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Dynamic Changes of Mineral Element in The Cell Wall of Growth Cells Detected by CSEM-EDX*

LIANG Feng[1,2], WANG Zhong[1], GUO Jian-Lin[1], SHEN Li-Zhen[1], YANG Qing[1]**
([1] *College of Life Sciences, Nanjing Agricultural University, Nanjing* 210095, China;
[2] *Department of Life Sciences, Shangqiu Normal College, Shangqiu* 476000, China)

Abstract Aerenchyma formation has been described in depth in a number of species at a histological level. But large gaps remain in our understanding of its regulation as a developmental process. It is attempted to analyse essential mineral elements like K, Mg, Cu, Zn, Ca and P in the cell wall of aerenchyma cells in petioles of *S. trifolia* at five different developmental stages by CSEM-EDX technique. At early stage, K and Cl concentrations in cell wall were high up to 36% and 4.3% of dry weight, respectively. It supported the hypotheses that aerenchyma spaces are filled with liquid at early developmental stages of aerenchyma in *S. trifolia* petiole. Mg concentration was high at stage 2, up to 0.86% of dry weight. Zinc and Cu were detected only at rapid expansion stages, during which the concentrations were up to 1.5% and 2.5%, respectively. Calcium was detected in the cell wall only at mature stages, the concentration was high up to 1.3% of dry weight at stages 4 and 5. These results confirmed that the element concentration of aerenchyma cell wall undergoes dynamic changes during different developmental stages, and a low Ca with high Zn and Cu concentration are needed for cell expansion. Copper and Zn deposition in the cell wall showed a significant positive linear correlation, suggesting that these two elements share same or similar uptake and transport mechanism in plants.

Key words aerenchyma, cell expansion, essential mineral element, cryo-scanning electron microscope, energy dispersive X-ray microanalysis

Aerenchyma is a plant tissue containing enlarged gas spaces exceeding those commonly found as intracellular spaces, and that make a pathway of gas conducting tissue extending from root to shoot[1~3]. It is formed in the roots and shoots of wetland species and in some dryland species under adverse conditions, either constitutively or because of abiotic stress[4~8]. Generally, according to their origin, aerenchyma can be identified into two different types, schizogenous (cell separation) and lysigenous (cell death through lysis)[9,10]. Programmed cell death (PCD) and cellular autolysis are involved in the development of lysigenous aerenchyma but not in schizogenous aerenchyma[11]. Both schizogenous and lysigenous aerenchyma are interesting development systems. Much is known about lysigenous aerenchyma at the cellular level, but many questions still remain about its developmental regulation[7]. While the process of schizogenous aerenchyma formation has been described in depth in a number of species at a histological level, large gaps remain in our understanding of its regulation as a developmental process.

Schizogenous aerenchyma is often complex and well ordered[9,12]. The petioles of *Sagittaria lancifalia* schizogenous aerenchyma, regular cylinders of aerenchyma develop[13]. The cylinders are formed by cells that divide both perpendicular and parallel to the petiole axis. So, that creates a gas space within the cylinder of cells and gives increasing volume during development. Another cell type, termed as diaphragm cells forms single cell thick partitions across the cylinders. The gaps between these partitions increase as the cylinder and diaphragm cells divide and enlarge[13,14]. Our examinations with *Sagittaria trifalia* leaf petioles showed that its aerenchyma was similar to that of

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** Corresponding author.
Tel: 86-25-84395221, E-mail: qyang19@njau.edu.cn
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Sagittaria lancifalia leaf petioles. Similar diaphragms have been described that originate from mother cells identifiable in intercalary meristerms in aerial internodes of aquatic species, such as *Scirpus validus*, *Sparganum eurycarpum*[15~17], *Nymphaeales odorata* and *Acorales calamus*[18]. Investigation of the developmental control mechanism of cell separation and the ordered growth and division of the cells surrounding and forming an air space is clearly ripe for further investigation[7].

It is well known that cell expansions or/and cell separations are closely related to the changes of their walls. Mathur [19] developed a simple cytoskeleton-based operational framework for plant cell morphogenesis. Cytoskeletal control of morphogenesis occurs indirectly, i.e. not by directly shaping the cell, but by regulating the orientation of newly synthesized wall elements (cellulose fibrils)[20]. The changes in cell wall cellulose fibrils in cell deform process, such as their synthesis, array direction and relaxation, are well studied[21]. However, little is known about the changes of other components of the wall, especially some ions, including potassium, chlorine, magnesium, copper and zinc. Since small ions are mobile and readily soluble in water, they are easily transferred from their original locations to the tissues by any cutting procedure, which can give rise to artifacts [22]. Cryo-sectioning largely preserves the plant tissues and prevents as much as possible ion movements during cuttings, and therefore appears to provide suitable samples for element determinations in biological material [23]. The cryo-scanning electron microscope (CSEM) was used to preserve the fragile structures of the dying cortical cells [24]. The energy dispersive X-ray microanalytical (EDX) capacity of the CSEM was used to analyze the elements in root liquids and to identify the viability of cells by the high ion (K^+) concentration of their vacuoles [25, 26]. Scanning electron microscopy coupled with energy dispersive X-ray microanalysis of tissue fractionations has been used to show Cd distribution patterns in leaf cells of *Thlaspi caerulescens* [27]. Thus, CSEM-EDX is one of the most reliable methods to measure different element concentrations within plant tissues at the same time in different locations.

