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Restricting the above ground sink corrects the root/shoot ratio and substantially boosts the yield potential per panicle in field-grown rice (*Oryza sativa* L.)

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Rice has shallow, weak roots, but it is unknown how much increase in yield potential could be achieved if the root/shoot ratio is corrected. Removing all tillers except the main one, in a japonica (Sakha 101) and an indica (IR64) rice cultivar, instantly increased the root/shoot ratio from 0.21 to 1.16 in Sakha 101 and from 0.16 to 1.46 in IR64. Over 30 days after detillering, the root/shoot ratios of the detillered plants decreased to 0.49 in Sakha 101 and 0.46 in IR64 but remained significantly higher than in the controls. The detillered plants showed two- or fourfold increase in the main tiller fresh weight, as a consequence of more positive midday leaf relative water content (RWC), and consistently higher rates of stomatal conductance and photosynthesis, but not transpiration, compared with the controls. The enhanced photosynthesis in Sakha 101 after detillering resulted from both improved water status and higher Rubisco contents whereas in IR64, increasing the Rubisco content did not contribute to improving photosynthesis. Detillering did not increase the carbohydrate contents of leaves but prevented starch depletion at the end of grain filling. The leaf protein content during vegetative and reproductive stages, the grain filling rate, the number of filled grains per panicle were greatly improved, bringing about 38.3 and 35.9% increase in the harvested grain dry weight per panicle in Sakha 101 and IR64, respectively. We provide evidence that improving the root performance by increasing the root/shoot ratio would eliminate the current limitations to photosynthesis and growth in rice.

Introduction

Agriculture consumes more than 70% of the fresh water resources worldwide (Shiklomanov 2000, Condon et al. 2004), about 30% of which is devoted for rice production (Barker et al. 1999). Because of its semi-aquatic ancestry, rice has the greatest water demands among all major cereals (Toorchi et al. 2003). As the world's population grows, rice production should be increased by at least 1% annually to meet the growing demand for food, in spite of the restricted water resources (Rosegrant et al. 1995, Otegui and Slafer 2004, Normile 2008). To achieve this, the yield potential of rice under favorable conditions should be improved and stable productivity in water-limited environments should be maintained.

In contrast to maize and sorghum in which breeding programs have developed uni-calm plants from ancestors that have many tillers, modern rice cultivars and hybrids are characterized by large numbers of tillers (Khush 1990) where each hill includes a few plants that produce 30–40 tillers but only about 20 of them produce panicles. Thus, limited tillering was suggested to avoid

Abbreviations – Dpa, days post anthesis; RGFP, rapid grain filling phase; Rubisco, ribulose-1,5-bisphosphate carboxylase oxygenase; RuBP, ribulose-1,5-bisphosphate; RWC, relative water content.

early closure of plant canopy and allow more efficient energy capture and nutrient partitioning by productive tillers, thereby increasing tiller size (Peng et al. 1994). It has been reported recently that late tillers (those that develop after the normal tillers have completed anthesis) do not increase yield because they do not produce panicles (Hazra and Chandra 2014). Nonetheless, restricting tillering, by manually cutting some tillers or by growing the plants in styrofoam slats which imposed physical restriction to tillering, was also found not to increase yield under field conditions (Ao et al. 2010), perhaps because reducing the number of tillers was not efficiently compensated by increase in the panicle size. However, in that study no reference was made to changes in root/shoot ratio as a result of restricting tillering. Because the root system of rice consists mainly of nodal roots and traditional rice plants with fewer tillers normally have smaller root systems (Fukai and Cooper 1995), it is possible that restricting tillering in the study of Ao et al. (2010) did not increase the root/shoot ratio possibly because it also led to comparable reduction in root size.

The hydraulic conductance of rice roots is lower than that of other crops such as maize because of intense suberization (Miyamoto et al. 2001, Schreiber et al. 2005). Moreover, the rate of stomatal and non-stomatal transpiration from rice leaves is higher than that in other crops such as soybean and maize (Tanguilig et al. 1987). This situation causes rice plants to experience water deficit under conditions of intense transpiration (e.g. at midday on sunny days) even if the soil is submerged with water (Ishihara and Saito 1987). Recently, we have shown that aquaporin expression profiles contribute largely to the unbalanced water relations in rice where aquaporins with high water transport activity were strongly induced in leaves but not in roots (Nada and Abogadallah 2014). Excessive transpiration from leaves coupled with inadequate water uptake by roots results in negative water status and subsequently reduces stomatal conductance particularly at midday (Ishihara and Saito 1987). Reduction of stomatal conductance and subsequently photosynthesis limits biomass accumulation (Murata et al. 2007, Iseki et al. 2013).

Therefore, the root system of rice has two critical functions, namely water and nutrient (particularly nitrogen) uptake. Modifying the roots with regard to these functions has been reported to bring about substantial changes in shoot growth. When rice plants were grown at the density of one plant per hill, the number of crown roots per stem as well the root length density were improved compared to those in plants grown at the density of three plants per hill. This resulted in improving the leaf nitrogen and Rubisco contents that collectively supported higher rates of photosynthesis during grain ripening (San-oh et al. 2004, 2006). However, no quantitative data were presented on changes in root biomass or on the kinetics of the response of gas exchange and tiller growth in relation to root/shoot ratio as a result of this treatment. Characterizing the high yielding indica rice cultivar Habataki that was able to maintain higher rates of photosynthesis during the midday because of higher hydraulic conductance from roots to leaves, as compared with the standard japonica cultivar Sasanishiki, Adachi et al. (2010) found that the higher hydraulic conductance of roots was the result of greater root surface area, but not from specifically higher conductivity per unit root surface area. Improving the depth of root growth (without changing total root length) by overexpressing a gene for deep rooting (*DRO1*) has been reported to improve rice plant growth and yield mainly by improving nitrogen uptake from soil (Arai-Sanoh et al. 2014). However, no data on water uptake or photosynthesis were presented in that study.

Decreasing the root/shoot ratio, by insertional mutation in the *CONSTITUTIVELY WILTED 1* (*COW1*) gene, in a traditional japonica cultivar resulted in limited water uptake, leaf rolling and growth retardation in the well-watered plants (Woo et al. 2007) and hence, the root/shoot ratio was described as critical for maintaining water homeostasis where the size of root system determines how much aboveground biomass could accumulate. Improving the root performance in terms of water uptake by overexpressing an aquaporin gene in rice allowed a root with reduced biomass (compared with the wild-type) to sustain more shoot biomass (Katsuhara et al. 2003). Moreover, it has been reported earlier that the root/shoot ratio is an important component of drought avoidance in rice (Champoux et al. 1995, Samson et al. 2002, Gowda et al. 2011). Nitrogen uptake efficiency of rice roots has been reported to vary from 30 to 80% with the rest of soil nitrogen fertilizer lost to the environment (Craswell and Godwin 1984). Enhancing ammonium or nitrate uptake by rice roots through overexpression of *AMT1;1* (Ranathunge et al. 2014) or *PTR6* (Fan et al. 2014) resulted in plants with improved growth under different nitrogen nutrition conditions indicating that the wild-type roots were performing below optimum in terms of nitrogen uptake from soil.

