

Effects of Formulated Fertilizer Synergist on Abscisic Acid Accumulation, Proline Content and Photosynthetic Characteristics of Rice under Drought

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Abstract: To investigate the effects of formulated fertilizer synergist on the drought tolerance in rice, pot experiment was conducted to analyze the photosynthetic characteristics and the accumulation of abscisic acid (ABA) and proline in middle-season rice variety Peiliangyou 93. The synergist could improve the net photosynthetic rate, and coordination between the water loss and the CO₂ absorption as well as reduce the harmful effect on photosynthetic process under drought conditions. Under drought, the ABA accumulated massively both in roots and leaves, while the ABA content in roots was far higher than that in leaves. The results indicate that synergist could increase the ABA accumulation, but reduce the proline accumulation in rice plant under drought.

Key words: fertilizer synergist; drought; abscisic acid; proline; photosynthetic characteristics

Drought, alkali and low temperature are three major abiotic factors severely causing losses in crop yield. Among them drought is the most severe. Plants have shown a series of responses against the water stress [1-4]. The plant endogenous hormone serves as a regulator of life activities and changes consequently under water stress to adapt to the environment change for the growth and development of plant [5-6]. ABA has been widely accepted as an important stress phytohormone, whose biosynthesis is triggered by stress in root and leaf [7-9]. It is generally suggested that root system perceives the stress signal and the signal triggers the ABA synthesis system to start massive ABA synthesis, and the ABA, acting as a signal of stress, arrives at different parts of the stem through tracheae and regulates plant transpiration and leaf growth [10-11]. Moreover, Yamada et al [12] found that *Petunias* (*Petunia hybrida* cv. 'Mitchell') accumulated free proline under drought conditions, which served as an osmoprotectant in plants subjected to drought conditions. The accumulation of proline has been observed in large number of plant species in response to drought and salt stresses, suggesting a key role of amino acid in plant stress adaptation [13-14]. In present experiment, the variations in ABA and proline

accumulation, and photosynthetic performances in rice were studied under drought conditions to investigate the effectiveness of formulated fertilizers synergist..

MATERIALS AND METHODS

Soil, rice variety and fertilizer synergist

The paddy soil was collected from the experimental station of Hunan Agricultural University. The soil characteristics were as follows: pH 6.5, organic substance of 20.3 g/kg, total nitrogen of 1.53 g/kg, total phosphorous of 1.13 g/kg; total potassium of 23.2 g/kg, alkaline hydrolysis nitrogen of 0.122 g/kg, available phosphorus of 0.011 g/kg, and available potassium of 0.082 g/kg.

The rice variety used was a middle-season hybrid rice Peiliangyou 93.

The fertilizers applied were rice formulated fertilizer (18% N, 2.67% P, 8.3% K), and rice synergistic formulated fertilizers (Containing 2% synergist, 18% N, 2.67% P and 8.3% K). The synergist mainly comprised urease inhibitor, nitrification inhibitor, rare earth elements and biological activity substance [12].

Experiment methods

The pot experiments were conducted in 2002.

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Each pot was filled with 12 kg soil. The rice was sowed on May 13 and transplanted on May 30, with one seedling per hill and three hills per pot. During experiment three treatments were used: A) Formulated fertilizer treatment, 3.4 g of rice formulated fertilizer was applied per pot; B) Synergistic formulated fertilizer treatment, 3.4 g of rice synergistic formulated fertilizer was applied per pot; C) Synergistic formulated fertilizer treatment, 2.72 g of rice synergistic formulated fertilizer was applied per pot. The experiment was replicated three times. The fertilizer applied rate in treatment B (3.4 g of rice formulated fertilizer) was similar to the rate in the field, in which 675 kg formulated fertilizer were applied in about 2.4×10^6 kg soil per hectare.

The pot was under submerged conditions until 5 July when rice plants were sampled. Then water was poured out of the pots and the pots were moved into the greenhouse. The plants were not supplied with water till the end of the experiments.

