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Stay-Green in Rice (*Oryza sativa* L.) of Drought-Prone Areas in Desiccated Soils

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Abstract : Stay-green in the post-anthesis period is thought to be an efficient drought-tolerance trait in crops, but its effectiveness in rice (*Oryza sativa* L.) is unknown. Our objectives were to determine whether the stay-green trait exists in rice cultivars in drought-prone areas. Twenty-four cultivars from Japan and Vietnam were grown in pots of 0.08 m in diameter and 1.00 m deep. At heading, irrigation was terminated in half of the pots and continued in the remaining pots. Every four days during the grain-filling period, we measured the leaf green color with a chlorophyll meter (SPAD), the green leaf area (GLA) and the fraction of transpirable soil water (FTSW). The capacity for maintenance of SPAD-value and GLA in desiccated soils was evaluated by determining the ratio of integrated SPAD-value and GLA in desiccated (D) plants to those in irrigated (I) plants ($SPAD_{D/I}$ or $GLA_{D/I}$, respectively). The $SPAD_{D/I}$ and $GLA_{D/I}$ in 24 cultivars showed diverse frequency distributions. Cultivars belonging to higher ranges of the distribution in $SPAD_{D/I}$ and $GLA_{D/I}$ tended to show higher ratios of plant dry weight at harvest in D to in I plants. $SPAD_{D/I}$ and $GLA_{D/I}$ in the grain-filling period were poorly correlated with those in the seedling period in desiccated soils, and hence the capacity for maintenance of green leaves in the grain-filling period would differ from that in the seedling period. These results suggest that the stay-green trait exists as the capacity for maintaining green leaves and benefits dry matter production in desiccated soils in rice cultivars in drought-prone areas.

Key words : Drought, Grain-filling period, Rice, Seedling period, Stay-green.

Drought is a serious environmental stress for rice production in rain-fed areas of Southeast Asia such as Vietnam (Barker and Herdt, 1979; Vietnam News, 2007). The development of new rice cultivars that can overcome the effects of drought on rice yield and yield stability in these areas has been expected (Barker and Herdt, 1979), but during the past several decades, the yield of rain-fed rice in Vietnam has increased only slightly from 2.7 to 3.3 t or less per ha (Yoshida, 1981; GSO, 2007).

Stay-green is the capacity to maintain green leaves (Thomas and Smart, 1993) and is considered to be effective in reducing the influence of drought in sorghum (Borrell et al., 2000a, 2000b; Borrell and Hammer, 2000; Mahalakshmi and Bidinger, 2002) and in wheat (Christopher et al., 2004). In sorghum, the stay-green ability has been identified as a secondary trait, and genotypes that possess this trait maintain more photosynthetically active leaves during post-flowering drought (Borrell et al., 2000a, 2000b; Thomas and Howarth, 2000). Green-leaf area in some rice cultivars decreased more slowly than in others during the grain-filling period under well water conditions, but these capacities for maintenance of green leaves did not affect photosynthetic activity or yield (Cha et al., 2002; Jang et al., 2004). Under drought conditions in Thailand, some rice cultivars were able to maintain green leaves longer than others, and showed higher dry-matter production and yield (Jearakongman et al., 1995). This suggests that stay-green contributes to drought resistance in rice, although it is unknown whether keeping away from plant dehydration and tolerance to dehydration of plant organs resulted in the maintenance of green leaves, because a direct comparison between soil or plant moisture conditions and the maintenance among cultivars was not shown.

The capacity for yield maintenance under drought conditions can be divided into several categories: drought escape, drought avoidance and drought tolerance (Turner, 1979; Kramer, 1980). Drought avoidance is the most effective mechanism for affecting yield maintenance in rice under drought conditions. The root distribution in wet deep soil layers can contribute to the absorption of water, and as a result for maintenance of plant growth under drought conditions (O'Toole, 1982; Kobata et al., 2000; Kato et al., 2007). However, during the seedling stage, with poor root development, drought tolerance plays a dominant role in seedling survival under dehydrated conditions (O'Toole, 1982; Ahmad et al., 1987). Several rice cultivars in the seedling stage can maintain green leaves or recover after re-watering even if the plants suffer from low leaf water potential (O'Toole and Chang, 1979; Ahmad et al., 1987; De Datta et al., 1988; Henderson et al., 1993; Cabuslay et al., 2002). Based on the categorization of drought-

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Table 1. Entryrice cultivars for stay-green capacity.

Cultivar No.	Cultivar name	Ecotypes	Improved/Local	Origin
Japonica cv.				
1	Nipponbare	Lowland	Improved	Japan
2	Hatakinumochi	Upland	Improved	Japan
3	IRAT13	Upland	Improved	Ivory Coast
4	Terisisazu	Upland	Local	Japan
5	Sensho	Upland	Local	Japan
Indica cv.				
6	IR72	Lowland	Improved	Philippines
7	M1-48	Upland	Improved	Philippines
8	Ma cha	Lowland	Local	Vietnam
9	Toa da No. 1	Lowland	Local	Vietnam
10	Nep nuong den	Lowland	Local	Vietnam
11	Khau tram tan	Lowland	Local	Vietnam
12	Khau mo khao	Lowland	Local	Vietnam
13	Khau say	Lowland	Local	Vietnam
14	Khau xien pan	Lowland	Local	Vietnam
15	Nep nuong	Lowland	Local	Vietnam
16	Khau ta	Lowland	Local	Vietnam
17	Nep dap	Lowland	Local	Vietnam
18	Khau nua han	Lowland	Local	Vietnam
19	Khau mo	Lowland	Local	Vietnam
20	Khau lech	Lowland	Local	Vietnam
21	Khau nua dam	Lowland	Local	Vietnam
22	Khau nu say	Lowland	Local	Vietnam
23	Tram vai	Lowland	Local	Vietnam
24	Muoi cai	Lowland	Local	Vietnam

resistance mechanisms, stay-green should be regarded as a drought tolerance mechanism. Therefore, the capacity for seedling survival as drought tolerance may be related to the stay-green trait during the terminal growth stage in rice. This assumption suggests the possibility of using conventional methods for assessing stay-green in diverse cultivars during the seedling period. Furthermore, conventional methods may solve the problem in difficulties of synchronizing water deficit treatments among diverse cultivars with different flowering times and root masses.

During the post-anthesis period, root development has almost finished, and water resources in the root zone are limited (Yoshida and Hasegawa, 1982; Harada and Yamazaki, 1993). Therefore, stay-green during the post-anthesis period appears to be quite important for rice plants to maintain grain yield in areas where drought occurs during the terminal growth stage, such as Vietnam (Vietnam News, 2007). The objectives of the present study were to determine whether stay-green in the grain-filling period under desiccated soil conditions exists in rice in drought-prone areas based on the responses of leaf color and green-leaf area to desiccated soils, and to test the availability of seedling plants for cultivar selection for stay-green.

