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Biological Characters of Root Border Cells Development in Maize (*Zea mays*)

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Abstract: The new inbred line Xiaohuangbaogu was used to investigate biological characters of root Border Cells (BCs) development in maize. The results showed that, maize border cells adnated on the 1-2 mm region of root cap, the shape was various, round, elliptical, bacilliform and so forth. The root cap PME activity, BCs number and viability varied with the primary root elongation and the general variation trend of the three traits was first up and then down. The PME activity was highest at 28.23 μmol H^+ root cap^{-1} h^{-1} when the primary root was 1 cm long. Root border cells developed synchronously with radicle growing, the number increased with primary root elongation, when the root was 2 cm length, the number reached a maximum of 4367, the maximum viability was about 90% when the root was 1.0 cm in length, the average viability was 76.16%. The aerial root border cells were smaller and fewer, but the viability was higher, the average BCs number was 2092 when the root was 1-4 cm in length and the average viability was 86.21%; low temperature was beneficial to maintain the detached BCs activity, the relative viabilities were both higher than 97% if the detached BCs were standing at 5 or 10°C for one day, while it was significantly different 4 days later, the relative viability at 5°C was still high up to 46.05% 5 days later, but at 10°C was as low as 13.50%. The 20/25°C was suitable for maize BCs redevelopment, high temperature 30°C promoted border cells to regenerate rapidly in 12-24 h. BCs number increased if root suffered from drought stress, but there was no obvious change in viability. It is possible that the increased border cells play an important role in reducing adversity damage to roots while maize is suffering from low temperature or drought stress.

Key words: Maize, root border cells, development, pectin methylesterase, temperature, drought stress

INTRODUCTION

Root Border Cells (BCs) are active cells that originate from root cap and are aggregated around the root cap by root tip mucus (Yu *et al*., 2004). These border cells have several biological functions. First, BCs play a significant mechanical role in decreasing frictional resistance to root penetration; second, border cells and their exudates can attract and immobilize nematode, repel bacteria, resist the infection of fungi (Hawes *et al*., 1998, 2003; Plancot *et al*., 2013) and relieve some adversity damage such as drought stress, aluminum toxicity and so on (Driouich *et al*., 2013; Miyasaka and Haves, 2001). Furthermore, the border cells secretion, the cell wall polyaccharide and other degraded fragments that generate during BCs released from root cap can enhance the validity of nutrition elements by changing the acidic or alkali conditions or redox potential of the rhizosphere to chelate some metal ions or improve their solubility. Hence, root border cells are actually a natural barrier between root cap and rhizosphere soil which build a stable and balanced ecosystem in the rhizosphere through adjusting cells quantity and activity after specific recognition of the rhizosphere conditions (Hawes *et al*., 2000, 2003), this is very important to plant growth and development.

It has been confirmed that maize root border cells can resist fungi invasion (Sherwood, 1987), attract potato rot nemotode (Rodger *et al*., 2003). Dettached maize BCs can actively absorb glucose in environment (Stubbs *et al*., 2004), HMC-toxin may lead detached border cells apoptosis (Ma *et al*., 2009, 2010, 2013). Maize BCs number and survival rate decrease under the stress of copper ions (Liu *et al*., 2012). Therefore it can be sure that maize root border cells still play the role of protecting root tip from biotic and abiotic stress, increasing nutrition absorption and improving rhizosphere conditions. However, maize BCs development characters and the influence factors just as temperature and moisture has not been investigated. In this study, we report the biological characters of maize root border cells development systematically, the expectation is to lay a foundation for

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further research on maize BCs biological function and the related mechanism while the roots are suffering from cold, drought, salinization, heavy metals and pathogens, etc.

MATERIALS AND METHODS

Material and culture methods: The new inbred line Xiaohuangbaogu (yellow flint type) was provided by the maize research laboratory of Xichang college. The seeds were surface-sterilized by immersion in 3% hydrogen peroxide for 5 min after being soaked in water 12 h, rinsed there times with sterile water and placed into a 15 cm-diameter Petri dish where the filter paper had been prepared the middle a little higher than the border (three-layer of 13 cm-diameter filter papers bottom and one-layer of 15 cm above) and moistened with sterile water and plates were placed into freshness protection packages and incubated in the dark at 25°C until radicles emerged after 1 to 2 days.

Microscopic observation on maize BCs adnation state and morphology: Different lengths of root tips were cut from seedlings and placed on clean slides, sterile water was added, the adnation state of root border cells was microscopically examined with light microscope Phenix100 and Motic BA300 under four times objective lens. The 0.5 cm lengths of root tip were cut, dispersed into sterile water for a few min, cells suspension was aspirated on to clean slides, stained with an equal volume of 0.4% trypan blue for 3-5 min (Wang *et al*., 2013; Zhang, 2011), after which the shape of the BCs was microscopically examined and photos were taken, the cells size of different shape was measured on twenty randomly selected cells by microscopic image processing software Phenix100.

