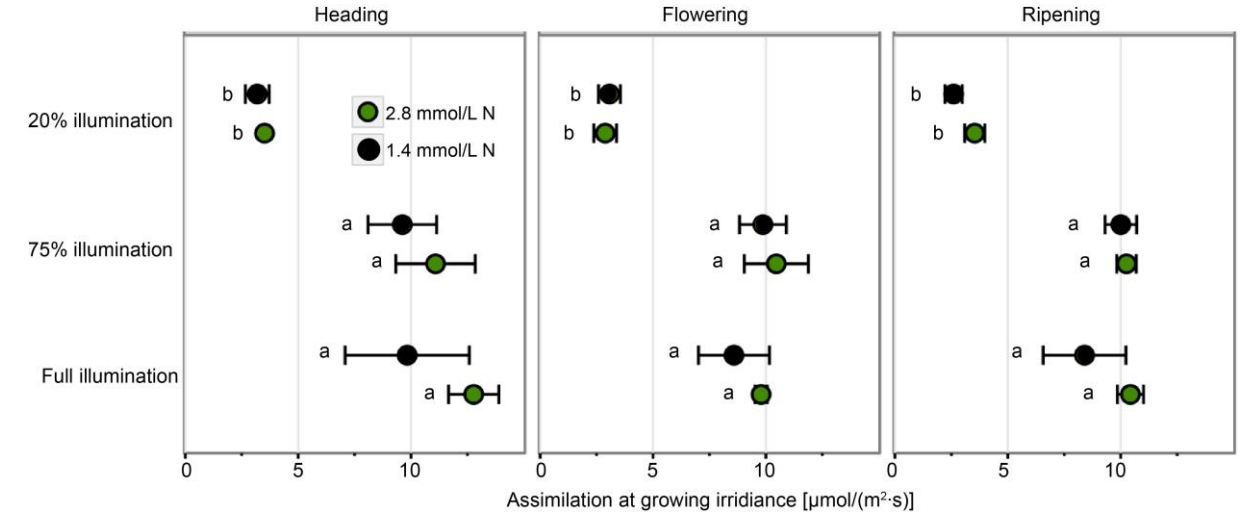

Fig. 1. Assimilation values of Super Dwarf Rice N71 at three different developmental stages under different treatments.
Data are Mean ± SD (n = 3). Different letters in each plot indicate significant differences at $P < 0.05$ measured by the Tukey's Honestly Significant Difference analysis.

showing the highest values. Neither light intensity nor nitrogen supply affected J_{max} or $V_{c(max)}$ during the earlier developmental stage (Table 3).

Fluorescence analysis

Light response curves for photochemical (ϕ_{PSII}), non-regulated (ϕ_{NO}) and non-photochemical (ϕ_{NPQ}) energy loss are shown in Fig. 2. ϕ_{PSII} linearly decreased when PPFD increased within 1000 µmol/(m^2·s), while ϕ_{NPQ} increased in the same manner, accompanied by a much smaller increase of ϕ_{NO}. Generally, under 2.8 mmol/L N-supply, leaves of plants under full illumination showed higher values of ϕ_{PSII}, especially under irradiances < 1000 µmol/(m^2·s). This difference was less pronounced under 1.4 mmol/L N-supply, whereas ϕ_{NPQ} of control plants tended to be the highest. Throughout the entire