Materials and Methods

1. Plant materials

Twenty-four rice cultivars were used, including two lowland and five upland cultivars from Japan, the Philippines and the Ivory Coast, and seventeen traditional lowland rice cultivars from the northern provinces of Vietnam (Table 1). Most of the Vietnam cultivars were grown under rain-fed conditions. Seeds of the cultivars were from the National Institute of Agrobiological Science in Japan. Experiments were conducted at the experimental farm of Shimane University, at 35º 28′ N, 133º 02′ E in Japan.

(1) Experiment for the post-anthesis period

Germinated seeds of the 24 cultivars were sown in seedling beds ($0.60 \times 0.30 \times 0.03$ m) on 13 May, 2005. On 6 June, 2005, four seedlings at the four-leaf stage were transplanted to pots of polyvinyl chloride (PVC) pipes with an inside diameter of 0.08 m and a height of 1.00 m, and thinned to two plants per pot one week later. The bottom of each pot was covered with a plastic mesh sheet to keep the soil from spilling out. Pots were filled with soil to a depth of 0.95 m. The outside wall of each pot was covered with a reflection sheet (Dry sheet, Daiken Kogyo Co., Tokyo) to protect the pot from the temperature increase caused by solar radiation. The soil used to fill the pots in this experiment was a mixture of Andosol for rice seedlings (Green Epoch Co., Izumo, Japan) and sandy soil (Masatuchi), which was sieved using a 0.5×10^{-2} m mesh, at a volume ratio of 1:1. Before seedlings were transplanted to the pots, a fertilizer consisting of 1.0 g ammonium sulfate, 2.0 g superphosphate of lime and 0.5 g potassium chloride was applied to the 0.10-m surface soil layer in the pots. All pots were placed in a non-temperature-controlled glasshouse and watered daily to keep soil water in the pots near field capacity.

The photoperiod treatment was carried out on Vietnam cultivars because of their photoperiodic sensitivity. The photoperiod treatment was from 6 July, 2005, to 3 Sept., 2005. The plants were put in a shelter at 1700 and taken out at 0700 the next day. This shelter was a steel frame ($3.60\times1.80\times2.20$ m) covered with a black vinyl sheet, and it had a temperature-controlled fan that drew air to the outside to lower the air temperature inside the shelter. When booting was observed, the treatment was finished and the pots were moved out of the shelter.

(2) Experiment for the seedling period

A fertilizer mixture of 0.6 g ammonium sulfate, 1.4 g superphosphate of lime and 0.4 g potassium chloride was mixed with soil for rice seedlings in round plastic pots with an inside diameter of 0.25 m and a height of 0.20 m, and then pots were filled with the rice seedling soil. Two germinated seeds each of the 24 cultivars were sown in the pots on 19 Sept., 2006. One seedling was removed after emergence, and one seedling was grown in each pot. On 27 Oct., 2006, when the seedlings were at the four-leaf stage, all pots were housed in three growth chambers (TGE-6H-4, Tabai Espec Co. Osaka, Japan), which were set up for a day/night temperature of 30/30°C, photosynthetic active radiation (PAR) of 354 μmol m^{-2} s^{-1}, relative day/night humidity of 75/90% and a day/night length of 10 hr/14hr. The plants were carried into the chambers 4 d before the start of soil desiccation to adapt to the growth chamber conditions. During this period, the pots were watered daily to keep the soil water content in the pots near field capacity.

2. Soil desiccation treatments

(1) Post-anthesis period

At the heading stage, one plant from each pot was harvested to measure the leaf areas of the five upper leaves, which was used as the area of each leaf at the start of soil desiccation, and then the surface of each pot was covered with foam polystyrene plates, and the spaces between the plants and plates were filled with oil clay soil. The bottom of each pot was closed with plastic sheeting to prevent direct soil evaporation. The pots were separated into two groups, irrigated and desiccated pots. The soil desiccation treatment was started at heading and continued for 40 d in the desiccated pots, while watering was continued in the irrigated pots through small pipes on the soil surface. When the soil water content decreased and the plants indicated a clear symptom of leaf wilting and were not able to recover from this wilting by the next morning, the amount of water needed to restore the pot weight to that of the previous day was added to slow down the soil desiccation rate. Position of the pots was changed at intervals of several days to reduce influence of plant positions.

(2) Seedling period

At the five-leaf stage, seedlings in 3 pots were harvested to measure the leaf area at various leaf positions, which was used as the area of each leaf at the start of soil desiccation. The surfaces of the pots were covered with plastic beads to prevent evaporation. The soil desiccation treatment was started when plants reached the five-leaf stage and continued for 15 days thereafter. During this period, watering to one-half of the pots in each growth cabinet was stopped while it was continued daily to the other half of the pots through small PVC tubes to keep the moisture level near field capacity.

3. Measurements of soil water content, leaf color and green leaf area

(1) Soil water content

The pots were weighed daily at about 1600 during the soil desiccation treatment. At the end of the experiments, fresh soil weight per pot (FSW, g pot^{-1}) was recorded, and parts of mixed soil were carefully sampled in vials. After weighing fresh soil weight, soils were oven-dried at 108°C for 48 h and weighed to record dry soil weight (DSW, g pot^{-1}). The soil water content (SWC, H_2O g dry soil g^{-1}) was determined by subtracting DSW from FSW/(SWC+1).

The fraction of transpirable soil water (FTSW) (Ray and Sinclair, 1997). FTSW was calculated using the following equation:

$$FTSW=(TSW-TSW_0)/(TSW_{max}-TSW_0) \quad (1)$$

where TSW is the observed total soil water weight (g pot^{-1}), TSW_0 is the minimum soil water weight that plants can use (g pot^{-1}) and TSW_{max} is the maximum holding soil water weight (g pot^{-1}). TSW was the difference between the observed pot weight and (dry soil+pot) weight, TSW_0 was calculated as SWC at the wilting point multiplied by DSW, and TSW_{max} was SWC at field capacity multiplied by DSW. To decide SWC at wilting point of −1.5MPa and at field capacity of

