

Kai LIU, Yuxiu YE, Cheng TANG, Zhiqin WANG, Jianchang YANG

Responses of ethylene and ACC in rice grains to soil moisture and their relations to grain filling

© Higher Education Press and Springer-Verlag 2008

Abstract The objectives of this study were to investigate ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) in rice grains and root bleeding sap during the grain filling period and their relationship to the grain filling rate. Two high lodging-resistant rice (*Oryza sativa* L.) cultivars were grown in pots or tanks. Three treatments, including well watered (WW), moderate soil-drying (MD) and severe soil-drying (SD), were conducted from 9 days of post-anthesis until maturity. The effects of chemical regulators on the concentrations of ethylene and ACC in the grains were also studied. The results show that MD significantly increased the grain-filling rate and grain weight, whereas SD significantly reduced the grain-filling rate and grain weight. Concentrations of ethylene and ACC in the grains were very high at the early grain filling stage and then sharply decreased during the linear period of grain growth. MD reduced the ACC concentrations and ethylene evolution rate, whereas SD remarkably increased the ACC concentrations and ethylene evolution rate. Both the ethylene evolution rate in rice grains and the ACC concentrations in the root-bleeding sap were significantly and positively correlated with the ACC concentrations in rice grains. The ethylene evolution rate was significantly and negatively correlated with the grain-filling rate. The application of amino-ethoxyvinylglycine (AVG), an inhibitor of ethylene synthesis, at 9–13 days of post-anthesis significantly reduced the ACC concentrations and ethylene evolution rate of grains, but significantly enhanced the activities of sucrose synthase, ADP glucose pyrophosphorylase and soluble starch synthase. The

results were reversed when ethephon, an ethylene-releasing agent, was applied. The results suggest that moderate soil drying during the grain-filling period in rice could inhibit the production of ethylene and ACC and therefore accelerate grain filling and increase grain weight.

Keywords rice, 1-aminocyclopropane-1-carboxylic acid (ACC), ethylene, grain filling, root-bleeding sap, soil moisture

1 Introduction

In cereals such as rice (*Oryza sativa* L.), drought stress during grain filling usually shortens the grain filling period and reduces the grain filling rate, leading to a reduction in grain yield (Kobata and Takami, 1983; Rahman and Yoshida, 1985; Ober et al., 1991). This process is suggested to be mediated by hormones and their interactions in plants (Davies, 1995, 2004). Ethylene is a plant hormone involved in the responses to stress (Apelbaum and Yang, 1981; Beltrano et al., 1999). Water stress enhances the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS, EC 4.4.1.14), leading to an increase in ACC concentration and promotes ethylene production (Chen and Yu, 1988; Song and Dai, 2002). An overproduction of ethylene induced by drought has frequently been related to fruit abortion in cotton (*Gossypium hirsutum*) (Guinn, 1976) and grain weight reduction in wheat (Xu et al., 1995) and maize (*Zea mays*) (Chen and Lur, 1996). But there are many reports showing that drought stress reduces, rather than increases ethylene production (Narayana et al., 1991; Feng and Barker, 1992; Morgan and Drew, 1997). In recent years, Yang et al. (2000, 2001a, 2001b, 2006) and Wang et al. (2004) have reported that a mild water stress imposed during grain filling can enhance the carbon remobilization from vegetative tissues to grains and accelerate the grain-filling rate in rice and wheat. However, little is known whether and how ethylene is

Translated from *Acta Agronomica Sinica*, 2007, 33(4): 539–546
[译自: 作物学报]

Kai LIU, Yuxiu YE, Cheng TANG, Zhiqin WANG, Jianchang YANG (✉)

Key Laboratory of Crop Genetics and Physiology of Jiangsu Province, Yangzhou University, Yangzhou 225009, China
E-mail: jcyang@yzu.edu.cn

Yuxiu YE

Department of Agricultural and Food Science, Huaiyin Institute of Technology, Huaiyin 223001, China

involved in the process. The purpose of this study was to test the hypothesis that ethylene may be involved in mediating the effects of soil drying on grain filling. The changing patterns of ethylene and ACC concentrations in rice grains and root bleedings subject to the soil drying during grain filling and their relations with grain filling rate were investigated and the effects of chemical regulators on the concentrations of ethylene and ACC in the grains were studied to verify the role of the hormones.

2 Materials and methods

2.1 Materials and cultivation

The experiment was conducted at a farm in Yangzhou University, Jiangsu Province, China (32°30' N, 119°25' E) during the rice growing season (May to October) of 2004, and repeated in 2005. Two rice (*Oryza sativa* L.) cultivars, Wuyunjing 8 (*japonica*) and Yangdao 6 (*indica*) were used and were grown in pots. Each porcelain pot (30-cm in height and 25-cm in diameter, 14.72-L in volume) was filled with 20 kg of sandy loam soil (Typic fluvaquents, Entisols (US taxonomy) that contained organic matter at 2.42% and available N, P and K at 110, 34.6 and 66.6 mg·kg⁻¹, respectively. The seeds were sown in the paddy field on May 8–10. Thirty-day-old seedlings were then transplanted to pots on June 8–10. Each pot was planted with three hills with two seedlings in each. 1 g N as urea, 0.3 g P as single super phosphate, and 0.5 g K as KCl were mixed into the soil in each pot before transplantation. N as urea was also applied at mid-tillering (0.5 g per pot), panicle initiation (1 g per pot) and early heading stage (0.8 g per pot). The water level in the pot was kept at 1–2 cm until 9 days of post-anthesis when the soil-drying treatments were initiated.