In the present communication, we report the results about the aerenchyma cell wall localization of K, Cl and the other elements, such as Mg, Cu, Zn, Ca and P, and analysis of the element concentration at the different developmental stages in leaf petioles of *S. trifolia* by using cryo-fractured samples and CSEM-EDX. The results showed that the different developmental stages of petioles had different elements in aerenchyma cell wall, and of elements have close relationships.

1 Materials and methods

1.1 Plant material and growth conditions

Tubers of *Sagittaria trifloia* L. were collected from plantation of Shuangzha-Town near Nanjing (southeast China). Tubers were grown in troughs filled with water solution containing 1/2 ×MS salts in the greenhouse, and the solution was replaced weekly. Plants with 5～6 leaves were used in this study.

1.2 Experimentation and data analysis

Different developmental stages of leaf petioles were designated as following: stage 1 with leaf buds; stage 2 with tender leaves inside sheath; stage 3 with tender leaves just out of sheath; stage 4 with leaves just opened; stage 5 with mature leaves opened for 3 days or longer. Measurement of the length of the two types of aerenchyma cells in petiole at different developmental stages was performed microscopically. The element concentration (minimal limited to 0.1%) of cell wall from two types of aerenchyma cells was detected by CSEM-EDX analyses. Statistical analyses were performed using SPSS software (SPSS, version 11, Inc., Chicago, IL, USA).

1.3 Anatomical preparation and measurement

Leaf petioles were cut into 2～3 mm pieces with razor blade for anatomical preparation. The samples were fixed in PBS buffer (pH 7.2) containing 2.5% glutaraldehyde for 2.5-h with vacuum, then, kept in fixing buffer for 3 days without vacuum. All samples were dehydrated in an ethanol series, and embedded in paraffin. Transverse and longitudinal sections of 10 μm thickness were sectioned with a rotary microtome. The sections were double-stained with safranin and fast green. Specimens were observed using an optical microscope (LEICA DMLS, Germany) connected to an image analyzer (LEICA DFC 320, Germany). Length of diaphragm cells and cylinder cells of aerenchyma were measured microscopically.

1.4 Cryo-scanning electron microscope and energy dispersive X-ray microanalysis (CSEM-EDX)

For CSEM, the leaf petioles were cut into 5～7 mm pieces using a razor blade and immediately plunged into a chamber of liquid N_2. After samples

were thoroughly cooled for about 10 min, each piece of petiole was broken into 2 pieces by a pre-cooled ram in liquid N_2, and new surfaces were exposed. The side of sample with new exposed surfaces was carefully identified and marked for further processing. Sections were dried with an ES-2030 Freeze Dryer (HITACHI, Japan). Dried sections were glued to the specimen stubs (marked face up), and then coated with gold-palladium in an ES-1010 ION SPUTTER (HITACHI, Japan). The sections were viewed and photographed using S-3000N SEM (HITACHI, Japan), while the element concentration of cell wall was detected by an energy dispersive X-ray microanalyzer (HORIBA, England). Analyses were carried out with the spot mode (50 nm spot size), and number of iterations is 5. The results of element concentration of the cell wall were reported as shown in Figure 1 and Table 1.

