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Abscisic Acid and Ethylene Interact in Rice Spikelets in Response to Water Stress During Meiosis

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Abstract This study investigated whether and how the interaction between abscisic acid (ABA) and ethylene is involved in the regulation of rice (*Oryza sativa* L.) spikelet sterility when subjected to water stress during meiosis. Two rice cultivars, HA-3 (drought-resistant) and WY-7 (drought-susceptible), were used and subjected to well-watered and water-stressed (WS) treatments during meiosis (15–2 days before heading). Leaf water potentials of both cultivars markedly decreased during the day as a result of the WS treatment, but panicle water potentials remained constant. The percentage of sterile spikelets in WS plants was increased by 49.7% for WJ-7 but only 12.7% for HA-3. ABA, ethylene, and 1-aminocyclopropane-1-carboxylic acid were all enhanced in spikelets by the water stress, but ethylene was enhanced more than ABA in WY-7 when compared with that in HA-3. Spikelet sterility was significantly reduced when ABA or amino-ethoxyvinylglycine, an inhibitor of ethylene synthesis, was applied to the panicles of WS plants at the early meiosis stage. Application of ethephon, an ethylene-releasing agent, or fluridone, an inhibitor of ABA synthesis, had the opposite effect, and sterility was increased. The results suggest that antagonistic interactions between ABA and ethylene may be involved in mediating the effect of water stress on spikelet fertility. A higher ratio of ABA to ethylene would be a physiologic trait of rice adaptation to water stress.

Keywords Abscisic acid (ABA) · 1-Aminocyclopropane-1-carboxylic acid (ACC) · Drought resistance · Ethylene · Meiosis · *Oryza sativa* (rice) · Spikelet sterility · Water stress

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Introduction

Water stress (commonly known as drought or water deficit) is a major abiotic stress and affects crop productivity nearly as much as all the other environmental factors combined (Saini and Westgate 2000; Sharp and others 2004). Rice (*Oryza sativa*) as a paddy field crop is particularly susceptible to water stress (Inthapan and Fukai 1988; Tao and others 2006). It is estimated that 50% of the world rice production is affected more or less by drought (Belder and others 2004; Hanson and others 1990).

Plant growth and development can be inhibited by water stress at any time during the crop life cycle, but sensitivity to water stress is particularly acute during the reproductive development because reproduction involves several processes that are extremely vulnerable to a change in plant water status (Boyer and Westgate 2004; Saini 1997). Meiosis is considered to be the most stress-sensitive period of reproduction in all species studied (Saini and Westgate 2000). A meiosis-stage water stress causes pollen sterility in self-pollinated cereals such as wheat (*Triticum aestivum*) (Saini and Aspinall 1981), maize (*Zea mays*) (Boyer and Westgate 2004), and rice (Sheoran and Saini 1996), leading to a reduction in grain set and yield (Saini and Westgate 2000). Although the sensitivity of crop species to water stress during reproductive growth is well documented, the

underlying biochemical mechanism is poorly understood (Saini and Westgate 2000).

It is generally believed that abscisic acid (ABA) and ethylene are two major plant hormones linking plant responses to stress (Cheng and Lur 1996; Davies 2004; Gazzarrini and McCourt 2001; Wilkinson and Davies 2002). In maize, drought can stimulate a significant ABA accumulation in aborted kernels (Ober and others 1991). Water-stress-induced decline in grain set in wheat is accompanied by increases in ABA levels in leaves and spikelets, and an application of exogenous ABA induces male sterility (Morgan 1980; Morgan and King 1984; Saini and others 1984; Waters and others 1984; Zeng and others 1985). More recent work (Andersen and others 2002) has shown, however, that aborted and developing ovaries in maize display no significant differences in ABA concentrations when subjected to drought stress during the critical, abortion-sensitive phase of young ovary development. Using a split root system to dry half the roots and keep the remainder watered, Dembinska and others (1992) have found that water-stress-induced changes in spikelet ABA levels do not affect grain set in wheat.

Various types of stresses have been reported to promote ethylene production from different tissues of a number of plant species (Morgan and Drew 1997; Narayana and others 1991; Yang and others 2004; Yang and Zhang 2006). An overproduction of ethylene induced by drought has frequently been related to fruit abortion in cotton (*Gossypium hirsutum*) (Guinn 1976) and in wheat (Narayana and others 1991). Although water stress is one of the commonly encountered stresses to cause an elevated release of ethylene, there are many reports that drought stress reduces, rather than increases, ethylene production (for example, Morgan and Drew 1997) Furthermore, there is no information about the relationship between internal ethylene production and spikelet sterility induced by water stress during meiosis of rice.

The purposes of this study were to test the hypothesis that the interaction between ABA and ethylene is involved in mediating the effects of water stress on spikelet sterility. The changes in ABA and ethylene concentrations in rice spikelets subjected to water stress during meiosis were investigated by using two cultivars differing in drought resistance. The effects of chemical regulators on sterility were also studied to verify the roles of the two hormones.

Materials and Methods

Plant Materials and Growth Conditions

The study was conducted at a farm belonging to 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 japonica rice (*Oryza sativa*) cultivars, HA-3 (Han A-03, drought-resistant) and WJ-7 (Wuyujing 7, high-yielding, drought-susceptible), were used. The seeds were sown in the paddy field on 9–10 May. Thirty-day-old seedlings were then transplanted to 16 concrete tanks, each with a surface area of 6.4 m^2, filled to 30 cm depth with sandy loam soil [Typic fluvaquents, Entisols (U.S. taxonomy)] that contained organic matter at 2.42% and available nitrogen (N), phosphorus (P), and potassium (K) at 112, 35.1, and 66.7 mg kg^{-1}, respectively. The hill spacing was 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 superphosphate), and K (3 g m^{-2} as KCl) were applied and incorporated before transplanting. N as urea was also applied at mid-tillering (3 g m^{-2}) and at panicle initiation (3 g m^{-2}). The tank was kept at the 1–2-cm water level until the onset of pollen mother cell (PMC) meiosis when water-stress treatments were initiated. Both cultivars headed on 24–26 August (50% of plants), flowered on 26–28 August, and were harvested on 15–16 October.

