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

Tien Ba Hoang and Tohru Kobata

(Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan)

**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 (SPADD/I or GLAD/I, respectively). The SPADD/I and GLAD/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. SPADD/I and GLAD/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-
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 35° 28' N, 133° 02' E in Japan.

1. Experiment for the post-anthesis period

Germinated seeds of the 24 cultivars were sown in seedling beds (0.60 × 0.30 × 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

| Cultivar No. | Cultivar name | Ecotypes | Improved/Local | Origin        |
|--------------|---------------|----------|----------------|---------------|
| 1            | Nipponbare    | Lowland  | Improved       | Japan         |
| 2            | Hatakinumochi | Upland   | Improved       | Japan         |
| 3            | IRAT13        | Upland   | Improved       | Ivory Coast   |
| 4            | Terissazu     | Upland   | Local          | Japan         |
| 5            | Semaho        | Upland   | Local          | Japan         |

| Cultivar No. | Cultivar name | Ecotypes | Improved/Local | Origin        |
|--------------|---------------|----------|----------------|---------------|
| 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       |
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 × 1.80 × 2.20 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 Exspec 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 105°C for 48 h and weighed to record dry soil weight (DSW, g pot⁻¹). The soil water content (SWC, H₂O g dry soil g⁻¹) was determined by subtracting DSW from FSW (SWC=FSW/DSW).

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

\[ FTSW = \frac{(TSW - TSW_0)}{(TSW_{max} - TSW_0)} \]  (1)

where TSW is the observed total soil water weight (g pot⁻¹), TSW₀ 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, TSW₀ 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
−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, cm²) 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:

\[
GLA = GLA_0 \times (1 - \text{SLAR}/100)
\]

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 \(\text{SLAR}\) 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. \(\text{SLAR}\) 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 (SPADD/1 and GLADD/1) was used to evaluate the capacity for maintenance of SPAD value and GLA on desiccated soils. The integration value (S) was obtained from the trapezoid area of SPAD value or GLA in every
measured interval was accumulated during the soil desiccation treatment.

\[ S = \sum \left( V_n + V_{n+1} \right) \times I_n/2 \]  

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 \( (1.06 \times 10^{-3} \text{ kg m}^{-2}) \) (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

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.
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 cm² plant⁻¹ at heading and 60.6 ± 7.7 in the irrigated and 36.0 ± 6.1 cm² 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).

(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

| 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 |
|-----------------|--------------------|-------------------------------|------------------------------|-----------------------------------|----------|------------|
| Toa da No. 1    | 10                 | + 5                           | 1.40 1.21                    | Terissazu                          | 10       | 0          |
| Terissazu       | 10                 | + 2                           | 1.35 1.07                    | Toa da No. 1                       | 10       | 0          |
| IRAT 13         | 10                 | + 2                           | 1.26 1.16                    | Sensho                            | 10       | – 1        |
| Nep nuong den   | 10                 | + 2                           | 1.24 1.13                    | Nep nuong den                      | 10       | 0          |
| Hatakinumochi   | 10                 | + 5                           | 1.23 1.03                    | Khau mo khoa                       | 10       | – 1        |
| Ma cha          | 10                 | + 1                           | 1.15 1.04                    | IRAT13                            | 10       | – 4        |
| Khau mo khoa    | 10                 | – 2                           | 1.13 1.07                    | Nipponbare                        | 10       | 0          |
| Sensho          | 10                 | + 3                           | 1.10 0.91                    | Ma cha                            | 10       | – 1        |
| Khau xien pan   | 10                 | + 2                           | 1.06 0.99                    | Khau xien pan                      | 10       | – 3        |
| Khau ta         | 10                 | 0                             | 1.04 1.03                    | IR72                              | 10       | 0          |
| Nipponbare      | 10                 | 0                             | 1.02 1.00                    | Khau tram tan                      | 10       | – 3        |
| Khau nu han     | 10                 | 0                             | 1.02 1.03                    | Hatakinumochi                      | 10       | – 1        |
| Khau mo         | 10                 | – 2                           | 1.00 0.95                    | Khau nu dam                       | 10       | – 5        |
| Nep dap         | 10                 | 0                             | 0.97 0.93                    | Khau mo                            | 10       | – 3        |
| IR72            | 10                 | 0                             | 0.97 0.88                    | Khau say (vo trung)                | 10       | – 5        |
| Khau nu say     | 10                 | – 1                           | 0.92 0.97                    | MI-48                             | 10       | – 2        |
| Muoi cai        | 10                 | – 2                           | 0.90 0.88                    | Nep dap                            | 10       | – 1        |
| Tram vai        | 10                 | 0                             | 0.88 0.86                    | Khau ta                            | 10       | – 8        |
| Khau tram tan   | 10                 | – 1                           | 0.87 0.86                    | Khau nu han                       | 10       | – 7        |
| Khau lech       | 10                 | 0                             | 0.86 0.94                    | Muoi cai                          | 10       | – 2        |
| Nep nuong       | 10                 | – 2                           | 0.85 0.98                    | Tram vai                          | 10       | – 8        |
| M1-48           | 10                 | – 1                           | 0.85 0.94                    | Khau nu say                       | 10       | – 8        |
| Khau nu dam     | 10                 | 0                             | 0.85 0.88                    | Khau lech                         | 10       | – 2        |
| Khau say (vo trung) | 10     | – 5                           | 0.85 0.97                    | Nep nuong                         | 10       | – 5        |
| Average         | 1.6                | 1.03 0.99                     | CV(%) 16.30 9.66             | Average                           | 2.9      | 0.80 0.91  
| CV(%)           |                     |                               |                               |                     | 15.77    | 6.33       |
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<sub>I</sub>/SPAD<sub>D</sub>) and GLA<sub>I</sub>/GLA<sub>D</sub>) 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<sub>I</sub> 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<sub>I</sub> varied from 1.13 to 0.59 and from 1.05 to 0.80, respectively. Variance among cultivars in SPAD<sub>I</sub> and GLA<sub>I</sub> 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<sub>I</sub> and GLA<sub>I</sub> (Table 2).