2.2 Soil-drying treatments

From 9 days of post-anthesis until maturity, three levels of soil water potential (Ψ_{soil}) were imposed by controlling water application. The well-watered (WW) treatment group was flooded at a 1–2 cm water level in the pot ($\psi_{\text{soil}} = 0$ MPa) by manually applying tap water. A moderate soil-drying (MD) treatment was maintained at -0.01 MPa to -0.03 MPa, and a severe soil-drying (SD) treatment was maintained at -0.04 MPa to -0.06 MPa. Soil water potential in the soil-drying treatments was monitored at the soil depth of 15–20 cm. A tension meter (Soil Science Research Institute, Nanjing, China) consisting of a sensor of 5-cm length was installed in each pot to monitor the water potential. The tension meter readings were recorded every day at 6:00–7:00, 12:00–13:00 and

17:00–18:00, respectively. When the readings dropped to the threshold, 0.4 L and 0.2 L of tap water per pot was added to the MD and SD treatments, respectively. The pots were placed in a field and sheltered from rain by a removable polyethylene shelter which was placed over them during rain. Each treatment had 60 pot replications.

2.3 Sampling and measurement

2.3.1 Sampling

Six hundred panicles that headed on the same day were chosen and tagged for each treatment. Thirty tagged panicles from each treatment were sampled at 3-day intervals from anthesis to maturity. All grains that developed from the fertilized spikelets were removed. Half of sampled grains were used for measurements of ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC). The other half of the grains were dried at 70°C to a constant weight and then weighed. The grain-filling process was calculated by the Richards' (Richards, 1959) growth equation as described by Zhu et al. (1988):

$$W = \frac{A}{(1 + Be^{-kt})^{\frac{1}{N}}}; \quad (1)$$

and the grain filling rate (G) was calculated as the derivative of Equation (1):

$$G = \frac{AkBe^{-kt}}{(1 + Be^{-kt})^{\frac{N+1}{N}}}, \quad (2)$$

where W is the grain weight (mg), A is the final grain weight (mg), t is the time after anthesis (d), and B , k , and N are coefficients determined by regression. The active grain filling period was defined as the days when W was from 5% (t_1) to 95% (t_2) of A . An average grain-filling rate during this period was therefore calculated from t_1 to t_2 .

2.3.2 Collection of root bleeding sap

Three hills of plants from each treatment were used in the collection of root bleeding sap. Each plant was cut at the stem height about 12 cm above the soil surface at 18:00. An absorbent cotton ball was placed on the top of each decapitated stem and each pot was covered with a polyethylene sheet. The cotton ball with bleeding sap was collected at 6:00 the next day. The volume of bleeding sap was estimated from the increase in cotton weight. Then the cotton ball was extracted with 95% ethanol. The extracts obtained from one hill of plants were pooled and then used for measurements of ACC. The collection of bleeding sap was made at 3-d intervals from anthesis to the 36th day of post-anthesis.

2.3.3 Measurement of leaf water potential (ψ_{leaf})

Leaf water potential of the flag leaves were measured at pre-dawn (6:00) and midday (11:30) on the days 0, 5, 9, 14, 18, 23, 27 and 31 after withholding water when the weather was clear. Well-illuminated flag leaves were chosen randomly for such measurements. A pressure chamber (Model 3000; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) was used for the measurement of leaf water potential, with six leaves for each treatment.

2.3.4 Analysis of ethylene and ACC

The ethylene evolved from the grains was determined according to Beltrano et al. (1994) with some modifications. Briefly, sampled grains were placed between two sheets of moist paper for 1 h at 27°C in darkness to allow wound ethylene to subside. Each sample contained 25–30 grains. The grains were then transferred into glass vials which were volume predefined and immediately sealed with airtight subseal stoppers, and incubated in the dark for 24 h at 27°C. A 1 mL gas sample was withdrawn through the subseal with a gas-tight syringe and ethylene was assayed by using gas chromatography (HP5890 Series II; Hewlett Packard Com, Palo Alto, CA, USA) equipped with a Porapak Q column (0.3 cm × 200 cm, 50–80 mesh) and flame ionization detector (FID). Temperatures for the injection port, column and detector were kept constant at 140°C, 100°C and 200°C, respectively. Nitrogen was used as carrier at a flow rate of 30 mL·min⁻¹, and hydrogen and air were used for FID at the rate of 30 mL·min⁻¹ and 300 mL·min⁻¹, respectively. The rate of ethylene evolution was expressed as a function of tissue fresh weight (FW).

ACC in the grains was determined according to Cheng and Lur (1996). Ethylene evolved from ACC was assayed by using gas chromatography as described above. The transformation rate as a percentage from ACC to ethylene was (87 ± 4.1)%, (93 ± 5.8)% and (77 ± 5.4)%, respectively, at early, mid and late grain filling stages.

2.3.5 Chemical applications

Both the cultivars were used for chemical application. Plants were grown in eight cement tanks under open field conditions. Each tank (0.3 m in height, 1.6 m in width and 8.8 m in length) was filled with sandy loam soil with the same nutrient contents as the pot experiment. The seeds were sown in the paddy field on May 10–11. Thirty-day-old seedlings were then transplanted into the tanks on June 10–11 at a hill spacing of 0.15 m × 0.20 m with two seedlings per hill. N (6 g·m⁻² as urea), P (3 g·m⁻² as single super-phosphate) and K (3 g·m⁻² as KCl) were applied and incorporated before transplantation. N as urea was also applied at mid-tillering (3 g·m⁻²), panicle initiation (5 g·m⁻²) and early heading stage (3 g·m⁻²).