Water-Stress Treatments

The experiment was a 2-by-2 (two cultivars and two levels of soil moisture) factorial design with four treatments. Each treatment had four plots (tanks) as replicates in a fully randomized arrangement. The development stage and size of panicles were determined as described by Namuco and O'Toole (1986) and through pilot experiments. From the onset of PMC meiosis [the distance of the ligule of the flag leaf was 10–11 cm below that of the penultimate leaf, 15 days before heading (DBH)] to the pollen completion stage (2 DBH, refer to Ling and others 1983), two levels of soil water potential (ψ_{soil}) were imposed by controlling water application. The well-watered (WW) treatment was flooded to 1–2 cm of water depth in the plot (ψ_{soil} = 0 MPa) by manually applying tap water, and the water-stressed (WS) treatment maintained ψ_{soil} at –0.05 MPa. The ψ_{soil} in the WS treatment plots was monitored with tension meters (Soil Science Research Institute, Nanjing, China) buried 15–20 cm in the soil, with four installed in each plot. Tension meter readings were recorded every 4 h from 0600 to 1800. When the readings dropped to the desired value, 20 L of tap water per WS treatment plot was manually added. A removable polyethylene shelter was used to protect the plot during rain. After the last sampling (3 DBH), all plots were flooded to 1–2-cm water depth.

Sampling

One hundred fifty main stems in each plot were tagged during the tillering period. Thirty panicles from the tagged

stems in each plot were sampled at 12, 9, 6, and 3 DBH, respectively. The sampling time of day was 1200 h when the leaf water potential was the lowest. All spikelets from the sampled panicles were removed and pooled. Replicates of 0.5 g fresh spikelets were dried at 70°C to constant weight and weighed. One third of the sampled spikelets were used for the determination of ethylene evolution rate. The remainder were frozen in liquid nitrogen and kept at –80°C for measurements of ABA and 1-aminocyclopropane-1-carboxylic acid (ACC). Twenty terminal leaf sheaths were also used for measurements of ethylene evolution rate and ACC concentration on the same dates that panicles were sampled. Spikelet fertility was determined from an average of ten comparable panicles grown to maturity in each plot and expressed as the percentage of aborted (sterile spikelets), partially filled, and fully filled grains compared to the total number of potentially fertilizable spikelets per panicle. Grain weight was determined from 20 panicles and grain yield was from all plants (except border ones) in each plot at maturity.

Measurement of Leaf and Panicle Water Potentials

Measurements of leaf and panicle water potentials were made at 2-h intervals at 4 and 3 DBH (11 days and 12 days after withholding water) for both cultivars. Well-illuminated flag leaves were chosen randomly for the measurement of leaf water potential. After cutting 1.5 cm below the panicle neck, panicles were immediately used to measure water potential. A pressure chamber (Model 3000, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) was used with six leaves per panicle for each treatment.

ABA Extraction, Purification, and Quantification

The methods for extraction and purification of (+)-ABA were modified from those described by Bollmark and others (1988) and He (1993). Samples of 0.8–1.0 g spikelets were ground in a mortar (at 0°C) in 10 ml 80% (v/v) methanol extraction medium containing 1 mM butylated hydroxytoluene as an antioxidant. The extract was incubated at 4°C for 4 h and centrifuged at 4800*g* for 15 min at the same temperature. The supernatants were passed through Chromosep C_{18} columns (C_{18} Sep-Pak Cartridge, Waters Corp, Millford, MA, USA), prewashed with 10 ml 100% and 5 ml 80% methanol, respectively. The hormone fractions were dried under N_2 and dissolved in 2 ml phosphate buffered saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for analysis by enzyme-linked immunosorbent assay (ELISA).

Mouse monoclonal antigen and antibody against ABA and immunoglobulin G–horse radish peroxidase (IgG–HRP) used in ELISA were produced at the Phytohormones Research Institute, China Agricultural University, China (see He 1993). The methods for quantification of ABA by ELISA and the recovery test were described previously (Yang and others 2001, 2003). The recovery percentage of ABA in spikelets was 85.0 ± 4.9. The specificity of the monoclonal antibody and other possible nonspecific immunoreactive interferences were checked previously and proved reliable (Xie and others 2003; Yang and others 2003).

Ethylene and ACC Analysis

Ethylene evolved from spikelets and sheaths was determined according to Beltrano and others (1994) with modifications. Briefly, sampled spikelets or sheaths 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 0.5–0.8 g spikelets or 1–2 g sheaths. Spikelets/sheaths were then transferred into 10-ml glass vials containing moist filter paper and immediately sealed with airtight subaseal stoppers and incubated in the dark for 24 h at 27°C. A 1-ml gas sample was withdrawn through the subaseal with a gas-tight syringe and ethylene was assayed by gas chromatography (HP5890 Series II, Hewlett Packard, 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, 100, and 200°C, respectively. Nitrogen was used as the carrier gas 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.

To examine the time-course changes in ethylene production by the spikelets after sampling, the spikelets sampled at 12 DBH were incubated for 6, 12, 18, 24, and 30 h, respectively, for ethylene measurement using the same method described above.

ACC in the spikelets and sheaths 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 percentage from ACC to ethylene was 90.3 ± 5.42 for spikelets and 88.5 ± 6.4 for sheaths, on average. ABA and ACC concentrations and the rate of ethylene evolution were expressed on a dry weight basis.

Chemical Applications

The cultivar WY-7 was used for chemical application. Plants were grown in porcelain pots in open-field

conditions. Each pot (30 cm in height, 25 cm in diameter, 14.72 L in volume) was filled with 20 kg sandy loam soil with the same nutrient contents as the tank experiment, and was planted with three hills with two seedlings per hill. On the day of transplanting (10–11 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. The pot was kept at the 1–2-cm water level until the onset of PMC meiosis when WS treatment was initiated.

From the onset of PMC meiosis to the pollen completion stage, either WW or WS treatments were imposed on the plants. Each treatment had 120 pots as replicates. The treatment details, water control, and rain prevention were the same as for the tank experiment.

Synthetic (±)-ABA, ethephon (an ethylene-releasing agent), amino-ethoxyvinylglycine (AVG, an inhibitor of ethylene synthesis by inhibiting ACC synthesis) (all from Sigma, St Louis, MO, USA), and fluridone (Fluka, Riedel-de Haën, Germany), an inhibitor of carotenoid biosynthesis and may indirectly also reduce ABA synthesis, were applied to the plants. Preparation of the chemical solutions was described elsewhere (Cheng and Lur 1996; Ober and Sharp 1994). Starting at the onset of PMC meiosis, either 20×10^{-6} M (±)-ABA, 20×10^{-6} M fluridone, 50×10^{-3} M ethephon, 5×10^{-5} M AVG, or 20×10^{-6} M fluridone + 20×10^{-6} M (±)-ABA were applied to the panicles of the main stems by injecting carefully from the top into the boot of the flag leaf with a 1-ml syringe. The chemicals were applied daily for 4 days at the rate of 0.5 ml per panicle at each application. All the solutions contained ethanol at final concentrations of 0.05% (v/v). Control plants received the same volume of deionized water containing the same ethanol concentration. Each chemical treatment had 90–100 main stems as replicates.

Concentrations of ABA and ethylene in the spikelets were determined at 2 and 6 days after the chemical treatment (9 and 5 DBH), respectively. Measurement methods were the same as described above. Spikelet number per panicle, percentages of sterile spikelets, partially filled grains, fully filled grains, and grain weight were measured from 20 main stems, and panicle weight was from 40 main stems in each treatment at maturity.