PME extraction and activity detection: PME was extracted from 40 excised root tips (0.5 cm) selected from these seeds just germinating with 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 cm length primary root. The enzyme extraction and measurement methods were derived from related literatures (Ma *et al*., 2005; Stephenson *et al*., 1994). The PME activity unit was μmol H^+ root cap^{-1} h^{-1}.

BCs number count and viability examination

Root border cells developed from primary roots: These seedlings that just germinated with 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 cm length root were selected, the root tips about 3-5 mm length were cut, 5 tips for each length, dispersed into 500 μL sterile water, standing 30 min, vortexed 60 sec, microscopic examination was done to ensure that the border cells had been cleaned completely. Eight micro liter aspiration of cells suspension was stained with 8 μL of 0.4% typanblue for 3-5 min (Wang *et al*., 2013; Zhang, 2011), under optical microscope Phenix100, viable cells were white, the dead were blue, the viable (N_L) and dead cells (N_D) were counted, respectively, BCs viability was calculated as:

$$(N_L)/(N_L+N_D)\times 100\%$$

The number for each root tip was expressed as:

$$12.5\times(N_L+N_D)$$

Root border cells developed from aerial root: These seedlings with 1-2 cm length primary root were placed into 250 mL erlenmeyer flask, 2 plants per flask and sterile water was added to keep the seedlings growing well. When the aerial roots generated, 0.5, 1.0, 2.0, 3.0 and 4.0 cm length root border cells were harvested, respectively and the BCs number and viability were tested as above.

Survival rate of detached root border cells: Twenty primary root tips were cut from the seedlings with 1 cm length root and immersed in 2.0 mL sterile water, standing 30 min, vortexed 60s, The cells suspension was divided into 300 uL per 1.5 mL microcentrifuge tube and incubated in dark at 5, 10, 15, 20, 25 and 30°C, without shaking, the viabilities were detected 1, 2, 3, 4 and 5 days later, the relative viability was described as the ratio of the current viability to the initial.

Temperature and drought stress experiment

Temperature experiment: Petri dishes were prepared just as described above and filter papers were fully moistened. These seedlings with 1 cm length primary root were selected, put into sterile water for 30 min, drained, rinsed three times with sterile water, the residual BCs and mucilage were wiped off with sterile paper and then the seedlings were placed into petri dishes, five plants each Petri dish, seedlings were grown in the dark at 10, 15, 20, 25 and 30°C, primary root lengths were measured at 6, 12, 24, 48 and 72 h after initiation of treatments and newly developed border cells of primary root were harvested, respectively. The total number of border cells and viability were calculated as described previously.

Drought stress experiment: An equivalent volume of sterile water and 15% polyethylene glycol6000 (PEG6000) solution (Li *et al*., 2011; Zhang *et al*., 2011) were used to moisten filter paper as control and treatment, respectively. Seedlings which had been cleaned of border cells were

placed on filter paper, five seedlings to each petri dish. Root lengths were measured at 1, 2 and 3 days after initiation of treatments and newly developed border cells of primary root were harvested, respectively the count and calculation methods were the same as described in previous section.

Statistical analysis: The whole experiment was repeated by three times independently and three to five operation repetitions among one experiment. The results in text represent the means of three independent experiments and the data from three independant experiments was subjected to analysis of variance and Duncan's single range test was applied using statistical software SPSS17.0, A probability level of 0.05 was considered to be statistically significant and figures were drawn in Excel 2003.

RESULTS

Maize BCs adnation state and morphology

BCs adnation state in different environment: Root border cells originate from root cap, namely they are mitosis products of root cap meristem. Maize root border cells developed with root growing, the mature border cells stayed at different state in various moisture conditions. If the root grew on moderate water moistened filter paper, root tip exuded mucus, when observed by 4 times in microscope, the mucilage was comprised of 2 layers, the outer was thin, while the inner was heavy and density, root border cells existed in the inner layer (Fig. 1a); if the root cap was directly contacted with visible state water, root border cells released into water with the mucus dilution. The release process could be seen by 4 times in microscope after 0.5 cm length root tip cut and placed on slide in water, border cells arranged in layers regularly around root cap at preliminary stage, the dispersed cells number increased with the time go (Fig. 1b), if stained with 0.4% typanblue the image was more clearer (Fig. 1c), eventually, these dispersed border cells distributed around the root cap radially and those far away from root cap dispersed in water rambling. If root tip was exposed to dry air minutes later, mucus and border cells were so cling to the root tip surface that the mucilage was invisible to the naked eye or microscopic observation (Fig. 1d), but they would disperse rapidly once the root tip was touched with water.