The tank was kept at a 1–2 cm water level until 9 days of post-anthesis when SD treatments were initiated. From 9 days of post-anthesis until maturity, either WW or SD treatments were imposed on the plants. Each of the treatments had four repetitions. The treatment details, water control and rain prevention were the same as the pot experiment. Ethephon (an ethylene-releasing agent) and amino-ethoxyvinylglycine (AVG, an inhibitor of ethylene synthesis) (all from Sigma, St Louis, MO, USA), were applied to the plants for both WW and SD treatments. Starting at 9 days of post-anthesis, either 50 × 10⁻³ mol·L⁻¹ ethephon, or 5 × 10⁻⁵ mol·L⁻¹ AVG, were sprayed at the rate of 800 mL·m⁻² on the top of plants (panicles) for 5 days. All the solutions contained ethanol and Tween-20 at final concentrations of 0.1% (v/v) and 0.01% (v/v), respectively. Control plants were sprayed with the same volume of deionized water containing the same concentrations of ethanol and Tween-20. Each chemical treatment was at an area of 1.2 m² with four replications.

The concentrations of ACC and ethylene in grains were determined on the 3rd and 7th day after the chemical treatment (16 and 20 days of post-anthesis), and the grain filling rate was measured by weighing grain every 6 days from heading to maturity. Measurement methods were the same as described above. Activities of three key enzymes involved in sucrose to starch conversion in rice grains, sucrose synthase (SuSase, EC 2.4.1.13), ADP glucose pyrophosphorylase (AGPase, EC 2.7.7.27) and soluble starch synthase (SSSase, EC 2.4.1.21) were also determined on the 18th and 24th days of post-anthesis. The enzyme activities were determined as described previously: SuSase (Ranwala and Miller, 1998), AGPase (Nakamura et al., 1989), and SSSase (Schaffer and Petreikov, 1997). Twenty plants (170–176 panicles) for each treatment were harvested at maturity for the determination of final grain weight.

2.3.6 Statistical analysis

The results were analyzed for variance using SAS statistical analysis package (version 6.12; SAS Institute, Cary, NC, USA). Data from each sampling date were analyzed separately. Means were tested by the least significant difference at a $P < 0.05$ level ($LSD_{0.05}$). The results from both years were very similar; therefore, data were averaged from both years.

3 Results and analysis

3.1 Leaf water potential (Ψ_{leaf})

Figure 1 illustrates the changes in Ψ_{leaf} during the first 35 days after withholding water. Midday Ψ_{leaf} of each

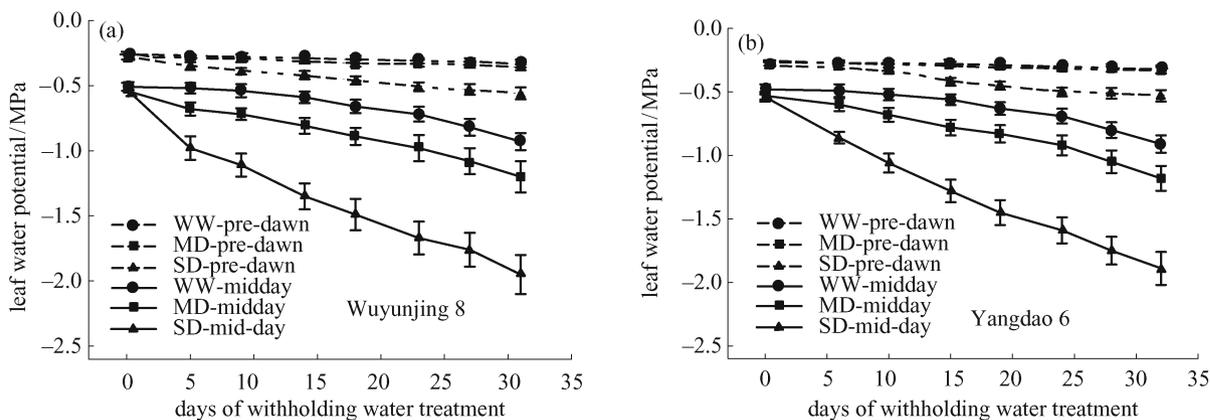


Fig. 1 Changes of leaf water potentials of rice

Note: Measurements were made on the flag leaves at pre-dawn (6:00 h, dashed lines) and midday (11:30, solid lines). WW, MD, and SD represent well-watered, moderate soil-drying and severe soil-drying treatments, respectively. The same applies below.

treatment decreased gradually during the grain filling. Water stress treatments substantially reduced Ψ_{leaf} and the reduction in SD treatments was much greater than that in MD treatments. There were no significant differences in pre-dawn Ψ_{leaf} between MD and WW plants, but it was much lower in SD plants, (Fig. 1), indicating MD plants could rehydrate overnight whereas SD plants could not. The two cultivars behaved similarly.

3.2 Effect of soil moisture on grain filling rate

MD treatment greatly increased the grain filling rate, but shortened the grain filling period (Fig. 2). The final grain weight in MD plants was significantly greater than that in WW plants, implying that the gain from the accelerated grain filling rate outweighed the possible loss of photosynthesis reduction and the shortened grain filling period. However, both the rate and duration of