Measurement

Determination of soil moisture content

The soil moisture contents were determined using the gravimetric method on July 5, 8, 12, and 16.

Photosynthetic characteristics and transpiration rate

The net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and transpiration rate of the first mature leaves were measured during 9:00-10:00 a.m. by a LI-6400 photosynthetic system, at photo flux density of 850 $\mu\text{mol}/\text{m}^2 \cdot \text{s}$ and 30°C.

Determination of free proline content

Free proline content was determined using ninhydrin reaction^[15].

Determination of ABA content

ABA extraction and purification were according to the procedure in HPLC method^[16]. Weigh 0.2000 g freeze rice root sample or 0.3000 g leaf sample (fresh

sample \rightarrow liquid nitrogen refrigeration \rightarrow vacuum-freeze drying), totally add pre-cooled 80% methanol 11 mL (5 mL, 2 mL, 2 mL and 2 mL respectively), then grind the sample very carefully in ice bath under weak light, continuously stir it in a 4°C refrigerator over night (15 h, avoiding light). Centrifugate the sample at $3077 \times g$ under 4°C for 10 min, decant the supernatant into a clean tube, repeat this step again, combine the supernatant, then condense the supernatant in cold-vacuum to get rid of the methanol, add 8 mL ammonium acetate (0.1 mol/L, pH 9.0) to dissolve the sample, centrifuge at $17624 \times g$ for 20 min, then transfer the supernatant into polyvinyl-pyrrolidone (PVPP) column and diethylamino-ethyl-sephadex A25, DEAE-Cross-linked dextran gel A25 column, collect the extracted endogenous phytohormones with SepPakC18 column, then elute the phytohormones from the column with 50% (volume percent) methanol, and determine the solution by the High-Performance Liquid Chromatography analysis.

Calculation of the stomatal limitation (L_s)

The stomatal limitation was calculated according to the method of Zou^[17]: $L_s = (C_a - C_i) / C_a$, where, C_a is the air CO₂ concentration, C_i is the intercellular CO₂ concentration.

RESULTS

Soil moisture content

The soil moisture content in treated pot rapidly dropped after pouring out of the water, almost similar to the air-dried soil at the end of the experiments (Table 1).

Effects of formulated fertilizers synergist on photosynthetic and transpiration characteristics of rice under drought

Effects on net photosynthetic rate of the top mature leaf

The net photosynthetic rate (P_n) of top mature

Table 1. Changes of soil moisture content (%).

Treatment	July 5	July 8	July 12	July 16
A (check)	36.8±1.2	20.6±0.9	10.6±0.5	4.6±0.3
B	37.2±0.8	19.7±1.3	9.5±0.5	5.1±0.2
C	36.6±1.0	21.3±1.2	11.5±0.7	4.8±0.3

leaf gradually decreased under drought conditions (Fig. 1). Before drought treatment, the Pn of the treatment B was similar to treatment A, but Pn of the treatment C was slightly lower than that of treatment A. After 7 days under drought conditions, the Pn of treatment A was only a half of the synergist treatments (B and C), while the treatment B showed the highest Pn. Eleven days after drought treatment, the Pn of treatment A decreased to $0.8 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, however, the synergist treatment B showed higher Pn at $1.6 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, while the treatment C remained higher Pn at $5.1 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$.

Effects on the stomata conductance to H_2O of the top mature leaf

The changes in stomata conductance showed a

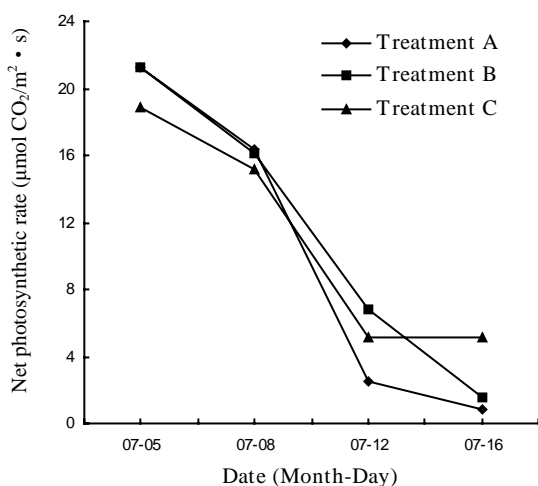


Fig. 1. Variation of net photosynthesis of rice plant.

