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Developmental characteristics and response to iron toxicity of root border cells in rice seedlings*

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Abstract: To investigate the Fe^{2+} effects on root tips in rice plant, experiments were carried out using border cells in vitro. The border cells were pre-planted in aeroponic culture and detached from root tips. Most border cells have a long elliptical shape. The number and the viability of border cells in situ reached the maxima of 1600 and 97.5%, respectively, at 20~25 mm root length. This mortality was more pronounced at the first 1~12 h exposure to 250 mg/L Fe^{2+} than at the last 12~36 h. After 36 h, the cell viability exposed to 250 mg/L Fe^{2+} decreased to nought, whereas it was 46.5% at 0 mg/L Fe^{2+}. Increased Fe^{2+} dosage stimulated the death of detached border cells from rice cultivars. After 4 h Fe^{2+} treatment, the cell viabilities were ≥80% at 0 and 50 mg/L Fe^{2+} treatment and were <62% at 150, 250 and 350 mg/L Fe^{2+} treatment; The viability of border cells decreased by 10% when the Fe^{2+} concentration increased by 100 mg/L. After 24 h Fe^{2+} treatment, the viabilities of border cells at all the Fe^{2+} levels were <65%; The viability of border cells decreased by 20% when the Fe^{2+} concentration increased by 100 mg/L. The decreased viabilities of border cells indicated that Fe^{2+} dosage and treatment time would cause deadly effect on the border cells. The increased cell death could protect the root tips from toxic harm. Therefore, it may protect root from the damage caused by harmful iron toxicity.

Key words: Rice plant, Border cells, Iron toxicity
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INTRODUCTION

Root border cells, which can be disengaged from root tips in water by mechanical stirring, come from meristematic tissue of root cap and consist of the water-solubility mucilage glue which is conjoint with root cap. Root border cells play an important role in regulating the root circumstance (Driouich *et al*., 2007; Wen *et al*., 2007). Release of Al-binding mucilage by border cells could play a role in protecting root tips from Al-induced cellular damages (Hawes *et al.*, 2000). It has also been shown that border cells have different responses to microorganism infection (Vicré *et al.*, 2005). At present, to research the growth control and biology functions of root border cells is one of the hotspots in this domain.

Iron toxicity is one of the main constraints to rice production in tropical and subtropical areas, where over 4 million hm^2 of land is adversely affected with yield reduction of approximately 30% to 60% (Cai *et al*., 2003; Majerus *et al*., 2007; Sahrawat, 2000). The direct iron toxicity that is harmful for the plant is Fe^{2+}. When the plant assimilates superfluous Fe^{2+}, it can cause illness. These years, root border cells involved in heavy metal tolerance have been largely studied in species such as pea (*Pisum sativum*) (Wen *et al*., 1999), snapbean (*Phaseolus vulgaris*) (Miyasaka and Hawes, 2001) and barley (*Hordeum vulgare* L.) (Zhu *et al*., 2003). The present investigation was undertaken to study the biotic characteristics and response to iron toxicity of root border cells in rice to search for iron tolerance mechanism at border cells.

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MATERIALS AND METHODS

Rice (*Oryza sativa* L.) seeds were surface sterilized with 0.1% (v/v) H_2O_2 solution, soaked in deionized water, and then germinated on wet filter paper in the dark at 30 °C until they sprouted. Then, the healthy and sterile seedlings were selected and transferred to 500 ml beakers, in which aeroponic culture with 200~250 ml dd H_2O was used to keep the surroundings humid (Zhu *et al*., 2003), and then the 500 ml beakers were put into the 1000 ml ones with 300 ml dd H_2O in the bottom to keep the surroundings warm (Cai *et al*., 2007). Nearly 80~100 seedlings were kept in each beaker.

To characterize the border cell number and viability, ten seedlings from eight categories (1, 2, 5, 10, 15, 20, 25 and 30 mm root length) were selected and the border cells from each of the seedlings were harvested into 1 μl FDA (fluorescein diacetate) and 2 μl PI (propidium iodide). After 10 min in the dark, the live cells showed green fluorescence and dead cells showed red fluorescence under fluorescent microscope (Motic BA400 EF-UPR, Motic Co., China). The ratio of live cells to all the cells counted was the activity of the root border cells.

Cells were harvested from 20~25 mm root length into water at approximately 24 border cells/μl. An equivalent volume of treatment solutions was added to result in a water control and 250 mg/L Fe^{2+} with 200 μmol/L $CaCl_2$ at pH 4.5. The cells were incubated in the dark at 25 °C, and cell viability was measured at 1, 4, 8, 12, 24 and 36 h after initiation of treatments. Five Fe^{2+} ($FeSO_4{\cdot}7H_2O$) levels (0, 50, 150, 250 and 350 mg/L) were treated with 0.1 mmol/L Ca^{2+} at pH 4.5, and cell viability was measured at 4 h and 24 h after initiation of treatment.

RESULTS AND DISCUSSION

Border cell number and viability

The border cells separated from root tips were observed as long ellipses and showed strong fluorescence, while the live cells showed green fluorescence after FDA-PI staining (Fig.1). As shown in Fig.2, the first formation of border cells occurred almost synchronously with primary root tip emergence in rice plant, and approximately 200 border cells were produced at 1 mm in root length. Cell number increased with root elongation and reached its maximum value (about 1600) when the root was 20~25 mm long. When the rice roots were only 1 mm, the viability of border cells can reach to 85.2%, and then remains higher than 95% after 5 mm long. The maximum value was 97.5% when the rice root grew up to 20 mm, and then decreased tardily.

