Root Border Cell Development is a Temperature-Insensitive and Al-Sensitive Process in Barley

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In vivo and in vitro experiments showed that border cell (BC) survival was dependent on root tip mucigel in barley (Hordeum vulgare L. cv. Hang 981). In aeroponic culture, BC development was an induced process in barley, whereas in hydroponic culture, it was a kinetic equilibrium process during which 300–400 BCs were released into water daily. The response of root elongation to temperatures (10–35°C) was very sensitive but temperature changes had no great effect on barley BC development. At 35°C, the root elongation ceased whereas BC production still continued, indicating that the two processes might be regulated independently under high temperature (35°C) stress. Fifty μM Al could inhibit significantly BC development by inhibiting pectin methylesterase activity in the root cap of cv. 2000–2 (Al-sensitive) and cv. Humai 16 (Al-tolerant), but 20 μM Al could not block BC development in cv. Humai 16. BCs and their mucigel of barley had a limited role in the protection of Al-induced inhibition of root elongation, but played a significant role in the prevention of Al from diffusing into the meristems of the root tip and the root cap. Together, these results suggested that BC development was a temperature-insensitive but Al-sensitive process, and that BCs and their mucigel played an important role in the protection of root tip and root cap meristems from Al toxicity.

Keywords: Al toxicity — Barley — Border cell development — Pectin methylesterase — Root cap — Temperature.

Abbreviations: BCs, border cells; FDA-PI, fluorescein diacetate-propidium iodide; MI, mitotic index; PME, pectin methylesterase.

Introduction

Root border cells (BCs) in plants, previously referred to as sloughed root cap cells, were traditionally considered as a constitutive product of root cap turnover (Clowes 1976, Clowes 1994), but recently much evidence has indicated that BC development is genetically regulated (Hawes and Lin 1990, Brigham et al. 1999, Woo et al. 1999). However, BC development in barley was a constitutive process during hydroponic culture. Hence, BC development regulation is an apparently complex process, particularly, in soil, how BC development is regulated is still unknown.

Temperature is a critical factor affecting root elongation. Hawes and Lin (1990) have observed that BC development of pea is a temperature-sensitive process that appears to be regulated independently of root elongation; in contrast, Stephenson and Hawes (1994) have indicated that the number of BCs in pea does not change at different temperatures (10–30°C). Hence, relationships of BC development and root elongation with temperature are also poorly understood.

Investigations of the molecular mechanisms of Al toxicity and tolerance in plants have been the focus of ongoing research in the area of stress phytophysiology (Ma et al. 2001). Al tolerance has been speculated to be the result of either external avoidance of Al or internal tolerance to Al in the root tips (Taylor 1991, Matsumoto 2000). Several possible mechanisms have been suggested to explain the exclusion of Al from the root tip. The first one is the chelation of Al by organic acids released by the root of Al-tolerant genotypes, which is supported by substantial experimental data (Miyasaka et al. 1991, Ryan et al. 1995a, Ryan et al. 1995b). The second is that Al tolerance in plants possibly results from a plant-induced pH increase in rhizosphere (Taylor and Foy 1985), which is supported by the results of Degenhardt et al. (1998) in Arabidopsis. The third possible mechanism is that mucigel secreted by root cap cells and/or BCs can adsorb Al, thereby blocking entry of Al into root-tip cells (Horst and Marschner 1982, Archambault et al. 1996, Li et al. 2000). Miyasaka and Hawes (2001) have examined the effect of Al toxicity on the viability of detached BCs from different Al-tolerant snapbean cultivars. Their results indicate that BCs have the capacity to protect root tips from Al toxicity. However, how Al toxicity affects BC development and the specific roles of BCs and their mucigel in the protection of root tips from Al toxicity still remains unclear.

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To better understand BC development and its responses to temperature and Al stress, and its functions during Al toxicity, we investigated BC viability, formation, and pectin methyl-esterase (PME) activity of the root caps during barley BC development. The results confirm that BC development is a temperature-insensitive but Al-sensitive process in barley.