Maize root border cells shape and size: It was difficult to find a small quantity of unstained maize root border cells under light microscope because they were colorless, but they were apparent even observed by 10 times in microscope if stained with 0.4% trypan blue, the viable cells were brilliant and white (Fig. 2a), but the dead were

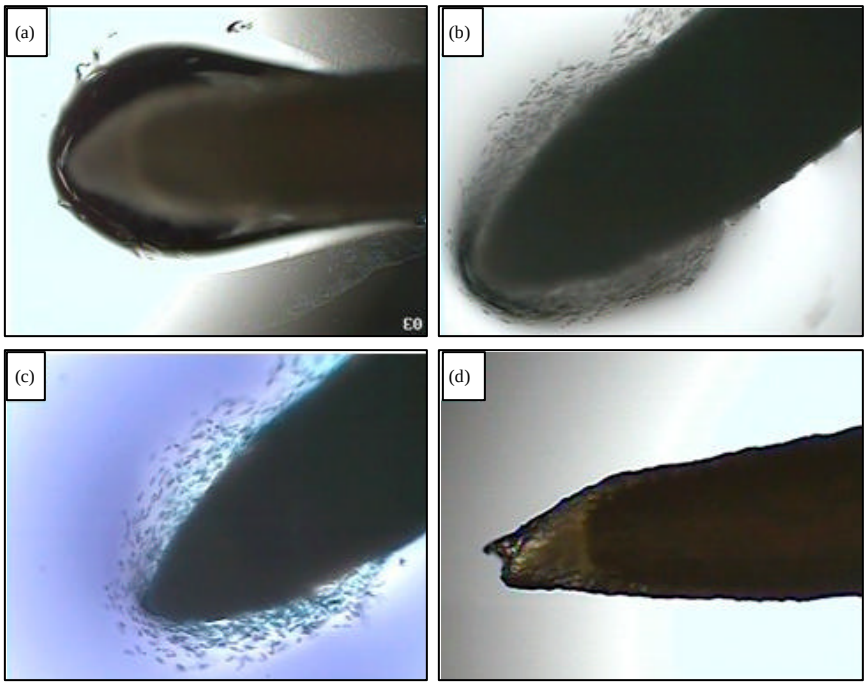

Fig. 1(a-d): Maize BCs adnation state, (a) Complete mucus layer around root tip, (b) BCs release in sterile water, (c) Add 0.4% trypan blue and (d) Root tip extends in dry air

Fig. 2(a-k): Maize BCs shape and activity, (a) Living Bcs, 10×, (b) Dead BCs, 10×; C-K, 40×: (c) Round dead BC, (d) Crooked bacilliform living BC, (e) Elliptical living BC, (f) Irregular living BC, (g) Glliptical dead BC, (h) Short bacilliform dead BC, (i) Bacilliform living BC, (j) Bacilliform dead BC and (k) Twisted living BC

bluish violet (Fig. 2b). When examined by 40 times, BCs shape and some entocytes were visible. Maize root border cells shape was various, round (Fig. 2c), elliptical (Fig. 2e, g), bacilliform (Fig. 2d, h-g), slim-twisted (Fig. 2k), irregular (Fig. 2f) and so on and the detached cells might continue to undergo changes in morphology and structure. Different shape cells with corresponding dimensions, the round cells diameter was in the range of 36.46-55.87 (44.68) μm, the elliptical was 48.87-69.64 (60.21)×20.75-41.23 (29.96) μm, the bacilliform was 84.06-322.4 (121.32)×14.5-30.55 (23.2) μm and the crooked bacilliform was 65.08-166.93 (103.97)×19.17-27.61 (22.57) μm. Border cells originated from different area of root cap were different of shape, the percentage of different shape cells varied with root growth. The cells originated from the root cap top were round or short-elliptical and small and might continue to grow after detached from root cap and turn elliptical; The cells originated from root cap near to the root hair zone were long-bacilliform and might become crooked bacilliform after separated from root surface. These border cells separated from the radicle with sheath were slim and some were twisted. The cells developed from 1-3 cm length primary root were large in dimension with standard shape of rod or ellipse; when primary root was longer than 4 cm, root grew faster, border cells separated from the root were long-bacilliform. The viable cell grew in suitable environments were smooth of surface with 1-2 vacuoles in cell; cytoplasm distributed evenly in dead cell and could be stained well by trypan blue (Fig. 2h). Unsuitable environment had somewhat influence on cells shape and activity, these cells derived from the roots suffered chilling injury or drought stress were irregular of shape, their vacuoles divided into a few littles, the cytoplasm of those died from drought stress shrinked and so the plasmolysis occurred (Fig. 2g, j).

PME activity, BCs number and viability varied with maize root elongation

PME activity in maize primary root cap, BCs number and viability: Root border cells separation correlates closely with the pectin methylesterase activity in root cap and the cells number and activity vary with root elongation which is significantly different in different species. This study revealed that, PME level, BCs number and viability varied with maize primary root elongation and the general variation trend of the three traits was first up and then down. PME activity increased with primary root elongation and reached the highest value of 28.23 μmol H^+ root cap^{-1} h^{-1} while the root was 1 cm long, then PME activity declined slowly and finally stabled at about 16 μmol H^+ root cap^{-1} h^{-1}. The average value of PME activity was 19.98 μmol H^+ root cap^{-1} h^{-1} while root length was in the range of 0-6.0 cm (Fig. 3a).