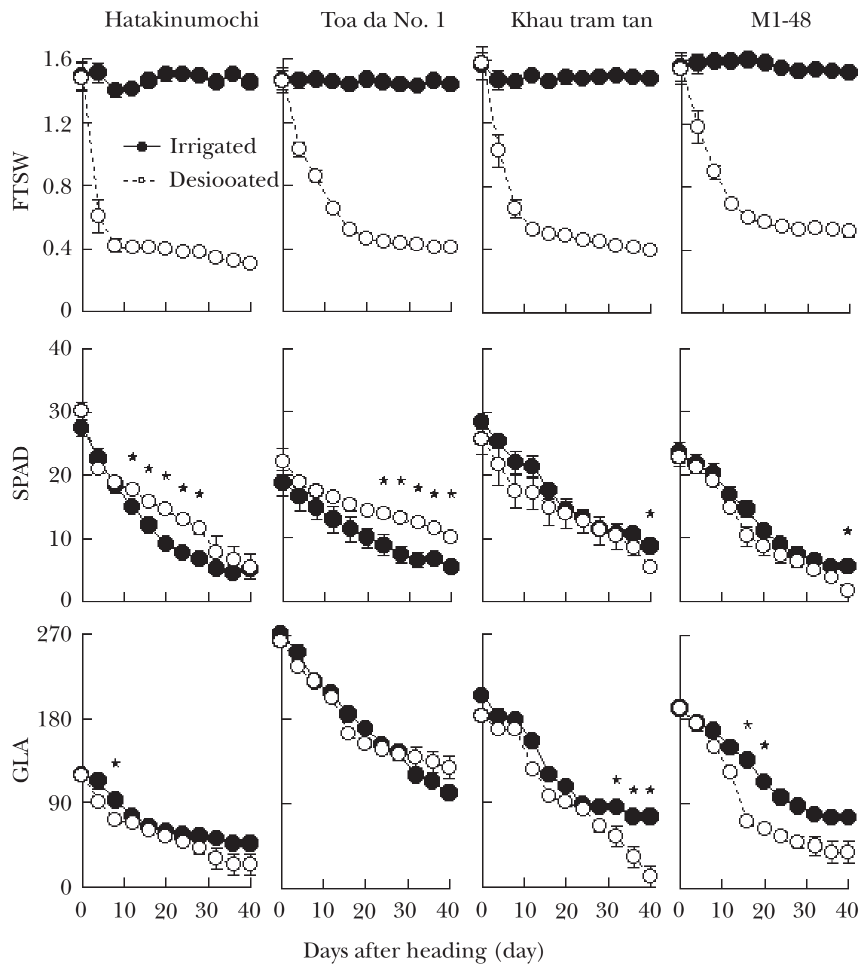

Fig. 1. Fraction of transpirable soil water (FTSW), SPAD value and green leaf area (GLA) in irrigated and desiccated plants after heading in rice cultivars, Hatakinumochi, Toa da No. 1, Khau tram tan and M1-48. All data are the mean ± standard error of three replications. * indicates significant differences at $P<0.05$ between the values in desiccated and irrigated conditions.

–0.03 MPa (Kramer and Boyer, 1995), we constructed a soil moisture characteristic curve for the soils using a psychrometer chamber with microvolt meters (C-52-SF and HR-33Tm, Wescor, Inc., Logan, UT, USA), as in previous experiments.

(2) Leaf color and green leaf area

Every four days in the post-flowering experiment and every three days in the seedling experiment after the start of the soil desiccation treatment, the leaf green color (SPAD value) and visual green color leaf area (GLA, cm^2) were measured. The SPAD value was measured in the same leaves that were used for the estimation of the green leaf area. The SPAD value was determined based on the average value of three positions of each leaf blade measured with a portable chlorophyll meter (SPAD-502, Minolta Co., Ltd., Tokyo).

The GLA was determined as follows:

$$GLA = GLA_0 \times (1 - SLAR/100) \quad (2)$$

where GLA_0 is GLA at the start of soil desiccation in each of five upper leaves measured with a leaf area meter (CI-203 area meter, CID, Inc., Vancouver, WA, USA), and SLAR is the percentage of senesced leaf area in each leaf. Senesced leaf area is defined as an area of leaf that is occupied by yellow or white portions resulting from degradation of chlorophyll pigment. SLAR was visually scored on a scale of 0 to 9 in each leaf from three tillers per plant, where 0 = 0% to 10% senesced leaf area and 9 = 90% to 100% senesced leaf area. The GLA in the five leaves was accumulated as the GLA in the whole plant.

The SPAD values and GLAs plotted against days after heading (day base) or FTSWs (FTSW base) were integrated, and the ratio of integrated value in desiccated to that in irrigated plants ($SPAD_{D/I}$ and $GLAD_{D/I}$) was used to evaluate the capacity for maintenance of SPAD value and GLA on desiccated soils. The integration value (S) was obtained from that the trapezoid area of SPAD value or GLA in every

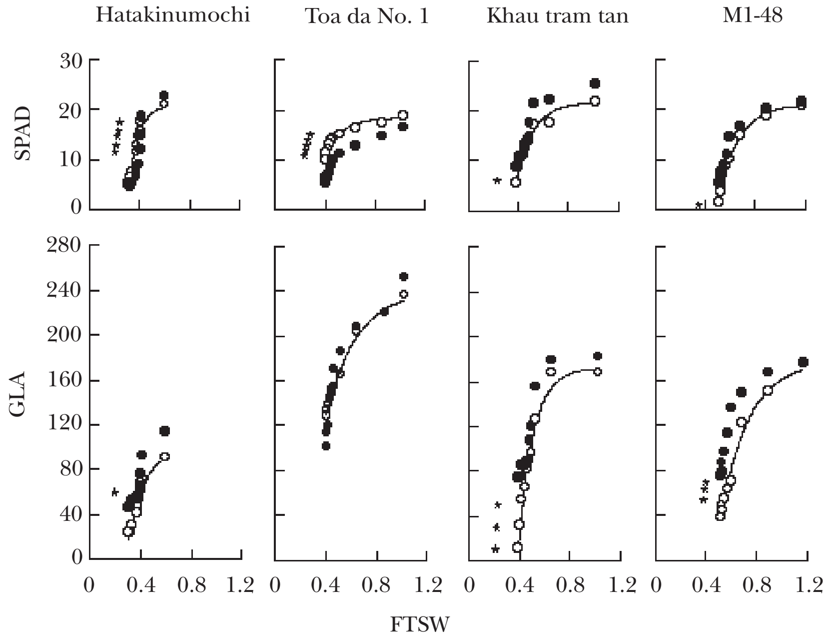

Fig. 2. Correlation of SPAD value and GLA with FTSW in the plants of rice cultivars, Hatakinumochi, Toa da No. 1, Khau tram tan and M1-48 subjected to the desiccated conditions for 40 d during the grain-filling period (open symbol). Values in irrigated plants are also shown by closed symbols. Each value is the mean of three replications. See Fig. 1 for *.

measured interval was accumulated during the soil desiccation treatment.

$$S=\Sigma\{(V_n+V_{n+1})\times I_n/2\} \quad (3)$$

where V_n is the value obtained at n th measurement and I_n is the interval between n th and n+1 th measurements. The interval of days for the integration was four days in the grain-filling period and three days in the seedling period, and that of FTSWs for integration was the difference in FTSW between n th and n+1 th measurements.