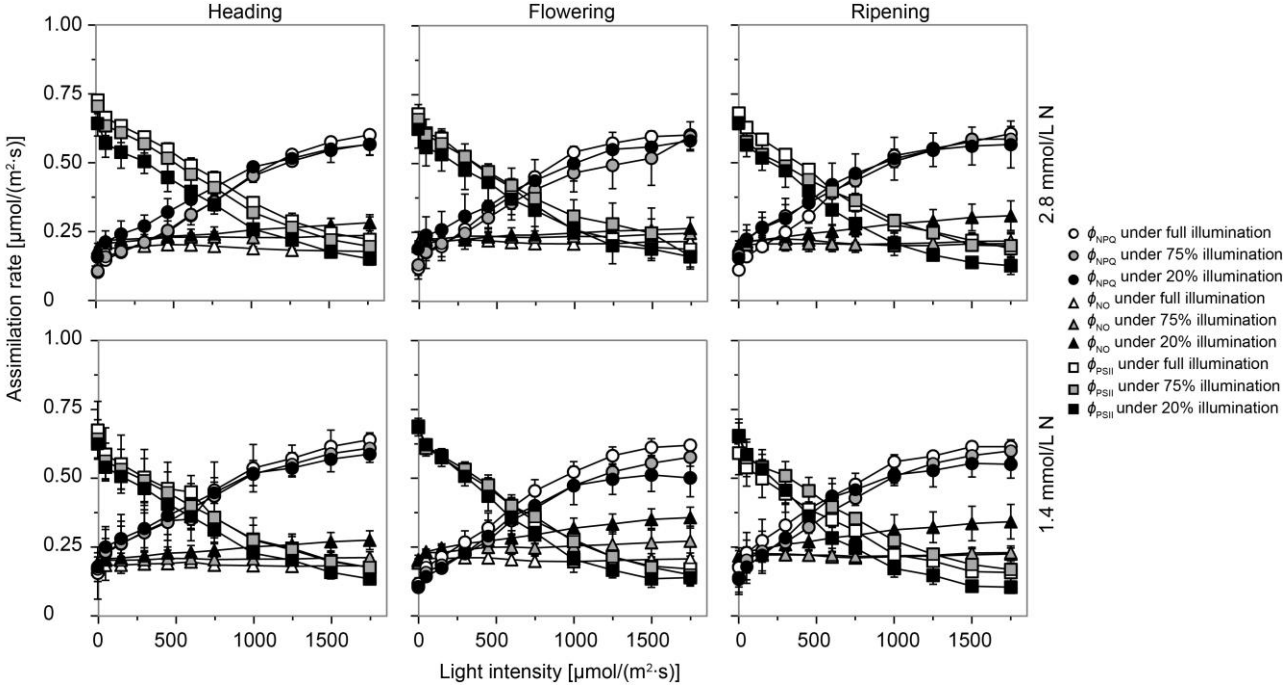

Fig. 2. Irradiance response curves of photochemical (ϕ_{PSII}), non-regulated (ϕ_{NO}) and non-photochemical (ϕ_{NPQ}) energy loss of Super Dwarf Rice N71 grown under three illumination levels and two nitrogen levels.
Data are Mean ± SD (n = 3). Different lowercase letters in each plot indicate significant differences at $P < 0.05$ measured by the Tukey's Honestly Significant Difference analysis.

Table 4. Photochemical (ϕ_{PSII}), non-regulated (ϕ_{NO}) and non-photochemical (ϕ_{NPQ}) energy loss of Super Dwarf Rice N71 under different treatments.

Developmental stage	Treatment		ϕ_{PSII}	ϕ_{NPQ}	ϕ_{NO}
	N (mmol/L)	Light (L)	[μmol/(m^2·s)]	[μmol/(m^2·s)]	[μmol/(m^2·s)]
Heading	2.8	Full light	0.44 ± 0.00 ab	0.36 ± 0.02 ab	0.20 ± 0.01 a
		75% illumination	0.46 ± 0.03 ab	0.31 ± 0.02 ab	0.23 ± 0.01 a
		20% illumination	0.54 ± 0.03 a	0.24 ± 0.05 b	0.22 ± 0.01 a
	1.4	Full light	0.36 ± 0.04 b	0.46 ± 0.04 a	0.19 ± 0.00 a
		75% illumination	0.40 ± 0.02 ab	0.39 ± 0.03 ab	0.21 ± 0.01 a
		20% illumination	0.51 ± 0.04 ab	0.28 ± 0.04 b	0.21 ± 0.00 a
	F value	F_L	6.29*	8.22**	5.37*
		F_N	3.92	5.82	3.23
		F_{LN}	0.30	0.32	0.20
Flowering	2.8	Full light	0.30 ± 0.01 c	14.10 ± 0.70 a	0.21 ± 0.00 b
		75% illumination	0.42 ± 0.03 bc	0.49 ± 0.01 a	0.23 ± 0.00 ab
		20% illumination	0.53 ± 0.02 ab	0.35 ± 0.03 bc	0.21 ± 0.01 ab
	1.4	Full light	0.35 ± 0.01 c	0.26 ± 0.02 cd	0.20 ± 0.01 b
		75% illumination	0.40 ± 0.01 c	0.45 ± 0.01 ab	0.25 ± 0.01 a
		20% illumination	0.58 ± 0.01 a	0.35 ± 0.00 bc	0.25 ± 0.00 a
	F value	F_L	39.29***	47.35***	11.34**
		F_N	1.43	6.64	5.77*
		F_{LN}	0.93	1.03	3.57
Ripening	2.8	Full light	0.35 ± 0.00 b	0.45 ± 0.01 a	0.20 ± 0.01 a
		75% illumination	0.40 ± 0.02 b	0.40 ± 0.03 a	0.21 ± 0.01 a
		20% illumination	0.52 ± 0.02 a	0.26 ± 0.02 bc	0.22 ± 0.01 a
	1.4	Full light	0.31 ± 0.02 b	0.48 ± 0.02 a	0.21 ± 0.01 a
		75% illumination	0.40 ± 0.03 b	0.38 ± 0.03 ab	0.22 ± 0.01 a
		20% illumination	0.53 ± 0.00 a	0.22 ± 0.01 c	0.24 ± 0.01 a
	F value	F_L	39.90***	34.11***	2.96
		F_N	0.14	0.16	3.43
		F_{LN}	0.73	0.84	0.34

Data are Mean ± SD (n = 3). Different lowercase letters indicate significant differences at $P < 0.05$ according to the Turkey's Honestly Significant Difference analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

reproductive phase, reduced light intensity increased ϕ_{NO}.

Table 4 shows mean values for ϕ_{PSII}, ϕ_{NPQ} and ϕ_{NO} extracted at the respective growing stage of all treatment groups. At all development stages, ϕ_{PSII} was always the highest for plants receiving 20% illumination and decreased with increased light intensity, whereas the opposite was the case for ϕ_{NPQ}. Plants grown under full light consistently showed lower values for ϕ_{NO} under their growing irradiance compared to plants under 75% and 20% illuminations.