Statistical Analysis

The results were analyzed for variance using the SAS/STAT 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 the $P_{0.05}$ level ($LSD_{0.05}$). The differences in data across years and in the interaction between treatments and years were not significant ($F < 1$), and therefore data from both years were averaged.

Results

Plant Water Status and Spikelet Sterility

The leaf water potential for WW plants showed a small change during the day, ranging from −0.21 MPa at predawn (0600 h) to −0.84 MPa at midday (1200 h). It was greatly reduced for WS plants, ranging from −0.38 MPa at predawn to −1.76 MPa at midday. Both cultivars exhibited similar diurnal changes (Figure 1A).

In contrast to a great reduction in leaf water potential for WS plants, the panicle water potential for these plants

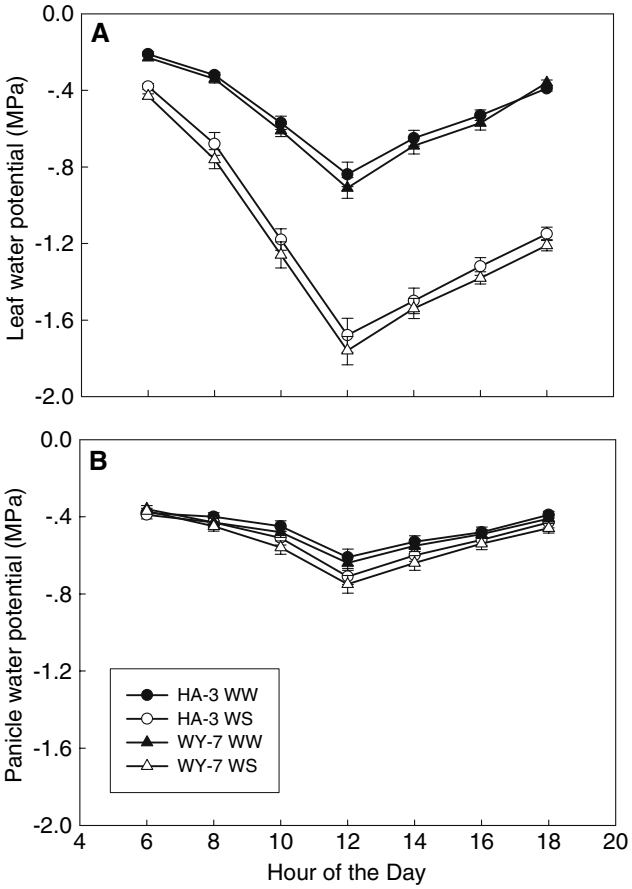

Fig. 1 Diurnal changes of water potentials of the flag leaf (**A**) and panicles (**B**) in rice. The treatments were well-watered (WW) and water-stressed (WS) during meiosis. Leaf water potential and panicle water potential were measured at 11 days and 12 days after withholding water (4 days and 3 days from heading), respectively. Data are presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$) and are means ± SE of 12 independent measurements

Table 1 Grain yield, spikelet number per panicle, percentages of sterile spikelets, partially filled grains and fully filled grains, and grain weight of rice

Year and cultivars	Water-deficit treatment	Grain yield (g m^{-2})	Spikelet number per panicle	Sterile spikelets (%)	Partially filled grains (%)	Fully filled grains (%)	Grain weight (mg $grain^{-1}$)
2004							
HA-3	WW	680 ± 20[b]	87 ± 4.4[a]	2.5 ± 0.3[c]	4.4 ± 0.5[a]	93.1 ± 4.8[a]	26.6 ± 0.5[a]
	WS	583 ± 16[c]	86 ± 4.8[a]	15.1 ± 1.5[b]	4.9 ± 0.8[a]	80.0 ± 3.7[b]	26.9 ± 0.4[a]
WY-7	WW	789 ± 29[a]	91 ± 5.3[a]	3.3 ± 0.6[c]	4.2 ± 0.4[a]	92.5 ± 4.3[a]	26.8 ± 0.5[a]
	WS	358 ± 11[d]	89 ± 4.7[a]	51.8 ± 3.4[a]	3.6 ± 0.6[a]	44.8 ± 2.6[c]	27.0 ± 0.6[a]
2005							
HA-3	WW	684 ± 23[b]	89 ± 4.5[a]	2.7 ± 0.4[c]	4.8 ± 0.8[a]	92.5 ± 4.6[a]	26.4 ± 0.5[a]
	WS	579 ± 19[c]	88 ± 4.8[a]	15.5 ± 1.2[b]	4.7 ± 0.6[a]	79.8 ± 3.4[b]	26.7 ± 0.5[a]
WY-7	WW	781 ± 34[a]	93 ± 6.2[a]	3.1 ± 0.5[c]	4.0 ± 0.5[a]	92.9 ± 4.5[a]	26.6 ± 0.6[a]
	WS	364 ± 15[d]	91 ± 5.4[a]	54.0 ± 4.3[a]	3.8 ± 0.4[a]	42.0 ± 2.1[c]	26.8 ± 0.4[a]

The treatments were well-watered (WW) and water-stressed (WS) during meiosis. Spikelet fertility was determined from an average of 40 comparable panicles grown to maturity in each treatment and expressed as the percentage of aborted (sterile spikelets), partially filled, and fully filled grains compared to the total number of potentially fertilizable spikelets per panicle. Grain weight was an average of 80 panicles, and grain yield was means of all plants harvested from each treatment

Data are means ± SE of four independent measurements, and within each column followed by dissimilar letters differ significantly at $p \leq 0.05$

remained constant during the water-stress treatment and ranged from −0.37 MPa at predawn to −0.75 MPa at midday (Figure 1B). There were no significant differences in panicle water potentials between the two cultivars, either under WW or WS treatment.

The difference in potential spikelet number per panicle was not significant either between the two cultivars or between the WW and WS treatments (Table 1). The WS treatment, however, substantially increased spikelet sterility in the drought-susceptible cultivar WY-7. The percentage of sterile spikelets was increased by 48.5–50.9% when compared with that of control plants, and filled grain percentage and grain yield were markedly reduced accordingly. In contrast, the percentage of sterile spikelets of the drought-resistant cultivar HA-3 was less affected by the water stress and was only 12.6–12.8% higher than that of the control, and therefore showed a higher filled grain percentage and grain yield than WY-7 (Table 1). The results from both years were very similar. Statistical analysis showed that the differences in grain yield and its components (spikelet number per panicle, percentage of fully filled grains, and grain weight) and percentages of sterile spikelets and partially filled grains across years and in the interaction between treatments and years were not significant ($F < 1$).