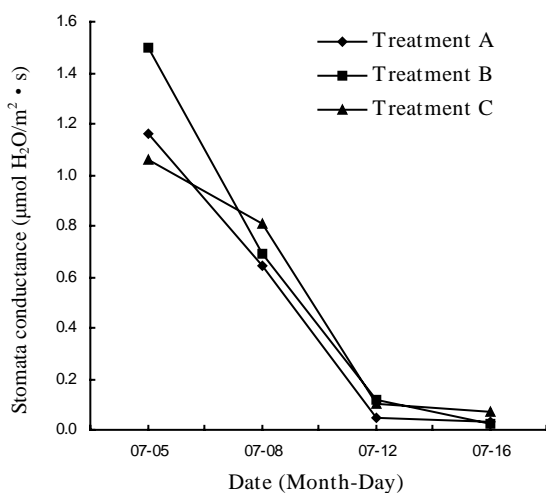


Fig. 2. Variation of stomata conductance to H_2O of rice plant.

decreasing trend during the experiment period, which was similar to that in net photosynthetic rate (Fig. 2). Before drought treatments, the stomata conductance to H_2O of treatment B was the highest among the treatments, and treatment A showed similar value as treatment C. After drought treatment, the stomata conductance to H_2O of treatments B and C was higher than that of treatment A, while 7 days later the stomata were almost closed.

Effects on the intercellular CO_2 concentration in the top mature leaf

At first the intercellular CO_2 concentration in top mature leaves of treatments A and B decreased and then increased, while the reduction was greater in treatment B than that in treatment A during early period, but the increase was less in treatment B than in treatment A during later period (Fig. 3). But there was a continuous decrease in the intercellular CO_2 concentration of top mature leaf in the treatment C.

Effects on the transpiration rate of the top mature leaf

The transpiration rates of the top mature leaves in the synergist treatments B and C were larger than that in treatment A (Fig. 4). The prolongation of drought resulted in a decrease of the transpiration rate with the highest values in treatment C at the 11 days of drought treatment.

Effects on the stomatal limitation (L_s) of the top mature leaf in rice

The stomatal limitation (L_s) of each treatment

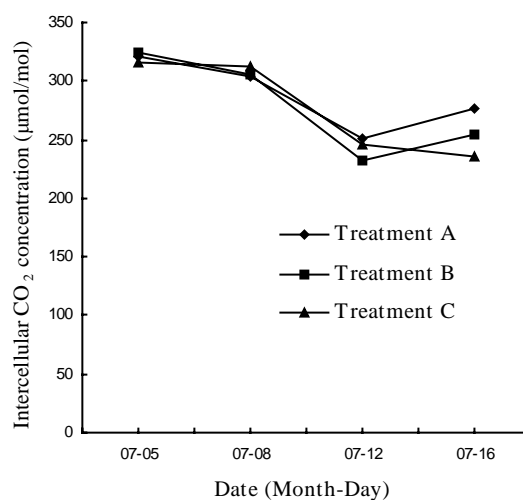


Fig. 3. Variation of intercellular CO_2 concentration.

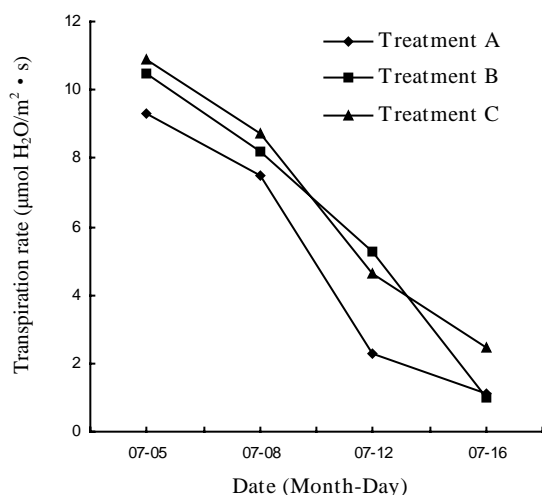


Fig. 4. Changes of transpiration rate of rice plant.