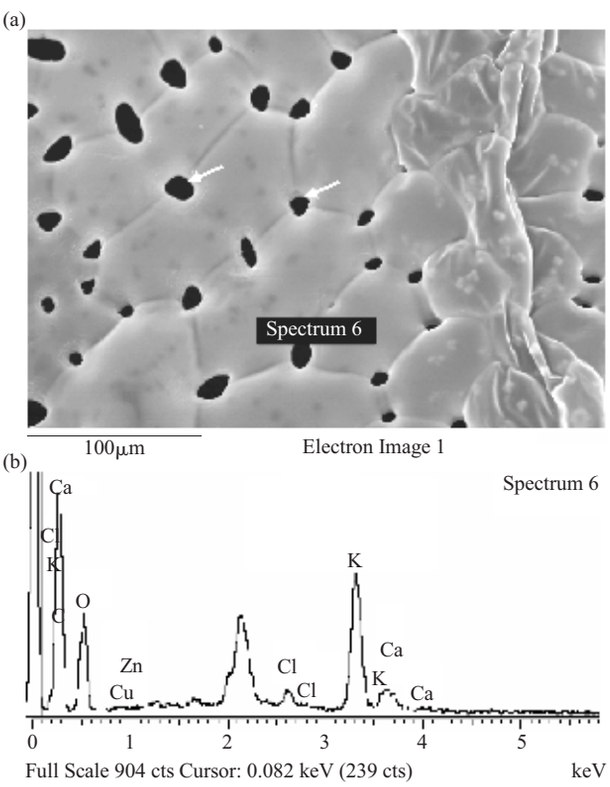

Fig. 1 Result reported by CSEM-DEX system

(a) The CSEM image and the local spot (cross). The image shows the diaphragma cells and the intercellular gas spaces shown by arrows. (b) The spectrum curve shows the individual element peak from one local spot analysis. (Spectrum processing: Number of iterations = 5. Peaks possibly omitted 1.665, 2.148 keV, are stand for Au (Aurum), which was introduced by sample preparative processes).

Table 1 One individual result reported by CSEM-DEX system displays the concentrations of all elements analyzed[1)]

Element	Dry weight/%	Atomic/%
C	52.95	65.66
O	31.38	29.22
Cl	0.75	0.32
K	8.20	3.12
Ca	0.94	0.35
Cu	2.95	0.69
Zn	2.81	0.64
Totals	100.00	100.00

[1)]These data show the same analysis result shown in Figure 1 by two ways: one by percent of dry weight, and the other by percent of atomic (Normalized, percent ⩾ 0.1%).

2 Results

2.1 Cell enlargement at different developmental stages of petiole

Cell division and cell expansion are primary factors of the aerenchyma air space formation in petioles of *S. trifolia*. The length of cells making up the cylinder varied from 25 μm at stage 1 to 119 μm at stage 5, and at stage 4 the length had the highest increase rate, but at stage 5 no increase was observed (Figure 2a). Like cylinder cells, the length of diaphragm cells varied from 27.5 μm at stage 1 to 114.5 μm at stage 5, and at stage 3 the length reached a peak increase rate, but it ceased at stage 5 (Figure 2b). Comparing the two cell types, the diaphragm cell rapid expansion at stage 3 and the diaphragm cell rapid expansion at stage 4, the rapid expansion period of diaphragm cells occurred little earlier than that of the cylinder cells.

2.2 Surface feature changes of aerenchyma cells during petiole development

The SEM image shows the surface features of diaphragm and cylinder cells at different developmental stages (Figure 3). Diaphragm cells were hexagon-shaped with few intercellular spaces at stage 1 (Figure 3a). At stage 2, the intercellular space formed at tri-cell-junction area, cell maintained the hexagon shape and, about six intercellular spaces were formed around each cell. The cells expanded slightly

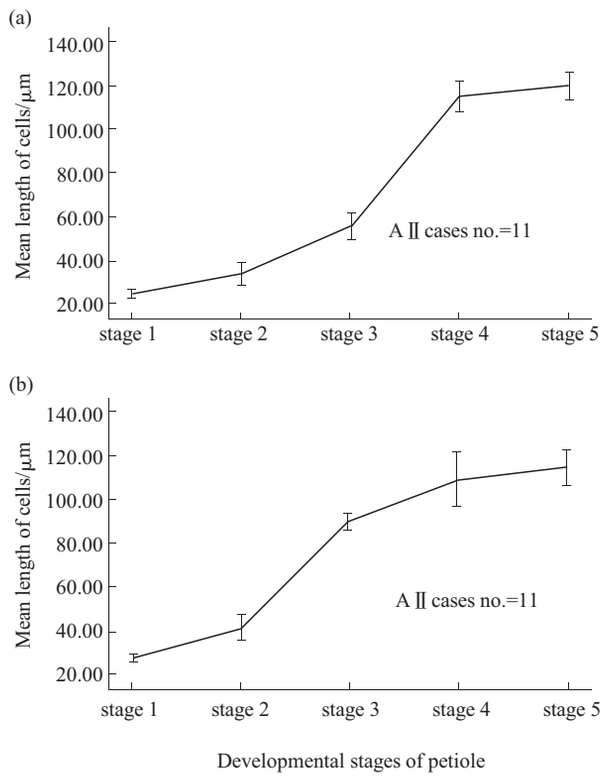

Fig. 2 Aerenchyma cell expansion with petiole development

Cell-type: (a) Cylinder; (b) Diaphragm. Error bars: +/–2.00 SE.

