

Effect of Various Intensities of Drought Stress on $\delta^{13}\text{C}$ Variation among Plant Organs in Rice: Comparison of Two Cultivars

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Abstract

The $\delta^{13}\text{C}$ value is widely used to assess the effects of drought on water status in plants. However, there is little information regarding the $\delta^{13}\text{C}$ signature in different organs of rice. We conducted a field study to examine whether the $\delta^{13}\text{C}$ among different plant parts would be affected by the intensities of drought, and to evaluate genotypic variation in $\delta^{13}\text{C}$ fluctuation among plant parts affected by drought intensities. Two cultivars, “Nipponbare” (*Oryza sativa* ssp. *japonica*) and “Kasalath” (*O. sativa* ssp. *indica*), were grown in the field with a line-source sprinkler system. The $\delta^{13}\text{C}$ values of panicles, flag leaves, straws, culms, and roots were measured from plant samples. The $\delta^{13}\text{C}$ value increased as drought stress increased, especially in the panicles and roots. “Nipponbare” showed higher values of $\delta^{13}\text{C}$ than “Kasalath” under the well-watered and mild drought stress conditions, but there was no significant difference between the genotypes in the $\delta^{13}\text{C}$ value under the severe drought stress condition. The variation in $\delta^{13}\text{C}$ value among different plant parts was also increased with increasing drought stress. In contrast, these variations were small under well-watered conditions. Furthermore, there was much greater variation in the $\delta^{13}\text{C}$ value among different plant parts in “Kasalath” than in “Nipponbare” when the plants were grown under drought stress conditions. A significant negative relationship was observed between the $\delta^{13}\text{C}$ value of panicles and shoot dry matter production, suggesting that the $\delta^{13}\text{C}$ value of panicles may be the best indicator of plant water status in rice.

Keywords

$\delta^{13}\text{C}$, Drought, Plant Parts, Rice

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1. Introduction

Drought is the major limitation to crop yields. The stable carbon isotope ratio ($\delta^{13}\text{C}$) or carbon isotope discrimination (CID), which corrects for variations in source air ^{13}C in plants, is a useful indicator to evaluate the impact of water stress on crop performance and to estimate water use efficiency (WUE) [1] [2]. It has been suggested by some authors that $\delta^{13}\text{C}$ or CID could be an effective selection criterion for grain yield under drought [3] [4]. In general, water stress increased $\delta^{13}\text{C}$ and decreased CID values in rice [5]-[8] and other crops, such as wheat [4] [9], barley [10], chickpea [11] and soybean [12]. Genotypic variation has been reported for $\delta^{13}\text{C}$ or CID values in rice; the genotypes for the *japonica* subspecies showed higher $\delta^{13}\text{C}$ values or lower CID values than the *indica* genotypes did [13]-[16]. Kondo *et al.* [5] also reported that CID values varied less in response to water regimes than they did between different genotypes in pot experiments. However, the impacts of the different intensities of drought stress may differ among different genotypes in the field. Therefore, special attention is required to improve understanding of genetic responses of $\delta^{13}\text{C}$ under various intensities of drought, which is considered to occur in the real field.

It is well known that significant differences in $\delta^{13}\text{C}$ may exist among different organs with the lower value of $\delta^{13}\text{C}$ in assimilating organs, such as leaves [17] [18]. Although understanding the $\delta^{13}\text{C}$ fluctuation among organs should be important, most of the studies that have been conducted measured $\delta^{13}\text{C}$ of a specific organ or of the whole plant, rather than comparing its value between organs [7] [8] [14]-[16]. Recently, much attention has been focused on the differences in $\delta^{13}\text{C}$ between plant organs in relation to carbon metabolism [19]-[21], but information on $\delta^{13}\text{C}$ in different organs is not well documented in rice. In addition, the effect of soil water conditions on the variation of ^{13}C among plant organs has not been examined in the field.

We previously used chromosome segment substitution lines derived from “Nipponbare” (*japonica*) and “Kasalath” (*indica*) crosses, and found that plant response in their shoot and root growth to various intensities of drought stress was different among genotypes under field conditions [22]. In that study, significant genotypic difference in dry matter production was observed among parent genotypes under drought stress conditions. “Kasalath” produced more shoot dry matter than “Nipponbare” through the greater photosynthetic rate and stomatal conductance due to the greater root development especially under mild drought stress conditions.