was very small under non-stress circumstance (Fig. 5). However, in all treatments the L_s increased with strengthening of the water stress. After 3 days under drought stress, a slight increase in L_s was noted, while the L_s of the synergistic treatments A and B were smaller than that of treatment C. At 7 days after stress, the L_s of treatment B was the highest, while after 11 days, the L_s of treatment C increased subsequently, but the L_s of the other two treatments decreased, and the decreasing degree of treatment A was larger than that of the treatment B.

Effect on the proline content in the top mature leaf

The proline contents in leaves of the synergistic treatments A and B were lower than that of treatment C under drought (Fig. 6). The proline content in top

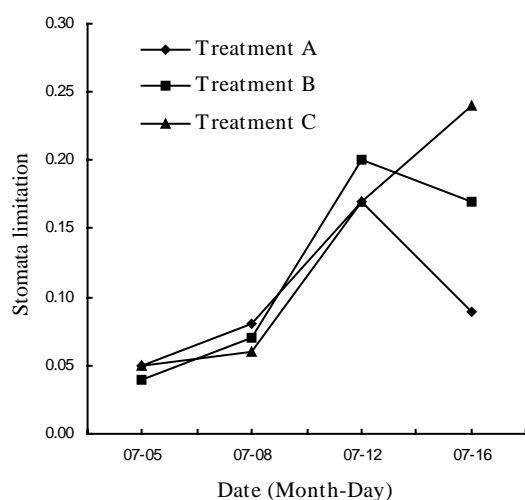


Fig. 5. Dynamics of stomata limitation.

mature leaves increased rapidly with the progress in drought conditions. Under less drought conditions, the proline content of all the treatments increased but the extent of increase was in smaller value. The proline content increased largely under the middle-level drought with the rapid increase in treatment C, followed by treatment A and B. However, the accumulation of proline in treatment C was the smallest under severe drought conditions. The accumulation of proline in synergistic treatments (A and B) was lower than that in treatment A at the 11th day after the stress.

Effects on ABA accumulation under drought

At first the ABA content in the root system increased greatly and then decreased (Fig. 7). Before drought treatment, the ABA content in root was very low. At third day after the stress, ABA content increased 58 to 101 times, and the accumulation degree of all synergistic treatments (B and C) was higher than that of treatment A, while the ABA content continuously increased. After 11 days of the stress a slight decrease of ABA content in treatment A was noted, but sharp decreases were observed in synergistic treatments.

The ABA contents in the leaves firstly increased and then decreased with increases in later period (Fig. 7). Before drought treatments, a minute difference was found in ABA contents among the treatments. After 3 days under drought, a large increase about 9 times in ABA content of treatment A was noted. Then 7 days

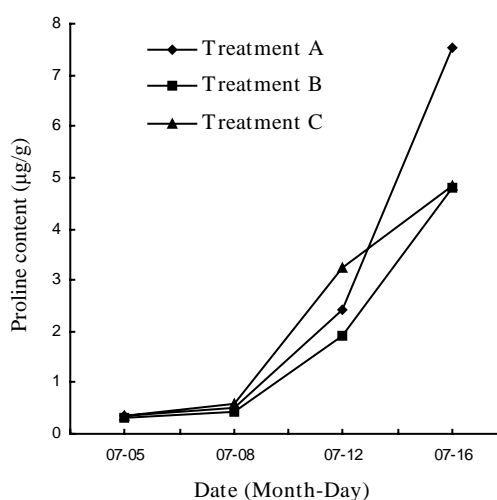


Fig. 6. Changes of proline content in rice plant.