(4) Relationships between SPAD<sub>I</sub> and GLA<sub>I</sub>

In all cultivars, the correlation coefficient (r) between SPAD<sub>I</sub> and GLA<sub>I</sub> on the day base was higher (0.970) than that on the FTSW base (0.563) (Table 3). The correlation coefficient of SPAD<sub>I</sub> and GLA<sub>I</sub> on the day base was 0.833 and 0.725, respectively. The coefficient of correlation of SPAD<sub>I</sub> and GLA<sub>I</sub> on the day base with those on the FTSW base was 0.853 and 0.725, respectively. The coefficient of correlation of SPAD<sub>I</sub> and GLA<sub>I</sub> on the day base with SPAD<sub>I</sub> and GLA<sub>I</sub> on the FTSW base was 0.758 and

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

| Parameters       | SPAD<sub>I</sub> | GLA<sub>I</sub> | SPAD<sub>D</sub> | GLA<sub>D</sub> |
|------------------|-----------------|----------------|-----------------|----------------|
| SPAD<sub>I</sub> | 0.970***        | 0.833***       | 0.758***        |                |
| GLA<sub>I</sub>  | 0.795***        | 0.725***       | 0.563***        |                |

*, ** 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.
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 nuong den were higher, while those in cultivars Khau mo and Ma cha were lower.

| Cultivars            | Whole plant DW (g plant$^{-1}$) | Significant difference I D | Brown rice weight (g plant$^{-1}$) | Significant difference I D | Harvest index | Significant difference I D |
|----------------------|---------------------------------|-----------------------------|------------------------------------|-----------------------------|---------------|-----------------------------|
| Sensho               | 13.88 16.15 ns                  | 1.16 3.43 2.71 *            | 0.50 0.39 0.17 ***                 | 0.43                        |               |                             |
| Terisirazu           | 13.80 13.53 ns                  | 1.13 4.37 2.53 *            | 0.58 0.32 0.16 ns                 | 0.32                        |               |                             |
| 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 3.45 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 khoa         | 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                        |               |                             |
| MI-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           | 0.83 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                             |                             |               |                             |

(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 nuong 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 the frequency distribution (Fig. 4). 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.
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_{day} value and GLA_{day} 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_{day} was smaller than in GLA_{day} (Table 5).