Photosynthetic limitation analysis

Fig. 3 shows the contributions of the stomatal (SL), mesophyll (ML) and biochemical limitations (BL) to the relative reduction in light saturated assimilation rate (A_{sat}) throughout the reproductive phase. In general, A_{sat} decreased between heading and flowering with ML contributing most to the decrease. Further, a decrease in A_{sat} was measured between flowering and ripening for plants under low N-supply. Here, the change was due to an increased BL under fully illuminated plants and an increased BL and ML in plants under 20% illumination. Accordingly, the strongest reduction in A_{sat} [6.4 μmol/(m^2·s)] was recorded for plants under 20% illumination and 1.4 mmol/L N-supply during ripening compared to that [18.5 μmol/(m^2·s)] under 75% illumination and high N-supply during heading. However,

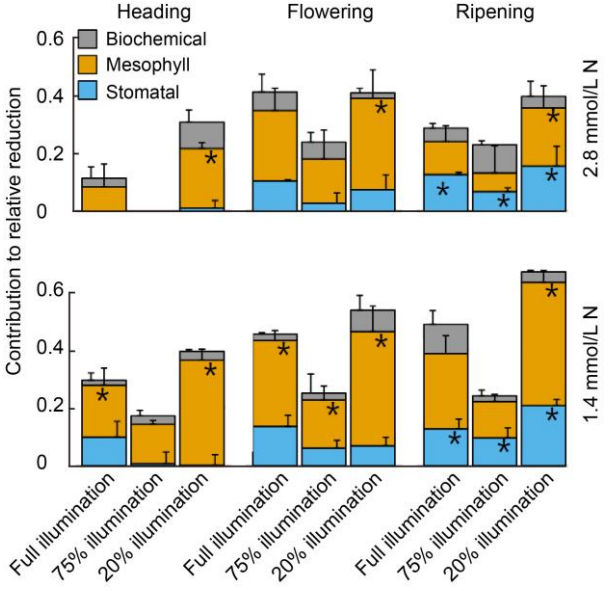

Fig. 3. Relative reduction of light saturated photosynthesis rates and contributions of different limiting components.
Stars indicate significant differences ($P < 0.05$) from the reference value.

A_{sat} values for 2.8 mmol/L-N plants increased after flowering due to a lower ML in all illumination treatments and that was not offset by a simultaneous, though smaller, increase in SL.

A mixed model analysis was carried out to identify significant differences for each of the limiting components during the growing phases from its corresponding reference value at the beginning of the reproductive phase. ML was significantly affected by N-supply, light intensity and development stage. SL was neither affected by N-supply nor by light intensity, but by developmental stage. For BL, the null model was not significantly different from the full model. Under high N-supply, higher light intensity significantly decreased ML at all development stages. Under low N-supply, ML was always higher for control plants and for plants receiving 20% illumination as well as during flowering for plants receiving 75% illumination. For both N treatments, SL was significantly increased during ripening for all light intensities.

No correlation between SLA of flag leaves was found (Fig. 4). Pooled over both N-levels, the average SLA of fully illuminated plants was 113 cm^2/g and g_m was 125 $mmol/(m^2 \cdot s)$. The values for 75% illuminated plants were 135 cm^2/g and 197 $mmol/(m^2 \cdot s)$, and 125 cm^2/g and 80 $mmol/(m^2 \cdot s)$ for plants under 20% illumination, respectively.

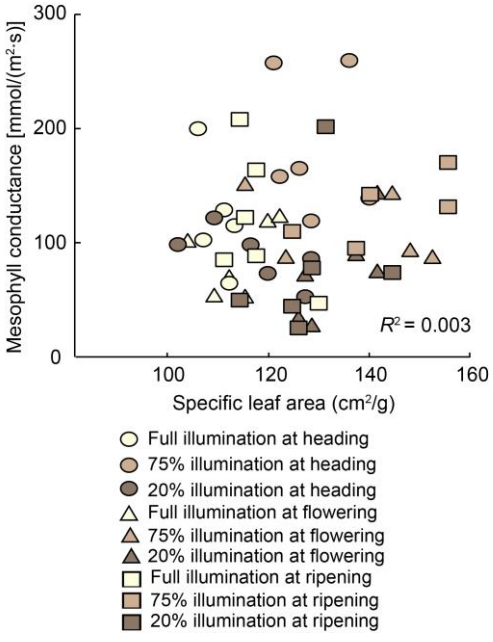

Fig. 4. Mesophyll conductance plotted vs. specific leaf area of Super Dwarf Rice N71 grown at different light intensities and nitrogen concentrations measured at three different developmental stages at growing irridiance.

DISCUSSION

Adjustments of leaf morphology and yield reduction

For most higher plants, leaves are the major organs for photosynthesis and assimilate production. Plants have a remarkable ability to adapt their morphology and biochemistry in response to the prevailing environmental conditions (Terashima et al, 2006; Samuolienė et al, 2012; Gong et al, 2015). Adaptations to reduced light intensities are generally categorized into shade avoidance or shade tolerance mechanisms (Gommers et al, 2013). Shade avoidance strategies include elongation of stems and petioles as well as reduced branching. Contrastingly, shade tolerance often results in a higher SLA and reduced chlorophyll a/b ratio as well as an increase in total leaf area and a higher leaf area ratio (Trapani et al, 1992). For Super Dwarf Rice to be used as a model crop in controlled environments that have limitations in space and energy, tolerance to low light conditions is required, rather than avoidance.

In this study, reduced light intensity significantly reduced leaf area via a strong reduction in tiller number, but increased the leaf area ratio. There were only minor effects of light intensity on SLA and leaf length on a whole plant level, and there were no significant effects on the size of older leaves or flag leaves. Based on the results on morphological adaptations, Super Dwarf Rice N71 exhibited pronounced shade tolerance strategies. The observed reduction in tiller number led to less self-shading, while the increase in leaf area ratio and SLA effectively increased total light capture. These features suggested Super Dwarf Rice is a suitable candidate for the growth in small-scale, low-light intensity environments.