(3) Plant and grain dry weight

Whole plant and grain dry weight at maturity in the post-anthesis period were measured. Forty days after heading, the aboveground part of plants in each pot was harvested. The plants were oven-dried at 80°C for 48 h and weighed. The spikelets were separated from each plant, counted and weighed. Ripened spikelets were selected by the gravitational method (1.06×10^{-3} kg m^{-2}) (Matsushima, 1959). The husks were removed, and the oven dried grain weight was determined. The mean single husk weight was determined by dividing the weight differences between the spikelets and grains in the control plants by the spikelet number. The husk weight per hill for each sample was calculated by multiplying the mean husk weight by the number of spikelets, and the grain weight estimated by eliminating the husk weight from the spikelet weight. The harvest index was defined as the ratio of grain to whole aboveground plant dry weight.

4. Experimental design and statistical analysis

The experimental design for the post-anthesis period was completely randomized with three replications. The experimental design in the seedling period was a randomized block with a split-plot arrangement. In the seedling period, the main plots comprised irrigated and soil desiccated treatments each consisting of three pots, with 24 cultivars as subplots, where the treatment was replicated three times with three growth chambers. ANOVA was used to test the significance of differences. The least-squares method was used to fit the curve expressing the relationship between the SPAD value or GLA and FTSW.

Results

1. SPAD value and GLA in desiccated soils in the post-anthesis period

(1) Changes in FTSW, SPAD value and GLA after withholding water

The heading date of the 24 cultivars was 98±12 days (mean±sd of 24 cultivars) after sowing. The earliest date was 3 August in Sensho and the latest date 26 Sept. in M1-48. FTSW in Hatakinumochi, Toa da No. 1, Khau tram tan and M1-48 decreased from around 1.50 to 0.40 within two weeks after heading and was then maintained in the lower level while rates of decrease in FTSW differed slightly among cultivars (Fig. 1). The FTSW at heading for the 24 cultivars was 1.49±0.03 (mean±se). It decreased to 0.39±0.01 two weeks after heading and was maintained at nearly the same level

Table 2. Number of measurements, effect of soil desiccation (+positive and–negative effects), and number of measurements in which significant effect of soil desiccation on SPAD or GLA was observed $SPAD_{D/I}$ and $GLA_{D/I}$ in 24 rice cultivars. See main text for calculation of $SPAD_{D/I}$ and $GLA_{D/I}$. Each value is the mean of three replications.

SPAD						GLA					
Cultivars	No. of observation	The effect of soil desiccation	No. of significant difference	Ratio of integrated rate in D to I		Cultivars	No. of observation	The effect of soil desiccation	No. of significant difference	Ratio of integrated rate in D to I	
				Day base	FTSW base					Day base	FTSW base
Toa da No. 1	10	+	5	1.40	1.21	Terisisazu	10		0	1.13	1.00
Terisisazu	10	+	2	1.35	1.07	Toa da No. 1	10		0	1.00	0.96
IRAT 13	10	+	2	1.26	1.16	Sensho	10	–	1	0.92	0.97
Nep nuong den	10	+	2	1.24	1.13	Nep nuong den	10		0	0.91	0.99
Hatakinumochi	10	+	5	1.23	1.03	Khau mo khao	10	–	1	0.90	0.98
Ma cha	10	+	1	1.15	1.04	IRAT13	10	–	4	0.89	1.03
Khau mo khao	10	–	2	1.13	1.07	Nipponbare	10		0	0.88	0.96
Sensho	10	+	3	1.10	0.91	Ma cha	10	–	1	0.88	0.95
Khau xien pan	10	+	2	1.06	0.99	Khau xien pan	10	–	3	0.84	0.89
Khau ta	10		0	1.04	1.03	IR72	10		0	0.83	0.85
Nipponbare	10		0	1.02	1.00	Khau tram tan	10	–	3	0.81	0.90
Khau nua han	10		0	1.02	1.03	Hatakinumochi	-10	–	1	0.80	0.87
Khau mo	10	–	2	1.00	0.95	Khau nua dam	10	–	5	0.77	0.92
Nep dap	10		0	0.97	0.93	Khau mo	10	–	3	0.76	0.86
IR72	10		0	0.97	0.88	Khau say (vo trung)	10	–	5	0.75	0.89
Khau nu say	10	–	1	0.92	0.97	M1-48	10	–	2	0.73	0.91
Muoi cai	10	–	2	0.90	0.88	Nep dap	10	–	1	0.71	0.87
Tram vai	10		0	0.88	0.86	Khau ta	10	–	8	0.70	0.87
Khau tram tan	10	–	1	0.87	0.86	Khau nua han	10	–	7	0.70	0.95
Khau lech	10		0	0.86	0.94	Muoi cai	10	–	2	0.68	0.84
Nep nuong	10	–	2	0.85	0.98	Tram vai	10	–	8	0.67	0.80
M1-48	10	–	1	0.85	0.94	Khau nu say	10	–	8	0.67	0.90
Khau nua dam	10		0	0.85	0.88	Khau lech	10	–	2	0.64	0.86
Khau say (vo trung)	10	–	5	0.85	0.97	Nep nuong	10	–	5	0.59	0.88
Average			1.6	1.03	0.99	Average			2.9	0.80	0.91
CV(%)				16.30	9.66	CV(%)				15.77	6.33

during the remaining 3 weeks in the desiccated plots, while the FTSW in the irrigated plots was kept about the same level as that at heading.

In all cultivars, the SPAD value and GLA in both the irrigated and desiccated plants started to decrease just after the start of soil desiccation treatment (the results for four cultivars are shown in Fig. 1). The average SPAD value in 24 cultivars was 24.1±0.7 (mean±se) at heading and 3.9±0.6 in the irrigated and 3.7±0.6 desiccated plants at harvest. The average GLA of the 24 cultivars was 194.6±9.4 cm^2 $plant^{-1}$ at heading and 60.6±7.7 in the irrigated and 36.0±6.1 cm^2 $plant^{-1}$ in the desiccated plants at harvest. The SPAD value of desiccated plants in Hatakinumochi and Toa da No. 1 decreased with time less than that in irrigated plants, but in Khau tram tan and M1-48 the SPAD value in desiccated plants decreased more than that in irrigated plants (Fig. 1). GLA in desiccated plants in Hatakinumochi and Toa da No. 1 decreased at almost the same rate as in irrigated plants, but GLA in Khau tram tan and M1-48 decreased much more than that in irrigated plants (Fig. 1).

