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Research article

Evaluation of rice drought stress response using carbon isotope discrimination

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ABSTRACT

Recent studies have used carbon isotope discrimination (△) to investigate crop water use efficiency in drought environments. The main objective of this study was to accurately evaluate the physiological responses to different degrees of drought stress in rice. For two planting patterns: plant pattern1 (mono-varietal), three rice shoots of each cultivar were planted in a pot and plant pattern2 (mixed-varietal), one rice shoot of three cultivars were planted in a pot), △ was used as a tool to analyze gas exchange parameters, dry matter (DM) and grain yield (GY) under different levels of water stress. We found that changes in △ were always consistent with changes in DM, GY and gas exchange parameters under different water supply condition and for all genotypes regardless of planting pattern. In both mixed-varietal and mono-varietal trials, △ at maturity was significantly associated with photosynthesis rate, stomatal conductance, transpiration rate and the ratio of internal CO_2 concentration to ambient CO_2 concentration. There were also stronger correlations between △ at maturity and DM and GY than between △ at other sampling stages and DM and GY. Based on these results we conclude that △ values at maturity are more accurate predictors for grain yield in rice cultivars grown under mild stress and moderate stress.

1. Introduction

Worldwide, water stress causes more reduction in crop yield than any other environmental stress (Cattivelli et al., 2008). Rice is one of the major cereal crops and is the staple food for almost half of the world's population (Lim et al., 2007). Water-use efficiency (WUE), expressed as the dry matter accumulation over time per unit of water used, has been considered an important indicator of productivity (Richards et al., 2002). However, it is difficult to assess this trait because screening WUE in large populations in the field is time- and labor-consuming and expensive. There are two WUE parameters: instantaneous WUE (WUE_{ins}), which is defined as the ratio between the photosynthesis rate (A) and transpiration (E), and intrinsic WUE (WUE_{int}), which is defined as the ratio of A and stomatal conductance (g_s) (Ali and Talukder, 2008). Because measurements of WUE_{ins} and WUE_{int} are instantaneous, they do not integrate diurnal and day-to-day variation (Impa et al., 2005). A study of two perennial grasses found that these two leaf-level indicators of WUE could not describe the whole plant WUE parameters (Gulías et al., 2012). Carbon isotope discrimination (△), which integrates the ratio of assimilation to transpiration over the period during which dry matter (DM) is assimilated, has the potential to overcome these difficulties (Farquhar et al., 1989).

In C_3 species, the isotopic ratio of ^{13}C to ^{12}C in plant tissues is less than the $^{13}C/^{12}C$ ratio in the atmosphere, indicating that plants discriminate against the heavier carbon isotope ^{13}C during photosynthesis. The correlation between △ and grain yield (GY) has been widely studied in several C_3 crop species, such as wheat, cotton, rice and barley (Araus et al., 2003; Merah et al., 2002; Monneveux et al., 2005; Read et al., 2006; Tambussi et al., 2007; Teulat et al., 2001; Wahbi and Shaaban, 2011; Zhu et al., 2010). These studies suggest that △ determined in plant tissues, especially in leaves or grain, may be used as a physiological tool to indirectly evaluate GY and as an indirect selection criterion to improve GY in crop breeding programs.

Studies in several species such as wheat and cowpea have shown that the rankings of genotypes based on △ in different parts of the plant are generally consistent when the same genotypes are grown either

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under well-watered or water-limited conditions, indicating low G × E for △ (Ismail and Hall, 1993; Yasir et al., 2013). Therefore, detailed studies of plants grown under different water availability conditions are essential to identify and dissect both constitutive and adaptive components affecting this trait and to develop the most appropriate phenotyping approaches (Ishitani et al., 2004; Reymond et al., 2004). To our knowledge, the effects of different water availability conditions on △ stability, gas exchange parameters, and GY and their relationships are still not clear in rice. The main objectives of the present study were as follows: (1) to measure the magnitude of genotypic variation in gas exchange parameters and △ in response to different water availability conditions; (2) to determine the stability of phenotypic expression of △ across multiple experimental environments; (3) to confirm the relationship between △ and gas exchange parameters, DM accumulation and GY; (4) to determine whether the use of △ as an indirect measure of rice yield is valid.

2. Materials and methods

2.1. Plant material

Three rice cultivars, Qishanzhan, Akihikari and Bendao, were used in this study. Qishanzhan is an indica rice. Akihikari and Bendao are japonica and landrace rice varieties released by Japan and China, respectively. All of the three cultivars as 3 genotypes have closed growth period.

2.2. Experimental conditions

Trials with two planting patterns were carried out in Shenyang, Northeast China (41.8'N, 123.38 E, and 45 m above sea level) during the 2014 and 2015 crop seasons.

