Planta (2002) 215: 645–652
DOI 10.1007/s00425-002-0789-2

ORIGINAL ARTICLE

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Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence and remobilization of carbon reserves in rice subjected to water stress during grain filling

Received: 20 October 2001 / Accepted: 7 January 2002 / Published online: 20 June 2002

Abstract The possible regulation of senescence-initiated remobilization of carbon reserves in rice (*Oryza sativa* L.) by abscisic acid (ABA) and cytokinins was studied using two rice cultivars with high lodging resistance and slow remobilization. The plants were grown in pots and either well-watered (WW, soil water potential = 0 MPa) or water-stressed (WS, soil water potential = –0.05 MPa) from 9 days after anthesis until they reached maturity. Leaf water potentials of both cultivars markedly decreased at midday as a result of water stress but completely recovered by early morning. Chlorophyll (Chl) and photosynthetic rate (Pr) of the flag leaves declined faster in WS plants than in WW plants, indicating that the water deficit enhanced senescence. Water stress accelerated starch remobilization in the stems, promoted the re-allocation of pre-fixed ^{14}C from the stems to grains, shortened the grain-filling period and increased the grain-filling rate. Sucrose phosphate synthase (SPS, EC 2.4.1.14) activity was enhanced by water stress and positively correlated with sucrose accumulation in both the stem and leaves. Water stress substantially increased ABA but reduced zeatin (Z) + zeatin riboside (ZR) concentrations in the root exudates and leaves. ABA significantly and negatively, while Z + ZR positively, correlated with Pr and Chl of the flag leaves. ABA, not Z + ZR, was positively and significantly correlated with SPS activity and remobilization of pre-stored carbon. Spraying ABA reduced Chl in the flag leaves, and enhanced SPS activity and remobilization of carbon reserves. Spraying kinetin had the opposite effect. The results suggest that both ABA and cytokinins are involved in controlling plant senescence, and an enhanced carbon remobilization is attributed to an elevated ABA level in rice plants subjected to water stress.

Keywords Abscisic acid · Carbon remobilization · Cytokinin · *Oryza* (water stress) · Senescence · Water stress

Abbreviations ABA: abscisic acid · Chl: chlorophyll · DAA: days after anthesis · NSC: nonstructural carbohydrate · Pr: photosynthetic rate · SPS: sucrose phosphate synthase · WS: water-stressed · WW: well-watered · Z: zeatin · ZR: zeatin riboside

Introduction

Senescence in monocarpic plants such as rice (*Oryza sativa* L.) is a genetically programmed process that involves remobilization of nutrients from vegetative tissues to grains (Kelly and Davies 1988a, b; Sklensky and Davies 1993; Buchanan-Wollaston 1997; Ori et al. 1999). Delayed senescence, which in practice is often induced by either too much nitrogen fertilizer or an adoption of strong lodging-resistant varieties that stay "green" for too long, delays such remobilization and can lead to slow grain filling and low harvest index. Our earlier work (Yang et al. 2000, 2001a, b) showed that remobilization of stored carbon reserves in wheat and rice is promoted by water stress and that water deficits imposed during grain filling enhance plant senescence and accelerate grain filling. However, very little is known about the mechanisms by which plant senescence facilitates the remobilization of assimilates and the relationship between these two processes.

It is generally assumed that abscisic acid (ABA) and cytokinins are two major regulators of plant senescence (Biswas and Choudhuri 1980; Nooden 1988; van Staden et al. 1988). Leaf senescence is usually correlated with a decrease in cytokinins in the leaves (Buchanan-Wollaston 1997; Nooden et al. 1997). Treatment with

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cytokinins has been shown to delay leaf senescence in many plants (van Staden et al. 1988). In contrast, ABA is considered a senescence promoter (Nooden 1988; Madhu et al. 1999; Tadas et al. 1999). Spraying with ABA promoted foliar senescence in rice (Ray et al. 1983). ABA has also been reported to be an important regulator in transporting assimilates to the developing seeds or fruits (Tietz et al. 1981; Clifford et al. 1986; Brenner and Cheikh 1995; Yang et al. 1999). However, the proposal that ABA is involved in regulating both senescence and assimilate mobilization has remained disputable (Jones and Brenner 1987; Nooden 1988; Barratt et al. 1989; Ober and Setter 1990; Brown et al. 1991; Schussler et al. 1991; de Bruijn and Vreugdenhil 1992; Yang et al. 2000).

The purposes of this study were to investigate the processes of senescence and remobilization of carbon reserves in rice subjected to water deficits during grain filling, and determine whether and how ABA and cytokinins in the root exudates and leaves were correlated with these processes.