(2) Changes of SPAD value and GLA by reductions of FTSW

Fig. 2 shows the responses of SPAD value and GLA to FTSW in desiccated and irrigated plants. FTSW in

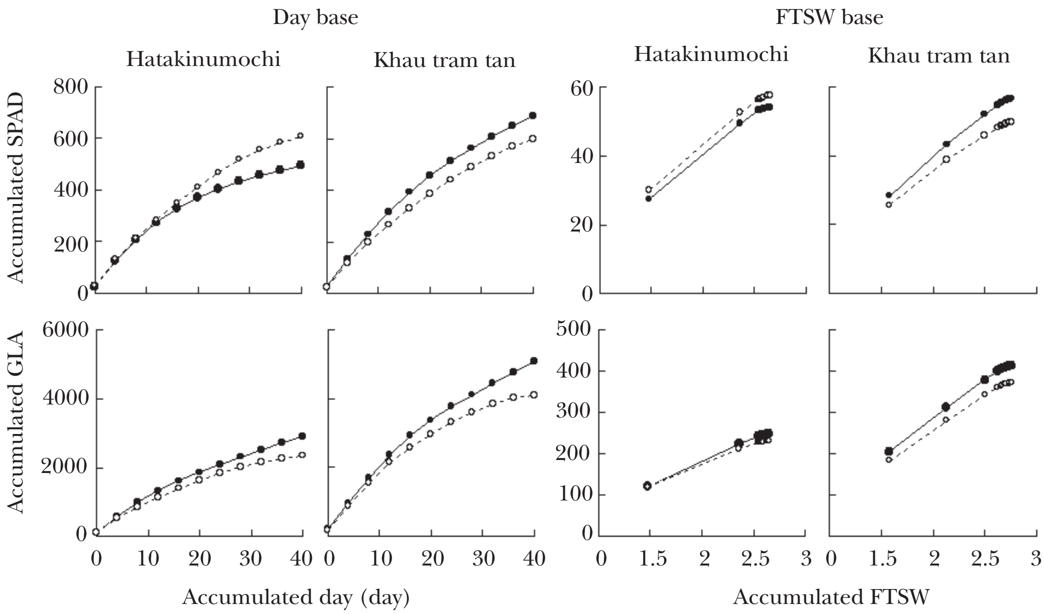

Fig. 3. SPAD value and GLA integrated for days or FTSWs in rice cultivars, Hatakinumochi and Khau tram tan during the grain-filling period in desiccated (open symbol) and in irrigated conditions (closed symbol). See main text for calculation of the integrated value.

Table 3. Correlation coefficient between parameters (Table 2) of capacity for maintenance of SPAD and GLA.

Parameters	$SPAD_{D/I}$ day base	$GLA_{D/I}$ day base	$SPAD_{D/I}$ FTSW base	$GLA_{D/I}$ FTSW base
$SPAD_{D/I}$ day base		0.970***	0.833***	0.758***
$GLA_{D/I}$ day base			0.795***	0.725***
$SPAD_{D/I}$ FTSW base				0.563**
$GLA_{D/I}$ FTSW base				

*, ** and *** indicate significance at $P<0.05$, $P<0.01$ and $P<0.001$, respectively; ns indicates no significance at $P<0.05$ between parameters with ANOVA.

the irrigated plants was maintained over 1.00 through the grain-filling period. The SPAD value and GLA decreases not only by soil desiccation but also by plant aging. The effect of soil desiccation on SPAD value and GLA is indicated by the differences between desiccated and irrigated plants. In the lower ranges of FTSW, the SPAD value and GLA in desiccated plants were significantly higher than or similar to those in irrigated plants in Hatakinumochi and Toa da No. 1, but significantly lower in Khau tram tan and M1-48 (Fig. 2 and Table 2).

(3) Comparison of cultivars in integrated SPAD value and GLA

The SPAD value and GLA plotted against days (Fig. 1) and FTSWs (Fig. 2) were integrated by [eq. (3)] (Fig. 3) and the ratio of integrated value in desiccated to irrigated plants ($SPAD_{D/I}$ and $GLA_{D/I}$) are shown in Table. 2. This table also shows the number of measurements, the significant positive or negative effect of soil desiccation, and the number of measurements in which significant differences in SPAD value or GLA between desiccated and irrigated plants were observed. $SPAD_{D/I}$ calculated by eq.(3) based on day interval (day base) and FTSW interval (FTSW base) varied from 1.40 to 0.85 and 1.21 to 0.86, respectively, and $GLA_{D/I}$ varied from 1.13 to 0.59 and from 1.03 to 0.80, respectively. Variance among cultivars in $SPAD_{D/I}$ and $GLA_{D/I}$ on the FTSW base was smaller than on the day base. Cultivars Terisisazu, Toa da No. 1 and Nep nuong den were ranked high and Khau lech and Nep nuong were ranked low in both $SPAD_{D/I}$ and $GLA_{D/I}$ (Table 2).

(4) Relationships between $SPAD_{D/I}$ and $GLA_{D/I}$

In all cultivars, the correlation coefficient (r) between $SPAD_{D/I}$ and $GLA_{D/I}$ on the day base was higher (0.970), than that on the FTSW base (0.563) (Table 3). The correlation coefficient of $SPAD_{D/I}$ and $GLA_{D/I}$ on the day base with those on the FTSW base was 0.833 and 0.725, respectively. The coefficient of correlation of $SPAD_{D/I}$ and $GLA_{D/I}$ on the day base with $SPAD_{D/I}$ and $GLA_{D/I}$ on the FTSW base was 0.758 and

Table 4. Dry weight of whole plant and rice grain and harvest index in irrigated (I) and desiccated (D) plants in 24 rice cultivars. D/I indicates the ratio of the value in D to that I in plants. Each value is the mean of three replications. Differences between I and D plants in dry weight and harvest index are indicated. See Table 3 for *, **, *** and ns.