Thus, we hypothesized that the differences in $\delta^{13}\text{C}$ values among different plant parts might be affected by drought intensities and there might be genotypic variation in $\delta^{13}\text{C}$ fluctuation among plant parts affected by drought intensities. To examine these hypotheses, we conducted field experiment using “Nipponbare” and “Kasalath”.

2. Material and Methods

2.1. Experimental Design

The field experiment was conducted at the experimental farm at Nagoya University, Nagoya, Japan (lat. 35°6'42"N, long. 137°4'57"E) during the summer, 2006. “Nipponbare” (*japonica*) and “Kasalath” (*indica*), which differed in their shoot growth and root development in our previous study [22], were grown in a water-tight experimental bed with a line source sprinkler system under a rain-out shelter to create a gradient in soil moisture, previously described [22].

Twenty-five-day-old seedlings of each cultivar were transplanted perpendicular to the water pipe so that they received different amounts of water. Each genotype was replicated for three rows along a 1.8 m row and spaced within a 45 × 20 cm area. The placements were randomized. Each row contained eight plants of a given genotype that received different amounts of water from the line source; the closest one received the most and the furthest one the least amount of water. The fields were kept well-watered by sprinkler irrigation for one week after transplanting during the period of establishment, after which drought stress was imposed. The soil moisture content (SMC) at a depth of 10 cm at the base of each plant in every line was measured by using Hydrosense (Decagon, Utah, USA) in % v/v, and then converted into the value in % w/w. **Figure 1** shows that the line source sprinkler system successfully created and maintained a soil moisture gradient ranging from 5% to 40% w/w of SMC, which is equivalent to a water potential of -0.163 MPa to 0 MPa.

2.2. Measurements of Gas Exchange

Several physiological traits—photosynthetic rate (Pn), transpiration rate, and stomatal conductance (gs)—were