Materials and methods

Plant materials

The experiment was conducted at a farm of Yangzhou University, Jiangsu Province, China (32°30′N, 119°25′E) during the rice growing season (May to October) of 1999, and repeated in 2000. Seeds of two highly lodging-resistant cultivars currently used in local rice production, *Oryza sativa* L. Yanjing 6 (japonica) and Yangdao 7 (indica), were sown in the paddy field on 10–11 May. Thirty-day-old seedlings were then transplanted to porcelain pots. Each porcelain pot (30 cm in height and 25 cm in diameter, 14.72 l in volume) was filled with 20 kg sandy loam soil [Typic fluvaquents, Entisols (U.S. taxonomy)] that contained organic matter at 2.43% and available N-P-K at 108, 33.9 and 66.3 mg kg^{-1}, respectively. Each pot was planted with three hills with two seedlings per hill. On the day of transplanting (9–10 June), 1 g N as urea, 0.3 g P as single superphosphate and 0.5 g K as KCl were mixed into the soil in each pot. N as urea was also applied at the mid-tillering (0.5 g per pot) and panicle initiation (0.8 g per pot) stages. Both cultivars headed on 21–22 August (50% of plants), flowered on 23–25 August, and were harvested on 9–10 October. The water level in the pots was kept at 1–2 cm until 9 days after anthesis (DAA) when water-stress treatments were initiated.

Water-stress treatments

The experiment was a 2×2 (two cultivars and two levels of soil moisture) factorial design with four treatments. Each treatment had 80 pots as replicates. From 9 DAA to maturity, two levels of soil water potential were imposed by controlling water application. The well-watered (WW) plants were kept at 1–2 cm water depth (soil water potential = 0 MPa) in the pot by manually applying tap water, and the water-stressed (WS) plants were maintained with a soil water potential at –0.05 MPa. Soil water potential in pots with WS plants was monitored at a soil depth of 15–20 cm using a tension meter consisting of a 5-cm-long sensor, which was installed in each pot. Tension-meter readings were recorded every 4 h from 0600 to 1800 hours. When the readings dropped to a designated value, 0.2 l of tap water per pot was added manually. The pots were placed in a field and sheltered from rain by covering with a removable polyethylene shelter.

Radioactive labeling

At the booting stage (11–12 August), 12 pots of plants from each treatment were labeled with $^{14}CO_2$. Flag leaves of main stems were used for labeling between 0900 and 1100 hours on a clear day with photosynthetically active radiation at the top of the canopy ranging between 1,000 and 1,100 μmol photons m^{-2} s^{-1}. The whole flag leaf was placed in a polyethylene chamber (25 cm long, 4 cm diameter) and sealed with tape. A 6-ml sample of air was drawn out of the chamber and the same volume of mixed gas containing $^{14}CO_2$ was injected into the chamber (0.01 mol CO_2 with specific radioactivity of ^{14}C of 1.48 MBq l^{-1}). The chamber was removed after a 30 min.

Labeled plants were harvested at 0 (50% anthesis), 9 (the initiation of water withholding) and 12 DAA, and from 15 to 39 DAA at 6-day intervals. Harvested plants were divided into leaf blades, stem (culm plus sheath), and panicles. Carbon-14 in the plants was assayed by the method described by Ge et al. (1996). Radioactivity distribution to each part of the plant was expressed as a percentage of total radioactivity remaining in the above-ground portion of the plant.

Measurement of grain filling and grain yield

Two hundred panicles that headed on the same day were chosen and tagged from 20 pots of each treatment. The flowering date and the position of each spikelet on the tagged panicles were recorded. Twenty tagged panicles from each treatment were sampled at 6-day intervals from anthesis to maturity. The sampled panicles were divided into two groups (10 panicles each) as sub-samples. Grains that developed from the spikelets that flowered on the same day were removed, dried at 70 °C to constant weight for 72 h, dehulled and weighed. The grain-filling process was fitted to the Richards' (1959) growth equation as described by Zhu et al. (1988): $W = A/(1 + Be^{-kt})^{1/N}$, where W is the grain weight (mg), A is the final grain weight (mg), t is the time after anthesis (days), and B, k, and N are coefficients determined by regression. The active grain-filling period is defined as that when W was from 5% ($t1$) to 95% ($t2$) of A. The average grain-filling rate during this period was calculated from $t1$ to $t2$.

Ten pots of plants were harvested at maturity for the determination of grain yield.

Collection of root exudate

Four pots of plants (total of 109–111 stems) from each treatment were moved into an air-conditioned darkroom with the temperature at 18 °C during collection of root exudate. Each plant was cut at an internode about 12 cm above the soil surface at 1200 hours. An absorbent cotton ball was placed on the top of each decapitated stem and each pot covered with a polyethylene sheet. The cotton ball with exudate was collected after 6 h. The volume of exudate was estimated from the increase in cotton weight with the assumption that the specific gravity of the exudation sap was 1.0. Then the cotton ball was extracted with 99% ethanol, and the exudate was extracted three times by squeezing on a suction filter as detailed by Soejima et al. (1992). The extracts obtained from one pot of plants were pooled and stored at –20 °C pending hormonal analysis. Exudate collection was made at 0, 9 and 12 DAA, and from 15 to 39 DAA at 6-day intervals. The stems (culm + sheath) and flag leaves of the cut plants were frozen in liquid nitrogen and then stored at –80 °C for hormonal and enzymatic measurements, as well as for the measurement of nonstructural carbohydrate (NSC) and chlorophyll (Chl).