Cultivars	Whole plant DW ($g\ plant^{-1}$)		Significant difference	D/I	Brown rice weight ($g\ plant^{-1}$)		Significant difference	D/I	Harvest index		Significant difference	D/I
	I	D			I	D			I	D		
Sensho	13.88	16.15	ns	1.16	5.43	2.71	*	0.50	0.39	0.17	***	0.43
Terisisazu	13.80	15.53	ns	1.13	4.37	2.53	*	0.58	0.32	0.16	ns	0.52
Khau nua han	12.97	13.60	ns	1.05	1.69	1.93	ns	1.14	0.13	0.14	ns	1.09
IR72	19.33	19.54	ns	1.01	6.75	4.18	*	0.62	0.35	0.21	*	0.61
Hatakinumochi	15.21	15.15	ns	1.00	4.09	2.88	ns	0.70	0.27	0.19	ns	0.71
Nep nuong den	12.54	12.23	ns	0.98	1.71	1.43	ns	0.84	0.14	0.12	ns	0.86
Nep dap	17.39	16.83	ns	0.97	1.97	1.54	ns	0.78	0.11	0.09	ns	0.81
Khau tram tan	14.56	13.19	ns	0.91	2.97	2.76	ns	0.93	0.20	0.21	ns	1.03
Toa da No. 1	13.01	11.75	ns	0.90	1.86	1.20	*	0.65	0.14	0.10	ns	0.71
Nipponbare	16.27	14.27	ns	0.88	3.32	2.55	ns	0.77	0.20	0.18	ns	0.88
Tram vai	13.88	11.90	ns	0.86	3.09	2.42	ns	0.78	0.22	0.20	ns	0.91
IRAT13	16.70	14.14	ns	0.85	4.50	3.43	ns	0.76	0.27	0.24	ns	0.90
Khau lech	13.64	11.43	ns	0.84	1.83	1.50	ns	0.82	0.13	0.13	ns	0.97
Khau say (vo trung)	19.14	15.95	ns	0.83	5.07	2.93	*	0.58	0.27	0.18	ns	0.69
Khau mo khao	16.75	13.88	*	0.83	4.16	2.91	*	0.70	0.25	0.21	ns	0.84
Nep nuong	14.00	11.38	ns	0.81	3.59	2.18	**	0.61	0.26	0.19	*	0.75
M1-48	21.10	17.15	ns	0.81	4.29	3.20	ns	0.75	0.20	0.19	ns	0.92
Khau nua dam	14.45	11.45	*	0.79	2.61	2.22	ns	0.85	0.18	0.19	ns	1.07
Muoi cai	21.74	17.20	***	0.79	7.16	5.77	ns	0.81	0.33	0.34	ns	1.02
Khau nu say	17.25	13.29	*	0.77	3.89	3.29	ns	0.85	0.23	0.25	ns	1.10
Khau xien pan	12.90	9.91	**	0.77	2.72	1.44	*	0.53	0.21	0.15	ns	0.69
Khau ta	18.41	13.90	*	0.75	3.21	1.60	*	0.50	0.17	0.12	ns	0.66
Khau mo	18.41	13.14	**	0.71	4.80	2.53	**	0.53	0.26	0.19	ns	0.74
Ma cha	16.19	10.78	**	0.67	3.71	1.35	***	0.36	0.23	0.13	*	0.55
Average				0.88				0.71				0.81
CV (%)				14.34				24.03				23.12

0.795, respectively.

(5) Plant and grain dry weight in desiccated soils

Dry weight of the whole plant (above-ground part) of irrigated plants at harvest ranged between 12.9 and 21.7 g $plant^{-1}$, and the reduction by soil desiccation was from 0% to 33% (Table 4). Grain dry weight in irrigated plants at harvest ranged between 1.7 and 7.2 g $plant^{-1}$, and reduction of grain dry weight by soil desiccation was from 0% to 64%. The harvest index in irrigated plants ranged between 0.11 and 0.39, and the reduction of harvest index by soil desiccation was from 0% to 57%.

The ratios of whole plant and grain dry weights of desiccated plants to those of irrigated plants ($DW_{D/I}$) in Sensho, Terisirazu, Khau nua han, Khau tra han, Hatakinumochi and Nep ruong den were higher, while those in cultivars Khau mo and Ma cha were lower.

(6) Cultivar distribution in $SPAD_{D/I}$, $GLA_{D/I}$ and $DW_{D/I}$

Based on the data in Table 2, all cultivars were classified into each range of the frequency distribution of $SPAD_{D/I}$ and $GLA_{D/I}$ (Figs. 4A, 5A), where the whole range of $GLA_{D/I}$ was less than half that of $SPAD_{D/I}$. The frequency distribution in $SPAD_{D/I}$ was biased within the low range of $SPAD_{D/I}$ (Fig. 4) and that in $GLA_{D/I}$ showed a normal distribution (Fig. 5). The average of $DW_{D/I}$ in all cultivars (Table 4) belonging to each range of $SPAD_{D/I}$ and $GLA_{D/I}$ are shown in Figs. 4 and 5. The average $DW_{D/I}$ in cultivars belonging to higher ranges of $SPAD_{D/I}$ or $GLA_{D/I}$ tended to show higher values than that in cultivars belonging to lower ranges. However, the ratio of D to I in grain dry weight and harvest index was not always high in the higher ranges of $SPAD_{D/I}$ or $GLA_{D/I}$.

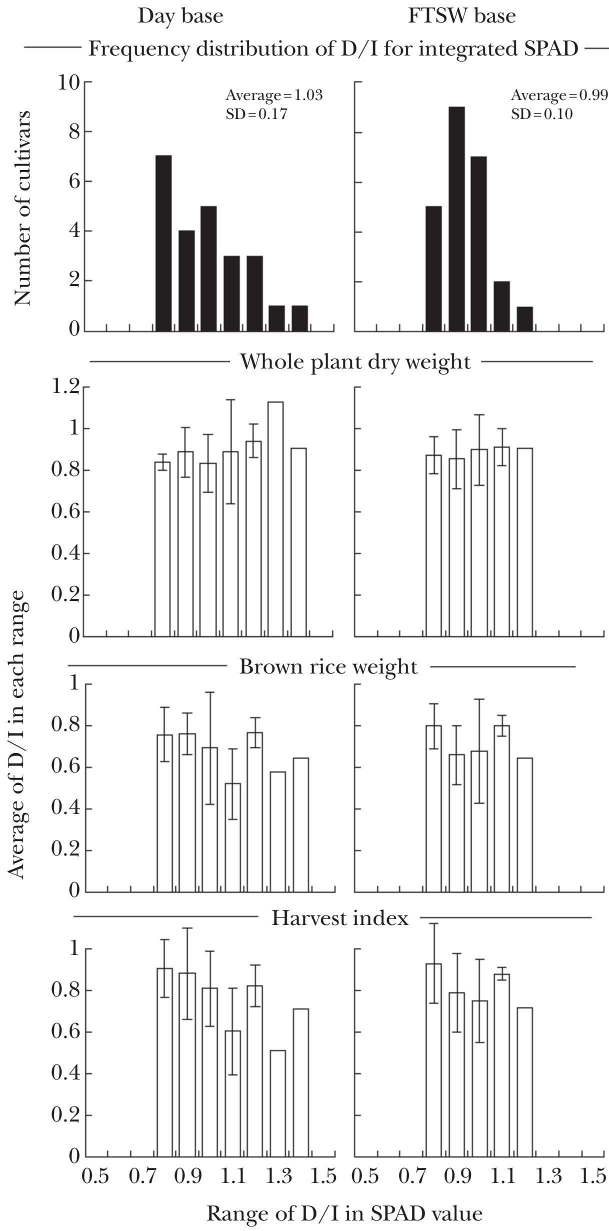

Fig. 4. Frequency distribution of the cultivars showing different $SPAD_{D/I}$ (shown in Table 2) during the grain-filling period in 24 rice cultivars, and frequency distribution of the average ratio of D to I in whole plant dry weight ($DW_{D/I}$), rice grain dry weight, and harvest index in each range of $SPAD_{D/I}$ are shown. Vertical bar indicates standard deviation (sd) of the mean.