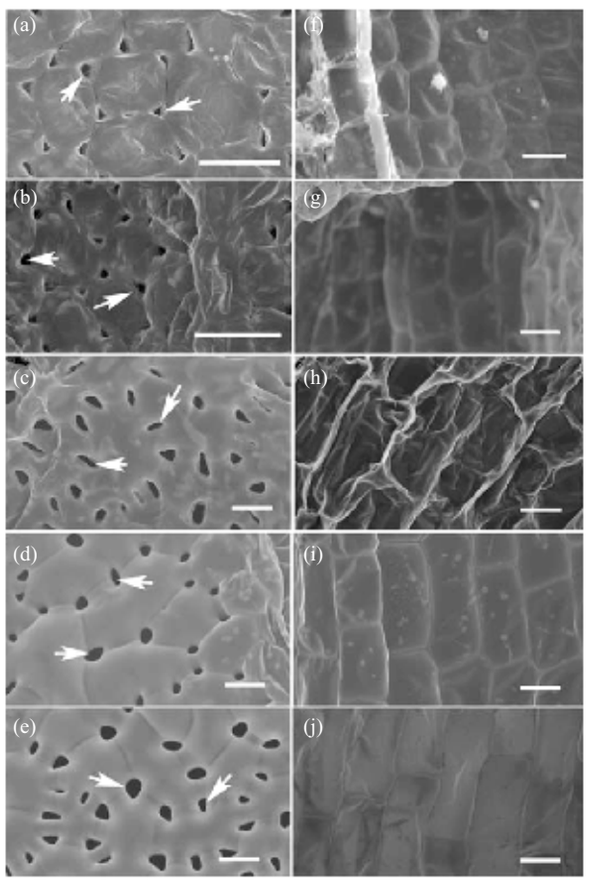

Fig. 3 The SEM images show surface of cells from five developmental stages of petiole aerenchyma

Diaphragm cells form intercellular spaces (arrows) and become irregular shape at latter developmental stages (image a ~e stand for stage 1~5, respectively), but the cylinder cells are with any intercellular spaces and maintain oblong shape in all developmental stages (image f~j stand for stage 1~5, respectively). Bars:20 μm.

compared with stage 1 (Figure 3b). As the diaphragms grow, at stage 3, new intercellular spaces were formed at the middle area of bi-cell-junction border, and the cell expanded rapidly (Figure 3c). As the cell walls was very thin at this stage, it was affected by the nucleus or by big organelles, and formed protuberances at the cell surface (cell wall caved in). It is clearly resulted from water lost during the freeze drying process. Up to stage 4, cells expanded with the intercellular space enlargement, but the protuberance in cell surface became smooth (Figure 3d). With the maturation of leaves at stage 5, cells further expanded with the intercellular space enlargement, while the cell surface became smooth (Figure 3e). The cylinder cells maintained an oblong shape with smooth surfaces during all developmental stages (Figure 3f～j), with no intercellular air space. But at rapid expansion stage, the surface of the cells shriveled. It is clear that this is due to the drying process of CSEM which makes the thin cell wall cave in. So, it can be easily imagined that the live cells are fully satiation at this stage.

2.3 Dynamic changes of element concentration in cell wall during petiole aerenchyma development

The concentrations of elements in the two types of aerenchyma cell walls presented different characteristics at different developmental stages (Figure 4 and 5). Some elements, such as C, O, Cl, K and Mg, were detected (limit to 0.1%) in the two cell types at all developmental stages of petiole development, while others such as Ca, Cu, Zn, P, were detected only in some of developmental stages. These elements are individually described below. As shown in Figure 4a, the concentration of C in cell wall varied from 39% of dry weight in stage 1 to 54% of dry weight in stage 2 and maintained at about 50% of dry weight during other stages. For oxygen (O), as shown in Figure 4b, the concentration varied from 23% (stage 1) to 35% (stage 3) of dry weight and mainly

maintained at about 30% during other stages. The concentration of Chlorine (Figure 4c) varied from 0.8% (stage 4) to 4.3% (stage 1) of dry weight and the lowest value presented at stage 4. Potassium concentration (K), as shown in Figure 4d, varied from 6% (stage 4) to 33% (stage 1) of dry weight, and, mainly remained at about 10% during other stages. Comparing the concentrations of C, O, Cl and K between diaphragm and cylinder cells demonstrated an analogous change tendency, but the cylinder cells showed variation in element concentration than that of diaphragm cells.