Measurement of photosynthetic rate

The photosynthetic rate (Pr) of the flag leaves was measured on the same date as exudate collection. A gas-exchange analyzer (CID-PS CO_2 Analyzer System; CID, Vancouver, Wash., USA) was used to measure Pr. Measurements were made during 0900–1100 hours

when photosynthetically active radiation above the canopy was 1,000–1,100 μmol photons m^{-2} s^{-1}. Six leaves were used for each treatment.

Measurement of leaf water potential

Leaf water potential of the flag leaves was measured at pre-dawn (0600 hours) and midday (1130 hours) on days 0, 6, 11, 17, 21 and 27 after withholding water, when the sky was clear. Well-illuminated flag leaves were chosen randomly for such measurements. A pressure chamber (Model 3000; Soil Moisture Equipment Corp., Santa Barbara, Calif., USA) was used for leaf water-potential measurement, with six leaves for each treatment.

Extraction, purification and quantification of hormones

Abscisic acid [(±) ABA], zeatin (Z) and zeatin riboside (ZR) were extracted and purified by the methods of Bollmark et al. (1988) and He (1993), and assayed by an enzyme-linked immunosorbent assay (ELISA). The mouse monoclonal antigens and antibodies against Z, ZR and ABA, and immunoglobulin G–horseradish peroxidase (IgG-HRP) used in the ELISA were produced at the Phytohormones Research Institute, China Agricultural University, China (see He 1993). The method for quantification of ABA, Z and ZR by ELISA was as described previously (Yang et al. 2001c). Recoveries of ABA, Z and ZR in root exudates were 77.6 ± 4.6, 79.8 ± 4.9 and 82.3 ± 5.1 respectively, and those in leaves were 75.7 ± 3.5, 76.3 ± 4.2 and 80.1 ± 4.7, respectively.

Extraction and assay of sucrose phosphate synthase (SPS)

The method for SPS (EC 2.4.1.14) extraction was modified from Hirano et al. (1997). The frozen stems and leaves [3–4 g fresh weight (FW)] were ground with a mortar and pestle in 10 ml of ice-cold 40 mM Hepes–NaOH (pH 7.5) buffer containing 10 mM $MgCl_2$, 1.5 mM EDTA, 3 mM DTT, 0.5 mg/ml BSA and 0.05%(v/v) Triton X-100. The homogenate was centrifuged at 12,000 *g* for 10 min (< 4 °C). The supernatant was immediately desalted by centrifugal filtration on a Sephadex G-25 (Pharmacia, Freiburg, Germany) column equilibrated with the grinding buffer minus EDTA and Triton X-100. SPS activity was assayed according to the method of Huber and Huber (1990). The enzyme activity was expressed as μmol sucrose synthesized g^{-1} FW min^{-1}.

NSC (soluble sugars and starch) and sucrose contents in stems and leaves were determined according to Yoshida et al. (1976) and Gong and Zhang (1995), respectively. The Chl content of the flag leaves was determined as described by Holden (1976).

Application of ABA and kinetin

An additional 90 pots of WW plants for each cultivar were arranged for ABA and kinetin application. Starting at 9 DAA, either 25×10^{-6} M ABA or 50×10^{-6} M kinetin (both from Sigma) was sprayed on the leaves at the rate of 50 ml per pot per day for 5 consecutive days, with 0.5% (v/v) Teepol (Fluka, Riedel-de-Haen, Germany) as surfactant. Control plants were sprayed with the same volume of 0.5% Teepol solution. Each treatment comprised 30 pots. Chl content and SPS activity in the flag leaves were measured as described above at 16 and 27 DAA, with five replications for each measurement. Ten pots of plants for each treatment were harvested for examination of grain weight and remobilization of carbon reserves in stems.

Statistical analysis

The results were analyzed for variance using the SAS statistical analysis package (version 6.12; SAS Institute, Cary, N.C., USA). Data from each sampling date were analyzed separately. Means were tested by least significant difference at the $P_{0.05}$ level ($LSD_{0.05}$). Linear regression was used to evaluate the relationship between hormonal contents in the root exudates and leaves with remobilization of carbon reserves and senescence parameters. The differences in the data between the two cultivars were not significant statistically but they are still presented separately for clarity.