3. Correlation of SPAD_{day} and GLA_{day} in the grain-filling period with those in the seedling period

Correlation of SPAD_{day} and GLA_{day} 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, \(n = 24\)).
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 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...
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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Table 5. Number of measurements, effect of soil desiccation (+ positive and – negative effects) and measurements in which significant differences in SPAD and GLA were 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.

| 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 |
|-------------------|--------------------|-------------------------------|--------------------------------|-----------------------------------|-------------------|--------------------|-------------------------------|-----------------------------------|-----------------------------------|
| Khau xien pan     | 5                  | 0                             | 1.06                           | 1.06                              | Khau mo khao      | 5                  | −                            | 1                                | 0.99                             |
| Ma cha            | 5                  | 0                             | 1.03                           | 1.03                              | Ma cha            | 5                  | −                            | 1                                | 0.98                             |
| Nep nuong den     | 5                  | 0                             | 1.03                           | 1.03                              | Khau ta           | 5                  | −                            | 0                                | 0.97                             |
| Muoi cai          | 5                  | 0                             | 1.02                           | 1.02                              | Toa da No.1       | 5                  | −                            | 2                                | 0.97                             |
| Khau ta           | 5                  | 0                             | 1.02                           | 1.02                              | Khau say (vo trung) | 5                  | −                            | 1                                | 0.97                             |
| Nep dap           | 5                  | 0                             | 1.02                           | 1.02                              | Khau xien pan     | 5                  | 0                            |                                 | 0.96                             |
| MI-48             | 5                  | 0                             | 1.02                           | 1.02                              | Nipponbare        | 5                  | −                            | 2                                | 0.96                             |
| Khau lech         | 5                  | 0                             | 1.01                           | 1.01                              | Nipponbare        | 5                  | 2                            | 0.96                             |
| Nep dap           | 5                  | 0                             | 1.01                           | 1.01                              | Khau ta           | 5                  | −                            | 2                                | 0.96                             |
| Toa da No.1       | 5                  | 0                             | 1.01                           | 1.01                              | Muoi cai          | 5                  | −                            | 2                                | 0.96                             |
| IR72              | 5                  | 0                             | 1.00                           | 1.00                              | Khau tran tan     | 5                  | −                            | 2                                | 0.95                             |
| Khau mo khao      | 5                  | 0                             | 1.00                           | 1.00                              | IR72              | 5                  | −                            | 3                                | 0.95                             |
| Nipponbare        | 5                  | 0                             | 1.00                           | 1.00                              | Khau nua han      | 5                  | −                            | 1                                | 0.95                             |
| Khau tran tan     | 5                  | 0                             | 1.00                           | 1.00                              | Khau lech         | 5                  | −                            | 3                                | 0.95                             |
| T tram            | 5                  | 0                             | 1.00                           | 1.00                              | T tram            | 5                  | −                            | 2                                | 0.94                             |
| IR713             | 5                  | 0                             | 0.99                           | 0.99                              | Khau nu say       | 5                  | −                            | 3                                | 0.93                             |
| Khau say (vo trung) | 5                | 0                             | 0.99                           | 0.99                              | Nep nuong den     | 5                  | −                            | 3                                | 0.93                             |
| Senso             | 5                  | 0                             | 0.99                           | 0.99                              | Khau mo           | 5                  | −                            | 1                                | 0.92                             |
| Terrisazu         | 5                  | 0                             | 0.99                           | 0.99                              | Nep nuong         | 5                  | −                            | 1                                | 0.92                             |
| Khau nu say       | 5                  | 0                             | 0.99                           | 0.99                              | Hatakinnuochi     | 5                  | −                            | 3                                | 0.92                             |
| Khau nua dam      | 5                  | 0                             | 0.98                           | 0.98                              | IRAT13            | 5                  | −                            | 3                                | 0.92                             |
| Khau nua han      | 5                  | 0                             | 0.97                           | 0.97                              | Tram vay          | 5                  | −                            | 1                                | 0.92                             |
| Nep nuong         | 5                  | 0                             | 0.97                           | 0.97                              | Terrisazu         | 5                  | −                            | 1                                | 0.90                             |
| Khau mo           | 5                  | 0                             | 0.97                           | 0.97                              | Khau nua dam      | 5                  | −                            | 3                                | 0.89                             |
| Hatakinnuochi     | 5                  | 0                             | 0.93                           | 0.94                              | MI-48             | 5                  | −                            | 3                                | 0.87                             |
| Average           | 0.0                | 1.00                          | 1.00                           | 1.00                              | Average           | 1.7                | 0.94                        | 0.95                             |
| CV(%)             | 2.52               | ns                            | ns                             | ns                                | CV(%)             | 3.15               | 2.40                        | ns                                |
| LSD<sub>0.05</sub> | ns                 | ns                            | ns                             | ns                                | LSD<sub>0.05</sub>| 0.11               | ns                          | ns                                |
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