Changes in ABA, Ethylene, and ACC Levels in Spikelets

Figure 2 shows the time-course changes in ethylene production by the spikelets after sampling. The concentration of ethylene increased with the sampling time and reached a maximum at 24 h. The changing pattern was similar for both cultivars and for both WW and WS treatments (Figure 2). The WS treatments significantly enhanced ethylene evolution from the spikelets, more for WY-7 than for HA-3. There was no significant difference in ethylene production between the two cultivars under the WW treatment (Figure 2).

The enhancement in ethylene production by water stress was also observed at different treatment dates (Figure 3A). The ethylene evolution rate (measured 24 h after sampling) was much greater for WY-7 than for HA-3 under the WS treatment, although its difference was not significant between the two cultivars under the WW condition. A similar pattern was observed for the ACC concentration in spikelets (Figure 3B). ACC concentration was significantly correlated with the ethylene evolution rate ($r = 0.99^{**}$, $p = 0.001$), suggesting that an increase in ethylene evolution is ascribed to an enhanced ACC level in the spikelets of WS plants.

Under WW conditions, ABA levels in spikelets changed little during meiosis (Figure 3C). Water stress significantly increased ABA concentrations in spikelets. There was no significant difference in ABA concentration between the two cultivars when ψ_{soil} was the same.

As shown in Figure 3, both cultivars showed increases in ABA, ethylene, and ACC levels in the spikelets under water stress. In comparison, ethylene or ACC was enhanced more than ABA in WY-7; as a result, the ratio of ABA to ACC (ABA/ACC) was significantly reduced when compared with the control (Figure 4), implying that ethylene production outperformed ABA accumulation in WY-

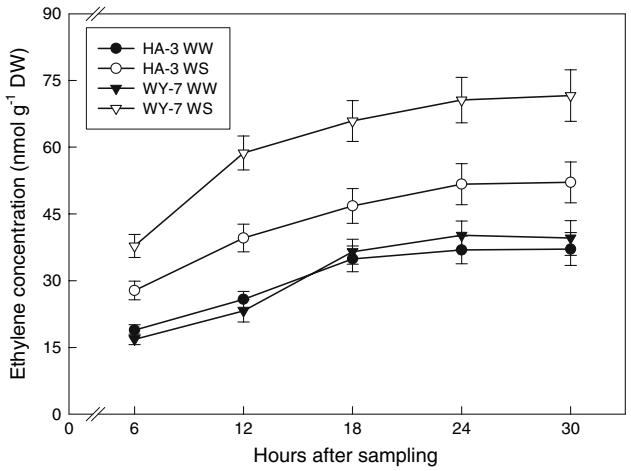

Fig. 2 Time-course changes in ethylene production by the spikelets of rice after sampling. The treatments were well-watered (WW) and water-stressed (WS) during meiosis. Spikelets were sampled at 12 days before heading. Data are presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$) and are means ± SE of eight independent measurements

7 when subjected to meiosis-stage water stress. In contrast, the differences in ABA/ACC in HA-3 were not significant between WW and WS plants, indicating that elevated ABA under water stress could balance ethylene overproduction (Figure 4).

To evaluate the role of ethylene produced from sheaths, the levels of ethylene and ACC in the terminal leaf sheaths were also examined. As shown in Figure 5A and B, the changes in ethylene evolution rate and ACC concentration in the sheaths were very similar to those in spikelets (refer to Figure 3A, B). The levels of ethylene and ACC in the terminal sheaths, however, were much lower than those in spikelets (Figure 5A, B).

Effects of Chemical Applications

To verify whether ethylene and ABA mediate the effect of water stress on spikelet sterility, chemicals involved in promoting or inhibiting both hormones were applied at early meiosis. As shown in Table 2, application of AVG, an inhibitor of ethylene synthesis, significantly reduced ethylene levels in spikelets of both WW and WS plants. Application of ethephon, an ethylene-releasing substance, exhibited the opposite effect. When fluridone, an indirect inhibitor of ABA synthesis, was applied to the panicle, the ABA concentration in spikelets was reduced, whereas the ethylene level was increased. The results were reversed when ABA was applied (Table 2).

Application of either ethephon or fluridone significantly increased spikelet sterility under both WW and WS

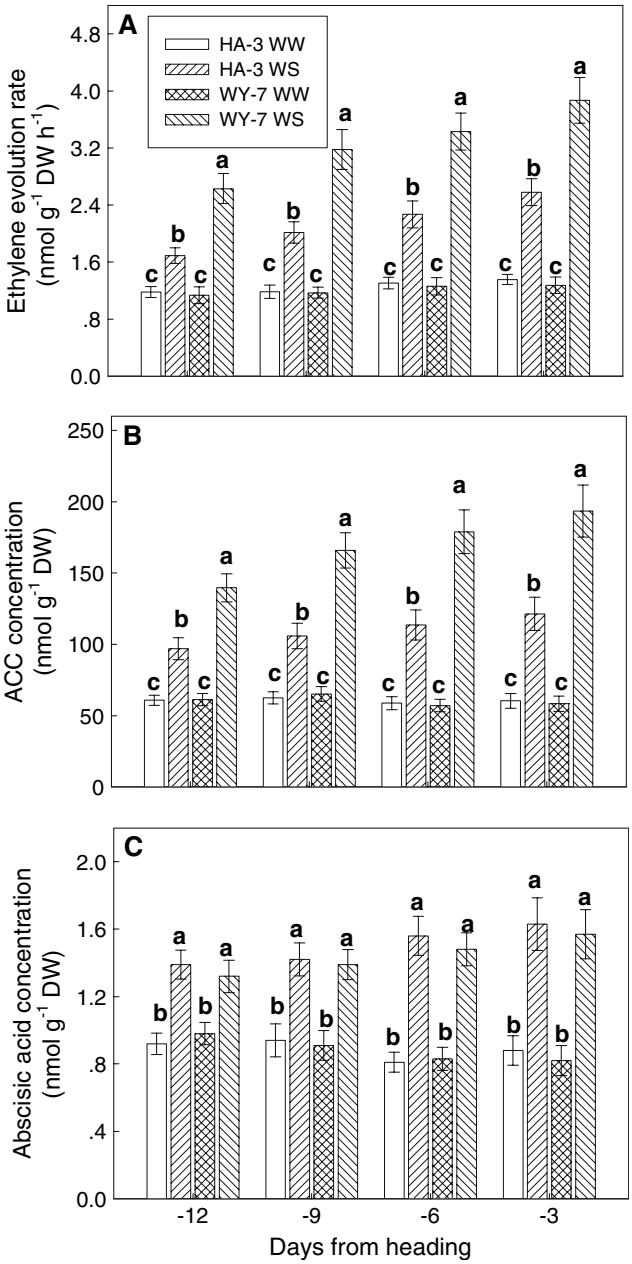

Fig. 3 Levels of ethylene (**A**), 1-aminocyclopropane-1-carboxylic acid (ACC) (**B**), and ABA (**C**) in the spikelets of rice. The treatments were well-watered (WW) and water-stressed (WS) during meiosis. Data are presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$) and are means ± SE of eight independent measurements. Dissimilar letters above bars differ significantly at $p \leq 0.05$ within the same measurement date

treatments, leading to a significant decrease in the fully filled grain percentage and panicle weight (Table 3). Application of AVG and ABA to WS panicles had the opposite effect. Applying ABA to WW plants increased spikelet sterility although the reduction in panicle weight was insignificant when compared with the control