The above findings provide good evidence that the rice root system is limiting to the growth of shoot system and that either structural or functional enhancements in the root performance lead to stable improvement in shoot growth. Interestingly, information on quantitative responses of leaf water status, gas exchange, nitrogen accumulation, shoot growth and grain yield per panicle, to changes in root performance in terms of quantitative variation in root/shoot ratio is lacking in most (if not all)

previous reports. Such information would provide more insights into how changes in the root performance regulate biomass accumulation in the shoot and ultimately in the grains as well as how much change in the root/shoot ratio is required to achieve the maximum performance of the shoot in terms of photosynthesis and biomass accumulation.

In this study, we hypothesized that rice plants do not achieve the tiller growth and/or yield potential per tiller due to inadequate water and nitrogen uptake by roots. We then investigated the consequences of restricting the shoot system, by manually removing the tillers except the main one when the plants were 40 day old in a japonica (Sakha 101) and an indica (IR64) rice cultivar, thereby greatly and transiently increasing the root/shoot ratio-on the subsequent growth, gas exchange and resource accumulation, at different values of root/shoot ratio. The data provide evidence that increasing the root/shoot ratio is essential for enhancing biomass accumulation and growth at the tiller level by improving water and nitrogen availability for shoot growth.

Materials and methods

Seeds of the rice (*Oryza sativa*) cultivar Sakha 101 (japonica) were obtained from the Agricultural Research Institute (Sakha research station, Kafr Elsheihk, Egypt). Seeds of IR64 (indica) were kindly supplied by the International Rice Research Institute (IRRI, Los Banos, Philippines). The two cultivars were chosen because they flower after the same period of time and have good yield potentials.

Plant growth and treatment

This work was carried out in the research field of Botany Department, Damietta University (New Damietta, Egypt) in summer (June to September 2014). The experiment location had coordinates of: 31.4391°N and 31.6821°E and altitude of about 5 m. The climatic conditions over the experiment period were: 27–31/22–25°C day/night temperature, 65–75% relative humidity during the day (RH), 12–13 h photoperiod and 2850 μmol m^{-2} s^{-1} maximum light intensity (full sunlight).

The seeds were soaked in tap water for 24 h and sown into dry clay soil. Each seed was covered with a thin layer of fine soil in a separate hole. The seeds were arranged in rows that were 30 cm apart. The soil was then saturated with water. The soil was kept at field capacity (saturated with water) by watering twice a day. When the plants were 15 day old, they were thinned to be 30 cm apart. The plants were then allowed to grow up to the age of 45 day where half of the plants of each cultivar was detillered by removing all tillers except the main one (oldest tiller). Care was taken not to remove basal leaves of the main tillers. The plants were detillered randomly so that each row included both intact (control) and detillered plants. The plants were then allowed to grow to maturity (or until they were sampled) where they received water regularly twice a day.

Sampling

Samples of the control and detillered plants were collected at 0, 10, 20 and 30 days after detillering, for measuring the numbers of tillers and root/shoot ratio, by removing whole plants with their complete root system from soil. The plants were then washed thoroughly with distilled water and used immediately for analysis as described below. Samples for measuring leaf relative water content (RWC) were collected 5 days after detillering at predawn, morning (09:00) and midday (13:00). When the plants started flowering, they were checked every morning and each tiller was labeled with the date of anthesis. Both cultivars started flowering 40 ± 1 days after detillering (i.e. 85 ± 1 days after planting). Samples for measurement of protein and sugar contents as well as panicle attributes, when applicable, were collected every 10 days after detillering (at 10, 20 and 30 days after detillering) and then every 5 days post anthesis (dpa) up to 25 dpa (i.e. at 45, 50, 55, 60 and 65 days after detillering). For measuring panicle attributes, whole panicle samples were collected from the control and intact plants into plastic bags and analyzed immediately. For measuring protein and sugars contents, samples from the second leaf (before anthesis) or from the flag leaf (after anthesis) were collected at 09:00, frozen immediately in liquid nitrogen and then stored at −80°C until use for subsequent analyses.

Measurement of growth parameters, root/shoot ratio and leaf RWC

The tillers of each of the control plants were counted. A part of shoot was considered as a tiller if it had a single leaf in the axil of an old leaf. Each plant was then separated into roots and shoots which were weighed in separate. The weights of individual tillers were calculated by dividing the weight of the whole shoot by the number of tillers for each plant. The root/shoot ratio was then calculated by dividing the fresh weights. Five plant replicates were used for each treatment. The RWC of leaf samples was determined as described in Nada and Abogadallah (2014).

Measurement of gas exchange parameters

The rate of photosynthesis (A), transpiration (E), stomatal conductance (g_s) and leaf internal CO_2

concentration (C_i) were measured for the second leaf (before anthesis) or for the flag leaf (after anthesis) in each plant by using LCi-SD gas exchange system (Analytical Development Company Ltd., Hoddesdon, UK). Each leaf was allowed for 2–4 min to acclimate inside the leaf chamber. Five measurements from different plants were made for each treatment. Measurement of gas exchange parameters started at 09:00 and lasted for about 90 min at light intensity of about 2130 μmol m^{-2} s^{-1}. Within each treatment, one plant was used a standard for which measurements were repeated every 20 min to make sure that gas exchange measurements did not change because of internal or external factors.

Measurement of panicle attributes

Panicles collected from the control and detillered plants as above were dried at 50°C for 2 days and weighed. The grains in each panicle were then counted. Grains that were totally empty were considered as unfilled. To find the dry weight per grain, the dry weight of grains from each panicle was divided by its number of grains. The harvest index (HI) per plant was calculated by dividing the grain dry weight by the shoot dry weight. Five replicates were made for each treatment.

Measurement of leaf protein and Rubisco contents

Soluble proteins were extracted from frozen leaf samples with 50 m*M* 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) buffer, pH 7.4, containing 2 m*M* ethylenediaminetetraacetic acid (EDTA) and 5 m*M* phenylmethanesulfonyl fluoride (PMSF). The protein concentration was then measured as described by Bradford (1976). To quantify Rubisco per unit protein, leaf protein samples were resolved on denaturing polyacrylamide gels as described by Laemmlli (1970) by using BioRad Tetra Cell equipment (BioRad Laboratories, Hercules, CA, USA). Five micrograms of protein was loaded onto each lane. The gels were stained with 0.1% brilliant blue R 250 in 40% methanol and destained with water. The gels were then scanned and the volume of Rubisco band was measured by using Image Studio v 12.0 software (Li-COR Biosciences, Lincoln, Nebraska, USA). For protein and Rubisco measurements, three replicates were made for each treatment. Data of leaf protein and Rubisco contents were then used to calculate the Rubisco contents per unit leaf fresh weight.

Measurement of leaf soluble sugars and starch

Total soluble sugars and starch contents were determined as described previously (Rose et al. 1991, Schluter and Crawford 2001). The sugar concentrations were calculated from a standard curve in the range 20–80 μg. For each treatment, three replicates from different plants were used.

Statistical analysis

All measurements were replicated as described in each section. The data was compared by running one-way ANOVA by using SPSS v 18 at significance level of $P < 0.05$. Whenever a significant difference is mentioned below, it means that the samples were compared at the significance level of $P < 0.05$.