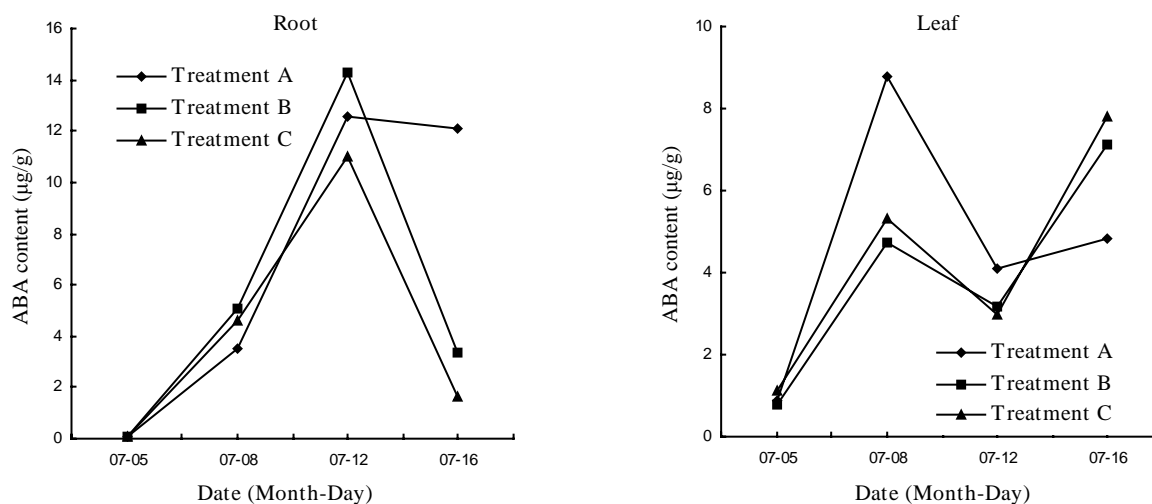


Fig. 7. ABA contents in roots and leaves of rice.

after the stress the ABA content decreased, while treatment A still had the highest value. But after 11 days of treatments, the ABA contents of two synergistic treatments were even higher than that of treatment A.

DISCUSSION

Effects of synergists on the photosynthesis of rice plant

The stomata are the channel of the air exchange and the water transpiration. Under drought conditions, the determining factors for regulating the opening and closure of the plant stomata are changed from light intensity and CO₂ concentration to water supply. In order to reduce the water loss from stomata, the stomatal conductance decrease, resulting in the increase in L_s under drought. Moreover, transpiration lessens and the transpiration rate decreases due to the stomata closure, while the intercellular CO₂ concentration also decreases at the same time by causing the decrease of the photosynthetic rate. Under slight drought, the key factor responsible for the decrease of the photosynthetic rate is stomata closure and the increase in L_s . But the damage due to the decreases in leaf photosynthesis and the L_s under the severe drought conditions are the major factors [14]. Our results showed that the net photosynthetic rate in the top mature leaves of the synergistic treatments were higher than that of the control under severe

drought stress, indicating that the synergist could improve the resistance capability to the stress. Simultaneously, after 11 days under drought stress, the intercellular CO₂ concentrations of treatment B and treatment A increased but the L_s decreased, suggesting that the major restraint factor of the photosynthesis might be the damage to the leaf photosynthetic functioning system. However, the intercellular CO₂ concentration of the synergistic treatment C continuously decreased, while the L_s showed an increasing trend, indicating that stomata closure is the major limiting factor and the damage to photosynthetic system is the secondary factor during photosynthetic process. This indicated that the synergists could improve the resistance capability of the rice plant by lightening the damage to photosynthetic system, while simultaneously improving the resistance by decreasing the fertilizer rate.