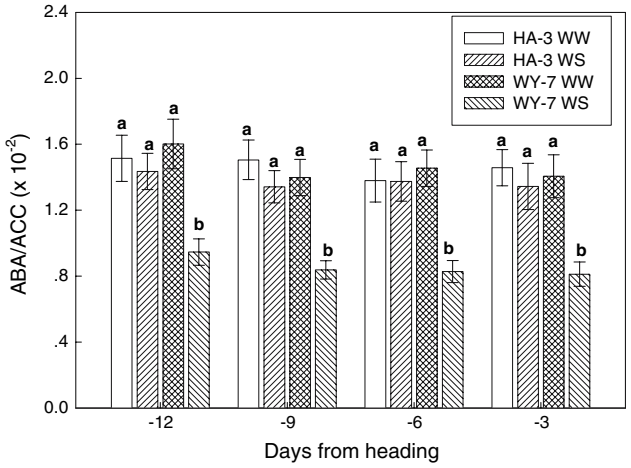

Fig. 4 The ratio of ABA to 1-aminocyclopropane-1-carboxylic acid (ACC) in the spikelets of rice. The treatments were well-watered (WW) and water-stressed (WS) during meiosis. Data are from Figure 3 and presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$) and are means ± SE of eight independent measurements. Dissimilar letters above bars differ significantly at $p \leq 0.05$ within the same measurement date

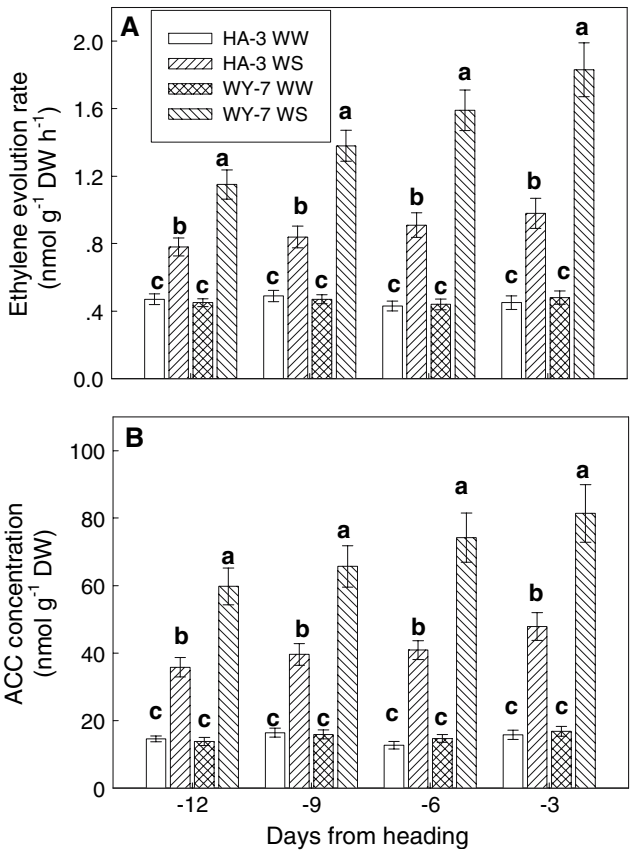

Fig. 5 Levels of ethylene (**A**) and 1-aminocyclopropane-1-carboxylic acid (ACC) (**B**) in the terminal sheaths of rice. The treatments were well-watered (WW) and water-stressed (WS) during meiosis. Data are presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$) and are means ± SE of eight independent measurements. Dissimilar letters above bars differ significantly at $p \leq 0.05$ within the same measurement date

(Table 3). All the chemicals had no significant effects on spikelet number per panicle, partially filled grain percentage, and per grain weight under both WW and WS treatments (Table 3).

Because the chemicals were injected into the terminal sheath, the effects of either fluridone or synthetic ABA on the chlorophyll content and conductance of leaves were not detected (data not shown). When plants were concomitantly treated with fluridone and ABA, the differences in all the measurements were not significant relative to the control (Tables 2 and 3), indicating that there are no indirect effects of fluridone on spikelet sterility.

Discussion

Present results confirmed early reports (Namuco and O'Toole 1986; Sheoran and Saini 1996) that water stress at the meiosis stage could cause serious spikelet sterility in rice. Our results showed, however, that the susceptibility to water stress during meiosis varied with cultivars. The percentage of sterile spikelets in WS plants for WY-7, a drought-susceptible cultivar, was increased by 48.5–50.9%, whereas that of HA-3, a drought-resistant cultivar, increased by only 12.6–12.8% when compared with their respective controls (Table 1). Genetic differences in drought tolerance may offer the unique opportunity to compare physiologic and biochemical responses to water stress that may be involved in drought tolerance. We observed that the difference in leaf water potential was not significant between the two cultivars under both WW and WS treatments (Figure 1A). Furthermore, panicle water potential was unaffected by water stress (Figure 1B). Similar results were also observed by others (Saini and Aspinall 1981; Tsuda and Takami 1993). The phenomenon that the water status of young panicles remains constant during meiotic-stage water stress may be partly due to limited transpiration within two enclosing leaf sheaths (Saini and Westgate 2000). It may be that xylem discontinuity between the floral stalk and the pericarp contributes to the apparent hydraulic isolation (Zee and O'Brien 1970). It may be concluded that the water status of panicles is attributed neither to spikelet sterility nor to the differences between the drought-resistant and drought-susceptible cultivars in response to water stress.