Results

Plant growth and leaf RWC

Biomass accumulation in the shoot and root increased progressively with time in both control and detillered plants (Fig. 1A, B). The shoot fresh weights were significantly lower in the detillered than in the control plants of Sakha 101 and IR64 after 10, 20 and 30 days of detillering. However, differences in root fresh weight between the control and detillered plants were less obvious, compared to these in the shoot, after 10 and 20 days (although significant at 20 days) of detillering but most obvious and significant after 30 days. The fresh weights shoots of the control plants in both cultivars were statistically similar and so were those of the roots. A similar trend was also observed for the detillered plants. The number of tillers in the control plants of both cultivars increased linearly with time where it was significantly higher in IR64 than in Sakha 101 at all time points (Fig. 1C), meanwhile, it was restricted manually to one tiller per plant in the detillered plants. Over a period of 30 days, the number of tillers per intact plant increased by 250 and 274% in Sakha 101 and IR64, respectively. The fresh weight of individual tillers increased progressively with time in both the control and detillered plants but was significantly higher in the detillered plants of both cultivars than in the controls (more than two- and fourfold of the control in Sakha 101 and IR64, respectively at 30 days after detillering) (Fig. 1D). Moreover, the control plants of Sakha 101 showed significantly heavier tillers than those of IR64 after 20 and 30 days after detillering. Over a period of 30 days, the individual tiller fresh weight increased by 265 and 281% in the control and by 649 and 705% in the detillered Sakha 101 and IR64, respectively. The root/shoot ratio per tiller of the control plants increased slightly over the course of experiment (from 0.21 to 0.28 in Sakha 101 and from 0.16 to 0.22 in IR64) where significant changes within cultivars were found at 20 and 30 days from those at 0 days

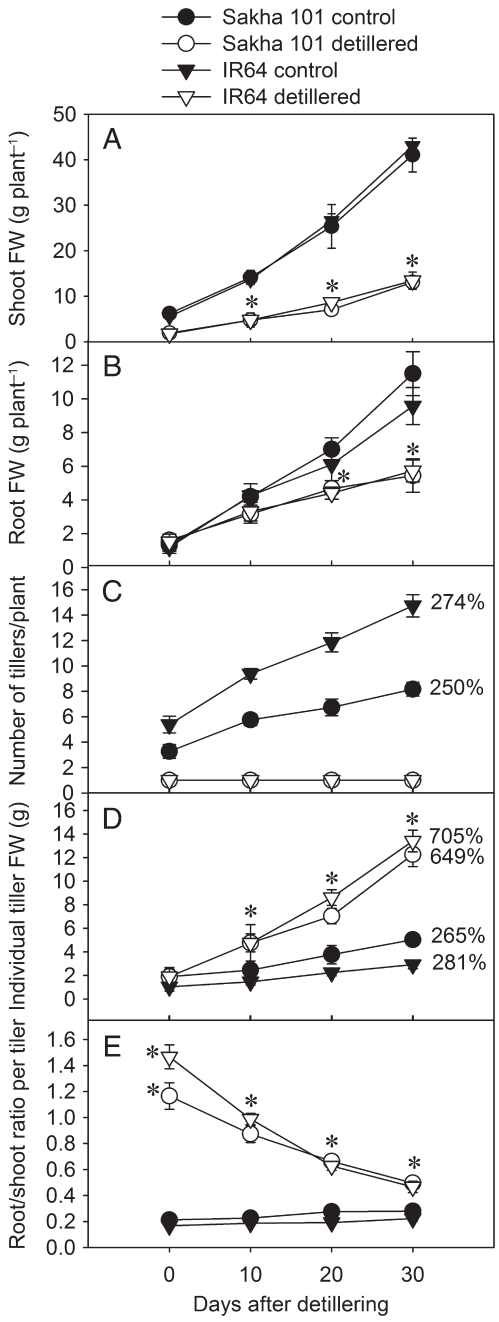

Fig. 1. Growth parameters of the intact control and detillered plants of Sakha 101 and IR64 over a period of 30 days after detillering. (A) Shoot fresh weight per plant; (B) root fresh weight per plant; (C) number of tillers per plant, which are restricted to one per plant in the detillered plants; (D) individual tiller fresh weight. In each of C and D, percentages at the end of each curve are the percent increase in the parameter over 30 days. (E) Root/shoot ratio. In all graphs, each point is the mean of five replicates ± SE. Means ± SE labeled with asterisks are significantly different from the corresponding controls at $P < 0.05$. An asterisk on two overlapping means ± SE indicates that both are significantly different from the controls. Some statistical comparisons are not shown on the graphs for simplicity.

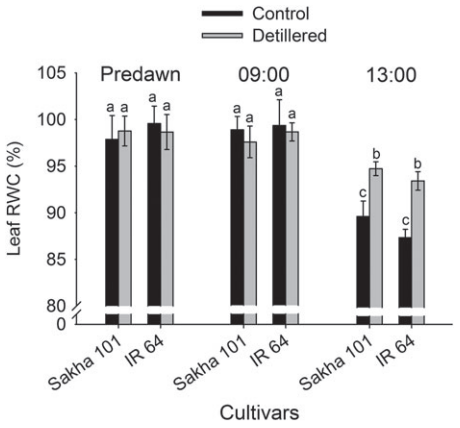

Fig. 2. Relative water content (RWC) of leaves in the control and detillered plants of Sakha 101 and IR64, measured 5 days after detillering at three time points during the day: predawn, 09:00 (morning) and 13:00 (midday). Bars are means of five replicates ± SE. Means ± SE labeled with different small letters are significantly different from the corresponding controls at $P < 0.05$.

after detillering (Fig. 1E). No significant differences in root/shoot ratio per tiller were found between cultivars. In the detillered plants of both cultivars, the root/shoot ratio per tiller was maximum at 0 days after detillering (1.16 and 1.46 in Sakha 101 and IR64, respectively) and decreased with time and reached the lowest values at 30 days (0.49 and 0.46 in Sakha 101 and IR64, respectively) but remained significantly higher than in the corresponding controls at all time points. In the detillered plants, significant difference between cultivars in the root/shoot ratio per tiller was only observed at 0 day of detillering.

The RWC of leaves was measured at 5 days after detillering. Fig. 2 shows that RWC of the control leaves of both cultivars decreased significantly at midday (13:00) compared to that at predawn or at 09:00. Detillering did not bring about significant changes in RWC at predawn or at 09:00 but did increase it significantly at midday in Sakha 101 and IR64 (94.7 and 93.4%, respectively) compared with the controls (89.6 and 87.4%, respectively). However, the RWC of the detillered plants remained slightly but significantly lower than that at predawn or at 09:00.