Effects of synergists on the relationship between photosynthesis and transpiration in rice plant

There has been a parallel and dependent relationship between the photosynthesis and the transpiration. The stomata closure can prevent water loss but also hinder the entrance of CO₂ from the external environment under drought, namely the reduced transpiration could lead to the lower CO₂ entrance, resulting in the lower photosynthesis. Therefore, it is of key importance to coordinate the

CO₂ absorption and water loss by regulating the movement of the stomata to adapt the plant to the adversities^[14,17-18]. Our study showed that synergists could improve the stomata conductance to H₂O and increase the transpiration rate, harmonize the relationship between the photosynthesis and the transpiration better than non-synergist treatment to lighten the damage of the photosynthetic function unit under drought.

Effects of synergists on the ABA accumulation in rice plants

At present, the role of ABA as a root-to-shoot stress signal is well established^[18]. It is suggested that roots are able to 'measure' decreasing soil water availability during a period of drought, which results in an increased release of ABA from the stelar tissues of roots to the xylem vessels. After xylem transporting ABA to the shoot, guard cells respond rapidly and sensitively to increased ABA concentrations resulting in the reduction of water loss due to transpiration. The rice roots contain enzymes and precursors that synthesize ABA, but the information is still lacking on the enzymatic step of ABA biosynthesis, which is responsible for the 'measurement' of reduced water availability. The synthesis of ABA in roots and transport to leaves is increased as a function of soil drying and shoot growth and physiology is modified as a function of this increased delivery^[18]. Previous studies have been reported that the synthesis of ABA is enhanced in response to soil water deficit, causing the rapid closure of stomata and thereby decreasing the intensity of the transpiration stream. The ABA functions to allow faster uptake of water by the roots and better transport within the plant. The effect of ABA is to dramatically reduce the apoplastic flow and to increase the cell-to-cell flow^[19].

The result of this experiment showed that ABA content in rice roots of synergist treatments was higher than that of the control at the beginning stage of drought, while the decrease in percentage was also higher than that of the control at the later stage of drought. However, ABA contents in leaves of the synergist treatments were lower than that of the control at the early stage of drought, and were higher than that of the control at the later stage of drought. It

may suggest that ABA accumulation in roots favored water absorption, thus increased the water efficiency, while the accumulation of ABA in leaves might cause the closure of stomata, thereby decrease water loss.

Effects of synergists on the proline accumulation in rice plants

To take the proline content as a result of drought resistance is still needed to be discussed, while it has been reported that the proline accumulation is only a response to drought^[7,20-21]. Our research showed that the proline content increased under drought, the proline content of the control was higher than that of the synergistic treatments under severe drought stress. However, the synergistic treatments showed higher tolerance to the stress than that of the control with slower decreases in photosynthetic rate. Thus, It can be suggested that the proline accumulation was not positively correlated with the tolerance to the stress, but responded to injury of drought.

Relationship between proline and ABA accumulation in rice plant

Previous report showed that ABA as a signal of adversity can be induced when roots suffered from drought stress, which resulted in proline production in order to help the plant to adapt environmental changes^[11]. Our results indicated that the accumulation of ABA in roots of the synergistic treatments was higher than that of the control, but the proline content in leaves was lower. During the last period of the stress, the accumulation of proline in leaves increased with the decrease in ABA content. This illuminated that ABA accumulation in roots was not positively correlated with proline content in leaves. It has been noted that the ABA accumulation in leaves first increased and then decreased, which was not positively correlated with the proline accumulation in leaves. But the results did not mean that the proline accumulation had no correlation with the ABA accumulation. The abundant accumulations of ABA and proline are due to the responses of plants to adverse conditions. It has been hypothesized by some researchers that ABA as a stress phytohormone is the signaling source of proline massive synthesis under drought to startup the biosynthesis of proline in the

plant, which plays an important role in modulating the cellular osmotic potential. Therefore, the relationship between proline and ABA accumulation needed to be further investigated.