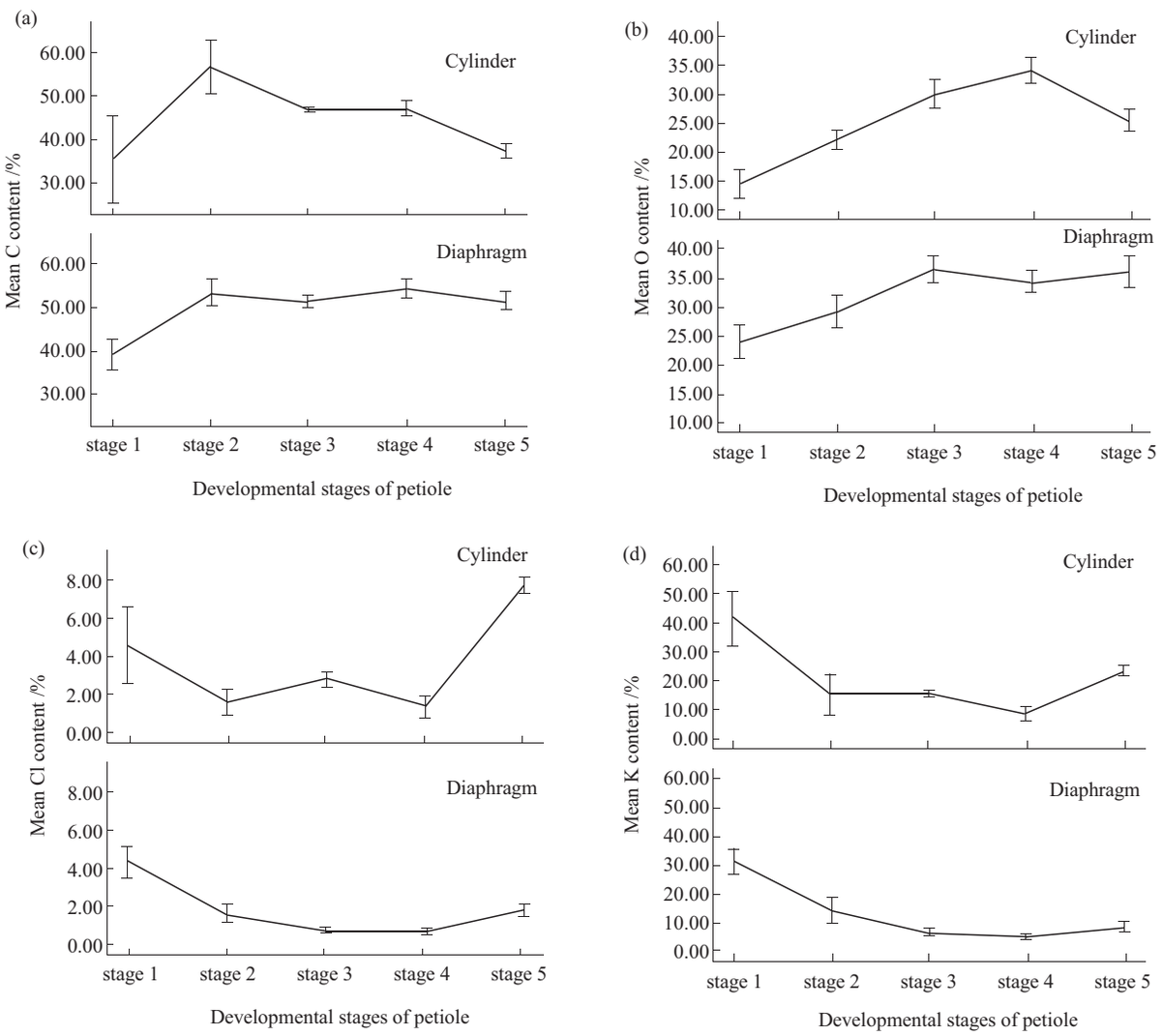

Fig. 4 The concentrations of C, O, Cl and K in cell wall of aerenchyma cells

(a) The content of C change with petiole developing. (b) The content of O change with petiole developing. (c) The content of Cl change with petiole developing. (d) The content of K change with petiole developing (n=11). Error bars: +/–2.00 SE.

For magnesium (Mg), as shown in Figure 5a, the concentration varied from 0.14% (stage 5) to 0.86% (stage 2) of dry weight and mainly maintained about 0.23% at other stages. Comparing the concentration of Mg between the diaphragm and cylinder cells, at early stages showed an analogous change tendency, with the highest value about 4% in cylinder cells at stage 4, while in diaphragm cell at the same stage the value was maintained at very low level (0.23%). For calcium (Ca), as shown in Figure 5b, it could be detected in cylinder cell only at stage 4 and in diaphragm cells at stages 4 and 5. Both at stage 4 and 5, the concentration of Ca in diaphragm cell wall was about 1.3% of dry weight, whereas at stage 3 the value was very low (0.03%). Copper (Cu), as shown in Figure 5c, could be detected only at stage 3 and 4 both in cylinder and

diaphragm cells. At these stages, the concentrations of Cu in diaphragm cell wall were 2.5% and 2.0% of dry weight at stage 3 and 4, respectively, while in cylinder cells at the same stages the values were 2.2% and 1.2%, respectively. Zinc (Zn), as shown in Figure 5d, could be detected out both in cylinder and diaphragm cells only at stages 3 and 4. The concentrations of Zn in diaphragm cell wall were 1.4% and 1.5% of dry weight at stage 3 and 4, respectively, while in cylinder cell at the same stages the values were 0.8% and 0.7%, respectively. In addition, Phosphorus (P) could be detected mainly at stage 1 only in fewer cases (data not shown). These results suggest that some elements, such as Mg, Ca, Cu and Zn, present dynamic changes during the cell expansion and cell maturation of petiole aerenchyma.