Results

Leaf water potential

Figure 1 illustrates the progression of leaf water potentials during the first 27 days after withholding water. Both cultivars exhibited a similar trend of leaf water potential changes either at pre-dawn (0600 hours) or at midday (1130 hours). In WW plants, midday leaf water potential decreased gradually during grain filling, from –0.53 to –0.59 MPa at the beginning to –0.98 to –1.01 MPa on day 27 after withholding water. In WS plants, midday leaf water potentials were substantially reduced from –0.56 to –0.58 MPa initially to –1.69 to –1.74 MPa 27 days after withholding water. The differences in pre-dawn leaf water potentials between WW and WS plants were insignificant, indicating that plants subjected to the water deficit could rehydrate completely overnight.

Senescence parameters

Photosynthetic rate and Chl content were monitored as an objective way to quantify senescence. Both Pr and Chl content in the flag leaf for WW plants gradually declined

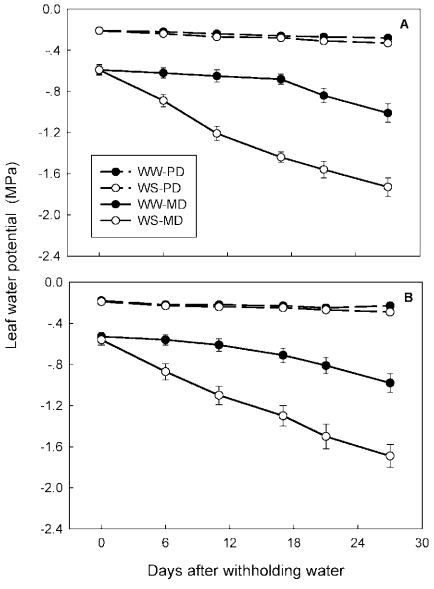

Fig. 1. Changes in leaf water potentials of the rice (*Oryza sativa*) japonica cultivar Yanjing 6 (**A**) and indica cultivar Yangdao 7 (**B**) during the first 27 days after withholding water. WW and WS are plants that were well-watered or water-stressed during grain filling. Measurements were made on the flag leaves at pre-dawn (*PD*, 0600 hours) and at midday (*MD*, 1130 hours). Vertical bars represent ± SE of the mean ($n = 6$) where these exceed the size of the symbol

during grain filling (Fig. 2). The rate of decline was increased after plants were exposed to a water deficit. The reduced Pr and Chl in the leaves of WS plants indicated that the water deficit promoted plant senescence.

Remobilization of carbon reserves and grain filling

Water stress facilitated the reallocation of pre-anthesis assimilates from the stems to grains. Figure 3 shows the disappearance of ^{14}C assimilated in the stems pre-anthesis and its appearance in the grains during grain filling. At the start of water withholding (9 DAA), about 75% of ^{14}C fed to the flag leaves at the booting stage was partitioned in the stems, and about 10% in the grains. After 18 days (27 DAA), ^{14}C in the stem was reduced to 17–21% under water stress and 44–48% in WW plants. Opposite to that observed in the stem, the ^{14}C in the grains increased by 59–65% in WS plants and only 27–33% in WW plants at 27 DAA.

Very similar to ^{14}C re-allocation, starch in the stems declined more quickly in WS than in WW plants (Fig. 4). At 27 DAA, starch in WS stems was 28–39 mg g^{-1} DW, i.e. 85–88 mg g^{-1} DW less than in WW stems. Remobilized carbon reserves (reduction in NSC from anthesis to maturity in the stems as a percentage of NSC in the stems at anthesis) and harvest index for WS plants were 56.4–59.1% and 13.0–15.9%, respectively, greater than values for WW plants (Table 1).

Water stress increased the grain-filling rate and shortened the grain-filling period (Table 1). The active grain-filling period was shortened by 5.9–9.4 days and the grain-filling rate increased by 0.28–0.36 mg $grain^{-1}$ day^{-1} compared with WW plants. Although final grain yield was higher under water stress it was not significantly different from that of WW plants, implying that the gain from accelerated grain-filling rate outweighed the loss of photosynthesis as a result of a shortened grain-filling period when water stress occurred during grain filling.

Sucrose and SPS

Water stress enhanced sucrose accumulation and SPS activity in the stems and leaves during the rapid period of starch remobilization in the stems (9–27 DAA;