Results

Maintenance of root tip mucigel to border cell viability in barley

After emerged seeds of barley cv. Hang 981 were cultured aeroponically for 24 h, BCs were collected in sterile water, and then were immediately stained with fluorescein diacetate-pyridium iodide (FDA-PI) for cell viability. Their viability was estimated to be 95±3% (Fig. 1A). When these BCs were cultured for 72 h in sterile water, their viability rapidly declined to 9.3% (Fig. 1B, C); however, BCs attached to root tips retained a higher viability (Fig. 1C, D). To determine whether this higher viability was due to high osmotic pressure of mucigel, collected BCs were cultured for 24 h in 0.5 M mannitol solution or sterile water. There was a significant difference (P <0.05) for BC viability between those in the mannitol solution and in the water alone (Fig. 1E). This result confirmed that higher osmotic pressure was beneficial to BC survival, suggesting that higher viability of BCs attached on root tips might result from higher osmotic pressure of mucigel on the root tips.
Border cell development under different cultural conditions

During culture under aeroponic conditions, the BC numbers of primary roots at different lengths (1, 2, 5, 10, 15, 20, 25, 30, and 35 mm) were counted (Fig. 2A). It was observed that the first BC formation in barley synchronously occurred with root tip emergence; there were approximately 70 BCs on 1 mm of the root tip (Fig. 1F), and the BC number reached a maximum value (~1,400) which coincided with a cessation of BC production when the root was 20–25 mm long (Fig. 2A). Only when BCs were removed, a new set of BCs formed again after 48 h (data not shown). We also observed the same phenomena in other monocotyledon species such as rice and wheat (data not shown).

To further study the biochemical basis of BC separation from the root cap, PME activity in root caps was examined during BC development. A close correlation between PME activity of root caps and BC separation was observed during BC development. PME activity in root caps was measured during primary root emergence in aeroponic culture. The root at 0-mm length represented one that had not emerged from the seed coat. ± SE values were less than 16% of the mean. PME activity in root caps after BC removal in aeroponic culture and hydroponic culture was also measured. ± SE values were less than 14% of the mean. The total number of BCs included BCs attached on the root tip and released into water. The BC number released into the water daily from the root cap was counted within 1–3 d, respectively. In A, D, and E, means ± SEs were from at least three independent replicates. In B and C, means ± SEs were from at least five independent experiments.

Fig. 2 Border cell development and PME activity of root caps in barley cv. Hang 891 in aeroponic and hydroponic cultures. (A) The formation and development of BCs during root emergence in aeroponic culture. (B) PME activity in root caps during primary root emergence in aeroponic culture conditions. The root at 0-mm length represented one that had not emerged from the seed coat. ± SE values were less than 16% of the mean. (C) PME activity in root caps after BC removal in aeroponic culture. ± SE values were less than 14% of the mean. (D) The formation and development of BCs during root emergence in hydroponic culture. The total number of BCs included BCs attached on the root tip and released into water. (E) The BC number released into the water daily from the root cap. The BC number in water and on the root tip was counted within 1–3 d, respectively. In A, D, and E, means ± SEs were from at least three independent replicates. In B and C, means ± SEs were from at least five independent experiments.
Root border cell development in barley BC development in aeroponic cultures. PME activity of the root cap was the highest when the root was 1 mm long (Fig. 2B). As soon as a full set of BCs on the root cap was removed, PME activity started to increase again within 1 h, and reached a maximum by 16 h (Fig. 2C). These results suggested that BC separation from the root cap was correlated to PME activity.

During culture under hydroponic conditions, when the root was 10 mm long, most of the BCs that had separated from the root cap were still attached to the root tip by mucigel (Fig. 2D). But when the root length exceeded 10 mm, the BC number attached on the root tip remained constant (~700), and the excess BCs were mainly released into water. When the root reached a length of 35 mm, the BC number (including BCs attached to the root tip and BCs released into water) also reached approximately 1,400. However, BC formation did not cease in hydroponic culture conditions, and 300–400 BCs were released into the water daily (Fig. 2E), suggesting that BC production was a kinetic equilibrium process only after BCs attached on the root tip reached a constant number in hydroponic culture.

**Responses of border cell development and root elongation to temperatures**

Temperature effect on barley root elongation was very obvious in aeroponic cultures (Fig. 3A). Root elongation was obviously inhibited at 10, 15, and 35°C, especially, after 24 h at 35°C, the root hardly elongated. However, among 20–30°C experiments, the root elongation gradually increased with increasing temperature. These results reflected that barley root elongation was very sensitive to temperature, particularly, to either low (10°C) or high temperatures (35°C).