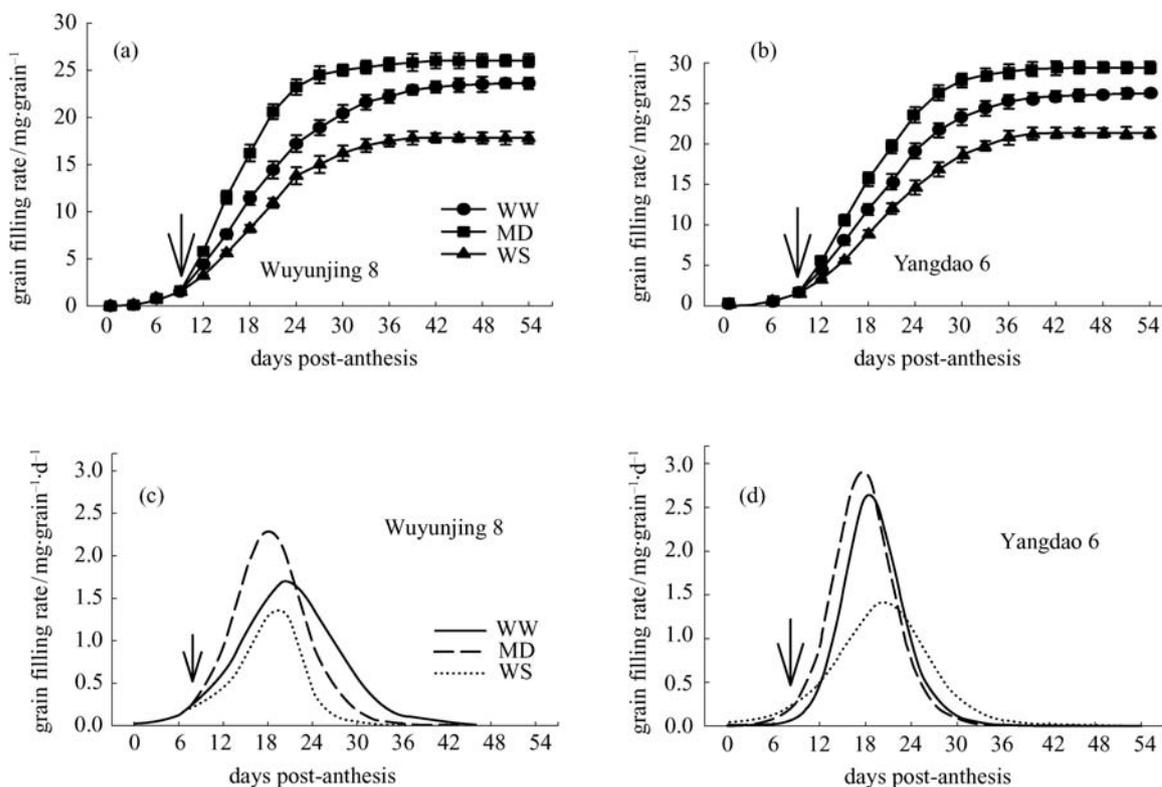


Fig. 2 Changes of grain weight (a, b) and grain filling rate (c, d) of rice

Note: Arrows in the figure indicate the start of withholding water. The same below.

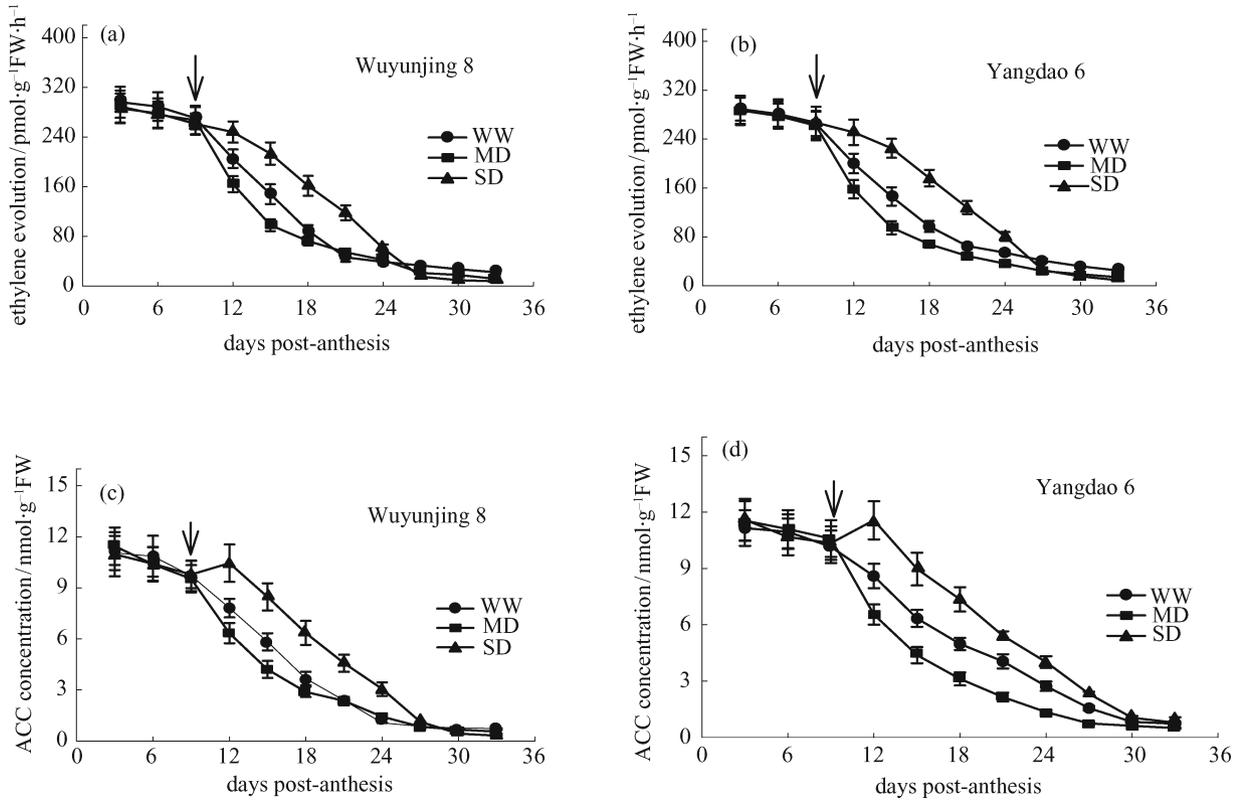


Fig. 3 Effect of water deficiencies on changes in ethylene evolution rate (a, b) and ACC concentrations (c, d) in the grains of rice

grain filling in SD plants were significantly reduced, leading to a lower grain weight, when compared with WW plants (Fig. 2).