The reduced grain yields observed in this study were consistent with previous studies on rice and other species (Cantagallo et al, 2004; Mu et al, 2010; Wang et al, 2015). When illumination was reduced to 75%, the reduction in tiller number was the main limitation for grain yield formation, followed by average grain weight, whereas 20% illumination significantly reduced all yield components between 72% and 100% compared to the fully illumination (Table 1). Due to inhibited GA synthesis and the resulting lack in apical dominance, N71 tillers excessively (Frantz et al, 2004). In our study, when plants growing under reduced light intensities, formation of new tillers was strongly reduced, whereas tiller formation in fully illuminated plants increased. Frantz et al (2004) found a positive relationship in N71 between

light intensity and fertile heads per square and grain yield, but did not specify whether this was caused by an increase in the total numbers of tillers or productive tillers. It was also reported that the number of tillers per plant decreases with increasing planting density and that the higher number of plants mitigates this effect. Reduction in tiller number is partly an effect of inter-canopy shading (Casal et al, 1986), as a result, we concluded that the positive relationship between grain yield and light intensity was caused by a change in the number of tillers per plant.

In a similar study conducted on field-grown rice over three growing seasons, Wang et al (2015) reported that 47% reduction in light intensity causes significant reductions of all yield components, except numbers of spikelets per panicle and spikelets per square, and showed that decreases in grain filling and 1000-grain weight have the largest effects on grain yield. In our study, we found significant effects of light intensity on number of filled grains per panicle due to the strong reduction of spikelet number per panicle under minimal illumination, but no significant difference was found between spikelet number per panicle in control plants and 75% illuminated plants. In our study, three plants were grown in one tube, resulting in self-shading conditions comparable to a canopy. Lower light levels decreased tiller number accompanied by a smaller reduction in the productive tiller number and of filled spikelet number per panicle. Therefore, it can be assumed that N71 would form a smaller number of spikelets per square when grown in a canopy scenario under sub-optimal light conditions. In summary, reducing tillering seems to be the dominant reaction of N71 to sub-optimal light supply. This can be beneficial in studies where high light supply is not applicable, e.g. due to technical limitations because the reduced self-shading comes along with a more homogenous light environment in the canopy and a higher light intensity at meristems like leaves and meristems could the observed reduction in kernel weight could result in decreased germination rates.

Parallel with the onset of the light intensity treatments, nitrogen concentration in the nutrient solution was reduced to 1.4 mmol/L for half of the plants (Yoshida et al, 1971). Since there was no further increase in yield when nitrogen concentration was doubled, we assumed that for yield formation, the nitrogen concentration of 1.4 mmol/L is sufficient for N71 under the given conditions. Accordingly, further research on light/nitrogen interactions incorporating this genotype should be carried out under lower N concentrations than used in this study.

Photosynthesis rates under decreased illumination and changes in chlorophyll content

Assimilation of CO_2 as part of photosynthesis involves both light dependent and light independent reactions. Plants have the ability to adjust and balance these reactions at a number of set points. Wang et al (2015) showed that lower illumination levels in some rice genotypes can decrease $P_{g(max)}$, J_{max} and $V_{c(max)}$, whereas in some genotypes, these parameters stayed constant or increased combined with increase of the efficiency of PSII photochemistry and reduction of non-photochemical quenching. Similar data exist in other species (Dai et al, 2009; Gong et al, 2015; Song and Li, 2016). In our study, $P_{g(max)}$ was consistently the highest when illumination was decreased to 75% of the control treatment. Accordingly, assimilation values compared from plants grown under full and 75% light intensity were not different, even when measured under the respective growing irradiances (Fig. 1).

Fig. 2 shows the complementary quantum yields of chlorophyll fluorescence quenching during light response curves. At the same light intensities, plants grown under 75% illumination did not transfer higher ratios of the excitation energy into photochemistry. As shown, this was the case across the entire light response curves. The only exception was measured during ripening for low-N plants, during which plants receiving 75% illumination had higher rates of photochemical quenching than the control plants. However, this was not accompanied by higher assimilation rates, as can be seen by the light-response curves. Under strong light, plants grown under the highest light intensity handled excessive light energy more efficiently, indicated by higher ratios of non-photochemical and lower rates of non-regulated-non-photochemical quenching, as the latter is considered a parameter indicating the inability of a plant to protect itself from light stress (Klughammer and Schreiber, 2008). Interestingly, the trend of the curves as well as the differences between the light treatments were in accordance with previously published reports, such as wheat and wintercreeper (*Euonymus fortunei*) (Zivcak et al, 2013; Song and Li, 2016). Quantum yields of photochemical and non-photochemical energy conversions indicate that plants grown under reduced light intensity exhibited a higher photochemical efficiency of PSII (Fig. 2). This partly explains why assimilation rates measured under the growing light intensities were not different between control plants and plants under 75% illumination.

In contrast, we found that reduced light intensity resulted in a decrease of the chlorophyll concentration as well as a increase of chlorophyll a/b ratio. Studies on rice and other species showed significant increases of chlorophyll a and b contents and a decrease of chlorophyll a/b ratio under reduced light intensity (Dai et al, 2009; Mu et al, 2010; Wang et al, 2015). This is usually interpreted as an adaptation process to improve light harvesting and funneling. To this moment, we have no explanation for this observation in N71. However, interactions between GA content in leaves and chlorophyll concentrations have been demonstrated in several studies (Li J R et al, 2010) and it is possible linked with GA deficiency in our study. It should be further investigated in future research.