However, there was a little temperature effect on BC formation in barley in aeroponic cultures (Fig. 3B). The BC numbers for different root lengths at 10–35°C were examined within 48 h. It was found that the BC number of barley was not significantly different at 10 or 30°C. After 48 h at 35°C, the longest root was only 15 mm but the BC number had reached about 1,500 (Fig. 3B). Further study showed that BCs were still continuously released from the root cap in spite of root elongation inhibition at 35°C (Fig. 3C). These results confirmed that large temperature changes had no great effect on barley BC development. It was concluded that root elongation of barley was much more sensitive to temperature (10–35°C) than was BC development.

**Fig. 3** The responses of border cell development and root elongation to temperatures in barley cv. Hang 981. (A) The effect of temperature changes on root elongation in aeroponic culture. ± SE values were less than 2.01. (B) Effect of temperature variation on BC development in aeroponic culture. ± SE values were less than 180. (C) Time course of BC development in 35°C aeroponic cultures. In these experiments, means ± SEs were calculated from at least three independent replicates.
Effect of Al toxicity on border cell viability and development

Al tolerance of the two cultivars used in this study was identified using relative elongation rates (RERs) (Fig. 4A) and thereby cv. Humai 16 (49.88%) was used as an Al-tolerant cultivar whereas cv. 2000–2 (5%) as an Al-sensitive one.

BCs were collected from cv. Humai 16 and cv. 2000–2, and were treated for 24 h with either 0, 20, or 50 µM Al (0.1 mM CaCl₂, pH 4.5). Detached BC viability was shown in Fig. 4B. Compared with the control without Al, BC viability of the two cultivars significantly declined after Al treatments. However, there was a significant difference (P < 0.05) for BC viability between 20 and 50 µM Al treatments in cv. 2000–2, but not in cv. Humai 16. This result suggested that the Al tolerance of BCs from Al-tolerant cultivar was higher than that of BCs from Al-sensitive one under Al stress, indicating that Al tolerance of BCs was consistent with that of plant levels.

When primary roots in cv. Humai 16 and cv. 2000–2 were 20–25 mm at length, 98% of BCs were removed (Fig. 1G), and these seedlings were treated for 24 h with 0, 20, or 50 µM Al (0.1 mM CaCl₂, pH 4.5). Compared with the control with no Al, BC formation was significantly inhibited (P < 0.05) by 20 µM Al treatment in cv. 2000–2, but not in cv. Humai 16 (P > 0.05).
(Fig. 4C). However, BC formation in the two cultivars was completely inhibited (significance \( P < 0.01 \)) by 50 \( \mu \)M Al treatment. These results suggested that Al toxicity could significantly inhibit barley BC development, but 20 \( \mu \)M Al could not inhibit BC development in the Al-tolerant cv. Humai 16.

To investigate mechanisms by which Al toxicity inhibits BC formation in barley, PME activity in root caps was examined during the course of Al treatments (Fig. 4D). Compared with the control without Al, PME activity in root caps of cv. 2000–2 was inhibited significantly \( (P < 0.01) \) in 20 and/or 50 \( \mu \)M Al treatment(s). However, there was no significant effect on PME activity in root caps of cv. Humai 16 in 20 \( \mu \)M Al treatment \( (P > 0.05) \), but a significant inhibition occurred upon treatment with 50 \( \mu \)M \( (P < 0.01) \). This result was closely correlated with Al-induced inhibition of BC development (Fig. 4C, D), suggesting that Al toxicity inhibited BC development possibly by inhibiting PME activity in root caps of barley.

**Possible roles of border cells in avoidance of Al toxicity**

Under laboratory conditions, BCs of seedlings readily dropped to the bottom of the vessel during Al solution treatments. This reduced the contribution of BCs to Al tolerance of plants, so that BC functions have been largely ignored in previous publications.

To examine the roles of BCs in avoidance of Al toxicity, seedlings of cv. 2000–2 and cv. Humai 16 either with BCs or after removal of BCs, whose root length was 20–25 mm, were treated for 36 h with 0, 5, and 20 \( \mu \)M Al (0.1 mM CaCl\(_2\), pH 4.5), and then root length was measured over time (Fig. 5A, B). In all treatments, there was no significant difference in the length of roots without BC removal and with BC removal in these two cultivars. This result confirmed that BCs had no significant role in the prevention of Al-induced inhibition of root elongation in barley.