Gas exchange

The rates of A, g_s, E and C_i were measured in the control and detillered plants over a period of 30 days after detillering (every 10 days) and over a period of 25 days into grain filling (every 5 days). In the control plants, A remained unchanged at all time points in both cultivars except that it decreased significantly at 20 and 25 dpa

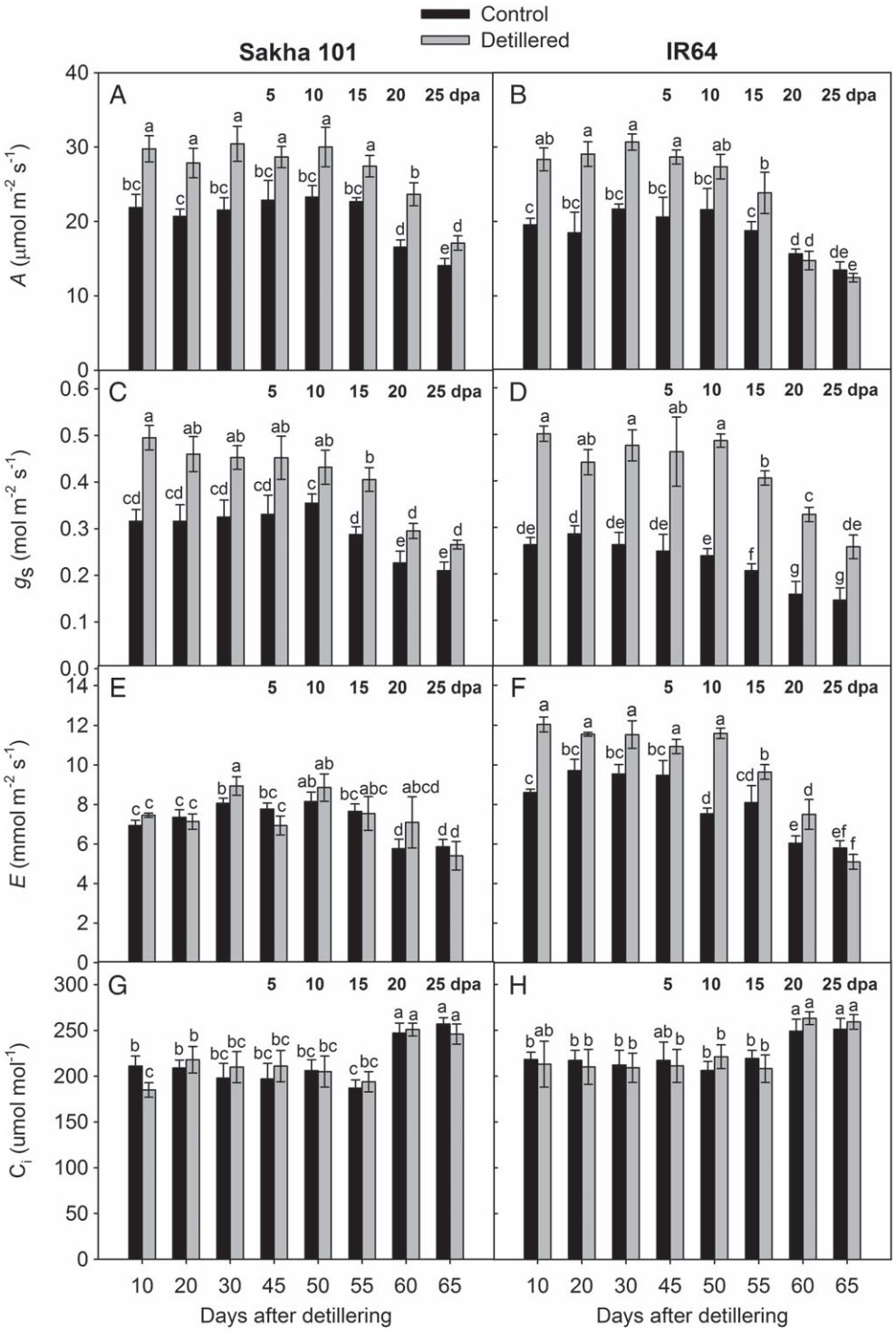

Fig. 3. Gas exchange parameters in the control and detillered plants of Sakha 101 and IR64 measured at 09:00. A and B: rate of photosynthesis (A); C and D: stomatal conductance (g_s); E and F: rate of transpiration (E); G and H: leaf internal CO_2 concentration (C_i). Gas exchange parameters were measured at 10 days intervals after detillering (10, 20 and 30 days) and then at 5 days intervals after anthesis (45, 50, 55, 60 and 65 days after detillering that are equivalent to 5, 10, 15, 20 and 25 dpa). Bars are means of five replicates ± SE. Within each graph, means ± SE labeled with different small letters are significantly different at $P < 0.05$.

(Fig. 3A, B). In the detillered plants, A was significantly higher than in the corresponding controls (up to 142 and 161% of the control in Sakha 101 and IR64, respectively) at all time points except that it was similar to the control at 20 and 25 dpa in IR64. A closely similar trend was also observed for g_s except that it was also significantly higher in the detillered than in the control plants of both cultivars at 20 and 25 dpa (Fig. 3C, D). The rates of transpiration showed smaller variation in the control plants of both cultivars starting from detillering and through the first 15 days of grain filling (Fig. 3E, F) but decreased significantly at 20 and 25 dpa. IR64 showed significantly higher E than Sakha 101 right from detillering and up to 5 dpa. Detillering caused no changes in E in Sakha 101 but increased it significantly in IR64 at all time points except 25 dpa. The C_i was maintained at statistically similar levels in both cultivars and treatments at all time points except it increased significantly at 20 and 25 dpa (Fig. 3G, H).

Panicle attributes in the control and detillered plants

Fig. 4A shows that the rate of increase in dry weight of grains varied significantly between the control and detillered plants of each cultivars and also between cultivars. The control grains of IR64 accumulated dry matter significantly faster than those of Sakha 101 after 10 and 15 dpa but dry matter accumulation over the next 5 days was faster in Sakha 101 so that the grain dry weights of the control plants of both cultivars were statistically similar after 20 and 25 dpa. A closely similar trend was also observed in the detillered plants but the grain dry weights of the detillered plants were significantly higher than those in the corresponding controls at 5, 10 and 15 dpa. All treatments had statistically similar grain dry weights after 20 and 25 dpa. Table 1 shows that the panicle size (number of grain per panicle) was statistically similar in the control plants of Sakha 101 and IR64, but was significantly higher in the detillered than in the corresponding controls of both cultivars (138 and 134% of the control in Sakha 101 and IR64, respectively). No significant differences were found in the numbers of unfilled grains in any treatment (Table 1). The harvested dry weight per panicle was significantly higher in the detillered plants than in the corresponding controls (138 and 135% of control in Sakha 101 and IR64, respectively). The HI of detillered plants was significantly higher than in the corresponding controls (117 and 122% of control in Sakha 101 and IR64, respectively) (Table 1). During the course of grain filling, 1–2 and 3–4 (significantly higher in IR64) of late tillers per plant were observed in Sakha 101 and IR64, respectively (Fig. 4B).