3.3 Changes in ethylene evolution rate and ACC concentrations in the grains and relationship with grain filling rate

Ethylene evolution from the grains was very high during the early grain filling stage and rapidly decreased during the linear period of grain growth (9–24 days of post-anthesis). Little changed thereafter (Fig. 3a,b).

Compared with WW, MD reduced the ethylene evolution from grains, whereas SD substantially increased the evolution. Changes in ACC concentration in the grains were similar to those in ethylene (Fig. 3 c, d), i.e. MD decreased ACC concentrations, while SD increased ACC concentration in grains. The two cultivars behaved similarly. ACC concentration was significantly correlated with ethylene evolution rate ($r = 0.992^{**}$ to 0.996^{**} , $P < 0.01$) (Fig. 4), suggesting that an increase in ethylene production is attributed to an enhanced ACC level in SD grains. The ethylene evolution was significantly and negatively correlated with the grain filling rate

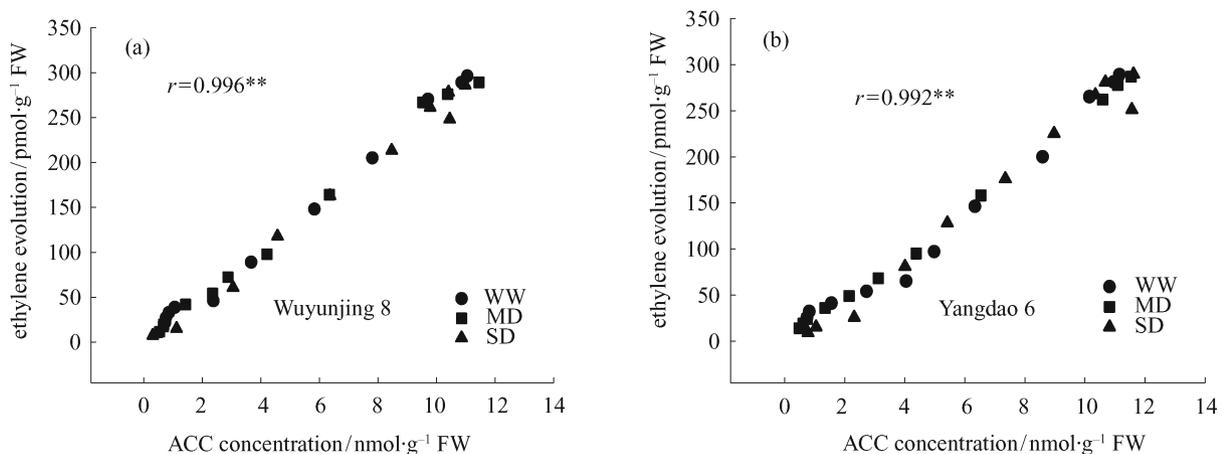


Fig. 4 Relationships of ethylene evolution rate with ACC concentrations in the grains of rice

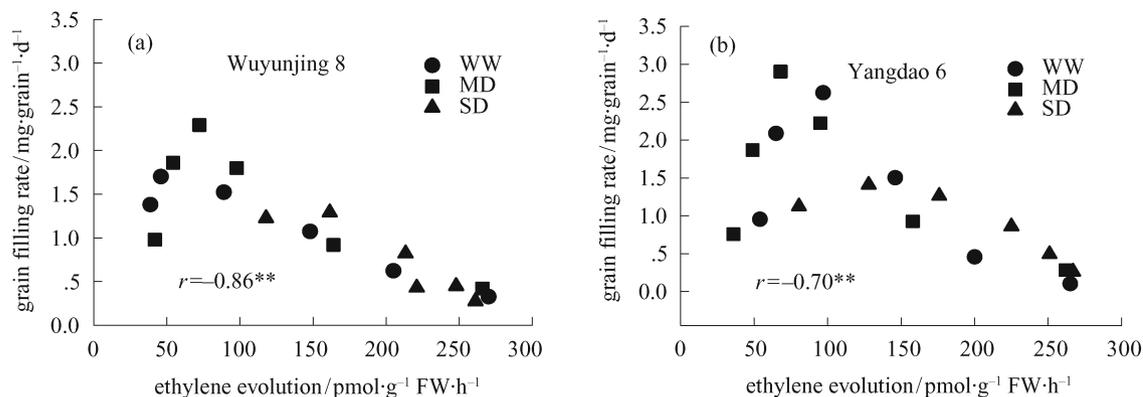


Fig. 5 Relationships of grain filling rate with ethylene evolution rate in the grains of rice

($r = -0.86^{**}$ to -0.70^{**} , $P < 0.01$) (Fig. 5), implying that ethylene plays a role in inhibiting grain filling.

3.4 Changes in ACC concentrations in root-bleeding sap

Changes in ACC concentrations in root-bleeding saps were similar to those in the grains under all the soil-drying treatments. ACC concentrations in root-bleeding saps were very high at early grain filling stage and rapidly decreased during the linear period of grain growth (9–24 days of post-anthesis). Little changed thereafter (Fig. 6 a, b). MD reduced ACC concentrations, while SD increased ACC concentrations in root-bleeding saps (Fig. 6 a, b). ACC concentrations in root-bleeding saps were significantly and positively correlated with ACC concentrations in grains ($r = 0.981^{**}$ to 0.983^{**} , $P < 0.01$) implying that ACC may act as a root-sourced signal by inducing ethylene production.