Hormones are likely to play important roles in the adaptation of plant growth and development to water stress, although the involvement of most of these compounds has not been elucidated (Sharp and others 2004). We observed

Table 2 Effects of applied ethephon, amino-ethoxyvinylglycine (AVG), abscisic acid (ABA), fluridone, and fluridone + ABA on levels of ABA and ethylene in the spikelets of rice

Treatment	9 days before heading		5 days before heading	
	ABA (nmol g^{-1} DW)	Ethylene (nmol g^{-1} DW h^{-1})	ABA (nmol g^{-1} DW)	Ethylene (nmol g^{-1} DW h^{-1})
WW				
Control	0.93 ± 0.07[b]	1.23 ± 0.12[c]	0.86 ± 0.05[b]	1.31 ± 0.09[b]
50×10^{-3} M ethephon	0.91 ± 0.06[b]	2.64 ± 0.18[a]	0.81 ± 0.06[b]	2.58 ± 0.26[a]
5×10^{-5} M AVG	0.95 ± 0.08[b]	0.81 ± 0.06[d]	0.88 ± 0.07[b]	0.73 ± 0.05[c]
20×10^{-6} M ABA	1.69 ± 0.11[a]	0.85 ± 0.07[d]	1.64 ± 0.12[a]	0.79 ± 0.06[c]
20×10^{-6} M fluridone	0.51 ± 0.04[c]	1.92 ± 0.13[b]	0.48 ± 0.03[c]	2.19 ± 0.29[a]
20×10^{-6} M fluridone + ABA	0.90 ± 0.08[b]	1.27 ± 0.14[c]	0.84 ± 0.06[b]	1.35 ± 0.08[b]
WS				
Control	1.43 ±0.09[b]	2.35 ± 0.19[b]	1.51 ± 0.09[b]	2.96 ± 0.32[b]
50×10^{-3} M ethephon	1.37 ± 0.12[b]	3.02 ± 0.21[a]	1.48 ± 0.12[b]	4.15 ± 0.37[a]
5×10^{-5} M AVG	1.45 ± 0.10[b]	1.42 ± 0.11[d]	1.56 ± 0.14[b]	1.69 ± 0.14[d]
20×10^{-6} M ABA	2.14 ±0.15[a]	1.86 ± 0.14[c]	2.03 ± 0.17[a]	2.03 ± 0.17[c]
20×10^{-6} M fluridone	0.82 ± 0.06[c]	2.96 ± 0.15[a]	0.77 ± 0.05[c]	3.98 ± 0.33[a]
20×10^{-6} M fluridone + ABA	1.41 ± 0.13[b]	2.29 ± 0.16[b]	1.54 ± 0.14[b]	2.87 ± 0.22[b]

The cultivar WY-7 was used. Two treatments of well-watered (WW) and water-stressed (WS) were conducted during meiosis. The young panicles received chemicals daily for 4 days starting at the onset of pollen mother cell meiosis. Control plants received deionized water. Levels of ABA and ethylene in panicles were determined 9 and 5 days before heading, respectively

Data are presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$). Data are means ± SE of eight independent measurements. Data within the same column and the same soil moisture treatment followed by dissimilar letters differ significantly at $p \leq 0.05$

that water stress substantially increased the ethylene evolution rate and ACC concentration in spikelets, with WY-7 enhanced more than HA-3 (Figures 2 and 3A, B). Application of an inhibitor of ethylene synthesis (AVG) to WS plants reduced and an ethylene-releasing agent (ethephon) to both WW and WS plants increased the percentage of sterile spikelets (Table 3), suggesting that ethylene plays a role in inducing spikelet sterility. Little is known about how ethylene affects spikelet fertility. Ethylene synthesis is reported to be closely associated with reactive oxygen species (ROS) levels in stressed plant cells (Overmyer and others 2000), which may lead to protein and nucleic acid degradation, lipid peroxidation, and membrane damage (Tambussi and others 2000). Naik and Mohapatra (1999, 2000) have observed that ethylene inhibitors applied to rice panicles at the booting stage could significantly enhance sucrose synthase activity in the spikelets and increase grain set. Their work may suggest that ethylene injures cells or limits pollen activity through an enhanced generation of ROS or an inhibition to the key enzymes involved.

It is worth noting that application of AVG to the panicle of WW plants reduced ethylene levels in spikelets (Table 2), but spikelet sterility remained constant (Table 3). A probable explanation is that under WW conditions, ethylene released from spikelets may not be at the damaging level, and therefore the suppression of ethylene production would not affect spikelet fertility.

It has been proposed that ethylene produced from the terminal sheath or the flag leaf may have an inhibitory effect on spikelet growth (Debata and Murty 1983; Khan and Choudhury 1992; Mohapatra and others 2000). We observed that water stress did enhance ethylene evolution from the terminal sheath and increase ACC concentration there (Figure 5A, B). However, ethylene and ACC levels in the sheath were much lower than in spikelets when subjected to water stress. Furthermore, the activities of ACC synthase and ACC oxidase enhanced by the water stress were much higher in spikelets than in the sheaths (data not shown). Such a result implies that increased spikelet sterility under the water stress might be attributed mainly to high ethylene and ACC levels in spikelets per se.

ABA has been regarded as an endogenous sporocide to induce spikelet sterility during meiosis-stage water stress (Morgan 1980; Saini and Westgate 2000). We observed that the ABA level did rise in spikelets under water stress, but it showed no significant difference between the two cultivars that differ in drought resistance (Figure 3C). Application of ABA to the panicles of WS plants significantly reduced spikelet sterility and increased panicle weight (Tables 2, 3). Moreover, the percentage of sterile

Table 3 Effects of applied ethephon, amino-ethoxyvinylglycine (AVG), abscisic acid (ABA), fluridone, and fluridone + ABA on panicle weight, spikelet number per panicle, percentages of sterile spikelet, partially filled grains, fully filled grains, and grain weight of rice

Treatment	Per panicle weight (g)	Spikelet number per panicle	Sterile spikelets (%)	Partially filled grains (%)	Filled grains (%)	Grain weight (mg grain^{-1})
WW						
Control	2.25 ± 0.16^{a}	93 ± 4.3^{a}	4.5 ± 0.6^{c}	3.9 ± 0.4^{a}	91.6 ±2.7^{a}	26.4 ± 0.6^{a}
50×10^{-3} M ethephon	1.58 ± 0.12^{b}	91 ± 3.9^{a}	30.6 ± 2.7^{a}	4.2 ± 0.3^{a}	65.2 ± 2.4^{c}	26.6 ± 0.5^{a}
5×10^{-5} M AVG	2.35 ± 0.18^{a}	94 ± 4.1^{a}	4.1 ± 0.7^{c}	4.0 ± 0.5^{a}	92.9 ± 3.2^{a}	26.9 ± 0.7^{a}
20×10^{-6} M ABA	2.12 ± 0.15^{a}	93 ± 5.2^{a}	10.8 ± 0.9^{b}	3.7 ± 0.4^{a}	85.5 ± 2.8^{b}	26.7 ± 0.5^{a}
20×10^{-6} M fluridone	1.51 ± 0.13^{b}	90 ± 4.6^{a}	33.6 ± 2.5^{a}	4.3 ± 0.4^{a}	62.1 ± 2.9^{c}	27.1 ± 0.7^{a}
20×10^{-6} M fluridone +ABA	2.17 ± 0.19^{a}	91 ± 4.3^{a}	5.3 ± 0.8^{c}	4.2 ± 0.5^{a}	90.5 ± 3.1^{a}	26.3 ± 0.4^{a}
WS						
Control	1.17 ± 0.09^{b}	90 ± 4.8^{a}	46.8 ± 3.9^{c}	3.0 ± 0.4^{a}	50.2 ± 2.5^{c}	25.8 ± 0.5^{a}
50×10^{-3} M ethephon	0.86 ± 0.06^{c}	88 ± 3.9^{a}	59.7 ± 3.2^{b}	3.1 ± 0.3^{a}	37.2 ± 2.1^{d}	26.4 ± 0.6^{a}
5×10^{-5} M AVG	1.79 ± 0.12^{a}	91 ± 4.5^{a}	20.5 ± 2.5^{e}	3.3 ± 0.4^{a}	76.2 ± 2.3^{a}	25.9 ± 0.5^{a}
20×10^{-6} M ABA	1.62 ± 0.13^{a}	89 ± 3.8^{a}	27.5 ± 2.2^{d}	2.9 ± 0.4^{a}	69.6 ± 1.8^{b}	26.2 ± 0.4^{a}
20×10^{-6} M fluridone	0.68 ± 0.05^{d}	87 ± 3.9^{a}	67.3 ± 3.7^{a}	3.4 ± 0.3^{a}	29.3 ± 1.2^{e}	26.5 ± 0.6^{a}
20×10^{-6} M fluridone +ABA	1.12 ± 0.10^{b}	89 ± 4.3^{a}	48.1 ± 2.8^{c}	3.3 ± 0.2^{a}	48.6 ± 1.9^{c}	26.1 ± 0.4^{a}