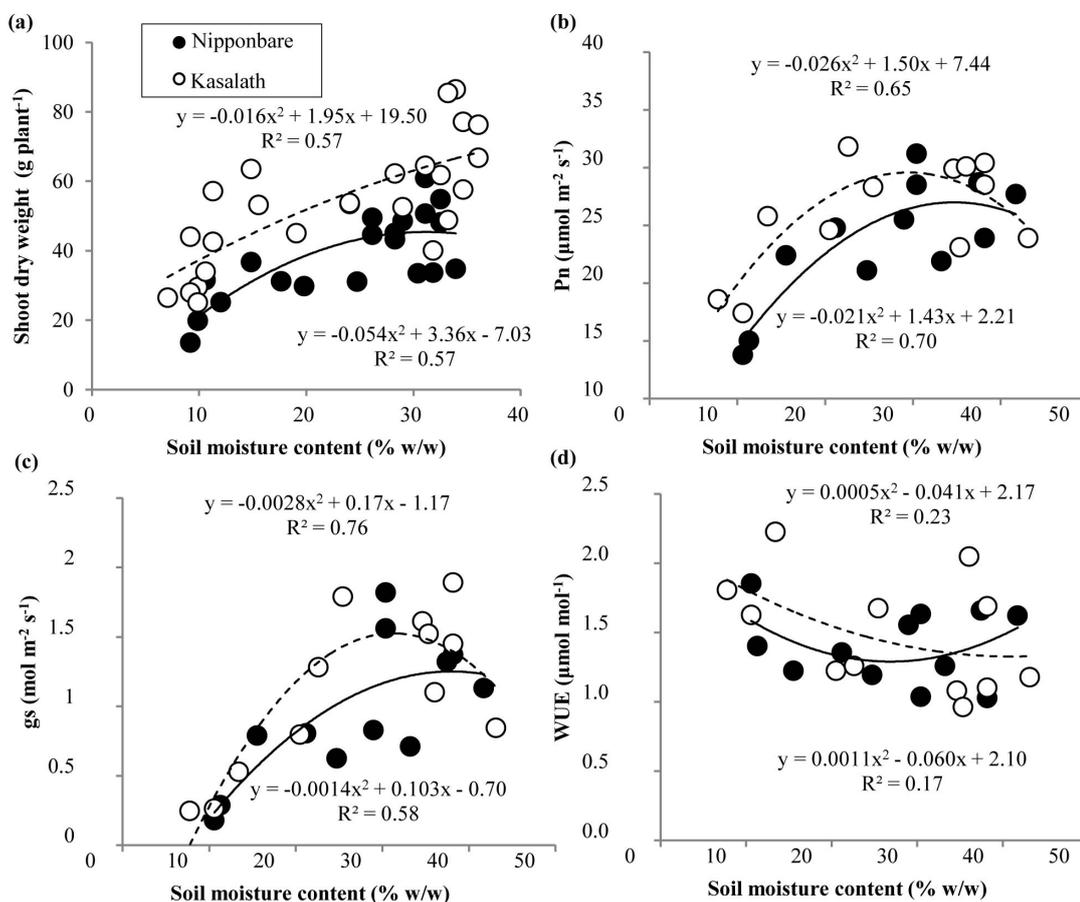


Figure 1. Relationships between shoot dry weight (a), photosynthetic rate (Pn) (b), stomatal conductance (gs) (c), and water use efficiency (WUE) (d) and soil moisture content for “Nipponbare” and “Kasalath”.

measured with a portable photosynthesis analyzer (LI-6400, LI-COR, USA) in the morning at 56 - 58 days after transplanting.

2.3. Plant Sampling and Analysis

Plants were harvested 70 days after transplanting. Harvested plants were separated into panicles (above the panicle base), flag leaves, straws (aboveground parts without panicles, flag leaves and culms), culms (5 cm long from the ground surface), and roots. All samples were wrapped with aluminum foil and immediately frozen in liquid nitrogen. Plant samples were oven dried at 70°C for two days and the dry weights were measured. Dried samples were milled into a fine powder using a sample mill (MF10, IKA, Germany). The $\delta^{13}\text{C}$ values of samples were analyzed by an isotope mass spectrometer (Finnigan MAT GmbH, Germany). The results of $\delta^{13}\text{C}$ analysis were expressed in per mil using the following equation:

$$\delta^{13}\text{C} = \left(R_{\text{sample}} / R_{\text{standard}} - 1 \right) \times 1000$$

where R refers to $^{13}\text{C}/^{12}\text{C}$ and standard refers to the international Pee Dee Belemnite standard (a fossil Belemnite from the Pee Dee formation in South Carolina). Measurements were performed with three repetitions for each sample.

2.4. Statistical Analysis

Fisher’s Least Significant Difference (LSD) test was used for mean comparison, and differences were considered significant at $p < 0.05$. The relationship between shoot dry weight and $\delta^{13}\text{C}$ values in different plant parts

were determined using regression analysis.

3. Results

Both “Nipponbare” and “Kasalath” apparently reduced their production of shoot dry matter with increasing drought stress. “Kasalath” showed higher shoot dry weight than “Nipponbare” at any SMC (Figure 1(a)). Similar trends were observed in Pn and gs with reduction of those values for both genotypes as drought stress increased (Figure 1(b), Figure 1(c)). However, there was no effect of drought stress on WUE for either cultivar (Figure 1(d)).

Figure 2 shows the effects of various SMC on the $\delta^{13}\text{C}$ in different plant parts for “Nipponbare” and “Kasalath”. The $\delta^{13}\text{C}$ value of the shoot tended to increase with increasing drought stress, and the variations in $\delta^{13}\text{C}$ in different plant parts were more pronounced as drought intensified in both “Nipponbare” and “Kasalath”. The effect of drought stress on $\delta^{13}\text{C}$ fluctuation among plant parts was greater in “Kasalath” than in “Nipponbare”. In “Kasalath”, the $\delta^{13}\text{C}$ value among plant parts ranged from -26.83% (panicle) to -30.78% (flag leaf), whereas in “Nipponbare” it ranged from -27.09% (panicle) to -29.69% (straw). Thus “Kasalath” showed 1.5 times larger fluctuation in ^{13}C due to drought stress as compared with “Nipponbare”.