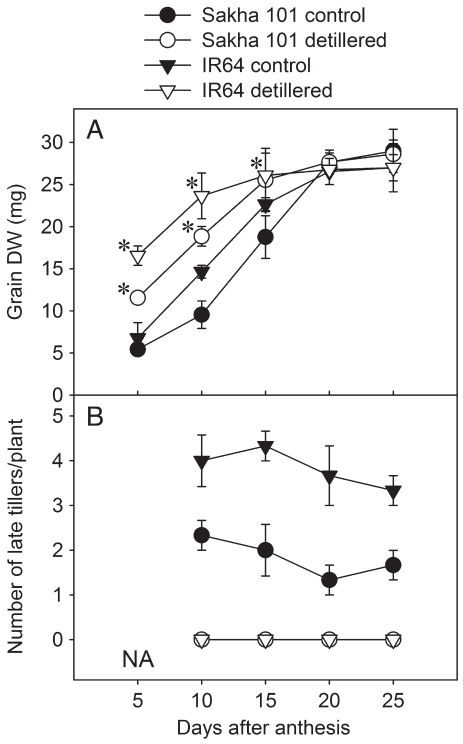

Fig. 4. (A) Kinetics of grain filling in the control and detillered plants of Sakha 101 and IR64 measured at 5 days intervals after anthesis. Bars are means of five replicates ± SE. (B) Numbers of late tillers in the control plants of Sakha 101 and IR64 measured at 5 days intervals after anthesis. Each point is the mean of five replicates ± SE. NA on x-axis means not applicable. Means ± SE labeled with asterisk are significantly different from the corresponding controls at $P < 0.05$. Some statistical comparisons are not shown on the graphs for simplicity

Protein and Rubisco content of leaves

Protein accumulation in the control plants varied with cultivar and time after detillering (Fig. 5). In the control plants, the protein content of the leaves of Sakha 101 remained unchanged over a period of 30 days after detillering and 15 dpa but decreased significantly at 20 and 25 dpa. Contrarily, IR64 showed significantly higher protein contents (compared to Sakha 101) that increased progressively at 10, 20 and 30 days after detillering, decreased steadily over the 25 days of grain filling but reached significantly higher values at 20 and 25 dpa compared with Sakha 101. Largely similar trends were found in the detillered plants except that the protein contents were significantly higher than those of the corresponding controls at all time points excluding IR64 before anthesis and 5, 20 and 25 dpa. The Rubisco contents per unit leaf protein showed no significant variation between cultivars or treatments either before or after anthesis except that it decreased significantly at 20 and 25 dpa in all treatments compared with earlier time

Table 1. Grain attributes per panicle in the control and detillered Sakha 101 and IR64; measured at 25 dpa. Numbers are means of five replicates ± SE. Numbers ± SE labeled with different small letter superscripts are significantly different at $P < 0.05$.

Treatment	Number of grains	Unfilled grains (% of total)	Harvested DW per panicle (g)	Panicle DW (% of control)	Harvest index (%)
Sakha 101					
Control	149 ± 23^{a}	4.65 ± 2.65^{a}	4.33 ± 0.23^{a}		47.26^{a}
Detillered	206 ± 11^{b}	3.87 ± 0.65^{a}	5.99 ± 0.16^{b}	138.33	55.50^{b}
IR64					
Control	158 ± 14^{a}	4.87 ± 2.56^{a}	4.76 ± 0.59^{a}		46.61^{a}
Detillered	212 ± 9^{b}	5.06 ± 0.61^{a}	6.47 ± 0.27^{b}	135.92	57.03^{b}

points (Fig. 6A, B). However, the Rubisco content per unit leaf fresh weight in Sakha 101 varied little over time until it significantly decreased at 20 and 25 dpa compared with previous time points (Fig. 6C). A similar trend was also observed in the detillered plants of Sakha 101 except that it showed significantly higher values than in the corresponding controls at all time points except at 20 days after detillering. IR64 showed different trends and contents of Rubisco per unit leaf fresh weight compared to Sakha 101 (Fig. 6D). In the control plants, the highest Rubisco contents were observed before anthesis (10, 20 and 30 days after detillering) and then they decreased progressively and significantly and reached the lowest contents at 20 and 25 dpa. In the detillered plants of IR64, the Rubisco contents were similar to the corresponding controls at all time points except at 10 and 15 dpa where they were significantly higher.

Soluble sugar and starch contents

The soluble sugar and starch contents were measured in the second leaf before anthesis and in the flag leaf after anthesis in all treatments. Fig. 7A, B shows that no consistent changes were observed in the soluble sugar contents of the control plants of Sakha 101 and IR64, where they were statistically similar for both cultivars at almost all time points. Moreover, detillering did not significantly affect the soluble sugar contents of both cultivars at any time point. A similar trend was also observed for starch contents except that they decreased significantly at 20 and 25 dpa in leaves of the control (but not in the detillered) plants of both cultivars, so that the detillered plants maintained significantly higher starch contents in their leaves at 20 and 25 dpa compared the corresponding controls (Fig. 7C, D).

Discussion

In traditional rice it is well-documented that the rice root system is limiting to biomass accumulation and yield due to the small root system (Yoshida and Hasegawa 1982, Cairns et al. 2009) and low root hydraulic conductance (Miyamoto et al. 2001, Schreiber et al. 2005) combined with high rates of stomatal and non-stomatal transpiration from leaves (Tanguilig et al. 1987, Nguyen et al. 1997). These features result in the midday stomatal depression under high transpiration demand even if the soil is saturated with water, which ultimately slows down biomass accumulation and reduces yield. Estimates of how much gain in biomass accumulation could be achieved, if the limitation from the root system is eliminated, do not exist. This gain is expected to be considerable given that Parent et al. (2010) have concluded that rice leaves are not specifically sensitive to evaporative demand (and water deficit) compared to maize leaves and that the exceptional sensitivity of rice to water imbalance is mainly attributed to its poor root system. They further suggested that differences between rice genotypes in the field with respect to sensitivity to water deficit could be the result of root system characteristics rather than physiology of the shoot system, but quantitative data that support this statement is lacking. In this study, we provide data on quantitative changes in photosynthesis and resource accumulation in the shoots and subsequently in the grains in response to quantitative changes in root/shoot ratio, over a period of 30 days of vegetative growth followed by 25 days of grain filling.

We transiently increased the root/shoot ratio of two field-grown rice cultivars (Sakha 101 and IR64) by removing all tillers except the main one and then compared the biomass accumulation of the detillered plants with the intact controls. In the control plants, the shoot biomass increased progressively along with the root biomass (Fig. 1A, B). The increase in shoot biomass was brought about by building up biomass in existing tillers and generating new ones in both cultivars (Fig. 1C, D). Fig. 1C, D show that emergence of new tillers was parallel to growth of the existing ones, although at different rates in the two cultivars (250, 265 in Sakha 101 and 274, 281% in IR64, respectively). Because the growth conditions were the same for both cultivars, these data indicate the growth rates and tillering are closely linked

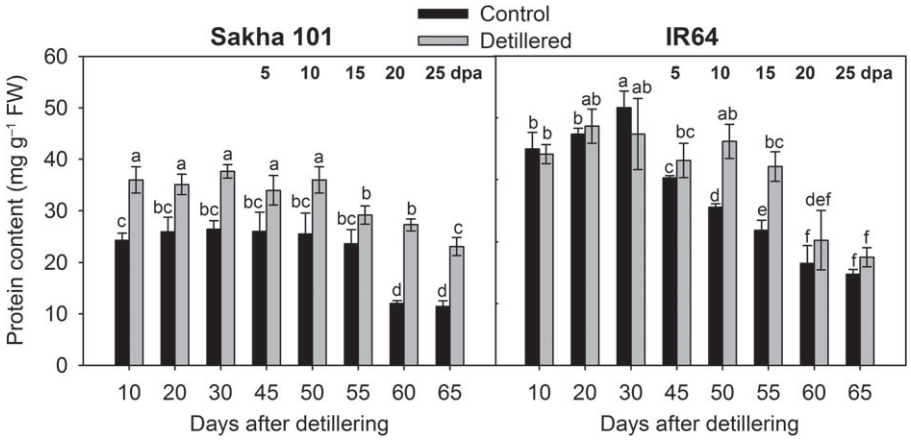

Fig. 5. Changes in leaf protein content in the control and detillered plants of Sakha 101 and IR64. Protein content was measured in second leaf samples before anthesis and in flag leaf samples after anthesis. Measurements were made at 10 days intervals after detillering (10, 20 and 30 days) and then at 5 days intervals after anthesis (45, 50, 55, 60 and 65 days after detillering that are equivalent to 5, 10, 15, 20 and 25 dpa). Bars are means of three replicates ± SE. Within each graph, means ± se labeled with different small letters are significantly different at $P < 0.05$.