2.3. Planting pattern

The two planting designs were used in both 2014 and 2015. For planting pattern 1 (mono-varietal), three rice shoots of the same cultivar were planted in a plastic pot (26.4 cm in height and 29.3 cm in diameter) containing 15 kg of clay-loam soil, and four water supply conditions-well watered, mild stress, moderate stress and severe stress were used for the treatments with 10 replicates per genotype. For planting pattern 2 (mixed-varietal), one rice shoot of each of the three cultivars was planted in the same plastic pot, and four water supply conditions were also used for the treatments with 30 replicates. There were 120 pots used in each planting pattern trail in each year. We used completely randomised design for sampling in the following experiments. The size of the pot and soil were the same used for planting pattern 1. A transparent shelter was used to shield the crops from rainfall. When there was no rain, the canopy cover was removed to allow for ventilation and exposure to ambient conditions. To each pot 1.06 g $(NH_4)_2HPO_4$, 1.65 g $CO(NH_2)_2$ and 2.31 g K_2SO_4 were added as base fertilizers, 0.82 g $CO(NH_2)_2$ was added at tillering, and 0.13 g $CO(NH_2)_2$ and 0.33 g K_2SO_4 were added at heading. Then each pot was watered to excess and mixed fully with a stick. Seeds of Qishanzhan, Akihikari and Bendao were seeded in April each year. Uniform plants were hand transplanted into the pots at the 3–4 leaf stage on day 45 after seeding. Water stress was implemented 23 days after transplanting. The soil water potentials were measured and monitored for six representative pots for each treatment in each planting pattern using tensiometers (the Institute of Soil Science, Chinese Academy of Sciences). Readings were recorded from the soil surface to adepth of 20 cm. Soil moisture was read 2-4 times a day, and then water was supplied to maintain water potentials of −6 to -8 KPa, −10 to −15 KPa, −30 to −35 KPa and −50 to −55 KPa for the well-watered, mild stress, moderate stress and severe stress conditions, respectively (Lu et al., 2012; Xue and Chen, 2016).

2.4. Measurements

2.4.1. Gas exchange measurements

Gas exchange measurements were taken for the topmost recently fully expanded leaf on the main stem at heading. Measurements were replicated three times for each genotype in each water treatment. The parameters, including A, g_s, intercellular CO_2 concentration (C_i), E and the ratio of internal CO_2 concentration to ambient CO_2 concentration (C_i/C_a) were measured and calculated using the Li-6400 portable photosynthesis system (Li-CorInc, USA). The LED light source of the Li-6400 was maintained at a PPFD of 1200 $\mu molm^{-2}s^{-1}$, and flow rate was set at 500 $\mu molm^{-2}s^{-1}$. All measurements were made between 09:00–11:00 on sunny days.

2.4.2. Carbon isotope discrimination

Carbon isotope discrimination (△) in the tip of fully expanded leaves was determined at the tillering, heading and maturity stages each year. For each genotype, about 45 leaves were collected from 6 plants and dried at 80 °C for 48 h. Then the dried leaves were ground to fine powder with a ball mill in a 0.15-mm sieve, and three 1 ± 0.1 mg replicates of each sample were transferred to stain capsules and sent to the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (Jilin) for carbon isotope analysis. The carbon stable isotope ratio ($^{13}C/^{12}C$) was analyzed using the stable isotope ratio mass spectrometer (MAT 253, Thermo Fisher Scientific, Inc., USA) connected to an elemental analyzer (Flash, 2000) for on-line sample preparation.

The $\delta^{13}C$ values were calculated as: $\delta^{13}C$ (‰) = $[(R_{sample}/R_{reference})-1] \times 10^3$, where R is the $^{13}C/^{12}C$ ratio relative to the universally accepted standard Vienna Pee Dee Belemnite (V-PDB) (Farquhar et al., 1982). Then the $\delta^{13}C$ values were converted to △ using the following formula (Farquhar et al., 1989): △ (‰) = $[(\delta^{13}C_a-\delta^{13}C_p)/(1+\delta^{13}C_p)] \times 10^3$, where $\delta^{13}C_p$ is the $\delta^{13}C$ value of the plant and $\delta^{13}C_a$ is the measured value of the atmospheric CO_2, which was approximately −8‰ on the PDB scale. Carbon isotope discrimination of the leaf at tillering, heading and maturity was denoted $△_t$, $△_h$, and $△_m$, respectively.

2.4.3. Agronomic traits

Three plants of each cultivar grown in different water supply conditions were cut at the base at tillering, heading and maturity. Then all plants were immediately dried in a forced-air drier at 105 °C for 30 min and at 80 °C for 48 h. The dry weight of the above-ground biomass at tillering, heading and maturity and GY at maturity were measured in each year.

2.4.4. Statistical analyses

An ANOVA for traits was carried out with the statistical soft ware package IBM SPSS 2.00 (IBM Crop., Armonk, NY, USA). When ANOVA showed significant, the significant differences between treatments and between cultivars were assessed using Dancan test ($P < 0.05$). Regression analysis was applied to determine the relationships between traits using Sigmaplot for EXCELL software. GraphPad Prism 5.0 was used for preparing graphs.

3. Results

3.1. Agronomic traits of rice subjected to different water supply conditions

For both the mono-varietal (planting pattern 1) and mixed-varietal (planting pattern 2) planting patterns and all stages of development there were significant differences in DM, GY and △ between the main factors (genotypes, treatments, planting patterns) based on ANOVA (Tables 1 and 2). For both planting patterns, GY, DM at tillering (DM_t), heading (DM_h) and maturity (DM_m), and △ at tillering ($△_t$), heading ($△_h$) and maturity ($△_m$) were higher under the well-watered condition