To examine the effects of various intensities of drought stress on the $\delta^{13}\text{C}$ value, the SMC was divided into the following three categories: the well-watered ($>30\%$ w/w of SMC), the mild drought stress ($15\% - 30\%$ w/w of SMC) and the severe drought stress ($<15\%$ w/w of SMC) [22] in Figure 3. At the well-watered, the $\delta^{13}\text{C}$ value of plant parts ranged within 0.6% from -29.7% (straw) to -29.1% (culm) in “Nipponbare”, and within 0.5% from -31.7% (panicle) to -31.2% (culm) in “Kasalath”. Genotypic variation was observed in $\delta^{13}\text{C}$ with significantly higher $\delta^{13}\text{C}$ value in “Nipponbare” than in “Kasalath” at well-watered condition. At the mild drought stress, the $\delta^{13}\text{C}$ value of plant parts ranged within 0.6% from -29.4% (flag leaf) to -28.8% (culm) in “Nipponbare”, and within 1.2% from -31.0% (flag leaf) to -29.8% (culm) in “Kasalath”. The $\delta^{13}\text{C}$ value was also significantly higher for “Nipponbare” than for “Kasalath” in any of plant parts except culms at mild drought stress condition. At the severe drought stress, the $\delta^{13}\text{C}$ value of plant parts ranged within 0.9% from -28.7% (straw) to -27.8% (panicle) in “Nipponbare”, and showed greater variation of 3.0% , ranging from -30.2% (flag leaf) to -27.2% (panicle) in “Kasalath”. Therefore, we confirmed statistically that drought effect on $\delta^{13}\text{C}$ fluctuation among plant parts was greater in “Kasalath” than in “Nipponbare”. In contrast, the $\delta^{13}\text{C}$ value did not significantly vary with the genotype under severe drought stress condition. Among the plant parts, panicles showed the greatest change in $\delta^{13}\text{C}$ induced by drought stress in both cultivars. In “Nipponbare”, the $\delta^{13}\text{C}$ values of panicles and roots significantly increased in severe drought stress conditions over the values in well-watered conditions, but there was no significant difference in the $\delta^{13}\text{C}$ value of panicles and roots between mild drought and well-watered conditions. However, “Kasalath” showed a significantly higher $\delta^{13}\text{C}$ value in panicles and roots in response not only to severe stress but also to mild drought stress. In contrast, the $\delta^{13}\text{C}$ values in flag leaves did not significantly increase in response to drought in either genotype. On the other hand, the effects of soil moisture on the $\delta^{13}\text{C}$ value of culm and straw were significant only in “Kasalath”.

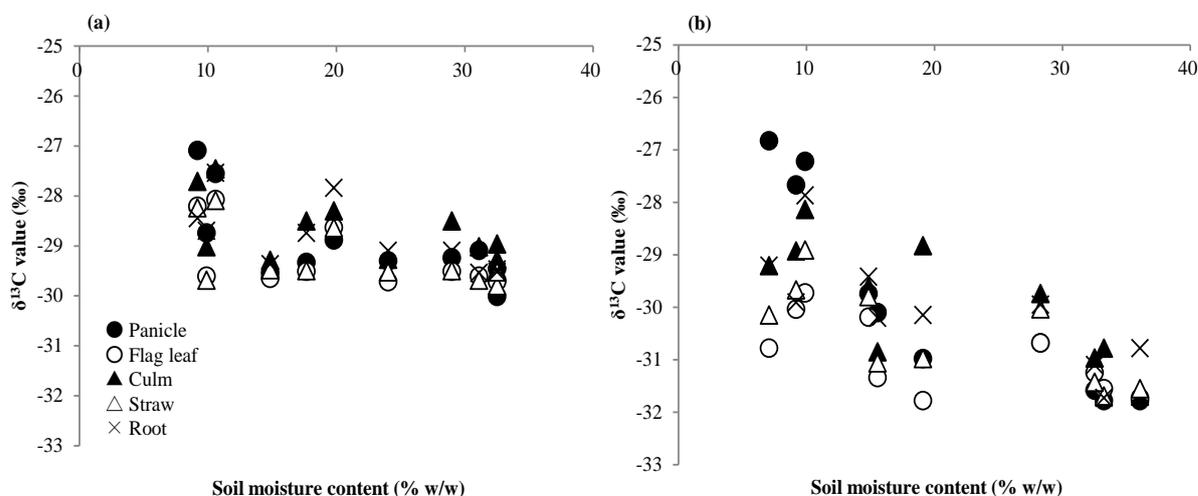


Figure 2. Relationships between $\delta^{13}\text{C}$ values of plant parts and soil moisture content for “Nipponbare” (a) and “Kasalath” (b).