and controlled genetically within each cultivar (Komatsu et al. 2003, Li et al. 2003, Zou et al. 2005). Restricting the shoot growth into one tiller and thereby transiently increasing the root/shoot ratio, resulted in a substantial increase in the growth of the main tiller, a change that was visible a few days after treatment and increased over the next 30 days (Fig. 1D). Interestingly, the root growth in the detillered plants did not cease or slow down but rather continued at slower rate (Fig. 1B). This suggests that root/shoot ratio that is believed to be genetically controlled (Woo et al. 2007), could be maintained, at least partially, if the shoot architecture is modified by reducing tillering through genetic modification. However, Fig. 1E shows that the root/shoot ratio of the detillered plants kept decreasing, although remained significantly higher than that of the control plants after 30 days (more than twofold in IR64). This difference was maintained because we kept removing the tiller re-growth. It is noteworthy here that in comparison to transient differences in shoot fresh weight (between the control and detillered plants) brought about by detillering, significant differences in root fresh weight were detected later at 20 and 30 days after detillering (Fig. 1B). This suggests that the roots arising from the removed tillers remained viable (and hence functioning), thus no sudden decrease in root biomass was observed. However, at 20 and 30 days after detillering, the control plants showed greater root biomass obviously due to development of new roots from the newly developed tillers (Fukai and Cooper 1995), a response that was not possible in the detillered plants in which the shoot system was manually restricted to a single tiller (Fig. 1C). In contrast to our findings, restricting tillering (to at least 13 tillers per hill) in field grown rice resulted in minor changes in tiller biomass and grain yield per panicle that ultimately did not bring about yield gain per unit area (Ao et al. 2010). However, we speculate that tillering was not restricted enough (compared to our study) to bring about a significant change in root/shoot ratio and hence eliminate root limitation to shoot growth. It should be mentioned here that the aim of our study was not to quantify yield per unit area after detillering but rather was to evaluate the potential gain (at single tiller level) in biomass and grain yield in response to quantitative changes in root/shoot ratio.

The boosted growth of the main tiller after detillering (more than two- and fourfold in Sakha 101 and IR64, respectively) was obviously the result of improved water status owing to increased water availability from roots as shown by the higher RWC of the detillered plants at midday compared to the control ones (Fig. 2). Consequently, the detillered plants had greater rates of photosynthesis and stomatal conductance in the vegetative stage that is over 30 days after detillering (Fig. 3A–D), although detillering increased transpiration in the vegetative stage (up to 30 days after detillering) only in IR64 (Fig. 3E, F). The persistent increase in A and g_s in Sakha 101 and IR64 in the vegetative stage, and for 15 days into grain filling (corresponding to 45, 50 and 55 days after detillering), suggests that both parameters are mainly limited by water availability from root and that sink capacity that may limit photosynthesis (Paul and Foyer 2001) could be increased relative to water availability. Recently, we have shown that IR64 has higher transpiration capacity as a result of abundant expression of aquaporins with high water transport activity in their leaves compared to the Sakha 101 (Nada and Abogadallah 2014). This suggests

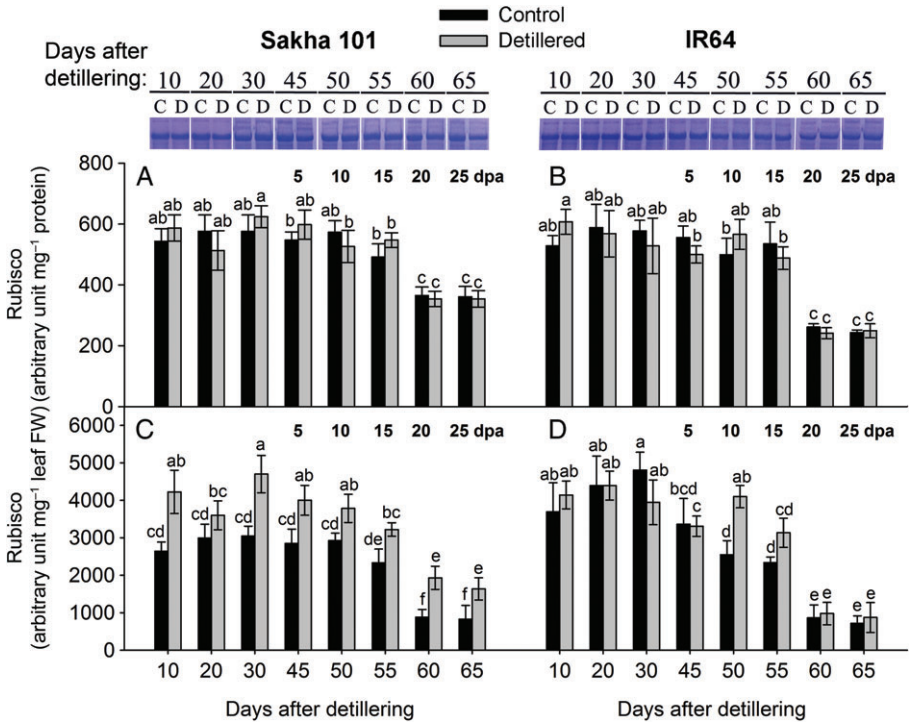

Fig. 6. Changes in leaf Rubisco content per unit leaf protein (A and B) and per unit leaf fresh weight (C and D) in the control and detillered plants of Sakha 101 and IR64. Rubisco content was measured in second leaf samples before anthesis and in flag leaf samples after anthesis. Measurements were made at 10 days intervals after detillering (10, 20 and 30 days) and then at 5 days intervals after anthesis (45, 50, 55, 60 and 65 days after detillering that are equivalent to 5, 10, 15, 20 and 25 dpa). Bars are means of three replicates ± SE. Within each graph, means ± SE labeled with different small letters are significantly different at $P < 0.05$.

that transpiration in the control plants of IR64 was below maximum due to limited water uptake by roots. These results are largely consistent with the previous findings that sensitivity of rice to evaporative demand and water deficit is attributed mainly to root limitation (Parent et al. 2010). However, the contrasting response of transpiration in the two cultivars suggests that physiological differences in leaves also contribute to plant growth in the field in relation to water availability. The lack of changes in C_i upon detillering in spite of the increased g_s suggests that the possible increase in CO_2 availability as a result of the enhanced g_s (and hence the expected increase in C_i) was offset by the enhanced rates of CO_2 fixation. However, the possible increased availability of substomatal CO_2 (C_i) does not appear to have contributed greatly to A in the detillered plants due to the lack of consistent changes in C_i relative to A. Recently, we have reported that C_i was not limiting to photosynthesis in rice (Nada and Abogadallah 2014). Similarly, Shimono and Bunce (2009) have reported that leaf photosynthesis in rice did not respond to elevated CO_2 concentration at the vegetative stage. However, a cultivar whose photosynthesis was enhanced by elevated CO_2 concentration, due to higher leaf mesophyll conductance to CO_2 and higher rates of carboxylation by Rubisco and electron transport, has been reported (Chen et al. 2014), indicating that C_i was limiting to photosynthesis in that specific cultivar. Overall, these results suggest that improving the water status of rice (even by minor increase in root/shoot ratio, see below) would support more efficient photosynthesis that in turn may break the current barriers of rice plant size (Sage 2000).