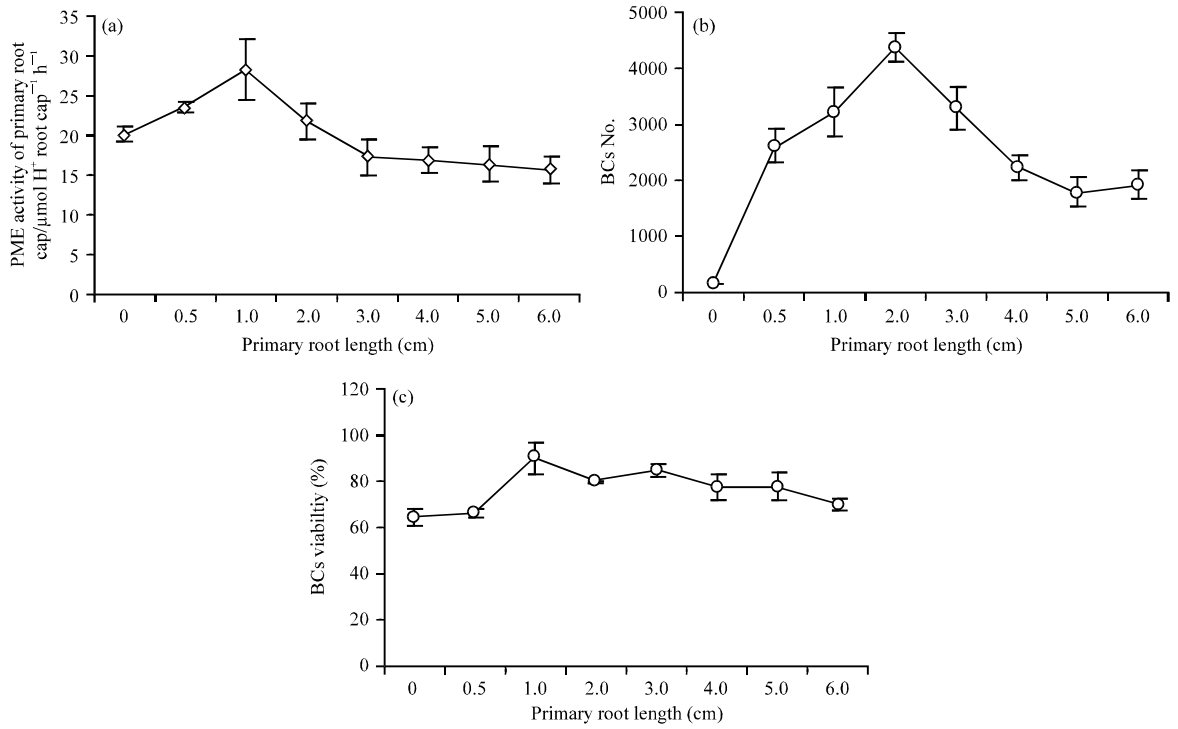

Fig. 3(a-c): PME activity, BCs number and viability varied with maize root elongation, (a) Changes of PME activity with the maize primary root elongation, (b) Maize BCs number with the primary root elongation and (c) Maize BCs viability with the primary root elongation. The error bars denote the standard error of the mean

Maize border cells developed synchronously with radicle generating, there were over 100 border cells per root cap at this time, then the number increased rapidly with primary root elongation and reached the maximum of 4367 while the root was 2 cm in length, afterwards the number decreased and maintained at about 1900 while the root was 5.0-6.0 cm in length (Fig. 3b). BCs viability showed a similar variation trend with PME activity with root elongation, the viabilities were all higher than 60%, the max viability was about 90% when the root was 1.0 cm in length and the average viability was 76.16% while the root was 0-6.0 cm length (Fig. 3c).

BCs number and viability of aerial root: While maize seedlings grew up with 2-3 leaves growing out, aerial root began differentiation. Border cells developed from maize aerial root cap were smaller than those from primary root, the BCs number showed a variation of downtrend overall (Fig. 4a) and averaged at about 2500 while the root was 1.0-3.0 cm length, then decreased sharply, there were only 447 BCs when the root was 4 cm length. When analyzed the variation trend carefully, there was a remarkable increase while the aerial root was 3 cm length, the possible cause was these 3 cm length roots were just long enough to get in touch with the bottom of culture bottle; maybe the solutions with root secretion and metabolite were

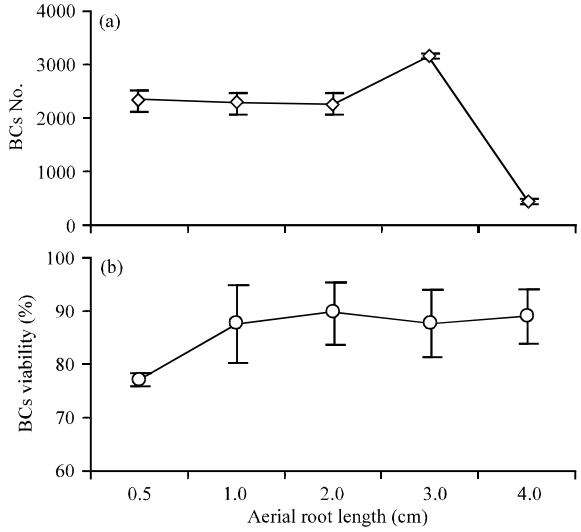

Fig. 4(a-b): Maize BCs development with the aerial root elongation, (a) Maize BCs number with the aerial root elongation and (b) Maize BCs viability with the aerial root elongation. The error bars denote the standard error of the mean

propitious to root growth and BCs development. However, while aerial roots were 4 cm length, they usually