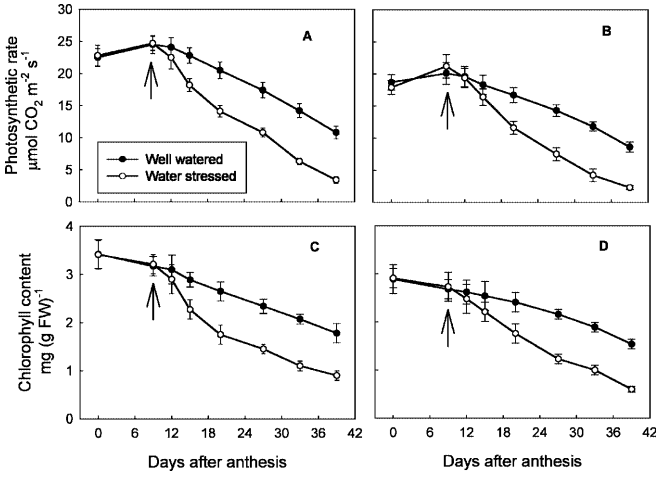

Fig. 2. Photosynthetic rate (**A, B**) and chlorophyll content (**C, D**) in flag leaves of well-watered and water-stressed plants of the rice japonica cultivar Yanjing 6 (**A, C**) and indica cultivar Yangdao 7 (**B, D**). *Arrows* in the figure indicate the start of withholding water. Vertical bars represent ± SE of the mean (n = 6) where these exceed the size of the symbol

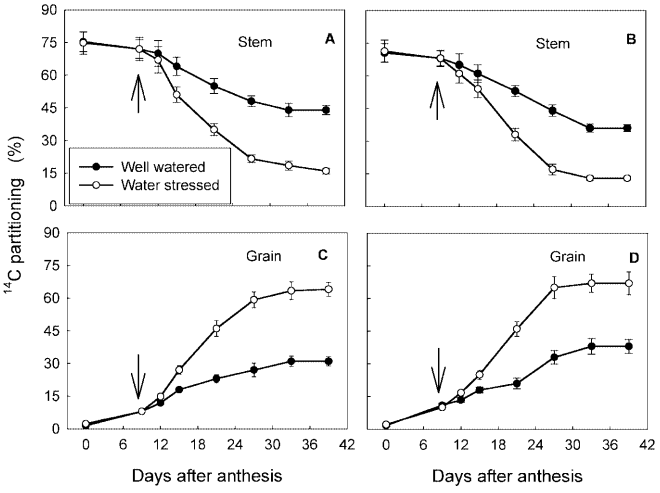

Fig. 3. Changes in ^{14}C partitioning in the stems (**A, B**) and grains (**C, D**) of well-watered and water-stressed plants of the rice japonica cultivar Yanjing 6 (**A, C**) and indica cultivar Yangdao 7 (**B, D**). The ^{14}C was fed to the flag leaves at the booting stage. *Arrows* in the figure indicate the start of withholding water. Vertical bars represent ± SE of the mean ($n = 6$) where these exceed the size of the symbol

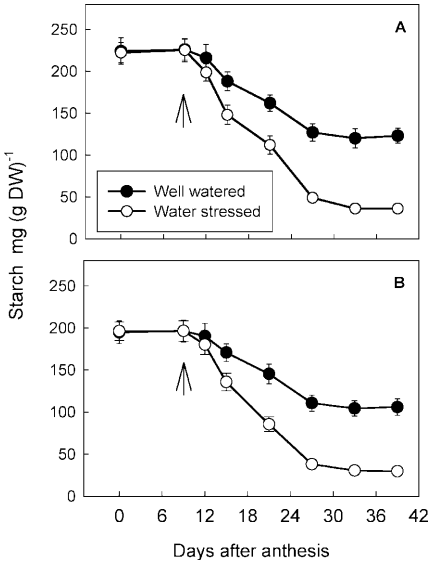

Fig. 4. Changes in starch content in the stems of well-watered and water-stressed plants of the rice japonica cultivar Yanjing 6 (**A**) and indica cultivar Yangdao 7 (**B**). *Arrows* in the figure indicate the start of withholding water. Vertical bars represent ± SE of the mean ($n = 4$) where these exceed the size of the symbol

Table 1. Remobilized carbon reserves, grain-filling rate and grain yield of rice (*Oryza sativa*). WW and WS plants were well-watered or water-stressed, respectively, during grain filling. The active grain-filling period and grain filling were calculated according to Richards' (1959) equation. Values of grain yield were means of the plants harvested from 10 pots of each treatment. Different letters within the same cultivar indicate statistically significant differences at $P_{0.05}$

Cultivars	Water deficit treatment	Remobilized C reserve[a] (%)	Active grain filling period (d)	Grain filling rate (mg d^{-1} $grain^{-1}$)	Grain yield (g pot^{-1})	Harvest index[b]
Yanjing 6	WW	46.8 b	33.8 a	0.78 b	84.5 a	0.44 b
	WS	73.2 a	24.4 b	1.14 a	87.6 a	0.51 a
$LSD_{0.05}$		6.2	1.5	0.15	9.8	0.02
Yangdao 7	WW	51.5 b	29.6 a	0.93 b	89.2 a	0.46 b
	WS	81.9 a	23.7 b	1.21 a	92.1 a	0.52 a
$LSD_{0.05}$		5.7	2.1	0.18	7.4	0.01

[a][NSC in stems at anthesis – NSC in stems at maturity]/NSC in stems at anthesis × 100
[b]Total grain weight/total above-ground dry weight

Fig. 5). Changes in SPS activity paralleled those in sucrose content. Regression analysis demonstrated that SPS activity was positively correlated with sucrose content with $r = 0.83$ ($P < 0.01$) in the stems and $r = 0.85$ ($P < 0.01$) in the leaves.