A remarkable finding in our study was that the improved RWC at midday and rates of photosynthesis in the detillered plants were maintained up to 30 days after detillering although the root/shoot ratio greatly decreased over this period in both cultivars (from 1.16 to 0.49 in Sakha 101 and from 1.46 to 0.46 in IR64) due to faster tiller growth coupled with slower root growth (compared to the control). The extremely high root/shoot ratio achieved instantly after detillering was suggested to support the maximum growth potential of a tiller by eliminating restrictions of water and nutrient supply from the root. If this is true, we thus speculate that the root resources (water and nitrogen) made available by the extremely high root/shoot ratio immediately after

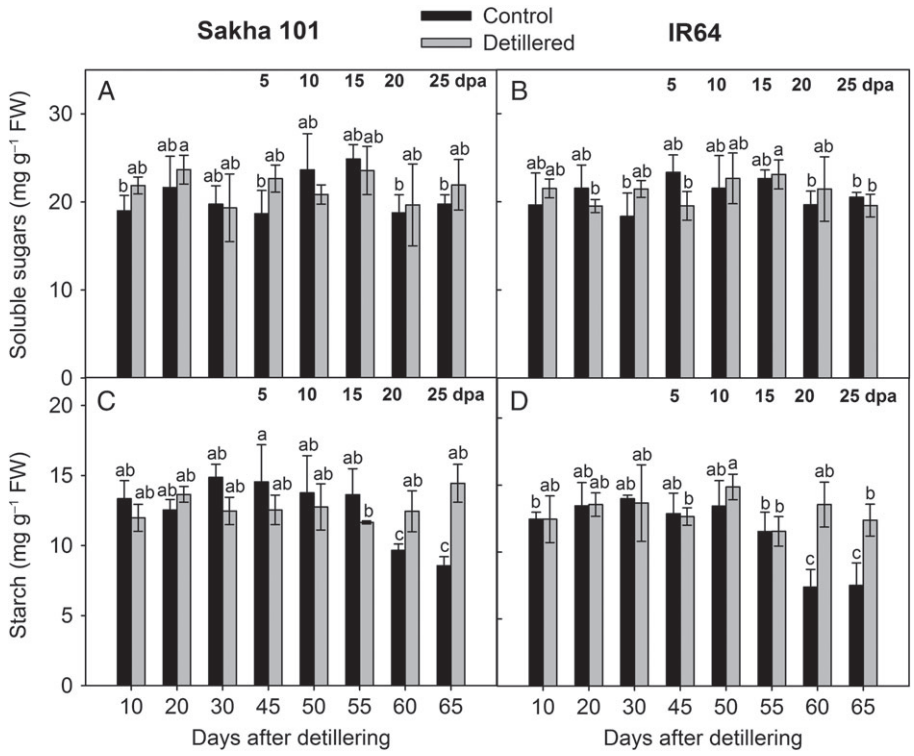

Fig. 7. Changes in leaf soluble sugars (A and B) and starch (C and D) contents in the control and detillered plants of Sakha 101 and IR64. Soluble sugars and starch contents were measured in second leaf samples before anthesis and in flag leaf samples after anthesis. Measurements were made at 10 days intervals after detillering (10, 20 and 30 days) and then at 5 days intervals after anthesis (45, 50, 55, 60 and 65 days after detillering that are equivalent to 5, 10, 15, 20 and 25 dpa). Bars are means of three replicates ± SE. Within each graph, means ± SE labeled with different small letters are significantly different at $P < 0.05$.

detillering were in excess of the requirements of the maximal growth potential in both cultivars and, hence increasing the root/shoot ratios up to these values observed 30 days after detillering (0.49 and 0.46 in Sakha 101 and IR64) would also provide sufficient resources to support the maximal growth potential per tiller in each cultivar, because the improved rates of photosynthesis and stomatal conductance brought about immediately after detillering were also maintained after 30 days of detillering although the root/shoot ratio decreased substantially.

The lowest root/shoot ratios shown in Fig. 1 after 30 days of detillering (i.e. 10 days before anthesis) were expected to be also performing during grain filling. It has been reported that root growth reaches a maximum around flowering and that increases in root biomass after flowering are minor if any (Yoshida and Hasegawa 1982, Cruz et al. 1986, Beyrouty et al. 1988). Therefore, grain filling and yield (per tiller) differences between the control and detillered plants can be explained on the basis of the root/shoot ratios recorded 30 days after detillering.

We hypothesized that enhancing the tiller size (and consequently leaf area, data not shown) by improving leaf photosynthesis and N source efficiency would result in improved grain filling and grain number per panicle (Janoria 1989, Khush 1995, Zhang et al. 2013). The data in Table 1 and Fig. 4A show that the boosted yield per panicle in Sakha 101 and IR64 after detillering (138 and 135% of the corresponding control, respectively) resulted from increase in panicle size (number of grains) but not from change in the filled grain dry weight (Fig. 4A) or the percentage of filled grains (Table 1). Although biomass accumulation in the grains (in the first 15 dpa) was faster in the detillered plants (Fig. 4A), the final grain dry weight was similar to that of the control in both cultivars, suggesting that the maximal grain size was achieved in the control plants and hence, no further gain in grain dry weight was possible even when the source capacity was improved by detillering. Previous reports have shown that grain size is tightly controlled by husk size regardless of resource availability (Yoshida 1981) and hence requires genetic modification if it is to be increased (Song et al. 2007, Shomura et al.

2008). This suggests that the grain number per panicle was more influenced by resource availability than was the final grain dry weight. The faster grain filling in the detillered plants (without significant changes in grain protein and starch contents, data not shown) apparently have resulted from the stably higher rates of photosynthesis as well as from higher leaf protein contents in the vegetative and reproductive stages, although specific increases in A during grain filling were not observed (Fig. 5). Murchie et al. (2002) found no consistent correlation between P_{max} (rates of light-saturated photosynthesis) and leaf protein and Rubisco contents on one side and the rapid grain filling phase (RGFP) on the other side in a traditional rice cultivar (IR72) and several new plant type (NPT) rice genotypes. Contrarily, Jiang et al. (1999) showed that P_{max} and leaf protein contents declined progressively right from flowering up to the end of grain filling. We suggest that the lack of consistent correlation of changes in A and leaf protein content to grain filling in the detillered plants, particularly the RGFP (between 5 and 15 dpa in this study), was because the panicle sink size was pre-determined (during panicle initiation phase) to fit the photosynthetic and N source strength, which were working at the maximum rate (and hence, no further increase in A is possible after anthesis) depending on the water and nitrogen availability from roots. This suggestion is supported by the findings of Ohsumi et al. (2011) who reported that increasing the panicle size does not increase yield unless the photosynthetic source capacity is improved to fully satisfy the increased sink size. However, a rather steep decline in leaf protein content during grain filling was observed in the intact IR64 but not in Sakha 101 (Fig. 5), indicating that a considerable proportion of leaf protein reserve was translocated to the grains earlier in the course of grain filling in IR64, presumably because leaf protein content in this cultivar at the onset of flowering was greatly in excess of photosynthesis and normal metabolism. It is noteworthy here that older leaves appear to contribute more than the flag leaf to the amount of mobilized nitrogen to grains, particularly during the RGFP in the control plants of both cultivars as indicated by the fast senescence of basal leaves as compared to the detillered plants in which senescence of older leaves was delayed (data not shown), presumably because of the more positive plant N budget.