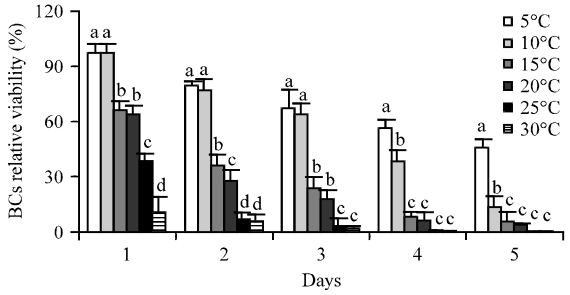

Fig. 5: The effect of temperature on detached BCs viability. Different small letters show significant difference at the 0.05 level during the same culture time

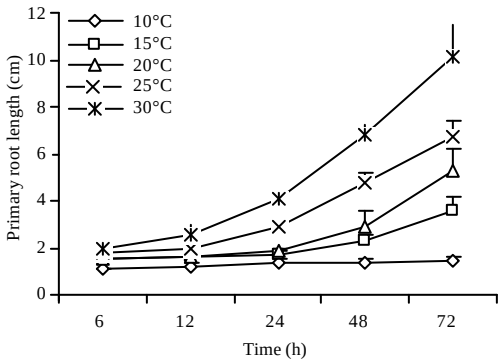

Fig. 6: Effect of temperature on maize primary root elongation

extended to the bottom of culture bottle and mixed with lateral roots, so it was difficult to take the root tip but no BCs loss, it was probably that the result got from the experimental condition was much less than field situation. With regard to BCs viabilities (Fig. 4b), they were higher generally and all were higher than 77%, there was no obvious difference between different length roots and the average viability was 86.21%. Compared with primary root, aerial root border cells were fewer in number but higher in viability.

The survival rate of dettached root border cells: Low temperature was beneficial to maintain detached BCs activity (Fig. 5). The Relative Viability (RV) of detached root border cells cultured at 5°C/10°C was significantly higher than at other temperatures in five days, there was no significant difference between 5 and 10°C in 1-3 days, relative viabilties were both higher than 97.0% at 5°C or 10°C for one day, while the RVs were significantly different 4 days later, the relative viabilty was still high up to 46.05 % 5 days later at 5°C, but was as low as 13.50% at 10°C. From 15-30°C, the higher the temperature was, the faster the detached BCs died, relative viabilty was lowest to 10.35% at 30°C only 1 day later which was significantly lower than others, furthermore, high temperature led the detached BCs entocyte outflow and cells disintegration, then cells suspension became turbid which might be because the detached BCs at higher temperature were at an active physiological metabolism state, but there was no oxygen and nutrition in culture solution, these cells must die quickly. On the contrary, physiological metabolism of these BCs at lower temperature almost stopped, it was just like hibernation state,so they survived longer.

Maize BCs regeneration was influenced by temperature and humidity

The effect of temperature on maize BCs regeneration: Temperature is a critical inflence factor on maize seed germination and root growth and also regulate root border cells development. In this study, these seedlings with 1 cm length primary root were washed off root cap mucus and border cells and cultured at different temperature, the root length and newly developed border cells were test, respectively. The result showed that (Fig. 6, Table 1), it was very slow that the primary root grew and border cells regenerated if cultured at 10°C at 15°C, roots grew faster and border cells regenerated relatively faster and there was no significant difference in BCs number between at 10/15°C and at 20/25°C 72 h later; primary roots of these cultured at 20/25°C grew moderate and strong, the newly developed BCs number was at a high level all along which reached the maximum about 24-48 h later; at 30°C, primary roots grew at a highest speed all the time, but the roots got slim and weak with the time go, border cells regenerated very fast in short time and at 12 h later the number was significantly more than at other temperatures, but which was significantly less than at other temperatures 72 h later. Thus it can be seen, high temperature could promote border cells to regenerate rapidly in short time, but was unfavourable for BCs sustainable development, yet the 20/25°C was beneficial to root growth and BCs regeneration for long. Whereas, at low temperature, maize root might be protected from chilling damage by developing more border cells.