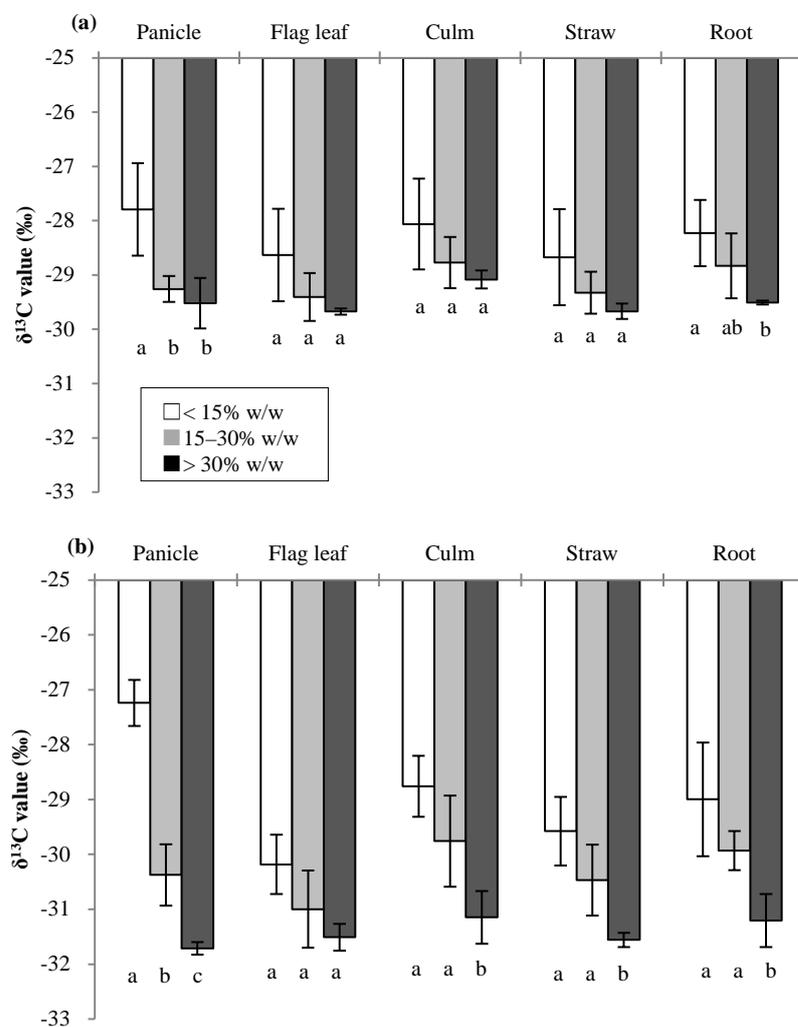


Figure 3. Differences in $\delta^{13}\text{C}$ values among plant parts under well-watered (>30% w/w of SMC), mild drought (15% - 30% w/w of SMC), and severe drought (<15% w/w of SMC) conditions for “Nipponbare” (a) and “Kasalath” (b). Within the same plant parts, values followed by the same letter genotype are not significantly different at the 5% level by Fisher’s LSD test. between ^{13}C values of plant parts and soil moisture content for “Nipponbare” (a) and “Kasalath” (b).

Correlation coefficient among shoot dry weight and $\delta^{13}\text{C}$ values of plant parts is shown in **Table 1**. The $\delta^{13}\text{C}$ values of panicles and roots were significantly correlated with shoot dry matter production in both cultivars and highest value of correlation coefficient was found between $\delta^{13}\text{C}$ in panicles and shoot dry weight. In contrast, there was no correlation between the $\delta^{13}\text{C}$ in flag leaf and shoot dry weight in either genotype. The $\delta^{13}\text{C}$ values of culm and straw were significantly correlated with dry matter production in “Kasalath”. In addition, correlation coefficients between $\delta^{13}\text{C}$ value and shoot dry matter production were higher in “Kasalath” than in “Nipponbare” in any of plant parts except flag leaves.