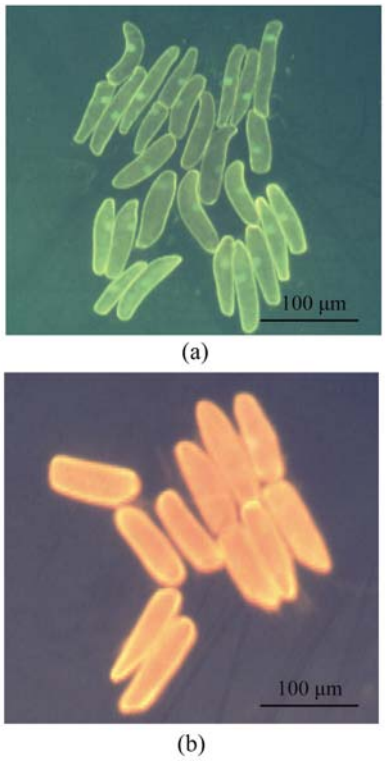

Fig.1 The dispersed border cells from root-tips of rice plant (FDA-PI staining, ×400). (a) The live border cells; (b) The dead border cells

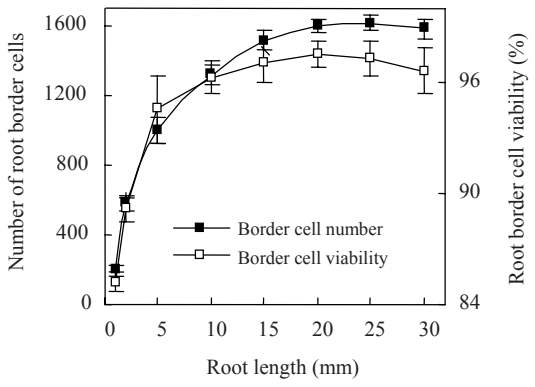

Fig.2 Number and viability of developed root border cells in rice

Responses of detached border cells to iron toxicity

The response of border cells to Fe^{2+} exposure time (1, 4, 8, 12, 24 and 36 h) is shown in Fig.3. Compared with the control, the increase in exposure time to Fe^{2+} significantly decreased the survival percentage of border cells. This decrease in viability of border cells was more pronounced at the first 1~12 h exposure to Fe^{2+} than at the last 12~36 h. The mortality of cells exposed to 250 mg/L Fe^{2+} was significantly lower than that of cells exposed to 0 mg/L Fe^{2+}. After 36 h, the cell viability of border cells at 250 mg/L Fe^{2+} decreased to nought, whereas it was 46.5% at 0 mg/L. As a result, high concentration Fe^{2+} solution is harmful to the rice border cells in vitro.

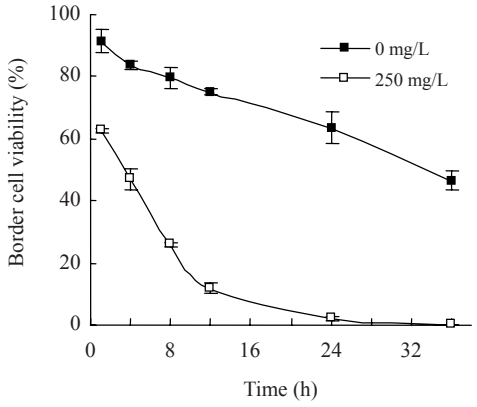

Fig.3 Effect of Fe^{2+} exposure time on relative viability of border cells in vitro

Fe^{2+} treatment stimulated the death of detached border cells from rice cultivars (Fig.4). After 4 h Fe^{2+} treatment, the viabilities of 0 and 50 mg/L Fe^{2+} treatment were ≥80% and showed obvious discrepancy with the others (P<0.01). For the 150, 250 and 350 mg/L Fe^{2+} treatment, the viability of border cells decreased by 10% when the Fe^{2+} concentration increased by 100 mg/L. After 24 h Fe^{2+} treatment, the viabilities of all the Fe^{2+} levels were <65%. Border cells exhibited greater cell mortality for 24 h exposure to Fe^{2+} treatment compared with 4 h Fe^{2+} treatment. The viability of border cells decreased by 20% when the Fe^{2+} concentration increased by 100 mg/L. There was great harm to the viability of border cells suffered from iron toxicity. Border cells may be affected by microdosage Fe^{2+}.

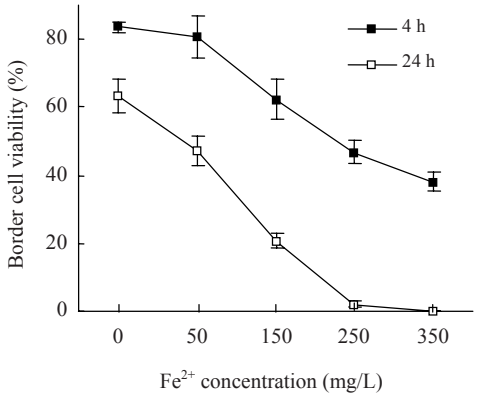

Fig.4 Effect of Fe^{2+} concentration on viability of border cells in vitro

CONCLUSION

The effects of Fe^{2+} concentration and exposure time on border cells in vitro were observed. High Fe^{2+} concentration and 24 h Fe^{2+} exposure had inconspicuous effects on the viability of root border cells. However, with the increase of Fe^{2+} concentration and time, each factor showed significantly inhibition to viability of border cells. The decreased viability of border cells indicated that Fe^{2+} dosage and treatment time would cause deadly effect on the border cells. The increased cell death could protect the roots from toxin harm. Therefore, it may protect root from the damage caused by harmful iron toxicity.

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