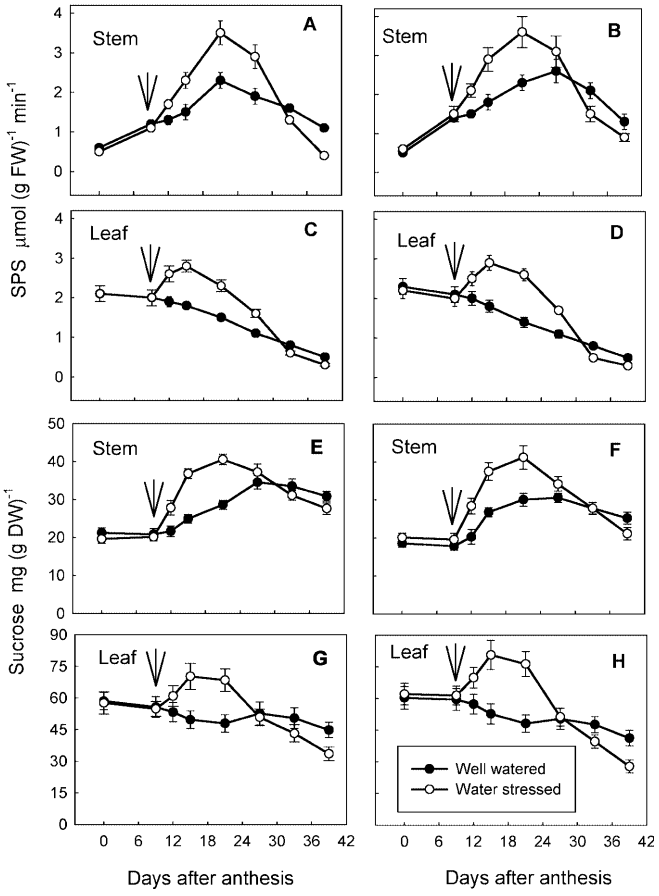

Fig. 5. Changes in SPS activity (**A–D**) and sucrose content (**E–H**) in the stems (**A, B, E, F**) and the flag leaves (**C, D, G, H**) of well-watered and water-stressed plants of the rice japonica cultivar Yanjing 6 (**A, C, E, G**) and indica cultivar Yangdao 7 (**B, D, F, H**). *Arrows* in the figure indicate the start of withholding water. Vertical bars represent ± SE of the mean ($n = 4$) where these exceed the size of the symbol

Hormonal changes in root exudates and leaves

The total volume of the root exudates collected from four pots of plants was 20.7–21.6 ml for WS plants, about two-thirds of that for WW plants (Table 2). The ABA concentration in the exudates of WS plants was 195–197 pmol ml^{-1}, about 3- to 4-fold greater than that for WW plants. In contrast to the increase in ABA concentration, Z and ZR concentrations decreased under water deficit, to about one-fourth to two-thirds of those for WW plants. At the same level of soil water potential, the Z concentration was about one-half that of ZR (Table 2), and both showed a similar changing pattern in the root exudates and leaves during grain filling (data not shown).

Changes in ABA and Z + ZR concentrations in the flag leaves exhibited very similar profiles to those in the root exudates (Fig. 6). The ABA concentration changed little in WW plants, but sharply increased as soon as a water deficit was imposed, reaching its maximum at 18 DAA and decreasing thereafter. Z + ZR concentrations declined during grain filling. They were affected little by water stress during the first week after withholding water, and declined rapidly thereafter.

Based on the relative values (WS plants/WW plants), the ABA concentration in both the root exudates and leaves was positively and significantly correlated with SPS activities, ^{14}C partitioning in grains, and starch remobilization in stems, and negatively and significantly correlated with Pr and Chl content of the flag leaves (Table 3). Opposite to ABA, the concentration of Z + ZR was positively and significantly correlated with the Pr and Chl content, and negatively and significantly correlated with ^{14}C partitioning in grains and starch remobilization in stems. The Z + ZR concentration was also negatively correlated with SPS activity, although the correlation was not statistically significant (Table 3).