Detillering resulted in a consistent increase in the leaf protein content in Sakha 101 in the vegetative and reproductive stages and in IR64 only in the reproductive stage (Fig. 5), presumably because IR64 in the vegetative stage has achieved the maximal protein content in the control plants (that was significantly higher than in Sakha 101). Consequently, the leaf protein, which supplies 70–90% of grain protein (Mae 1997), was below-optimum in the intact plants of Sakha 101 during the vegetative and reproductive stage as well as in IR64 during the reproductive stage. Thus, adjusting the leaf protein content by detillering supported faster grain filling in both cultivars and delayed the drop in leaf protein content at the final phase of grain filling (Fig. 5). The implication of this is that nitrogen supply from root during either vegetative or reproductive stages (or both) could limit grain filling and/or grain number per panicle. It has been estimated that in order to achieve a grain yield of 10 t ha^{-1}, a leaf area index (LAI: total one-sided leaf area per m^2 land area) of 7 is needed to supply the required amount of nitrogen for the grains (Horton and Murchie 2000), indicating that the nitrogen reserve in leaf biomass before heading is a determinant of grain yield. One limiting factor to achieving the required LAI at the optimum protein content could be the nitrogen uptake capacity of roots. Consistently with this, Ranathunge et al. (2014) reported that overexpression of the ammonia transporter *AMT1;1* in rice resulted in greater nitrogen (and hence, carbon) assimilate levels compared with the wild-type, which led to increasing grain yield under sub-optimal and optimal soil nitrogen conditions. The increase in protein content of the detillered plants thus, appears to have resulted from improving the nitrogen supply from roots when the root/shoot ratio was increased.

Fig. 5 shows that leaf proteins in the intact plants were depleted in both cultivars – although at different kinetics – at 20 and 25 dpa, a response that was prevented greatly in Sakha 101 but slightly (if any) in IR64 by detillering. Depletion of leaf proteins could mark the onset of leaf senescence (Rubia et al. 2014) and if so, then detillering could have acted to delay leaf senescence by maintaining higher leaf protein contents. Detillering did not bring about specific increase in the Rubisco contents relative to total leaf soluble proteins in either cultivar. This contrasts with the findings of Mae (1997) who reported that both specific and absolute (per unit leaf fresh weight) contents of Rubisco increased when nitrogen supply to plants was improved. Such difference could be attributed to plant growth conditions (specifically soil nitrogen) before applying more nitrogen to plants. The decrease in leaf protein content in the intact IR64 did not coincide with a decrease in Rubisco content. This suggests that Rubisco may not be a main source of remobilized nitrogen from leaves to grains (Murchie et al. 2002). Contrarily, a consistent increase in Rubisco content per unit leaf fresh weight was observed in the detillered plants of Sakha 101 but not IR64. Data in Fig. 6C, D indicate that the Rubisco contents per unit leaf fresh weight in Sakha 101 control plants were below maximum and as a result, such contents were enhanced in the detillered plants presumably as a result

of improved nitrogen supply from roots. In contrast, the control plants of IR64 at most time points appeared to have the maximum Rubisco content that was not further improved by improving nitrogen supply from roots. In IR64 detillered plants, the changes in Rubisco content shown in Fig. 6 appear to have contributed little if any to the enhanced rates of photosynthesis shown in Fig. 3A, B, because A was enhanced in IR64 before anthesis without a change in Rubisco content. However, in Sakha 101, the consistent increases in Rubisco content strongly correlate with increases in A suggesting a causal relationship (Figs 3A, 6C), and implying that earlier suggestions that the rate of CO_2 assimilation under ambient air and saturating light is limited by the amount of Rubisco (Hudson et al. 1992, Makino et al. 1997), does not equally apply to all rice cultivars under all physiological conditions. The differential response of Sakha 101 and IR64 to changes in Rubisco content and its consequences on rate of photosynthesis, was apparently because IR64 had significantly higher Rubisco content in the intact plants (compared to Sakha 101) that may be already in excess of the amount for ribulose-1,5-bisphosphate (RuBP) regeneration (Mae 1997). Overall, our data suggest that in the detillered Sakha 101, A was improved as a result of improved water status (see above) as well as increased Rubisco content; whereas in IR64, A was enhanced essentially because of the more positive water status during the midday (Fig. 2).

The soluble sugar contents of leaves in Sakha 101 and IR64 showed no response to grain filling or to detillering. Starch contents of leaves responded similarly except that they were depleted in the control but not in the detillered plants of the two cultivars at 20 and 25 dpa. A largely similar response in intact rice plants was reported by Murchie et al. (2002). The stability of sugar contents of leaves before and after flowering in the control and detillered plants (although rates of photosynthesis were different) suggests that sink size was continually adjusted to fit the carbon source strength so that no excessive carbohydrates accumulate in leaves. In this case, the increase of protein but not carbohydrate contents (in spite of higher rates of photosynthesis, Fig. 3A, B) in the leaves of the detillered plants suggests that sugar (but not protein) translocation to sink(s) was enhanced as a result of the more positive water status (Quick et al. 1992) and/or as a result of the expanded sink capacity in the form of bigger leaves (Fig. 1D) during the vegetative stage or bigger panicles (Table 1) during the grain filling. However, the decrease in starch content of leaves at 20 and 25 dpa in the control but not in the detillered plants coupled with the accelerated depletion of leaf proteins indicates that these resources have been transported to a secondary alternative sink (late tillers, Fig. 3B) that does not exist in the detillered plants. Contrarily, sugar and protein resources seem to be in excess of the grain filling requirements in the detillered plants. Overall, these data suggest that carbohydrate accumulation in leaves of the tested rice cultivars seems to be more tightly controlled than that of proteins whose levels were modified after detillering. Consequently, the size of existing or developing sinks may be adjusted predominantly based on carbohydrate availability.

We conclude that the rice root system in the tested traditional rice cultivars is limiting to biomass accumulation and grain yield at the tiller level because of insufficient water and nitrogen uptake. Increasing the root/shoot ratio was shown experimentally to instantly increase stomatal conductance, photosynthesis and leaf protein content during the vegetative and reproductive stages, which resulted in a substantial boost in vegetative biomass accumulation and grain dry weight per panicle. Therefore, the yield potential of the current traditional rice cultivars could be substantially improved by enhancing the root system performance presumably through increasing the root growth relative to that of the shoot or improving water and nitrogen uptake capacity of root tissues by gene transformation.

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