3.5 Effects of chemical regulators on ethylene evolution, ACC concentration and grain filling

The application of AVG, an inhibitor of ethylene synthesis, at 9–13 days of post-anthesis significantly reduced the ACC concentrations and ethylene evolution

rate in grains under both WW and SD conditions. The application of ethephon, an ethylene-releasing substance, shows an opposite effect (Table 1). The promoting or inhibiting effects of chemical regulators on the ethylene evolution and ACC production were higher in WW than those in SD. When compared with the control (no application of chemical regulators), the application of ethephon had no significant effect on ethylene evolution in grains in SD plants.

Additionally, the application of AVG significantly increased the activities of SuSase, AGPase, and SSSase in grains when compared with the control under both WW and SD conditions (Table 2). The application of ethephon to WW plants reduced the activities of enzymes mentioned above and that of ethephon to SD plants had no significant effect on the activities of SuSase, AGPase and SSSase (Table 2).

When Table 1 was compared with Table 2, it was noted that the increase or decrease in activities of SuSase, AGPase and SSSase was closely associated with the decrease or increase in ethylene evolution in grains indicating that the ethylene regulated the activities of enzymes involved in starch synthesis in grains.

Apart from the above, the application of AVG significantly increased grain filling rate, lengthened active

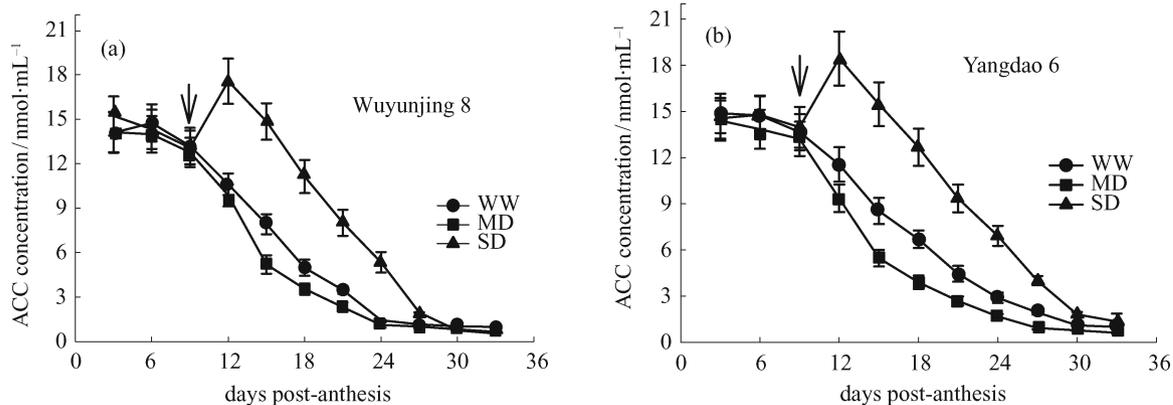


Fig. 6 Changes of ACC concentrations in root-bleeding sap in rice

Table 1 Effects of chemical regulators on ACC concentration and ethylene evolution rate in the grains of rice

treatment		16 days of post-anthesis		20 days of post-anthesis	
		ACC/nmol·g ⁻¹ FW	ethylene/pmol·g ⁻¹ FW·h ⁻¹	ACC/nmol·g ⁻¹ FW	ethylene/pmol·g ⁻¹ FW·h ⁻¹
well-watered	control	5.53 b	146 b	2.59 b	78.6 b
	5 × 10 ⁻⁴ mol·L ⁻¹ ethephon	8.57 a	212 a	6.42 a	153 a
	5 × 10 ⁻⁵ mol·L ⁻¹ AVG	3.26 c	85.5 c	1.11 c	46.3 c
soil-drying	control	8.98 a	229 a	5.49 a	129 a
	5 × 10 ⁻⁴ mol·L ⁻¹ ethephon	10.3 a	246 a	6.15 a	137 a
	5 × 10 ⁻⁵ mol·L ⁻¹ AVG	5.13 b	148 b	3.23 b	75.3 b

Note: The test cultivar is Yangdao 6, and the values followed by a different letter are significantly different at $P = 0.05$ within a column in the same soil moisture treatment (the same below).

grain filling period and significantly increased the grain weight under both WW and SD conditions. However, the application of ethephon to WW plants showed an opposite effect on grain filling rate, active grain filling period and grain weight in WW plants, with no significant effects observed when the ethephon was applied to SD plants (Table 3).

4 Discussion

Usually, a water deficit imposed during grain filling can reduce grain filling rate, shorten grain filling period and lead to a reduction in grain weight (Richards, 1959; Kobata and Takami, 1983; Rahman and Yoshida, 1985; Ober et al., 1991; Ahmadi and Baker, 1999; Wardlaw and Willenbrink, 2000). Similar results were observed under the severe soil-drying conditions in this study. However,

we observed that if a moderate soil-drying (MD, maintained at -0.01 MPa to -0.03 MPa) was imposed during the mid and late grain filling periods and plants could rehydrate overnight (Fig. 1), the remobilization of pre-stored carbon from vegetative tissues to the grains could be enhanced with the grain filling rate and grain weight increased (Fig. 2). The results were consistent with those of Yang et al. (2000, 2001a, 2001b, 2006) and Wang et al. (2004). They have reported that a moderate water-stress imposed during the grain filling period could enhance the activities of sucrose-phosphate synthase, α/β -amylase in stems and sheaths and the physiological activity of grain sink. It promoted the remobilization of stored assimilates from vegetative tissues to grains. Simultaneously, a moderate water deficit could enhance the activities of key enzymes (SuSase, AGPase and SSSase) involved in sucrose-to-starch and the gain from the accelerated grain filling rate and the enhanced