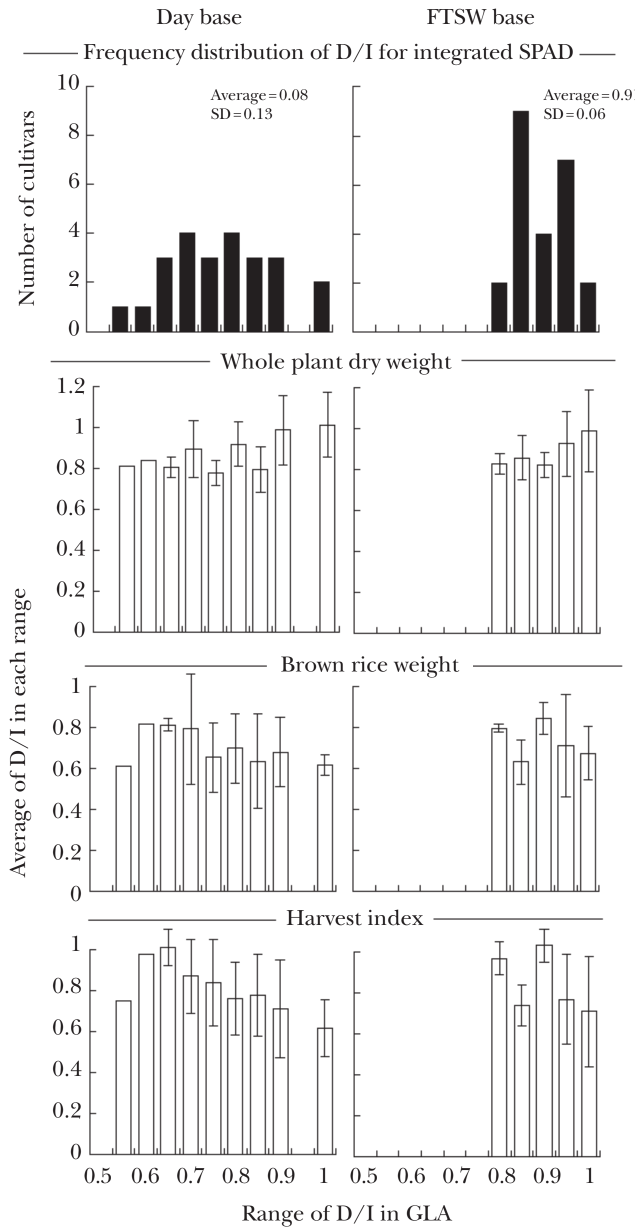

Fig. 5. Frequency distribution of the cultivars showing different $GLA_{D/I}$ (shown in Table 2) during the grain-filling period in 24 rice cultivars, and the frequency distribution of the average ratios of D to I in the whole plant dry weight ($DW_{D/I}$), rice grain dry weight and harvest index in each range of $GLA_{D/I}$ are shown. Vertical bar indicates sd of the mean.

2. SPAD value and GLA in desiccated soils in the seedling period

In seedling plants suffering from desiccated soils for 15 d, the SPAD value was scarcely decreased with a reduction in FTSW, while GLA was reduced in some cultivars (Fig. 6). All cultivars in the seedling period suffered from soil desiccation at the same time in the same pot under a controlled environment and hence the FTSW in the non-irrigated pots was expected to be similar in all cultivars (Fig. 6). The $SPAD_{D/I}$ value and $GLA_{D/I}$ on day and FTSW bases were calculated in the same manner as in the grain-filling period [eq. (3)]. The variation among cultivars in $SPAD_{D/I}$ was smaller than in $GLA_{D/I}$ (Table 5).

3. Correlation of $SPAD_{D/I}$ and $GLA_{D/I}$ in the grain-filling period with those in the seedling period

Correlation of $SPAD_{D/I}$ and $GLA_{D/I}$ in the seedling period (Table 5) with those in the grain-filling (Table 2) period in 24 cultivars was very low (r=–0.01 to 0.06, n=24).

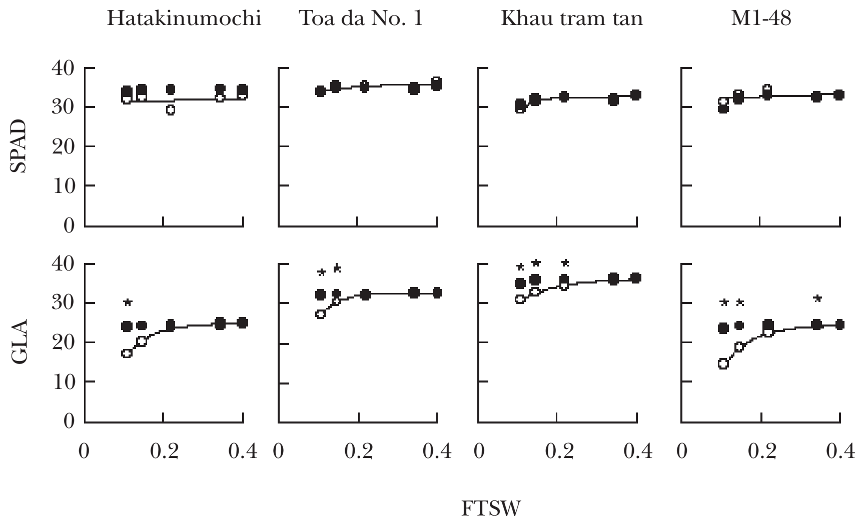

Fig. 6. SPAD value and GLA plotted against FTSW in desiccated and irrigated plants of rice cultivars, Hatakinumochi, Toa da No. 1, Khau tram tan and M1-48. Values in the plants subjected to desiccated conditions for 15 d during the seedling period (open symbol), and in irrigated plants (closed symbol) are shown. See Fig. 1 for *.

Discussion

1. Does stay-green under desiccated soil conditions exist in rice cultivars?

The 24 rice cultivars subjected to soil desiccation in the post-anthesis period showed diverse frequency distribution in $SPAD_{D/I}$ and $GLA_{D/I}$ (Figs. 4, 5). Furthermore, the capacity for maintenance of green leaf color and that of green leaf area seems to be compatible because of higher correlations between $SPAD_{D/I}$ and $GLA_{D/I}$ (Table 3). The changes of FTSW during the grain-filling period were similar in most of the cultivars (Fig. 1) and hence the cultivars we tested would suffer from a similar soil drying cycle, and furthermore, differences in $SPAD_{D/I}$ and $GLA_{D/I}$ among cultivars were observed (Table 2). These results suggest that there is a fairly wide variance in the capacity for maintenance of green leaf color and green leaf areas among the rice cultivars in the grain-filling period under soil desiccated conditions.