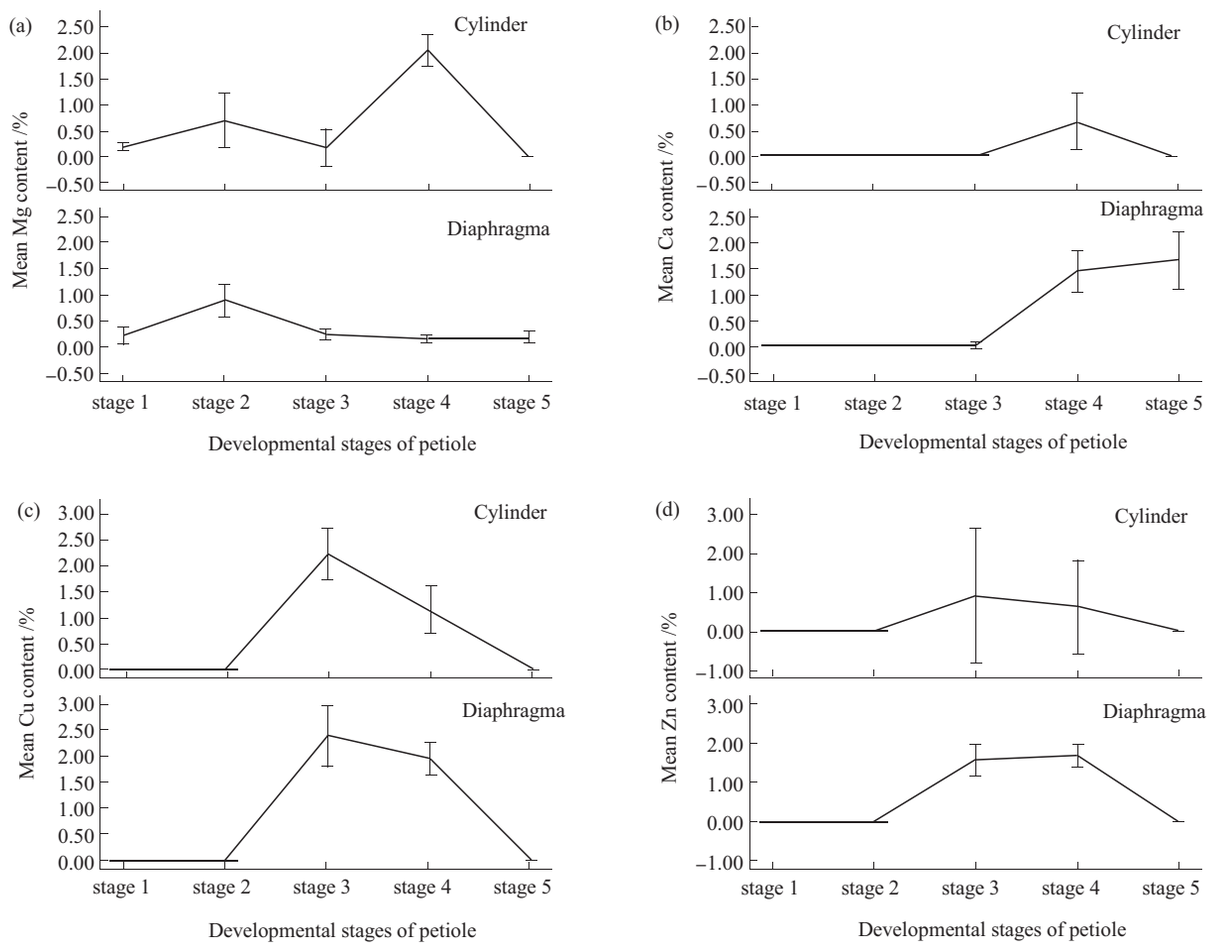

Fig. 5 The concentration changes of Mg, Ca, Cu and Zn in cell wall of aerenchyma cells

(a) Mg content in cell wall of aerenchyma cells of petiole. (b) Ca content in cell wall of aerenchyma cells of petiole. (c) Cu content in cell wall of aerenchyma cells of petiole. (d) Zn content in cell wall of aerenchyma cells of petiole (n=11). Error bars: +/–2.00 SE.

2.4 Some elements presented close relationships

The concentration of each element as an individual variable was detected from all cases by CSEM-EDX, so that it brings a series of variable, which are interrelated to each other. Table 2 displays the results of bivariate correlation analyses performed by SPSS software. Cl showed a significant positive correlation (r = 0.819, P < 0.01) with K, Cu showed a significant positive correlation of 0.963 at P <0.01 with Zn. Interestingly, both Cl and K between other elements presented significant negative correlation. Furthermore, C and O were negatively correlated with a coefficient of –0.208, while C and Ca, O and Cu and O and Zn were positively with coefficients of 0.190, 0.259 and 0.262, respectively.