The cultivar WY-7 was used. Two treatments of well-watered (WW) and water-stressed (WS) were conducted during meiosis. The young panicles received chemicals daily for 4 days starting at the onset of pollen mother cell meiosis. Control plants received deionized water. Spikelet number per panicle, percentages of sterile spikelets, partially filled grains, fully filled grains, and grain weight were measured from 20 main stems, and panicle weight was from 40 main stems in each treatment at maturity

Data are presented as averages between two years because the differences in them across years and in the interaction between treatments and years were not significant ($F < 1$). Data are means ± SE of eight independent measurements. Data within the same column and the same soil moisture treatment followed by dissimilar letters differ significantly at $p \leq 0.05$

spikelets was remarkably increased when ABA was reduced in spikelets through the application of fluridone, an indirect inhibitor of ABA synthesis (Tables 2, 3). suggesting that a higher ABA level is required to maintain spikelet growth during meiosis-stage water stress. The mechanism by which ABA in WS plants protects spikelets from sterility is not understood. An important function of increased ABA concentrations in water-stressed plants may be to prevent excess ethylene production and thereby to maintain appropriate plant growth (LeNoble and others 2004; Sharp and others 2000, 2004; Spollen and others 2000). There are many reports that elevated ABA in stressed plants could play protective roles. These include reduction in membrane damage (Rajasekaran and Blake 1999), expression of many stress-related genes (Guan and Scandalios 1998; Zhu 2002), and upregulation of antioxidant enzyme activities (Bellaire and others 2000; Guan and others 2000). These results may imply that, in contrast to ethylene, ABA in WS plants may maintain spikelet growth through inhibition of ethylene production and reduction in the generation of ROS or activation of antioxidant enzymes.

It is noteworthy, however, that spikelet sterility was increased when ABA was applied to the WW panicles (Table 3). In wheat, application of ABA was reported to affect fertility, whereas an increase in the endogenous level of the hormone did not (Dembinska and others 1992). The reason that the application of ABA to WW plants increases spikelet sterility is not clear. Sharp and others (2004) observed that root elongation of maize was severely inhibited when ABA accumulation in water-stressed roots was reduced, whereas in well-watered seedlings treated with ABA, root growth was substantially inhibited at an ABA content that occurs in water-stressed roots. Sharp and others (2004) speculated that the maintenance of plant growth under water stress by ABA is not solely a function of increased ABA content, but also requires the change in environmental conditions that modifies the growth response to ABA.

Plant hormones can act either synergistically or antagonistically and it is the balance between promoting and inhibiting agents that ultimately determines plant growth and development (Davies 1995, 2004). Our results showed that both ABA and ethylene levels were enhanced in spikelets in both cultivars under water stress. Ethylene was enhanced more than ABA in the drought-susceptible cultivar WY-7, whereas elevated ABA levels balanced ethylene production in the drought-resistant cultivar HA-3 (Figure 4). As a result, HA-3 exhibited a higher ratio of ABA to ACC than WY-7. These results may suggest that

antagonistic interactions between ABA and ethylene mediate spikelet fertility in rice when subjected to water stress during meiosis.

In conclusion, spikelet sterility in rice induced by water stress at the meiosis stage varies largely with the drought resistance of the cultivar. Panicle water status is not attributed to spikelet sterility. Enhanced ABA in WS plants may maintain, rather than inhibit, spikelet growth. Overproduced ethylene under water stress plays a role in inducing spikelet sterility. Antagonistic interactions between ABA and ethylene in WS plants may be involved in mediating spikelet development. A higher ratio of ABA to ethylene would be a physiologic trait of rice adaptation to water stress.

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References

Andersen MN, Asch F, Wu F, Jensen CR, Naested H, Mogensen VO, Koch KE (2002) Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. Plant Physiol 130:591–604

Belder P, Bouman BAM, Cabangon R, Guoan L, Quilang EJP, Li Y, Spiertz JHJ, Tuong TP (2004) Effect of water-saving irrigation on rice yield and water use in typical lowland conditions in Asia. Agric Water Manag 65:193–210

Bellarire BA, Carmody J, Braud J, Gossett DR, Banks SW, Lucas TE, Fowler TE (2000) Involvement of abscisic acid-dependent and independent pathways in the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. Free Radic Res 33:531–545

Beltrano J, Carbone A, Montaldi ER, Guiamet JJ (1994) Ethylene as promoter of wheat grain maturation and ear senescence. Plant Growth Regul 15:107–112

Bollmark M, Kubat B, Eliasson L (1988) Variations in endogenous cytokinin content during adventitious root formation in pea cuttings. J Plant Physiol 132:262–265

Boyer JS, Westgate ME (2004) Grain yield with limited water. J Exp Bot 55:2385–2349

Cheng CY, Lur HS (1996) Ethylene may be involved in abortion of the maize caryopsis. Physiol Plant 98:245–252

Davies PJ (1995) Introduction. In: Davies PJ (ed), Plant hormones, physiology, biochemistry and molecular biology. Dordrecht, The Netherlands: Kluwer Academic Publishers, pp 1–12

Davies PJ (2004) The plant hormones: their nature, occurrence and function. In: Davies PJ (ed), Plant Hormones, biosynthesis, signal transduction, action! Dordrecht, The Netherlands: Kluwer Academic Publishers, pp 1–15