REFERENCES

- 1 Parry M A J, John A P, Khan S, Lea P J, Keys A J. Rubisco activity: Effects of drought stress. *Ann Bot*, 2002, **89**: 833-839.
- 2 Griffiths H, Parry M A J. Plant responses to water stress. *Ann Bot*, 2002, **89**: 801-802.
- 3 Wang Z L, Mambelli S, Setter T L. Abscisic acid catabolism in maize kernels in response to water deficit at early endosperm development. *Ann Bot*, 2002, **90**: 623-630.
- 4 Yu L X, Setter T L. Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. *Plant Physiol*, 2003, **131**: 568-582.
- 5 Fan X R, Sheng Q R, Cui G X, Xu G H. Effect of soil water regime on dynamic levels of endogenous hormones and relationship between hormones and physio-biochemistry and morphology of rice of different cultivars cultivated in upland soil. *Acta Ped Sin*, 2002, **39**(2): 206-213. (in Chinese with English abstract)
- 6 Chaves M M, Pereira J S, Maroco J, Rodrigues M L, Ricardo C P P, Osorio M L, Carvalho I, Faria T, Pinheiro C. How plants cope with water stress in the field? Photosynthesis and growth. *Ann Bot*, 2002, **89**: 907-916.
- 7 Jia W S, Wang Y Q, Zhang S Q, Zhang J H. Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. *J Exp Bot*, 2002, **53**: 2201-2206.
- 8 Borel C, Audran C, Frey A, Marion-Poll A, Tardieu F, Simonneau T. *N. Plumbaginifolia* zeaxanthin epoxidase transgenic lines have unaltered baseline ABA accumulations in roots and xylem sap, but contrasting sensitivities of ABA accumulation to water deficit. *J Exp Bot*, 2001, **52**: 427-434.
- 9 Sharp R E, Lenoble M E. ABA, ethylene and the control of shoot and root growth under water stress. *J Exp Bot*, 2002, **53**: 33-37.
- 10 Wang S X, Peng K Q. Advances of study in mechanism of ABA biosynthesis and its signal transduction under stresses. *J Hunan Agric Univ: Nat Sci*, 2002, **28**: 55-61. (in Chinese with English abstract)
- 11 Hare P D, Cress W A, van Staden J. Proline synthesis and degradation: A model system for elucidating stress-related signal transduction. *J Exp Bot*, 1999, **50**(333): 413-434.
- 12 Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, Yoshida Y. Effects of free proline accumulation in petunias under drought stress. *J Exp Bot*, 2005, **56**(417): 1975-1981.
- 13 Thiery L, Leprince A S, Lefebvre D, Ghars M A, Debarbieux E, Savoure A. Phospholipase D is a negative regulator of proline biosynthesis in *Arabidopsis thaliana*. *J Biol Chem*, 2004, **15**(279): 14812-14818.
- 14 Wang S X, Peng K Q, Xiao L T, Tong J H, Wang R Z. Study on application of synergist to rice special fertilizers and its mechanism. *Plant Nutr Fert Sci*, 2003, **9**(3): 294-298.
- 15 Li H S. Principle and Technology for Plant Physiological and Biochemical Experiment. Beijing: Higher Education Press, 2001.
- 16 Wang R Z, Xiao L T, Lin W H, Cao Y. High performance liquid chromatographic determination of internal hormones in inter subspecific hybrid rice. *Chinese J Chromatography*, 2002, **20**(2): 148-150. (in Chinese with English abstract)
- 17 Zou Q. The photosynthesis of crop in water stresses. *Crops*, 1994(5): 263-267. (in Chinese)
- 18 Sauter A, Davies W J, Hartung W. The long-distance abscisic acid signal in the droughted plant: The fate of the hormone on its way from root to shoot. *J Exp Bot*, 2001, **52**(363): 1991-1997.
- 19 Morillon R, Chrispeels M J. The role of ABA and the transpiration stream in the regulation of the osmotic water permeability of leaf cells. *PNAS*, 2001, **98**(24): 14138-14143.
- 20 Xia Y. Effects of water stress on changes of proline and chlorophyll on fruit crops. *J Gansu Agric Univ*, 1993(1): 26-31. (in Chinese with English abstract)
- 21 Wang S J, Guo B. The relationship between the accumulations of the abscisic acid and free proline in wheatgrass under water stress. *J Gansu Agric Univ*, 1993(1): 36-40. (in Chinese with English abstract)