Table 2 Pearson correlation among concentrations of different elements

	C	O	Cl	K	Mg	Ca	Cu	Zn
C	1	–0.208*	–0.649***	–0.629***	0.107	**0.190***	0.180	0.184
O		1	–0.410***	–0.608***	–0.129	0.178	**0.259****	**0.262****
Cl			1	**0.819*****	–0.194*	–0.258**	–0.409***	–0.399***
K				1	–0.029	–0.318***	–0.406***	–0.394***
Mg					1	–0.142	–0.090	–0.154
Ca						1	0.118	0.156
Cu							1	**0.963*****
Zn								1

*** Correlation is significant at the 0.001 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed). n=109.

2.5 Cu and Zn concentration in the cell wall showed a significant positive linear correlation

As shown in Table 2, the bivariate correlations analyses between Cu and Zn, showed a significant positive correlation (Pearson correlation coefficients was 0.963). In order to understand the relationship of these elements, further analyses of regression were performed by SPSS software. As Cu and Zn were detected only at stage 3 and 4 (all cases number was 42), the data of these cases were used for regression analyses of Cu and Zn (Figure 6). The concentration of atomic Zn was assigned as a dependent variable, while the concentration of atomic Cu as an independent variable. The histogram showed a normal distribution of standardized residuals of dependent variable Zn (Figure 6a). The normal P-P plot of regression standardized residual showed that all expected cumulative probabilities were nearly around a beeline (Figure 6b). From the result of model summary, regression equation was given as: Y_{Zn}=0.062+0.642X_{Cu}, r^2=0.739 (Figure 6b). The scatter plot of dependent variable: Zn shown that all cases formed a linear tendency (Figure 6c).

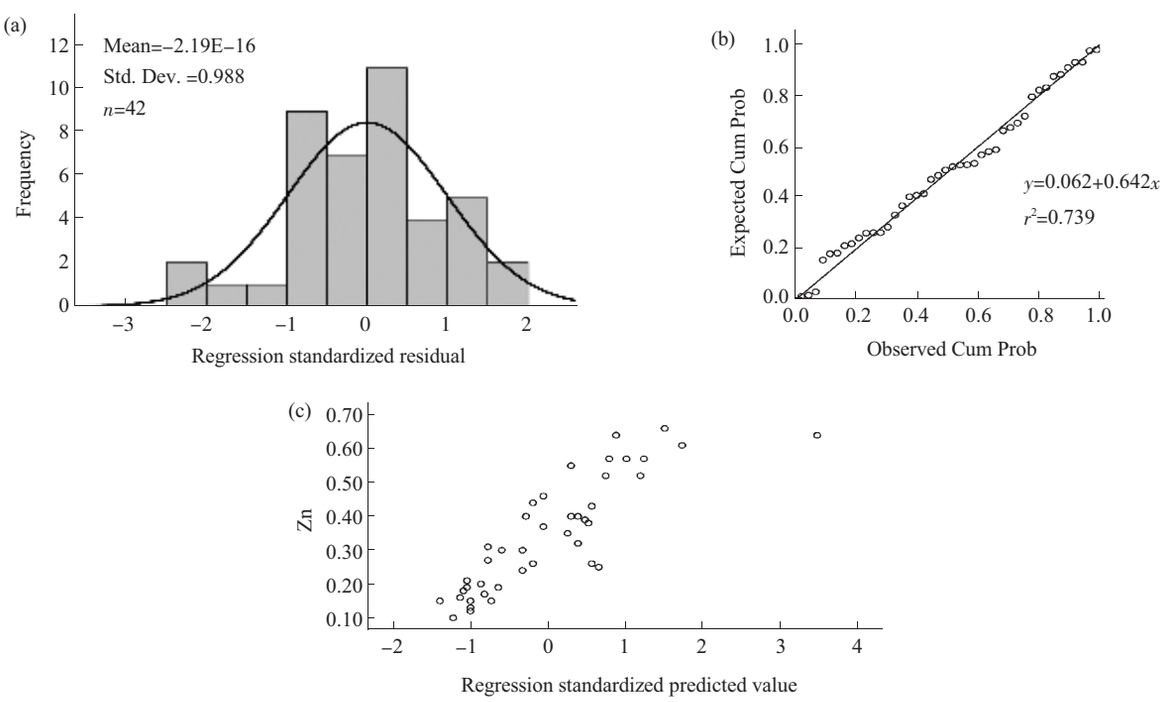

Fig. 6 Regression analyses results of Zn & Cu concentrations

The concentration of Zn was assigned as dependent variable while the concentration of Cu as independent variable. (a) Histogram shows the dependent variable Zn presented a normal distribution of regression standardized residual. (b) Normal P-P plot of regression standardized residual. Regression equation was given by regression model. (c) Scatter plot of dependent variable Zn.