Debata A, Murty KS (1983) Endogenous ethylene content in rice leaves during senescence. Indian J Plant Physiol 26:425–427

Dembinska O, Lalonde S, Saini HS (1992) Evidence against the regulation of grain set by spikelet abscisic acid levels in water-stressed wheat. Plant Physiol 100:1599–1602

Gazzarrini S, McCourt P (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. Curr Opin Plant Biol 4:387–391

Guan LQ, Scandalios JG (1998) Effects of the plant growth regulator abscisic acid and high osmoticum on the developmental expression of the maize catalase genes. Physiol Plant 104:413–422

Guan LM, Zhao J, Scandalios JG (2000) Cis-elements and trans-factors that regulate expression of maize *Catl* antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for response. Plant J 22:87–95

Guinn G (1976) Water stress and ethylene evolution by young cotton bolls. Plant Physiol 57:403–405

Hanson AD, Peacock WJ, Evans LT, Arntzen CJ, Khush GS (1990) Drought resistance in rice. Nature 345:26–27

He Z (1993) Method for an indirect enzyme-linked immunosorbent asssay. In: He ZP (ed), Guidance to experiment on chemical control in crop plants. Beijing, China: Beijing Agricultural University Publishers, pp 60–68

Inthapan P, Fukai S (1988) Growth and yield of rice cultivars under sprinkler irrigation in south-eastern Queensland. 2. Comparison with maize and grain sorghum under wet and dry conditions. Aust J Exp Agric 28:243–248

Khan RI, Choudhury MA (1992) Role of endogenous hormones in the regulation of whole plant senescence in rice. Indian J Exp Biol 30:131–134

LeNoble ME, Spollen WG, Sharp RE (2004) Maintenance of shoot growth by endogenous ABA: genetic assessment of the involvement of ethylene suppression. J Exp Bot 55:237–245

Ling Q, Su Z, Chang H, Cai J, Ho J (1983) The leaf-age model of development process in different varieties of rice. Sci Agric Sin 16:9–18

Mohapatra PK, Naik PK, Patel R (2000) Ethylene inhibitors improve dry matter partitioning and development of late flowering spikelets on rice panicles. Aust J Plant Physiol 27:311–323

Morgan JM (1980) Possible role of abscisic acid in reducing seed set in water-stressed wheat plants. Nature 285:655–657

Morgan JM, King RW (1984) Association between loss of leaf turgor, abscisic acid levels and seed set in two wheat cultivars. Aust J Plant Physiol 11:143–150

Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. Physiol Plant 100:620–630

Naik PK, Mohapatra PK (1999) Ethylene inhibitors promote male gametophyte survival in rice. Plant Growth Regul 28:29–39

Naik PK, Mohapatra PK (2000) Ethylene inhibitors enhanced sucrose synthase activity and promoted grain filling of basal rice kernels. Aust J Plant Physiol 27:997–1008

Namuco OS, O'Toole JC (1986) Reproductive stage water-stress and sterility. 1. Effect of stress during meiosis. Crop Sci 26:317–321

Narayana I, Lalonde S, Saini HS (1991) Water-stress-induced ethylene production in wheat. Plant Physiol 96:406–410

Ober ES, Sharp RE (1994) Proline accumulation in maize (*Zea mays* L.) primary roots at low water potentials. I. Requirement for increased levels of abscisic acid. Plant Physiol 105:981–987

Ober ES, Setter TL, Madison JT, Thompson JF, Shapiro PS (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 Physiol 97:154–164

Overmyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H Jr, Kangasjarvi J (2000) Ozone-sensitive *Arabidopsis* rcdl mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12:1849–1862

Rajasekaran LR, Blake TJ (1999) New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. J Plant Growth Regul 18:175–181

Saini HS (1997) Effects of water stress on male gametophyte development in plants. Sex Plant Reprod 10:67–73

Saini HS, Aspinall D (1981) Effect of water deficit on sporogenesis in wheat (*Triticum aestivum* L.). Ann Bot 48:623–633

Saini HS, Westgate ME (2000) Reproductive development in grain crops during drought. Adv Agron 68:59–96

Saini HS, Sedgley M, Aspinall D (1984) Developmental anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. Aust J Plant Physiol 11:243–253

Sharp RE, LeNoble ME, Else MA, Thorne ET, Gherardi F (2000) Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. J Exp Bot 51:1575–1584

Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT (2004) Root growth maintenance during water deficits: Physiology to functional genomics. J Exp Bot 55:2343–2351

Sheoran IS, Saini HS (1996) Drought-induced male sterility in rice: Changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. Sex Plant Reprod 9:161–169

Spollen WG, Noble ME, Samuels TD, Bernstein N, Sharp RE (2000) Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. Plant Physiol 122:967–976

Tambussi EA, Bartoli CG, Beltrano J, Guiamet JJ, Araus JL (2000) Oxidative damage to thylakoid proteins in water stressed leaves of wheat (*Triticum asetivum*). Physiol Plant 108:398–404

Tao H, Brueck H, Dittert K, Kreye C, Lin S, Sattelmacher B (2006) Growth and yield formation for rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). Field Crops Res 95:1–12

Tsuda M, Takami S (1993) Changes of water potential in rice panicle under increasing drought stress at various stages. Jpn J Crop Sci 62:41–46

Waters SP, Martin P, Lee BT (1984) Influence of sucrose and abscisic acid on the determination of grain number in wheat. J Exp Bot 35:829–840

Wilkinson S, Davies WJ (2002) ABA-based chemical signaling: the co-ordination of responses to stress in plants. Plant Cell Environ 25:195–210

Xie ZJ, Jiang D, Cao WX, Dai TB, Jing Q (2003) Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statuses. Plant Growth Regul 41:117–127

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New Phytol 169:223–236

Yang J, Zhang J, Wang Z, Zhu Q, Wang W (2001) Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiol 127:315–323

Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2003) Involvement of abscisic acid and cytokinins in the senescence and remobilization of carbon reserves in wheat subjected to water stress during grain filling. Plant Cell Environ 26:1621–1631

Yang J, Zhang J, Ye Y, Wang Z, Zhu Q, Liu L (2004) Involvement of abscisic acid and ethylene in the responses of rice grains to water stress during filling. Plant Cell Environ 27:1055–1064

Zee SY, O'Brien (1970) A special type of tracheary element associated with 'xylem discontinuity' in the floral axis of wheat. Aust J Biol Sci 23:783–791

Zeng ZR, Morgan JM, King RW (1985) Regulation of grain number in wheat: geneotypic difference and response to applied abscisic acid and to high temperature. Aust J Plant Physiol 13:347–352

Zhu JK (2002) Salt and drought stress signal transduction in plants. Ann Rev Plant Biol 53:247–273