The actual photosynthesis rate under light saturating conditions is limited by the concentration of CO_2 at the site of carboxylation and the biochemical capacity of enzymatic apparatus of the Calvin Cycle, represented by the parameter $V_{c(max)}$. The CO_2 concentration is a result of the strength of photosynthesis itself, the CO_2 concentration of the ambient air, the conductance of stomatal and mesophyll components between the ambient air and the inside of the chloroplast. In our study, stomatal and mesophyll limitations of plants under 75% illumination were always smaller compared to the control plants and plants receiving 20% illumination. In all treatments, mesophyll conductance was the dominant contributor to reductions in photosynthesis. No clear pattern emerged for the contribution of the biochemical limitations between the treatments and over time. The results from this study regarding Super Dwarf Rice support earlier results by Martins et al (2014) on shade-tolerant coffee leaves but are in contrast with results published on non-shade tolerant trees species and sugar beet (Grassi and Magnani, 2005; Grassi et al, 2009; Sagardoy et al, 2010) where biochemical and stomatal limitations dominated.

In studies on *Juglans regia* and several *Acer* species, Piel et al (2002) and Hanba et al (2002) found a positive relationship between light and mesophyll conductance, but these results were confounded by several environmental and physiological factors, especially a higher leaf thickness resulting in a higher mesophyll surface area exposed to intercellular air spaces. In Super Dwarf Rice, no correlation between mesophyll conductance and specific leaf area was found (Fig. 4). In summary, photosynthesis of N71 under low light intensities is characterized by shade-tolerance mechanisms.

Several studies on low-N supply have linked reduced photosynthesis rates to a decrease in mesophyll conductance (Caemmerer and Evans, 1991; Warren, 2004). In this study, reducing the nitrogen concentration in the nutrient solution by 50% neither led to marked reductions in photosynthesis rates nor differences in the ratio of the different limiting components compared to 2.8 µmol/L IV-apply. There was no clear contribution of different N-supply levels to changes in PSII chlorophyll fluorescence patterns as was formerly demonstrated (Verhoeven et al, 1997; Cheng, 2003). However, we assumed that the tested N-levels were not sufficiently low enough to induce any changes in PSII efficiency (Shrestha et al, 2012).

In this study, we demonstrated that Super Dwarf Rice only undergoes marginal morphological and anatomical changes under low light conditions. The strongest morphological constraint under low illumination was a strong reduction in tiller number, but even under light intensities as low as 150 µmol/(m^2 s), tillering still took place, allowing researchers to examine carbon allocation or pooling during plant development. The fact that leaf elongation is strongly suppressed could ease the growth of this genotype in low-light, low-height growing racks. In summary, this rice genotype seems to be a promising candidate for experiments on microgravity. However, when N71 is grown for food production, light levels should be high since strong reductions in yield can occur under low light conditions. When grown in Yoshida nutrient solution, a nitrogen concentration of 1.4 mmol/L was shown to be sufficient, and doubling it to 2.8 mmol/L during tillering, as usually proposed, is not necessary as indicated by the lack of changes in yield, morphology or photosynthesis in our study. Photosynthesis of N71 was also remarkably stable under reduced illumination, which was mostly due to a higher mesophyll conductance under reduced light.

METHODS

Plant cultivation, treatment and sampling

Super Dwarf Rice N71 plants from the Konoshita Collection (seeds provided by Dale Bumpers National Rice Research Center, AR, USA) were used and grown in a hydroponic system using an adapted Yoshida nutrient solution (Yoshida et al, 1971) in a climate chamber (Percival E-75L1, CLF PlantClimatics GmbH, Wertingen, Germany) at the University of Hohenheim, Germany. For the solution, the macronutrient element composition (mmol/L) was 2.8 N (full nitrogen) as NH_4NO_3, 0.32 P as $NaH_2PO_4{\cdot}2H_2O$, 1.02 K as K_2SO_4, 1.00 Ca as $CaCl_2$ and 1.65 Mg as $MgSO_4{\cdot}7H_2O$. And the micronutrient element composition (µmol/L) was 9.10 Mn as $MnSO_4{\cdot}H_2O$, 0.05 Mo as $(NH_4)_6{\cdot}Mo_7O_{24}{\cdot}4H_2O$, 18.50 B as H_3BO_3, 0.15 Zn as $ZnSO_4{\cdot}7H_2O$, 0.16 Cu as $CuSO_4{\cdot}5H_2O$ and 35.82 Fe as FeNa-EDTA. Photoperiod was set to 14 h/10 h for light and

dark as suggested by Bugbee (1999), and temperature to 30 ℃ and 28 ℃ during light and dark periods, respectively. Relative humidity inside the growth chamber was set to 70%.

To provide anaerobic conditions during germination as proposed by Frantz and Bugbee (2002), seeds were transferred into a polyethylene bottle and covered with approximately 15 cm of tap water. Germination took place in darkness at 30 ℃. After germination, about 200 seeds were transferred into plastic boxes (20.0 cm × 20.0 cm × 5.5 cm) with moist tissue paper. Light was supplied at 8 d after germination (DAG) when seedlings reached a height of 5 cm. Seedlings were transferred into 3 hydroponic systems consisting of 60.0 cm × 3.5 cm PVC-pipes that were placed into a 10 L plastic container (Georg Utz AG, Bremgarten, Switzerland) filled with nutrient solution at 16 DAG. Ceapren plugs (Greiner Bio-One GmbH, Frickenhausen, Germany) were used to fix the seedlings into the PCV-pipes. The position of plants was changed randomly every 2 d to prevent border effects.

After onset of tillering, the main tiller of the randomly selected plants was cut open with a razor blade and checked for panicle formation with an optical microscope (Stemi 2000-C, Carl Zeiss AG, Oberkochen, Germany). When the onset of panicle formation at 52 DAG was observed, 54 homogenous plants were transferred into 18 pots (1.1 L) each, with three plants per pot. The remaining plants were transferred into the 2nd climate chamber and kept as dummy plants for replacing plants used for destructive analyses during the experiment. Different illumination levels were established by 15 cm diameter PVC-U pipes with 50 cm height that were placed bottom-open over the pots. Pipes and pots were standing on a metal grate fixed at half-height inside the climate chamber. The inside of the pipes was covered with a highly reflecting light-scattering foil (Diamond ECO, Easy Grow Ltd., Grimsby, UK).