Cultivars classified into the higher ranges of the frequency distribution in $SPAD_{D/I}$ and $GLA_{D/I}$ tended to show higher $DW_{D/I}$ (Figs. 4, 5). This suggests that the capacity for maintenance of green leaves can contribute to high dry matter production in rice. Thomas and Howarth (2000) identified two types of stay-green; one is a functional stay-green, which maintains both green leaf area and photosynthetic capacity, and the other is a non-functional stay-green, which maintains only a green leaf area. The functional stay-green has been identified and is a meaningful trait under drought conditions for sorghum (Borrell et al., 2000a, 2000b; Borrell and Hammer, 2000; Mahalakshmi and Bidinger, 2002) and wheat (Christopher et al., 2004). We did not monitor the photosynthetic rate of cultivars, but the higher maintenance of plant dry matter at harvest in higher ranges of $SPAD_{D/I}$ and $GLA_{D/I}$ suggests that the rice cultivars have the functional stay-green trait. Based on the results of our experiments, we classified Terisisazu, Hatakinumochi, Sensho and IRAT13 into japonica rice of Japan or Ivory Coast and Toa da No. 1 and Nep nuong den into indica rice of Vietnam.

The grain weight in cultivars showing a higher $SPAD_{D/I}$, $GLA_{D/I}$ and $DW_{D/I}$ was not always heavy (Figs. 4, 5). This might be because the grain weight is decided not only by mass production but by assimilate distribution into grains. The distribution of assimilate during the grain-filling period should be highly related to the fertilization of spikelets (Kobata and Takami, 1979). Furthermore the contributions of stay-green to fertilization as a key factor for assimilate partition into grains in desiccated soils should be investigated in rice.

2. Is stay-green a stable trait during the whole growing season in rice?

During the seedling period, variation among cultivars in $SPAD_{D/I}$ was very small, while a relatively larger variation was observed in $GLA_{D/I}$ (Table 5). Moreover, the correlation of $SPAD_{D/I}$ and $GLA_{D/I}$ in the seedling period (Table 5) with those in the grain-filling period (Table 2) in the 24 tested cultivars was very poor. In addition, there was a weak to moderate correlation between drought score in the seedling period and grain yield, and this correlation was significant only in particular drought conditions (Patuwan et al., 2004). Therefore, the stay-green trait should differ from seedling survival, which is observed in diverse rice cultivars (O'Toole and Chang, 1979; Ahmad et al., 1987; De Datta et al., 1988; Henderson

Table 5. Number of measurements, effect of soil desiccation (+ positive and – negative effects) and measurements in which significant differences in SPAD and GLAwas observed and $SPAD_{D/I}$ and $GLA_{D/I}$ in 24 rice cultivars. See main text for calculation of $SPAD_{D/I}$ and $GLA_{D/I}$. Each value is the mean of three replications. See Table 3 for note.

SPAD						GLA					
Cultivars	No. of observation	The effect of soil desiccation	No. of significant difference	Ratio of integrated rate in D to I		Cultivars	No. of observation	The effect of soil desiccation	No. of significant difference	Ratio of integrated rate in D to I	
				Day base	FTSW base					Day base	FTSW base
Khau xien pan	5		0	1.06	1.06	Khau mo khao	5	–	1	0.99	0.99
Ma cha	5		0	1.03	1.03	Ma cha	5	–	1	0.98	0.98
Nep nuong den	5		0	1.03	1.03	Khau ta	5		0	0.97	0.98
Muoi cai	5		0	1.02	1.02	Toa da No.1	5	–	2	0.97	0.98
Khau ta	5		0	1.02	1.02	Khau say (vo trung)	5	–	1	0.97	0.98
Nep dap	5		0	1.02	1.02	Khau xien pan	5		0	0.96	0.97
M1-48	5		0	1.02	1.02	Nipponbare	5		2	0.96	0.97
Khau lech	5		0	1.01	1.01	Nep dap	5	–	2	0.96	0.97
Toa da No. 1	5		0	1.01	1.01	Muoi cai	5	–	2	0.96	0.96
IR72	5		0	1.00	1.01	Khau tram tan	5	–	2	0.95	0.96
Khau mo khao	5		0	1.00	1.01	IR72	5	–	3	0.95	0.95
Nipponbare	5		0	1.00	1.00	Khau nua han	5	–	1	0.95	0.96
Khau tram tan	5		0	1.00	1.00	Khau lech	5	–	3	0.95	0.95
Tram vai	5		0	1.00	1.00	Sensho	5	–	2	0.94	0.95
IRAT13	5		0	0.99	0.99	Khau nu say	5	–	3	0.93	0.94
Khau say (vo trung)	5		0	0.99	0.99	Nep nuong den	5	–	3	0.93	0.95
Sensho	5		0	0.99	0.99	Khau mo	5	–	1	0.92	0.94
Terisisazu	5		0	0.99	0.99	Nep nuong	5	–	1	0.92	0.93
Khau nu say	5		0	0.99	0.99	Hatakinumochi	5	–	3	0.92	0.95
Khau nua dam	5		0	0.98	0.98	IRAT13	5	–	3	0.92	0.94
Khau nua han	5		0	0.97	0.97	Tram vai	5	–	1	0.92	0.94
Nep nuong	5		0	0.97	0.97	Terisisazu	5	–	1	0.90	0.92
Khau mo	5		0	0.97	0.96	Khau nua dam	5	–	3	0.89	0.91
Hatakinumochi	5		0	0.93	0.94	M1-48	5	–	3	0.87	0.90
Average			0.0	1.00	1.00	Average			1.7	0.94	0.95
CV(%)				2.52	2.52	CV(%)				3.13	2.40
$LSD_{0.05}$				ns	ns	$LSD_{0.05}$				0.11	ns

et al., 1993). In the seedling period, the dominant growth organs are the leaves and roots, but in the grain-filling period the grain growth is predominant in rice (Kobata and Takami, 1979; Takami et al., 1990). Rice grains have a high requirement for assimilate and nitrogen from straws, and hence leaves would suffer from severe nutrient deficits under desiccated soil conditions during the grain-filling period. Stay-green would be affected not only by an individual capacity for maintenance of green leaves but also by the sink function in other plant organs such as grains.

Our results suggested that the stay-green trait exists in rice cultivars in drought-prone areas such as Vietnam. The stay-green trait in the terminal growth stage would differ from that in the seedling stage. Furthermore, the availability and evidence of stay-green in rice in desiccated soils should be investigated through measurements of assimilation, photosynthetic capacity, yield and water-use efficiency under growth conditions equivalent to the crop stand.

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