However, BCs and their mucigel are located at the place where the root tip and root cap meristems separate (Fig. 1H, I). Therefore we considered whether BCs and/or the mucigel were able to protect the two meristems from Al toxicity. Hence, under same experimental conditions, the mitotic index (MI) of the root tips including root tip mitoses and root cap mitoses

**Fig. 5** The possible roles of border cells in Al toxicity. Three Al levels containing 0, 5, and 20 \( \mu \)M (0.1 mM CaCl\(_2\), pH 4.5) were used in these experiments. (A and B) Effect of BCs and mucigel on Al-induced inhibition of root elongation in cv. 2000–2 and cv. Humai 16, respectively. The roots with and without BC removal were exposed for 36 h to three Al solutions, respectively, and then root length (mm) was measured at indicated time points. ± SE values were less than 8% of the mean. (C) The role of BCs in the protection of root tip meristem in cv. 2000–2 from Al toxicity. The roots with and without BC removal were exposed for 24 h to three Al solutions, respectively, and then were cultured with no Al for 8 h. MI of root tips was examined at indicated time points. ± SE values were less than 15% of the mean. In these experiments above, means ± SEs were from at least three independent experiments.
Root border cell development in barley was examined at different time points during Al treatments (Fig. 5C). In 8-h treatments with 5 or 20 μM Al (0.1 mM CaCl₂, pH 4.5), the MI of root tips without BC removal was clearly higher than that of root tips with BC removal. Furthermore, there was no significant difference for the MI between 20-Al-treated root tips without BC removal and the 5-Al-treated root tips with BC removal. This result fully reflected that in 8-h Al treatments, BCs and their mucigel had a significant role in the protection of cell division activity of root tips from Al toxicity. However, during 8–24 h 20 μM Al treatments, all the MI of root tips rapidly declined and there was no obvious difference between the MIs with and without BC removal. During 5 μM Al treatments, the MI of root tips without BC removal remained relatively high compared with that of root tips with BC removal. In 4–8 h recovery culture with no Al, the MI of 20-Al-treated root tips with or without BC removal did not obviously recover, but the MI of 5-Al-treated root tips without BC removal more significantly recovered than that of root tips with BC removal did. These results showed that BCs and their mucigel played a significant role in the protection of root-tip mitoses during low concentration of Al or short treatment periods, suggesting that they had ability to prevent Al from diffusing into meristems of the root tip and the root cap.

Discussion

Border cell survival dependent on mucigel

In the present experiments, barley BCs cultured in water survived for only 3 d, whereas BCs attached on root tips remained viable for much longer (Fig. 1C). There are two possible explanations for this phenomenon. (1) Mucigel of the root tip in aeroponic culture has higher osmotic pressure than free water does, and is beneficial to BC survival. This is supported by the present experiment (Fig. 1E). (2) There is “a survival factor” in mucigel. This factor secreted by root-cap cells can inhibit BC cell death. When root tips are immersed into water or BCs are isolated, BCs easily die as a result of dilution or removal of this factor. However, whether this factor really exists in mucigel still remains to be confirmed.

Patterns of border cell development

At present, there are two viewpoints on the pattern of BC development. It was previously assumed that BC formation was a constitutive process, and that the root cap meristem was always mitotically active so that the root tip continuously released BCs into the soil as the root elongated (Clowes 1976, Clowes 1994). However, Hawes’ research group has found that BC production in some species such as cereals, legumes and cotton, is an induced process that is controlled by endogenous and environmental signals (Hawes and Lin 1990, Brigham et al. 1998, Hawes et al. 1998, Hawes et al. 2000). They first proposed the hypothesis that BCs could secrete a specific extracellular suppressor factor which could feedback inhibit new BC production when the BC number reached a species-specific maximum value. This hypothesis could satisfactorily explain an induced process of BC development. However, the chemical nature of this factor remains unknown.

Our present finding in barley shows that BC development is an induced process in aeroponic culture, whereas it is a kinetic equilibrium process in hydroponic culture (Fig. 2). During hydroponic culture, the detachment of BCs from root tip mucigel layer can activate further BC production. One possible explanation is that the decrease of BC number reduces a BC-secreted suppressor factor concentration in root tip mucigel, leading to activation of root cap meristem. We attempted to look for this suppressor factor in BC exudates, but failed to succeed. Under natural conditions, continuous elongation of roots produces the friction between the root tips and the soil; newly produced BCs are continuously transferred to behind the root tip (Fig. 6A). Hence, we speculate that BC development more possibly takes on a constitutive process in soil.
The response of border cell development to temperature stress

Many environmental stress factors such as bacteria, fungi, nematodes, CO₂, could directly and indirectly regulate the production of BCs (Hawes and Pueppke 1987, Hawes et al. 1996, Zhao et al. 2000a, Zhao et al. 2000b, Pan et al. 2002a). Present results showed that BC development was insensitive to temperature changes in barley (Fig. 3), whereas root elongation was a temperature-sensitive process, indicating that these two processes might be regulated independently. Insensitivity of BC development to temperature is possibly due to insensitivity of PME activity of root caps and/or root cap meristem to temperature (Stephenson and Hawes 1994), but which remains to be confirmed with more evidence. Our next objective is to screen for a temperature-sensitive mutant of BC development in barley, which would aid our understanding of the temperature-insensitive mechanism of BC development and allow us to decipher the relationship between BC development and root elongation.