Six pots were placed under tubes that were covered with a wire mesh covering the upper opening (mesh size 0.63 mm × 0.16 mm), resulting in a light intensity of 553 μmol/(m^2·s), and six pots were covered with a plastic mesh resulting in a light intensity of 157 μmol/(m^2·s), and another six pots were not covered at all, receiving a light intensity of about 745 μmol/(m^2·s) full light, as the control. Thus, the illumination levels were 75% and 20% of the control light intensity, respectively. The light intensities were measured with a SP2 Lite photometer (Kipp and Zonen, 2628 XH Delft, Netherlands) and referred to half plant-height with respect to fully-grown plants. Three pots in each light treatment group received 50% nitrogen concentration in the nutrient solution. Sampling took place at the following phenological stages: panicle emergence, flowering, ripening/onset of senescence.

Gas exchange and chlorophyll fluorescence measurements

Gas exchange and chlorophyll fluorescence of fully expanded flag leaves in six randomly selected plants were measured simultaneously with a GFS-3000/3055-F (Heinz Walz GmbH, Effeltrich, Germany). Plants were dark-adapted for 60 min prior to the measurement. Minimal (F_0) and maximal (F_m) fluorescences in the dark-adapted state were measured at a modulated light intensity of 1.2 μmol/(m^2·s) and a saturating light pulse (SLP) of about 4 500 μmol/(m^2·s) light intensity for 0.8 s. Subsequently, actinic light of an intensity of 1 500 μmol/(m^2·s) was imposed until photosynthesis, stomatal conductance and transient chlorophyll fluorescence (F_s) reached steady state. Then, the light intensity was increased to 2 000 μmol/(m^2·s), and gas exchange and minimal and maximal fluorescences in the light-adapted state (F_0' and F_m') of light saturated photosynthesis and chlorophyll fluorescence were measured. Then, a light response curve for PPFD (photosynthetically active photon flux density) values of 1 750, 1 500, 1 250, 1 000, 750, 500, 300, 150, 50 and 0 μmol/(m^2·s) was recorded. For measurements of F_0', the actinic light was switched off directly after the SLP, and a far red light of 17 μmol/(m^2·s) light intensity was supplied for 2 s followed by measurement of F_m' at a modulated light intensity of 1.2 μmol/(m^2·s). F_s' was measured prior to the SLP together with gas exchange.

After the light response measurements, CO_2-response curves of gas exchange and chlorophyll fluorescence were recorded for CO_2-concentrations of 1 200, 1 000, 800, 600, 400, 300, 200, 100 and 50 μmol/mol, respectively, following the same protocol as given above. For all measurements, steady state of photosynthesis and F_s were reached in 10 to 20 min. Temperature inside the cuvette was 30 ℃ and relative humidity ranged from 50% to 60%, depending on the stomatal conductance of the sample. Light response curves were fitted to an irradiance response model given by Ye (2007). Maximum gross photosynthesis [$P_{(g)max}$, μmol/(m^2·s)], compensation irradiance [I_{comp}, μmol/(m^2·s)], the quantum yield of photosynthesis at zero irradiance [$f_{I(0)}$, mmol/mol] and dark respiration rate (R_{Dark}) were calculated accordantly.

For the CO_2- and light-response curves, the values for stomatal conductance for water vapor (g_s) and CO_2 (g_{sc}) and intercellular CO_2 concentrations were calculated according to von Caemmerer and Farquhar (1981).

Maximum and actual quantum yield of PSII photochemistry in the dark and light adapted state respectively [$F_v / F_m = (F_m - F_0) / F_m$) and $\phi_{PSII} = (F_m' - F_s) / F_m']$, the quantum yield of non-regulated ($\phi_{NO} = F_s / F_m$), and regulated non-photochemical energy loss in PSII ($\phi_{NPQ} = F_s / F_m' - F_s / F_m$) were derived from the fluorescence measurements according to Genty et al (1989) and Hendrickson et al (2004).

Mesophyll conductance to CO_2 (g_m), chloroplastic CO_2-concentations (C_c), the product of leaf absorption and ratio of photons absorbed by PSII (τ), maximum carboxylation capacity [$V_{c(max)}$], maximum electron transport capacity (J_{max}) and triose phosphate release rate (*TPU*) were calculated A/C_i by using the curve-fitting approach proposed by Moualeu-Ngangue et al (2017).

Leaf pigment analysis

The area of the flag leaf used for gas exchange measurements was measured with a Ll-3000C leaf area meter (Ll-Cor Inc., Lincoln, USA). Leaf chlorophyll and carotenoid contents were determined with a Beckman DU-640 UV-VIS spectrophotometer (Beckman Instruments Inc., Fullerton, USA) following 24 h dimethylsulfoxid (DMSO) extraction at room temperature as

described by Sumanta et al (2014). Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry weight.

Flag leaf photosynthesis was measured at three phenological stages to see how Super Dwarf Rice adapts to low-light conditions. The measurements were combined with destructive sampling to assess biomass and leaf morphological data. Additionally, we performed a yield component analysis at the end of the experiment.

Biomass and yield component analysis

After the gas exchange measurements, plants were separated into stems, leaves and roots, and dry weights were determined after drying at 70 °C to constant weight. Number of tillers per plant, number of productive tillers per plant, and number of grains per plant were determined, and, if generative material was present, weight and number of filled and unfilled spikelets per plant were determined. Additionally, leaf area of the entire plants was measured. Whole plant SLA and leaf area ratio were calculated.