As regards the root border cells viability, the newly developed BCs viability at 10°C was significantly lower than other temperature 6 h after regeneration culture, but no significant difference 72 h later when compared to 15/20/25°C culture, viabilities of these cultured at 15/20/25°C were higher all the time; viabilities at 30°C, 12 to 24 h declined apparently, but at that time, the newly developed BCs number arrivered at the maximum, the presumed reason was that a large number of newly developed border cells released much heat and with the high enviroment temperature which was all make against Bcs survival. Given consideration to

Table 1: Effect of temperature on maize root border cells regeneration (Mean±SD)

	Culture time (h)				
Culture temperature (°C)	6	12	24	48	72
BCs number (No.)					
10	137±23^{b}	297±144^{be}	707±347^{c}	1967±274^{abc}	1693±220^{a}
15	661±307^{b}	1034±67^{cd}	1257±204^{c}	2047±462^{ab}	1747±172^{a}
20	1302±213^{a}	1454±267^{bcd}	2344±164^{ab}	2624±437^{a}	1987±431^{a}
25	1460±122^{a}	1592±139^{bc}	2087±340^{b}	2553±404^{a}	1910±185^{a}
30	1660±400^{a}	2854±234^{a}	2982±512^{a}	1487±420^{bc}	627±46^{b}
BCs viability (%)					
10	71.08±2.35^{c}	84.08±5.15^{b}	82.99±4.40^{bc}	82.75 ±4.03^{c}	95.53 ±3.35^{a}
15	90.77±1.96^{ab}	93.29±3.27^{ab}	88.01±0.47^{ab}	96.57 ±2.96^{a}	95.90 ±2.65^{a}
20	90.50±1.27^{ab}	88.82±3.06^{ab}	89.91±5.15^{ab}	94.07 ±3.17^{ab}	95.86 ±0.81^{a}
25	88.84±3.11^{ab}	94.96±1.20^{a}	88.81±0.98^{ab}	96.21 ±1.71^{a}	94.53 ±2.59^{ab}
30	88.66±2.24^{ab}	68.57±6.65^{c}	77.68±6.37^{c}	88.36 ±3.06^{abc}	77.12 ±3.88^{d}

Different small letters show significant difference at the 0.05 level among different temperatures at the same time

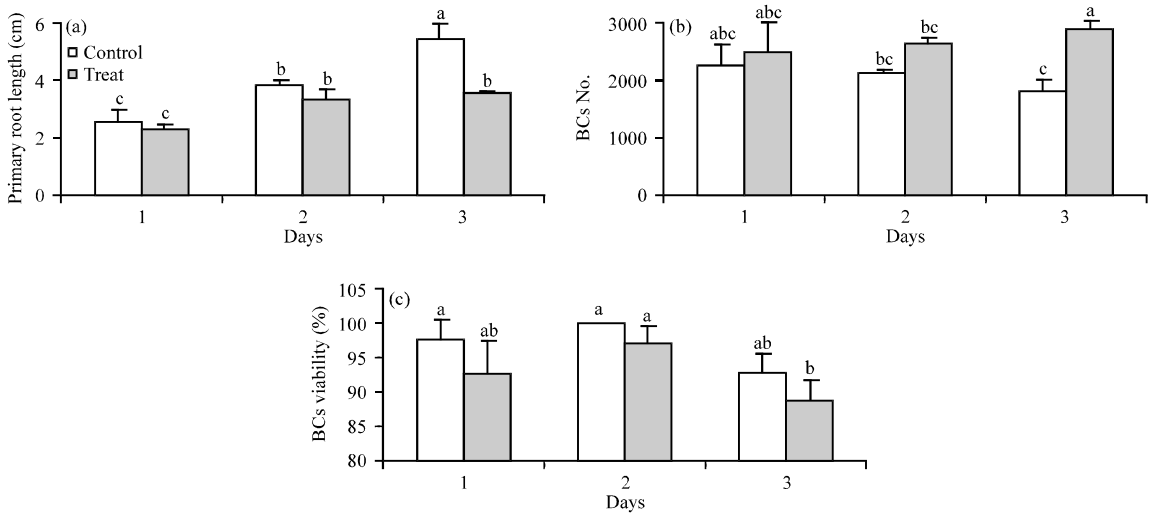

Fig. 7(a-c): Effect of drought stress on maize primary root elongation and BCs regeneration, (a) Effect of drought stress on maize primary root elongation, (b) Effect of drought stress on regenerative BCs number and (c) Effect of drought stress on regenerative BCs viability. Different small letters show significant difference at the 0.05 level

root growth and BCs regeneration, 20/25°C was suitable for maize root border cells redevelopment (Table 1).

Effect of moisture on maize BCs regeneration: It is well known that humidity is an important factor to inflence seed germination and seedling growth. The experiment was carried out under analogue drought condition with 15% PEG6000. It showed that, drought stress slowed maize primary root elongation and the inhibition effect became more and more obvious as the treat time extension (Fig. 7a), the primary root length was only 64.02% to the control 3 days later. However, the root cap mucilage increased after 12 h drought stress and the BCs number increased, as the culture time went on, the promotion effect got apparent, BCs number in treatment was 1.6 times to the control after 72 h. Compared with control, newly developed BCs under drought stress were relatively smaller, but there was no significant difference in viability. Based on the result, it was presumed that border cells might play an important role in maize reaction to drought stress, the thicker mucilage and increased BCs number might help plant in resisting drought stress by protecting root tip from drought injury and the thicker mucilage might be resulted from root tip secretion increase or BCs mucous layer thickened.

DISCUSSION

Culture and testing methods: Root border cells may disperse into water easily, in previous studies, aerial-culture method was usuallly adopted to ensure the seedlings grew well and with no Bcs dispersion.