Table 2 Effects of chemical regulators on sucrose synthase(SuSase), adenine diphosphoglucose pyrophosphorylase (AGPase) and Insoluble starch synthase(SSSase) in the grains of rice (activities of enzymes in $\mu\text{mol}\cdot\text{grain}^{-1}\cdot\text{h}^{-1}$)

treatment		16 days of post-anthesis			20 days of post-anthesis		
		SuSase	AGPase	SSSase	SuSase	AGPase	SSSase
well-watered	control	2.34 b	1.12 b	0.12 b	3.12 b	1.46 b	0.17 b
	5 × 10 ⁻⁴ mol·L ⁻¹ ethephon	1.17 c	0.69 c	0.06 c	1.78 c	0.84 c	0.09 c
	5 × 10 ⁻⁵ mol·L ⁻¹ AVG	3.11 a	1.63 a	0.18 a	4.39 a	1.89 a	0.27 a
soil-drying	control	1.72 b	0.78 b	0.08 b	1.43 b	0.67 b	0.06 b
	5 × 10 ⁻⁴ mol·L ⁻¹ ethephon	1.67 b	0.73 b	0.07 b	1.39 b	0.61 b	0.05 b
	5 × 10 ⁻⁵ mol·L ⁻¹ AVG	2.86 a	1.36 a	0.13 a	2.41 a	1.14 a	0.11 a

Table 3 Effects of chemical regulators on grain weight, active grain filling period, and grain filling rate of rice

treatment		grain weight /mg·grain ⁻¹			active grain filling period /d	grain filling rate/ mg·grain ⁻¹ ·d ⁻¹
		16 days of post-anthesis	20 days of post-anthesis	maturity		
well-watered	control	7.45 b	13.4 b	25.2 b	26 b	0.88 b
	5 × 10 ⁻⁴ mol·L ⁻¹ ethephon	4.65 c	9.75 c	18.8 c	20 c	0.85 c
	5 × 10 ⁻⁵ mol·L ⁻¹ AVG	10.4 a	17.9 a	28.5 a	28 a	0.92 a
soil drying	control	5.69 b	10.4 b	19.6 b	20 b	0.88 b
	5 × 10 ⁻⁴ mol·L ⁻¹ ethephon	5.53 b	10.2 b	18.8 b	20 b	0.85 b
	5 × 10 ⁻⁵ mol·L ⁻¹ AVG	8.57 a	16.5 a	26.9 a	24 a	1.01 a

remobilization could outweigh the possible losses in a shortened grain filling period resulting in the increase in the grain filling rate, grain weight and grain yield (Yang et al., 2000, 2001a, 2001b, 2005, 2006; Wang et al., 2004). A mild water deficit imposed during the grain filling period also could improve the grain quality of rice (Cai et al., 2002; Yang et al., 2005). All the results suggested that a mild soil-drying imposed during the grain filling period could benefit the grain filling of rice. The soil water potential of -0.01 MPa to -0.03 MPa could be the irrigation indices during grain filling for both high yield and high quality of rice.

The effect of water stress on ethylene production remains in dispute (Morgan and Drew, 1997). Our results show that the concentration of ethylene release from grains decreased under the MD and remarkably increased under SD treatments. The ethylene evolution was closely associated with the elevated ACC levels in grains and the ACC concentration in grains were highly correlated with that in root bleeding sap implying that ACC may act as a root-sourced signal by inducing ethylene production to limit grain filling under a severe water stress.

Our results show that ethylene inhibits grain filling. There are few reports about the regulation of ethylene on grain filling. One such study reported that ethylene could act as a signal to induce the expression of the gene coding α -amylase in nutrient-stored organs leading to a decrease in starch accumulation in sinks (Rook et al., 2001). Naik and Mohapatra (2000) observed that the application of inhibitor of ethylene synthesis significantly enhanced the activities of sucrose synthase in rice grains at booting and heading stages. We also observed that the application of AVG, as an inhibitor of ethylene synthesis, at 9–13 days of post-anthesis significantly enhanced the activities of SuSase, AGPase and SSSase. The results were reversed when the ethephon, as an ethylene-releasing agent, was applied. The results implied that ethylene may inhibit the grain filling by weakening sink activity probably through regulating key enzymes involved in sucrose-to-starch conversion, especially, SuSase, AGPase and SSSase in rice grains.

5 Conclusions

A moderate water deficit or soil-drying imposed during the grain filling period of rice can accelerate the grain filling rate and increase the filled-grain percentage and grain weight. Severe drying can reduce the grain filling rate and grain weight. Such changes are closely associated with the ethylene evolution and ACC concentration in grains or roots. A lower ethylene and ACC concentration benefits the grain filling, whereas a higher ethylene and ACC concentration

exhibits an opposite effect. The mediation of ethylene to grain filling is probably through regulating the key enzymes involved in the sucrose-to-starch pathway. The physiological mechanism needs to be studied further.

Acknowledgements The research was financially supported by the National Natural Science Foundation of China (Grant Nos. 30671225 and 30771274), and the Natural Science Foundation of Jiangsu Province, China (No. BK2006069).