4. Discussion

As shown in **Figure 1**, the drought-induced decrease in biomass production was largely associated with reduced Pn in both cultivars. This decrease could be explained by reductions in gs. On the other hand, WUE remained relatively stable at all levels of SMC, and WUE was not highly correlated with shoot dry matter production in this study (data not shown). Water uptake capacity may be more significant than WUE in determining biomass production under drought conditions, as reported by previous studies [23] [24].

Table 1. Correlation coefficient among shoot dry weight and $\delta^{13}\text{C}$ values of plant parts.

Shoot dry weight	$\delta^{13}\text{C}$ value				
	Panicle	Flag leaf	Culm	Straw	Root
“Nipponbare”	-0.68*	-0.61	-0.57	-0.60	-0.67*
“Kasalath”	-0.83**	-0.61	-0.80**	-0.77**	-0.76*

* and ** indicate significant correlation at the 5% and 1% level, respectively.

The general trend of increasing ^{13}C in the shoot with drought stress found in the present study agrees with the results of previous studies on rice [5]-[8]. A tendency for higher values of $\delta^{13}\text{C}$ (lower CID) to appear in the *jaпонica* genotype than in the *indica* genotype shown in the present results also was consistent with previous reports [13]-[16]. Under the well-watered and mild drought stress conditions, higher $\delta^{13}\text{C}$ values of the shoot in “Nipponbare” indicate that “Nipponbare” assimilated CO_2 more enriched in ^{13}C than “Kasalath”, and this accords with that Pn and gs in Nipponbare was lower than Kasalath. However, such genotypic variation in $\delta^{13}\text{C}$ was not observed in any of the individual plant parts under the severe drought stress condition in this study (Figure 3). This finding indicates that the cultivar effects on $\delta^{13}\text{C}$ values become less pronounced as drought stress intensifies. Therefore, the differences in $\delta^{13}\text{C}$ value between drought stress and non-stressed conditions are critical.

There was a general trend toward enrichment in ^{13}C in grains and roots, compared with flag leaves and stems, in previous studies on rice [5] [6] [13]. In this study, a similar trend in $\delta^{13}\text{C}$ variation among different plant parts was also observed, but such variations were affected by SMC and by genotypes (Figure 2, Figure 3). Drought had stronger effects on $\delta^{13}\text{C}$ levels in panicles and roots than in other plant parts (Figure 3). In the present study the panicles and roots were the prominent sink for photo-assimilated carbon. The translocation of photo-assimilated carbohydrates from leaves to sink organs may be largely prevented by drought stress. The difference in carbon isotope composition among plant parts is related to the differences in fractionation processes during transport, the synthesis of metabolites, and the chemical composition of different organs, such as the amounts of lipids and lignin [25]. However, the reason for these differences is not clear.

Interestingly, the variance in $\delta^{13}\text{C}$ value among different plant parts was much higher in “Kasalath” than in “Nipponbare” when the plants were grown under drought stress conditions (Figure 2, Figure 3). “Kasalath” is considered to be well adapted to mild drought stress conditions, compared with “Nipponbare”, in our previous studies [22] [26]. Therefore, the $\delta^{13}\text{C}$ value in response to mild drought may be related to plant adaptations to such stress conditions.

The stable isotope ^{13}C technique has been used for screening plants with higher tolerance under drought stress conditions. In rice (*Oryza sativa*), putative quantitative trait loci were detected for $\delta^{13}\text{C}$ or CID values [13]-[16]. So far, despite various efforts, $\delta^{13}\text{C}$ values have not been used to produce drought-resistant varieties of any crop. In this study, the $\delta^{13}\text{C}$ value of panicles was better correlated with shoot dry matter production than was the $\delta^{13}\text{C}$ of other plant parts in both “Nipponbare” and “Kasalath” (Table 1). This result indicates that the $\delta^{13}\text{C}$ value of panicles may be the best indicator of plant water status in rice, although further research is needed to evaluate how $\delta^{13}\text{C}$ variation in different plant parts can related to plant adaptation to drought stress in rice.

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