However, it is difficult to maintain the maize seedlings growth by aerial-culture with spraying because maize roots and germs grow very fast and if the seedlings are suspended in bottles with water, roots might elongated into water soon and BCs dispersion happened soon after. In this study, the seedlings were cultured in Petri dish with filter papers middle a little higher than edge and about 1 mL sterile water was droped to just fully moist the papers in morning and evening everyday, by the way, the seedlings grew well and the root tip mucilage was intact with no border cells loss.

Maize roots generate a large number of border cells, BCs of different degree of ripeness disperse with different degree of difficulty and they are always combined with mucus, so they are difficult to be dispersed uniformly in water. Whereas, the degree of uniformity of cells suspension have a great impact on count result, so BCs sampling and count method is a critical factor to the accuracy of experiment. In this study, in order to improve the accuracy of the experiment result, one new inbred line that was homozygous and at a large quantity of seeds was selected as material to enture the consistence of the tested seeds, full vortex and microscopic examination was done to make sure that the border cells were cleaned completely from root cap, each sample was detected by 3-5 times repetition and the whole experiment was repeated 3 times independently.

The methods FDA-PI stained with fluorescence microscopy or trypan blue stained with light microscopy might be used to detect border cells activity and the former was adopted generally in previous studies, but FDA-PI preparation was complicated and the available period is short, the stain time was long and the stain and microscopy examination must be done in dark room, besides, the fluorescence microscopes were limited in number in common laboratory, examination technology was somewhat difficult, so which was not suitable for a large number of samples detection. Inversely, 0.4% trypan blue can be prepared easily and available for long time, the stain time was short in 3-5 min and the stain and microscopy examination was simple, 3 times repetitions can be done on one glass slide, one sample can be counted in 3-5 min, so the trypan blue stain method was used in this study. Yet, it is necessary to control the pipette sample volume in the range of 5-8 μL in case cells overflow from cover slip.

Effect of enviromental conditions on root border cells development: It has been proved, if root border cells are removed or the extracellular suppressor is diluted by dipping the root into water, new cells can be collected from cap periphery within 1 h, after 24 h a new set of cells is complete (Hawes *et al*., 2000). Environmental condition might influence border cells development, low temperature delayed the border cells development in soybean, but at high temperature increased BCs number and mucus might prevent soybean roots from high-temperature injury (Ma *et al*., 2005). In this study, maize border cells regenerated slowly at low temperatures 10/15°C in 6-24 h, but 72 h later there was no significant difference in BCs number and viability when compared with 20/25°C; border cells regenerated very fast in 6-12 h when cultured at high temperature 30°C, but with the time go, the regeneration speed decreased and 72 h later the BCs number and viability were all significantly lower than other treatments, namely high-temperature wasn't permanently favorable for maize BCs development. So, the result was different from the former study on soybean, it might be because of the differences of the culture method and examination time, in the former study, the just germinating seedlings were cultured at different temperatures, the BCs number was counted by the roots with identical lengths; in our study, the seedlings wipped off BCs were cultured at different temperatures, yet the redeveloped BCs number and viability at one point were counted by roots with different lengths. However it can't be denied that maybe the effect of temperature on different species is different.

The previous studies reported that (Miyasaka and Haves, 2001; Zhao *et al*., 2000), plant root could generate more border cells and heavier mucilage layer to resist adversity stress and there was coherence between reactions at the level of border cells and plant. As to maize research, BCs survival rate declined with the concentrations of copper ions in solution increased, BCs number decreased with a similar variation trend and the cells morphology turned abnormal (Liu *et al*., 2012). Nevertheless, BCs' number increased when maize root suffered from osmotic stress, but no significant change occurred in the cells viability (Zhang, 2011), the result of the drought experiment in this study was in accordance with Zhang's report, as regards the reason why the BCs number and viability reacted differently to copper ions and drought stress, the possible explanation was because of the different essence of the stresses, the former is heavy metal toxicity to root, but the latter is physiological damage, however, it might also because of the differences of intensity and persistence time of the stresses. Therefore, it is necessary to investigate the maize BCs reaction pattern on adversity stresses further, such as salt stress, nutrition deficiency, pathogen invasion and to elucidate these related mechanisms thoroughly, we expect the result of this study can lay a foundation for future research.

CONCLUSION

Maize root border cells adnated on the 1-2 mm region of root tip, the shape was various, PME level, BCs number and viability varied with primary root elongation and the general variation trend of the three traits was first up and then down. PME activity was highest of 28.23 μmol H^+ root cap^{-1} h^{-1} when primary root was 1cm length and averaged at 19.98 μmol H^+ root cap^{-1} h^{-1} while root length was in the range of 0-6 cm. BCs developed synchronously with radicle growing, the number of border cells increased with primary root elongation and reached a maximum of 4367 when root was 2 cm length, the max viability was about 90% when root was 1.0 cm length, the average viability was 76.16%. Aerial root border cells were smaller and less, but the viability was higher, the average BCs number was 2092 when aerial root was 1-4 cm length and the average viability was 86.21%. Low temperature was beneficial to maintain detached BCs activity, 20/25°C was suitable for maize BCs redevelopment. BCs number increased when maize root suffered from drought stress, but no obvious changes in viability.