Al-induced inhibition of border cell development

How Al toxicity affects BC development has not been previously investigated. According to our results, Al toxicity significantly inhibited BC development in barley (Fig. 4C), but the mechanism of Al-induced inhibition remains unclear. According to our findings, we propose that Al toxicity may inhibit the early stage of BC development by inhibiting mitoses in the root-cap meristem (Fig. 5C). Alternatively, Al toxicity may inhibit the late stage of BC development by inhibiting PME activity in the root cap. The binding of Al to the pectin matrix in cell walls of root cap periphery may induce cross-linking, which makes the cell wall rigid (Schmohl et al. 2000, Schmohl and Horst 2000, Willats et al. 2001), leading to inhibition of enzyme activity and secretion; including PME, polygalacturonases, and pectate lyases. Al-induced inhibition of PME activity can block BC separation from the root cap periphery, which has been confirmed by present experiments (Fig. 4C, D).

Al toxicity of BCs was in line with that of individual plant levels in snapbean (Miyasaka and Hawes 2001) and barley (Fig. 4B). Therefore it is likely that BCs will provide a convenient in vitro model system for studying the molecular mechanism of Al tolerance in higher plants. This implies that understanding the mechanism of Al tolerance in BCs could be very significant for understanding the Al-tolerance mechanism in plant levels.

Roles of border cells and their mucigel in Al toxicity

On the basis of our present results in barley, at least, under laboratory conditions, BCs and their mucigel plays only a minor role in the protection of root elongation from Al toxicity (Fig. 5A, B). BCs and their mucigel are mainly located at 1-mm region from the root tip in barley (Fig. 1H, I), wheat, rice, and maize (data not shown), whereas the main site of Al-induced inhibition of root elongation is located in a region at 2–3 mm from the root tip (Fig. 6B) (Ryan et al. 1993, Sivaguru and Horst 1998, Sivaguru et al. 1999). However, BCs in legumes (such as pea, snapbean) and cotton are distributed in a region up to 10 mm behind the root cap (Hawes and Lin 1990, Hawes et al. 2000), indicating that BCs have the capacity to protect root elongation from Al toxicity in these species. Hence, this capacity of BCs and their mucigel may depend upon their distribution region on the root tip.

However, under natural conditions, the elongating root tips are subjected to the obstruction from soil particles, resulting in a portion of BCs and their mucigel being pushed behind the root cap (Fig. 6A). So, the terminal 2–3 mm region in the root tip is covered with plenty of BCs and mucigel, possibly leading to extra protection of root elongation from Al toxicity.

The present results suggest that BCs and their mucigel have an important role for protection of root tip or root cap meristems, providing a physical and/or chemical barrier for Al toxicity (Fig. 1H, I, 5C). This barrier is partially provided by ionic acids such as galacturonic acids in mucigel and BC-secreted exudates such as organic acids and/or other unknown constituents (Bacic et al. 1986, Li et al. 2000). Once Al is bound to the carboxyl groups of uronic acids and organic acids, it becomes inactivated and lacks toxicity for root tip cells (Bacic et al. 1986, Li et al. 2000). Hence, the contribution of BCs to the protection of root tip and root cap meristems from Al toxicity is also dependent on contents of the carboxyl groups in mucigel.

Materials and Methods

Plant materials and seed germination

Three barley cultivars (Hordeum vulgare L. cv. Hang 981, cv. Humai 16, and cv. 2000–2) were used in the present studies. Seeds were sterilized with 95% (v/v) ethanol for 10 min, and then with 10% sodium hypochloride (v/v) for 30 min, and rinsed with sterile ddH₂O. These seeds were germinated for 1 d in the dark at 25°C, on sterile filter paper (Whatman No. 102, Hangzhou, China) placed on absorbent cotton moistened with 0.1 mM CaCl₂ solution.