Effects of exogenous ABA and kinetin

Application of ABA to WW plants at the early grain-filling stage (9–13 DAA) significantly reduced Chl in the leaves (Table 4). However, SPS activity, remobilized

Table 2. Volumes of the root exudates collected over a 6-h period and concentrations of ABA, Z and ZR in the exudates during grain filling of rice. WW and WS plants were well-watered or water-stressed, respectively, during grain filling. The exudate was collected from 1200 to 1800 hours at 0, 9, 12, 15, 21, 27, 33 and 39 DAA. Data are expressed as means ± SE of four pots of plants at eight collection times

Cultivar	Treatment	Total volume (ml)	Concentration (pmol ml^{-1})		
			ABA	Z	ZR
Yanjing 6	WW	34.4 ± 2.6	55.4 ± 3.7	4.9 ± 0.4	10.4 ± 1.1
	WS	21.6 ± 1.4	195 ± 12.4	3.7 ± 0.3	7.6 ± 0.5
Yangdao 7	WW	35.9 ± 2.4	63.8 ± 4.3	4.8 ± 0.4	11.4 ± 1.2
	WS	20.7 ± 1.7	197 ± 15.8	3.2 ± 0.3	7.1 ± 0.4

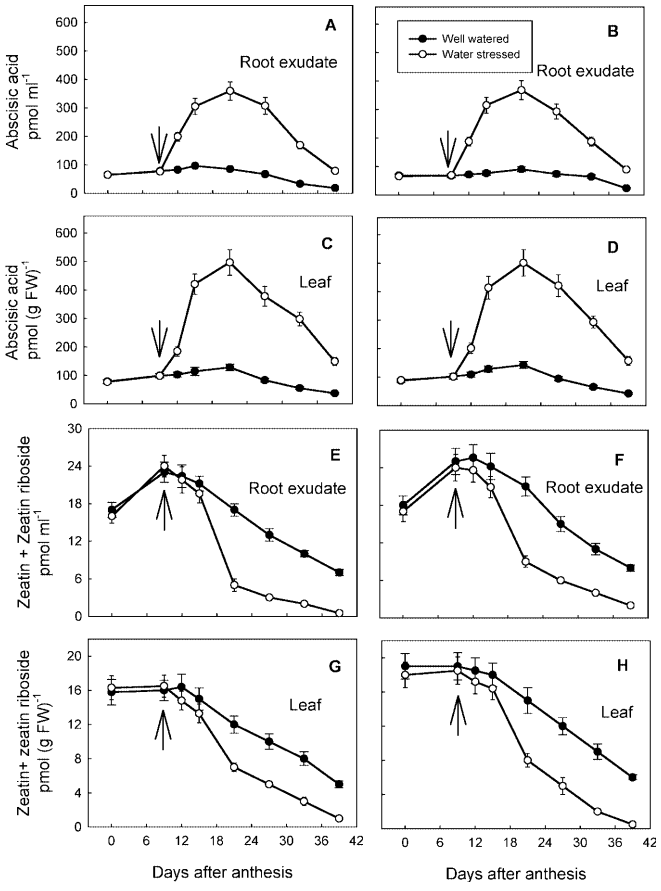

Fig. 6. Changes in ABA (**A–D**) and Z + ZR riboside (**E–H**) concentrations in the root exudates (**A, B, E, F**) and the flag leaves (**C, D, G, H**) of well-watered and water-stressed plants of the rice japonica cultivar Yanjing 6 (**A, C, E, G**) and indica cultivar Yangdao 7 (**B, D, F, H**). *Arrows* in the figure indicate the start of withholding water. Vertical bars represent ± SE of the mean ($n = 4$) where these exceed the size of the symbol

carbon reserves and grain weight for ABA-treated plants were increased by 101–128%, 62–63%, and 4.2–4.5%, respectively, compared with the control. Application of kinetin had the opposite effect.

Discussion

Water stress imposed during grain filling, especially at the early filling stage, usually results in a reduction in grain weight (Ober et al. 1991; Setter 1993; Mambelli and Setter 1998). We observed, however, that if the water deficit is controlled properly during grain filling, plants can rehydrate overnight (Fig. 1). A benefit from such a water deficit is that it can enhance plant senescence (Fig. 2) and lead to faster and better remobilization of pre-stored carbon from vegetative tissues to the grains (Fig. 3, 4), and an acceleration of the grain-filling rate (Table 1). The gain from the enhanced remobilization and accelerated grain-filling rate may outweigh the loss of photosynthesis and shortened grain-filling period, and increase the grain yield and harvest index. This practice would be of great importance in cases where heavy use of nitrogen or adoption of strong lodging-resistant varieties delays senescence. Also, such practice may benefit rice production in areas where water shortage is a problem.