Relative reductions of yield components (RR) for each plant in all treatment groups were calculated [*RR* = 1 – (Yield component of treatment group / Yield component of control)]. Further, the dynamics of yield formation was analyzed by calculating the contribution of the specific yield components to total yield loss compared to control.

Statistical analysis

Statistical analysis was performed using a two-way analysis of variances with the Statsmodels module (0.8.6) (Seabold and Perktold, 2010) for Python. Treatment means were compared according to the Tukey's HSD (Honestly Significant Difference) test at the 5% level. Data for yield components, leaf and photosynthetic traits were analyzed separately for every phenological stage. For statistical analysis of the photosynthetic limitations, a mixed model analysis was performed with the lmerTest package Version 3.1.0 (Kuznetsova et al, 2017) in *R* Version 3.5.2, (https://cran.r-project.org), followed by a *post hoc* analysis using the emmeans package Version1.3.3 (Searle et al, 1980) to detect significant deviations for each of the limiting components during each growing stage from its respective reference value.

ACKNOWLEDGEMENT

The authors thank the Dale Bumpers National Rice Research Center, Arkansas, USA for providing the seeds.

REFERENCES

Bugbee B. 1992. Determining the potential productivity of food crops in controlled environments. *Adv Space Res*, **12**(5): 85–95.

Bugbee B. 1999. Engineering plants for spaceflight environments. *Gravit Space Biol Bull*, **12**(2): 67–74.

Caemmerer S V, Evans J R. 1991. Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C3 plants. *Aust J Plant Physiol*, **18**(3): 287–305.

Cantagallo J E, Medan D, Hall A J. 2004. Grain number in sunflower as affected by shading during floret growth, anthesis and grain setting. *Field Crops Res*, **85**: 191–202.

Casal J J, Sanchez R A, Deregibus V A. 1986. The effect of plant density on tillering: The involvement of R/FR ratio and the proportion of radiation intercepted per plant. *Environ Exp Bot*, **26**(4): 365–371.

Cheng L L. 2003. Xanthophyll cycle pool size and composition in relation to the nitrogen content of apple leaves. *J Exp Bot*, **54**: 385–393.

Dai Y J, Shen Z G, Liu Y, Wang L L, Hannaway D, Lu H F. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environ Exp Bot*, **65**: 177–182.

de Souza I R P, MacAdam J W. 2001. Gibberellic acid and dwarfism effects on the growth dynamics of B73 maize (*Zea mays* L.) leaf blades: A transient increase in apoplastic peroxidase activity precedes cessation of cell elongation. *J Exp Bot*, **52**: 1673–1682.

Frantz J M, Bugbee B. 2002. Anaerobic conditions improve germination of a gibberellic acid deficient rice. *Crop Sci*, **42**(2): 651–654.

Frantz J M, Pinnock D, Klassen S, Bugbee B. 2004. Characterizing the environmental response of a gibberellic acid-deficient rice for use as a model crop. *Agron J*, **96**(4): 1172–1181.

Genty B, Briantais J M, Baker N R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta*, **990**(1): 87–92.

Germer J, Sauerborn J, Asch F, de Boer J, Schreiber J, Weber G, Müller J. 2011. Skyfarming an ecological innovation to enhance global food security. *J Verbr Lebensm*, **6**(2): 237–251.

Gommers C M M, Visser E J W, Onge K R S, Voesenek L A C J, Pierik R. 2013. Shade tolerance: When growing tall is not an option. *Trends Plant Sci*, **18**(2): 65–71.

Gong W Z, Jiang C D, Wu Y S, Chen H H, Liu W Y, Yang W Y. 2015. Tolerance vs. avoidance: Two strategies of soybean (*Glycine max*) seedlings in response to shade in intercropping. *Photosynthetica*, **53**(2): 259–268.

Grassi G, Magnani F. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ*, **28**(7): 834–849.

Grassi G, Ripullone F, Borghetti M, Raddi S, Magnani F. 2009. Contribution of diffusional and non-diffusional limitations to midday depression of photosynthesis in *Arbutus unedo* L. *Trees*, **23**(6): 1149–1161.

Hanba Y T, Kogami H, Terashima I. 2002. The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. *Plant Cell Environ*, **25**(8): 1021–1030.

Hendrickson L, Furbank R T, Chow W S. 2004. A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. *Photosynth Res*, **82**(1): 73–81.

Klughammer C, Schreiber U. 2008. Complementary PSII quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. *PAM Appl Notes*, **1**: 27–35.

Kölling K, George G M, Künzli R, Flütsch P, Zeeman S C. 2015. A whole-plant chamber system for parallel gas exchange measurements of Arabidopsis and other herbaceous species. *Plant Methods*, **11**(1): 48.

Kuznetsova A, Brockhoff P B, Christensen R H B. 2017. lmerTest package: Tests in linear mixed effects models. *J Stat Softw*,

82(13): 1–26.

Li H W, Jiang D, Wollenweber B, Dai T B, Cao W X. 2010. Effects of shading on morphology, physiology and grain yield of winter wheat. *Eur J Agron*, **33**(4): 267–275.

Li J R, Yu K, Wei J R, Ma Q, Wang B Q, Yu D. 2010. Gibberellin retards chlorophyll degradation during senescence of paris polyphylla. *Biol Plant*, **54**(2): 395–399.

Livingston N J, Davies G J, Eby B M, Filek G, Fuchs E E, Pepin S, Percy R E. 1994. A whole-plant cuvette system to measure short-term responses of conifer seedlings to environmental change. *Tree Physiol*, **14**: 759–768.