Aeroponic culture and hydroponic culture

Barley cv. Hang 981 seeds were cultured aeroponically. Briefly, a plastic net, on which a 10-hole filter paper moistened with 0.1 mM CaCl₂ was placed, was fixed to a 50 ml beaker containing 200 ml ddH₂O. This beaker was sealed with plastic film and autoclaved. Emerged seeds were planted in holes on the mesh of filter paper and net, so that roots could grow through mesh into the water-saturated air. BCs on the root tips were counted at different root lengths. Preliminary experiments showed that it was difficult to count BC number when emerged seeds were cultured hydroponically, because a portion of BCs dropped from root tips to the bottom of beaker. Hence, it is rather difficult to study BC development in hydroponic culture. In this study, three primary roots, which appeared earliest during emergence, were used to study BC development in hydroponic cultures. Each emerged seed only with three primary roots was independently cultured in 1 ml sterile ddH₂O. The BCs present in water and on the root tips were counted at different root lengths. To examine the number of BC released from the root cap daily, each seedling with only three 25-mm primary roots, was cultured in 1 ml fresh sterile ddH₂O. The BC number in water and on the root tip was counted at 1, 2, and 3 d.
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Number and viability detection of border cells

In aeroponic cultures, BC number was examined when primary roots were 1, 2, 5, 10, 15, 20, 25, 30, and 35 mm long. Ten primary root tips at each length were cut and immersed into 1 ml ddH2O. After 1–2 min, the water was gently agitated with a Pasteur pipette. The BCs in five 20-µl aliquots (100 µl) were counted under a light microscope, and the total number gave an estimation of the average BC number per root tip. In hydroponic cultures, the method to count BCs was the same as the method above, but the total number of BCs in five 20-µl aliquots (100 µl) times 10/3 was the average BC number per root tip because of only three primary root tips in 1 ml ddH2O.

BC viability was determined using FDA-PI according to the method by Pan et al. (2001).

PME in root caps was extracted using a high-salt buffer (Ren and Kermode 2000). Sixty excised root tips (1–2 mm) were placed into 200 µl PME extraction buffer (0.1 M citrate acid, 0.2 M Na2HPO4, and 1 M NaCl, pH 5.0), and were fully ground at 4°C. The homogenized slurry was transferred to an Eppendorf, and then was incubated on ice for 1 h, during which it was vibrated three times at 20-min intervals, and centrifuged for 10 min at 15,000×g at 4°C. The supernatant was collected and stored at −20°C.

PME activity was examined according to the methods described by Richard et al. (1994). Eight µl of PME extract were added to 4 ml substrate solution [0.5% (w/v) citrus pectin (Sigma), 0.2 M NaCl, 0.15% (w/v) methyl red, pH 6.8], and incubated for 2 h at 37°C. The OD value of the solution was measured with a spectrophotometer (UV-265 FS/FW, Shimadzu, Japan) at 525 nm. A calibration curve was obtained by adding 80–240 µl 0.01 M HCl to 4-ml substrate solution and measuring the respective OD values at 525 nm. PME activity of different PME extracts (nmol H+ (root cap)−1 h−1) was obtained according to the calibration curve.

Responses of border cell development and root elongation to temperatures

Emerged seeds were cultured aeroponically at 10, 15, 20, 25, 30, and 35°C, respectively. Root lengths and BC numbers were measured at different time points and at different root lengths within 48 h at the specific temperature. Preliminary experiments showed that all roots ceased to elongate whereas BC production still continued after 24-h 35°C treatment. Hence, we examined BC numbers of 5- and 10-mm roots at 12, 24, 36, and 48 h under 35°C.

The effects of Al toxicity on border cell viability and development

Two barley cultivars (cv. Humai 16 and cv. 2000–2) differing in Al tolerance were selected from 30 barley cultivars according to root RERs (Pan et al. unpublished data). One-day-old merged seeds were cultured for 1 d in 0.1 mM CaCl2 solution (pH 5.8) and then for 2 d in 0.1 mM CaCl2 solution (pH 4.5). These 4-day-old seedlings were exposed for 24 h to the control solution (0.1 mM CaCl2, pH 4.5) or Al solution (20 µM Al, 0.1 mM CaCl2, pH 4.5) in the dark at 25°C. Before Al treatments, the longest primary roots of 10 seedlings in the exposure Foundation of Zhejiang Province (grant no. 300255 to J.W.P) and the Provincial Natural Science Foundation of Zhejiang Province (grant no. 300255 to J.W.P).

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