References

- Ahmadi A, Baker D A (1999). Effects of abscisic acid (ABA) on grain filling processes in wheat. *Plant Growth Regulation*, 28: 187–197
- Apelbaum A, Yang S F (1981). Biosynthesis of stress-ethylene induced by water deficit. *Plant Physiology*, 68: 594–596
- Beltrano J, Carbone A, Montaldi E R, Guiamet J J (1994). Ethylene as promoter of wheat grain maturation and ear senescence. *Plant Growth Regulation*, 15: 107–117
- Beltrano J, Ronco M G, Montaldi E R (1999). Drought stress syndrome in wheat is provoked ethylene evolution imbalance and reversed by rewatering, amin-oethoxyvinylglycine, or sodium benzoate. *Journal of Plant Growth Regulation*, 18: 59–64
- Cai Y X, Zhu Q S, Wang Z Q, Yang J C, Zhen L, Qian W C (2002). Effects of soil moisture on rice quality during grain-filling period. *Acta Agronomica Sinica*, 28: 601–608 (in Chinese)
- Chen Y M, Yu S W (1988). Effects of different type of water stress on ethylene production, contents of ACC and MACC in wheat plants. *Acta Phytophysiological Sinica*, 14: 281–288 (in Chinese)
- Cheng C Y, Lur H S (1996). Ethylene may be involved in abortion of the maize caryopsis. *Physiologia Plantarum*, 98: 245–252
- Davies P J (1995). Introduction. In: Davies P J, ed. *Plant Hormones, Physiology, Biochemistry and Molecular Biology*. The Netherlands: Kluwer Academic Publishers, 1–12
- Davies P J (2004). Introduction. In: Davies P J, ed. *Plant Hormones, Biosynthesis, Signal Transduction, Action!* The Netherlands: Kluwer Academic Publishers, 1–35
- Feng J, Barker A V (1992). Ethylene evolution and ammonium accumulation by tomato plants under water and salinity stresses, Part II. *Journal of Plant Nutrition*, 15: 2471–2490
- Guinn G (1976). Water deficit and ethylene evolution by young cotton bolls. *Plant Physiology*, 57: 403–405
- Kobata T, Takami S (1983). Grain production and dry matter partitioning in rice (*Oryza sativa* L.) in response to water deficits during the whole grain filling period. *Japanese Journal of Crop Science*, 53: 283–290
- Morgan P W, Drew M C (1997). Ethylene and plant responses to stress. *Physiologia Plantarum*, 100: 620–630
- Naik P K, Mohapatra P K (2000). Ethylene inhibitors enhanced sucrose synthase activity and promoted grain filling of basal rice kernels. *Australian Journal of Plant Physiology*, 27: 997–1008
- Nakamura Y, Yuki K, Park S Y (1989). Carbohydrate metabolism in the developing endosperm of rice grains. *Plant Cell Physiology*, 30: 833–839
- Narayana I, Lalonde S, Saini H S (1991). Water-stress-induced ethylene production in wheat: A fact or artifact? *Plant Physiology*, 96: 406–410

- Ober E S, Setter T L, Madison J T, Thompson J F, Shapiro P S (1991). Influence of water deficit on maize endosperm development. Enzyme activities and RNA transcripts of starch and zein synthesis, abscisic acid, and cell division. *Plant Physiology*, 97: 154–164
- Rahman M S, Yoshida S (1985). Effect of water stress on grain filling in rice. *Soil Science and Plant Nutrition*, 31: 497–511
- Ranwala A P, Miller W B (1998). Sucrose-cleaving enzymes and carbohydrate pools in *Lilium longiflorum* floral organs. *Physiologia Plantarum*, 103: 541–550
- Richards F J (1959). A flexible growth functions for empirical use. *Journal of Experimental Botany*, 10: 290–300
- Rook F, Corke F, Card R, Munz G, Smith C, Bevan M W (2001). Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signaling. *Plant Journal*, 26: 421–433
- Schaffer A A, Petreikov M (1997). Source-to-starch metabolism in tomato fruit undergoing transient starch accumulation. *Plant Physiology*, 13: 739–746
- Song F B, Dai J Y (2002). Relationship between changes of ethylene release and polyamines content in maize leaves and drought-tolerance in maize under water stress. *Journal of Northeast Agricultural University*, 33(4): 345–352 (in Chinese)
- Wang W, Zhang J H, Yang J C, Zhu Q S (2004). Effect of water stress on metabolism of stored carbon-hydrate of stem and yield in rice grown under unfavorable-delayed senescence. *Acta Agronomica Sinica*, 30(3): 196–204 (in Chinese)
- Wardlaw I F, Willenbrink J (2000). Mobilization of fructan reserves and changes in enzymes activities in wheat stems correlate with water stress during kernel filling. *New Phytologist*, 148: 413–422
- Xu Z Z, Yu Z W, Qi X H, Yu S L (1995). Effect of soil drought on ethylene evolution, polyamine accumulation and cell membrane in flag leaf of winter wheat. *Acta Phytophysiological Sinica*, 21: 295–301 (in Chinese)
- Yang J C, Yuan L M, Chang E H, Wang Z Q, Liu L J, Zhu Q S (2005). Effect of dry-wet alternate irrigation on rice quality and activities of some enzymes in grains during the filling. *Acta Agronomica Sinica*, 31(8): 1052–1057 (in Chinese)
- Yang J, Zhang J (2006). Grain filling of cereals under soil drying. *New Phytologist*, 169: 223–236
- Yang J, Zhang J, Huang Z, Zhu Q, Wang L (2000). Remobilization of carbon reserves is improved by controlled soil drying during grain filling of wheat. *Crop Science*, 40(6): 1645–1655
- Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2001a). Water deficit-induced senescence and its relationship to remobilization of pre-stored carbon in wheat during grain filling. *Agronomy Journal*, 93: 196–206
- Yang J, Zhang J, Wang Z, Zhu Q, Wang W (2001b). Remobilization of carbon reserves in response to water deficit during grain filling of rice. *Field Crops Research*, 71: 47–55
- Zhu Q S, Cao X Z, Luo Y Q (1988). Growth analysis in the process of grain filling in rice. *Acta Agronomica Sinica*, 14: 182–192 (in Chinese)