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REFERENCES

Driouich, A., M.L. Follet-Gueye, M. Vicre-Gibouin and M. Hawes, 2013. Root border cells and secretions as critical elements in plant host defense. Curr. Opin. Plant Biol., 16: 489-495.

Hawes, M.C., L.A. Brigham, F. Wen, H.H. Woo and Y. Zhu, 1998. Function of root border cells in plant health: Pioneers 1 in the rhizosphere. Annu. Rev. Phytopathol., 36: 311-327.

Hawes, M.C., U. Gunwardena, S. Miyasaa and X.W. Zhao, 2000. The role of root border cells in plant defense. Trends Plant Sci., 5: 128-133.

Hawes, M.C., G.A. Bengough, G. Cassab and G. Ponce, 2003. Root caps and rhizosphere. J. Plant Growth Regul., 21: 352-367.

Li, L., Z.F. Hao, J.F. Weng, D.G. Zhang and S.H. Zhang, 2011. Evaluation of maize drought tolerance at germination and seedling stages. J. Maize Sci., 19: 5-9.

Liu, T.T., F. Li, X. Zhang, J.Y. Shi and Y.X. Chen, 2012. The number and surviral rate of corn root border cells under the stress of copper ions. Plant Physiol. J., 48: 669-675.

Ma, B.J., J.W. Pan, Z.J. Fu, P.H. Zhang and M.Y. Zhu, 2005. Development and nfluencing factors of soybean root border cell. Acta Agronomica Sinica, 31: 165-169.

Ma, C.H., Q.L. Zheng, W.Q. Dong, X.L. Li and H.X. Zhang *et al*., 2009. Apoptosis in detached corn root cap cells treated by hmc-toxin under fluorescent microscope. Acta Agriculturae Boreali-Sinica, 24: 135-139.

Ma, C.H., X.L. Li, W.Q. Dong, Y.C. Li, S.P. Cui, Y.S. Jia and L.A. Wang, 2010. Detection of apoptosis in detached maize root cap cells induced by HMC-toxin. J. Huazhong Agric. Univ., 29: 138-142.

Ma, C.H., X.L. Li, W.Q. Dong, H.X. Zhang, Z.G. Dai, L.A. Wang and Y.S. Jia, 2013. Different fluorescent staining methods forinducing of apoptosisin homocaryon with B37 of Maize by *Helminthosporium maydis* Race C(HMC) Toxin. J. Maize Sci., 21: 981-1001.

Miyasaka, S.C. and M.C. Hawes, 2001. Possible role of root border cells in detection and avoidance of aluminum toxicity. Plant Physiol., 125: 1978-1987.

Plancot, B., C. Santaella, R. Jaber, M.C. Kiefer-Meyer and M.L. Follet-Gueye *et al*., 2013. Deciphering the responses of root border-like cells of Arabidopsis thaliana and *Linum usitatissimum* to pathogen-derived elicitors. Plant Physiol., 163: 1584-1597.

Rodger, S., A.G. Bengough, B.S. Griffiths, V. Stubbs and I.M. Young, 2003. Does the presence of detached root border cells of Zea mays alter the activity of the pathogenic nematode *Meloidogyne incognita*? Phytopathology, 93: 1111-1114.

Sherwood, R.T., 1987. Papilla formation in corn root-cap cells and leaves inoculated with *Colletotrichum graminicola*. Phytopathology, 77: 930-934.

Stephenson, M.B. and M.C. Hawes, 1994. Correlation of pectin methylesterase activity in root caps of pea with root border cell separation. Plant physiol., 106: 739-745.

Stubbs, V.E.C., D. Standing, O.G.G. Knox, K. Killham, A.G Bengoughand and B. Griffiths, 2004. Root border cells take up and release glucose-C. Ann. Bot., 93: 221-224.

Wang, Y.N., H. Jiang, Y. Wang, D.W. Mang, X.X. Li and L.S. Wang, 2013. Response of mung bean root border cells to cadmium in relation to their status. Acta Pedologica Sinica, 50: 165-170.

Yu, M., Z. Cui, H. Wen, H. Xiao, Y. Zhang, L. He and H. Liang, 2004. Root border cells: A recently defined population of alive cells in rhizosphere. J. Huazhong Agric. Univ., 23: 275-280.

Zhang, Q., H.S. Zhang, W.L. Gai, M.T. Duan and W. Jiang, 2011. Drought resistance analysis of maize varieties at germ ination stage. Shandong Agric. Sci., 2: 21-23.

Zhang, Y.P., 2011. Effects of seed-soaking with paclobutrazol solution on generation of maize root border cells under osmotic stress. Acta Botanica Boreali-Occidentalia Sinica, 31: 2503-2508.

Zhao, X.W., I.J. Misaghi and M.C. Hawes, 2000. Stimulation of border cell production in response to increased carbon dioxide levels. Plant Physiol., 122: 181-188.