Mackill D J, Khush G S. 2018. IR64: A high-quality and high-yielding mega variety. *Rice*, **11**: 18.

Martins S C V, Galmés J, Cavatte P C, Pereira L F, Ventrella M C, DaMatta F M. 2014. Understanding the low photosynthetic rates of sun and shade coffee leaves: Bridging the gap on the relative roles of hydraulic, diffusive and biochemical constraints to photosynthesis. *PLoS One*, **9**(4): e95571.

Matsukura C, Itoh S I, Nemoto K, Tanimoto E, Yamaguchi J. 1998. Promotion of leaf sheath growth by gibberellic acid in a dwarf mutant of rice. *Planta*, **205**(2): 145–152.

Moualeu-Ngangue D P, Chen T W, Stützel H. 2017. A new method to estimate photosynthetic parameters through net assimilation rate-intercellular space CO_2 concentration (A-Ci) curve and chlorophyll fluorescence measurements. *New Phytol*, **213**(3): 1543–1554.

Mu H, Jiang D, Wollenweber B, Dai T, Jing Q, Cao W. 2010. Long-term low radiation decreases leaf photosynthesis, photochemical efficiency and grain yield in winter wheat. *J Agron Crop Sci*, **196**(1): 38–47.

Piel C, Frak E, Le Roux X, Genty B. 2002. Effect of local irradiance on CO_2 transfer conductance of mesophyll in walnut. *J Exp Bot*, **53**: 2423–2430.

Pinstrup-Andersen P. 2018. Is it time to take vertical indoor farming seriously? *Glob Food Sec*, **17**: 233–235.

Russo E. 2003. Special report: The birth of biotechnology. *Nature*, **421**: 456–457.

Sagardoy R, Vázquez S, Florez-Sarasa I D, Albacete A, Ribas-Carbó M, Flexas J, Abadía J, Morales F. 2010. Stomatal and mesophyll conductances to CO_2 are the main limitations to photosynthesis in sugar beet (*Beta vulgaris*) plants grown with excess zinc. *New Phytol*, **187**(1): 145–158.

Samuolienė G, Sirtautas R, Brazaitytė A, Duchovskis P. 2012. LED lighting and seasonality effects antioxidant properties of baby leaf lettuce. *Food Chem*, **134**(3): 1494–1499.

Seabold S, Perktold J. 2010. Statsmodels: Econometric and statistical modeling with python. *In*: van der Walt S, Millman J. Proceedings of the 9th Python in Science Conference: 57–61.

Searle S R, Speed F M, Milliken G A. 1980. Population marginal means in the linear model: An alternative to least squares means. *Am Stat*, **34**(4): 216–221.

Shrestha S, Brueck H, Asch F. 2012. Chlorophyll index, photochemical reflectance index and chlorophyll fluorescence measurements of rice leaves supplied with different N levels. *J Photochem Photobiol B*, **113**: 7–13.

Song X H, Li H. 2016. Effects of building shade on photosynthesis and chlorophyll fluorescence of *Euonymus fortunei*. *Acta Ecol Sin*, **36**(5): 350–355.

Sumanta N, Haque C I, Nishika J, Suprakash R. 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Res J Chem Sci Res*, **4**(9): 63–69.

Sun S, Moravek A, von der Heyden L, Held A, Sörgel M, Kesselmeier J. 2016. Twin-cuvette measurement technique for investigation of dry deposition of O_3 and PAN to plant leaves under controlled humidity conditions. *Atmos Meas Tech Discuss*, **9**(2): 599–617.

Terashima I, Hanba Y T, Tazoe Y, Vyas P, Yano S. 2006. Irradiance and phenotype: Comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. *J Exp Bot*, **57**(2): 343–354.

Thomas S G, Sun T P. 2004. Update on gibberellin signaling: A tale of the tall and the short. *Plant Physiol*, **135**(2): 668–676.

Trapani N, Hall A J, Sadras V O, Vilella F. 1992. Ontogenetic changes in radiation use efficiency of sunflower (*Helianthus annuus* L.) crops. *Field Crops Res*, **29**(4): 301–316.

van Norman J M, Benfey P N. 2009. *Arabidopsis thaliana* as a model organism in systems biology. *Wires Syst Biol Med*, **1**(3): 372–379.

Verhoeven A S, Demmig-Adams B, Adams W W. 1997. Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach exposed to high light and N stress. *Plant Physiol*, **113**(3): 817–824.

von Caemmerer S, Farquhar G D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**(4): 376–387.

Wang L, Deng F, Ren W J. 2015. Shading tolerance in rice is related to better light harvesting and use efficiency and grain filling rate during grain filling period. *Field Crops Res*, **180**: 54–62.

Warren C R. 2004. The photosynthetic limitation posed by internal conductance to CO_2 movement is increased by nutrient supply. *J Exp Bot*, **55**: 2313–2321.

Ye Z P. 2007. A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. *Photosynthetica*, **45**(4): 637–640.

Yoshida S, Forno D A, Cock J. 1971. Laboratory Manual for Physiological Studies of Rice. Los Baños, the Philippines: International Rice Research Institute.

Zabel P, Bamsey M, Schubert D, Tajmar M. 2016. Review and analysis of over 40 years of space plant growth systems. *Life Sci Space Res*, **10**: 1–16.

Zivcak M, Brestic M, Balatova Z, Drevenakova P, Olsovska K, Kalaji H M, Yang X H, Allakhverdiev S I. 2013. Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. *Photosynth Res*, **117**: 529–546.

(Managing Editor: WANG Caihong)