Our results showed that water stress increased ABA and decreased Z + ZR concentrations in the root exudates (Fig. 6, Table 2), and changes in ABA and Z + ZR concentrations in the flag leaves were very closely associated with those in the root exudates. This result supports the earlier proposal that both ABA and cytokinins can act as stress signals that are produced in roots and carried through the xylem into shoots where physiology is regulated (Nooden et al. 1990; Davies and Zhang

Table 3. Coefficients for correlation of ABA and cytokinin concentrations in root exudates and flag leaves with SPS activity, starch mobilization in the stem (starch at anthesis minus starch at 9, 12, 15, 21, 27, 33 and 39 DAA), ^{14}C partitioning in grains, photosynthetic rate and chlorophyll content of the flag leaves during grain filling of rice. Data used for calculations are from Figs. 2, 3, 4, 5 and 6 and are expressed as relative values (WS plants/WW plants). *, ** Correlation significant at $P = 0.05$ and $P = 0.01$ levels, respectively

Correlation with:	Abscisic acid in:		Cytokinin in:	
	Exudate	Flag leaf	Exudate	Flag leaf
SPS in stems	0.82**	0.78*	–0.46	–0.39
SPS in the flag leaf	0.85**	0.81*	–0.49	–0.51
Starch remobilization in stems	0.87**	0.91**	–0.89**	–0.90**
^{14}C in grains	0.94**	0.93**	–0.86**	–0.81**
Photosynthetic rate of flag leaf	–0.83**	–0.92**	0.89**	0.96**
Chlorophyll content of flag leaf	–0.87**	–0.97**	0.88**	0.92**

Table 4. Effects of exogenous ABA and kinetin on chlorophyll content and SPS activity of the flag leaves, and remobilization of carbon reserves and grain weight of rice. The plants were grown in well-watered pots. The leaves were sprayed either with 25×10^{-6} M ABA or with 50×10^{-6} M kinetin daily for 5 days starting at 9 DAA. Chlorophyll and SPS values are the means of measurements made 16 and 27 DAA, respectively, with five replications for each measurement. The remobilization of carbon reserves and grain weight were determined at maturity with 10 replications. Statistical comparison is within the same column and the same cultivar. *, ** Values significantly different from the control at $P=0.05$ and $P=0.01$ levels, respectively

Cultivar	Treatment	Chlorophyll content (mg g^{-1} FW)	SPS activity (μmol g^{-1} FW min^{-1})	Remobilized carbon reserve[a] (%)	Grain weight (mg $kernel^{-1}$)
Yanjing 6	Control	2.49	1.23	45.9	26.4
	ABA	1.34**	2.81**	74.8**	27.5*
	Kinetin	2.78*	1.21	32.4**	24.8*
Yangdao 7	Control	2.29	1.34	49.7	26.7
	ABA	1.27**	2.69**	80.4**	27.9*
	Kinetin	2.69*	1.29	30.8**	25.8*

[a][NSC in stems at anthesis – NSC in stems at maturity]/NSC in stems at anthesis × 100

1991). We observed that ABA sharply increased as soon as the water deficit was imposed, whereas Z and ZR showed almost no response to water-deficit treatment during the initial treatment days (Fig. 6). This implies that in rice plants under mild water stress ABA may act as a more sensitive signal than cytokinins.

Although major plant hormones have been implicated in the senescence process, only cytokinins and ethylene have been shown definitively to have a role in the regulation of senescence (Smart 1994). Our results showed that ABA was significantly and negatively correlated with Pr and Chl of the flag leaves, while Z + ZR was positively correlated (Table 3). Exogenous ABA significantly reduced, while kinetin increased, the Chl content (Table 4). These results suggest that both ABA and cytokinins are involved in controlling senescence in rice plants subjected to water stress.

Water stress enhanced SPS activity both in the stems and leaves (Fig. 5). The activity was significantly correlated with sucrose accumulation in stems, in agreement with the view that SPS plays a major role in the resynthesis of sucrose (Whittingham et al. 1979; Wardlaw and Willenbrink 1994). The significance of an enhanced SPS activity under water stress is that not only does the plant accumulate the disaccharide as a response to the stress (Quick et al. 1989; Toroser and Huber 1997; Escobar-Gutierrez et al. 1998), but it also sustains the assimilatory carbon fluxes from source to sink (Isopp et al. 2000).

It is notable that ABA, but not cytokinins (Z + ZR), was positively and significantly correlated with SPS activity in both the stems and leaves (Table 3). When ABA was applied to the leaves, SPS activity was increased, whereas kinetin application had the opposite effect (Table 4). This may indicate that ABA in plants acts as a regulator of SPS activity. Since ABA, not cytokinins, was positively correlated with remobilization of prestored carbon, and such remobilization was enhanced by exogenous ABA (Table 4), we conclude that the enhanced remobilization can be attributed, at least partly, to an elevated ABA level under water stress in rice plants, probably through the regulation of key enzymes.

Acknowledgements We are grateful for grants from the Faculty Research Grant of Hong Kong Baptist University, Research Grant Council of Hong Kong University Grants Council (HKBU2052/00 M), the Area of Excellence for Plant and Fungal Biotechnology in the Chinese University of Hong Kong, the National Natural Science Foundation of China (Project No. 39970424), and the State Key Basic Research and Development Plan (grant No. G1999011704).

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