3 Discussions

There are 17 essential mineral elements in plants, nine of which are designated macronutrient elements. Of these macronutrients, C, H and O are most abundant elements in plants and the total content occupy over 95% of plant dry weight. Some macronutrients, such as Ca, K and Mg, exist in plant as free ions, while others elements such as N, P and S are assimilated into organic compounds (Table 3), the concentrations of these elements were regarded as normal data [28]. On comparing the concentrations of some elements in cell wall detected by CSEM-EDX in this study with that of the concentrations that were regarded as normal and adequate in plants, we found higher concentrations than those regarded as normal. For example, the concentrations of K was 6～36 folds higher than that of normal concentration (Table 3). At early stages the K content of cell wall constituted up to 36% of dry weight, but it rapidly decreased during later stages to lower than 10%. Because the content of K in the living cell is about 5～10 times that in whole plant, at the later stages the K content was near normal level. The question that arises here is why so high concentration of K and Cl in cell wall at early stages? Our further analyses found that the highest content of K and Cl was mainly present at stage 1 or 2, were from leaf buds or tender leaves inside sheath under water. The intercellular spaces of aerenchyma at these stages were filled with liquid, as the material was under a freeze dryer, water sublimated out and the solute deposited on the cell wall surface，resulting in high content of K and Cl in cell wall. Several studies have that root aerenchyma spaces are filled with liquid[26, 29]. Our results support the views of root aerenchyma space filled with liquid , but aerenchma spaces filled with liquid in shoot has not been reported.

Table 3 Essential mineral elements concentration data compared between normal and this study

	Normal concentration[1)] (μg •g^{-1} dry weight)	Concentration in this study[2)] (μg •g^{-1} dry weight)
Macronutrient		
N (organic compound)	15 000	ND
K (K^+)	10 000	60 000～360 000
Ca (Ca^{2+})	5000	13 000
Mg (Mg^{2+})	2000	2 000～9 000
P (organic compound)	2000	2 000～11 000
S (organic compound)	1000	ND
Micronutrient		
Cl	100	8 000～40 000
B	20	ND
Fe	100	ND
Mn	50	ND
Zn	20	15 000～16 000
Cu	6	18 000～24 000
Mu	0.1	ND
Ni	0.005	ND

[1)] Normal data which were regarded as adequate concentration in plan[28]. [2)]Data of concentrations were conversed from percent of dry weight in this study.

At stage 2, cell wall contained Mg at high level (Figure 5a), while at stages 4 and 5, Ca concentrations maintained a high level in cell wall (Figure 5b). Interestingly, the high level of Mg was present from the beginning of rapid petiole enlargement, but the high level of Ca was present until complete petiole enlargement. The presence of a high level of Mg in the cell wall just before the beginning of rapid cell expansion, implied that Mg was involved in the regulation of cell expansion and also in enzyme activation for relaxing the primary cell wall. As the petiole matured the cell wall needed more Ca

deposition to increase its rigidity. Our results are in agreement with previous works, where they reported that primary cell wall has very low concentration of Ca, that increases with maturity [21].

Some heavy metals such as Cu and Zn are essential for normal plant growth, although elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms. Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus allow some tolerance to metal stress. These mechanisms include for mycorrhiza and for binding to cell wall and extracellular exudates; reduced uptake or efflux pumping of metals at the plasma membrane; chelation of metals in the cytosol by peptides such as phytochelatins; repair of stress-damaged proteins; compartmentation of metals in the vacuole by tonoplast-located transporters [30]. The normal concentrations of Zn and Cu are 20 μg/g and 6 μg/g of dry weight respectively (Table 3). At stages 3 and 4 of petiole development, the concentrations of Zn and Cu in cell wall were 16 000 μg/g and 24 000 μg/g of dry weight respectively. These concentrations are 800 and 4 000 times higher the normal concentration of Zn and Cu in plants respectively. This result suggested that at cell expansion stages, cells could deposit high level of Zn and Cu in the cell wall. Interestingly, as the petiole matured, Zn and Cu disappeared from the cell wall, indicating that Zn and Cu were imbibed by cell or/and transported to other parts of the plant where they were needed, hence presented a dynamic process. The functions of Cu or/and Zn in cell expansion remain unclear. While Ca, Zn and Cu efflux as cell wall expansion ceases, Ca is still present in the cell wall for rigidity. Therefore, we suggest that Zn or/and Cu may perform the function of relaxing the cell wall or compete with Ca to prevent Ca deposition.

Complex interactions of transport and chelating activities control the rates of metal uptake and storage [31]. A synergetic effect of Cu and Zn on each other in terms of root levels has been reported [32]. Our results showed that there was a significant positive linear correlation between Cu & Zn concentrations (regression equation is: $Y_{Zn}=0.062+0.642X_{Cu}$, $r^2=0.739$). It is possible that Cu and Zn have a synergetic effect for their deposition in cell wall. We suggest that these metals may share same or similar uptake and transport mechanism in the plant.

Integrating all above data and analyses, we conclude that element concentration of cell wall forming aerenchyma presents dynamic changes at different developmental stages, which are more significant than we considered previously. Our results also support the view that aerenchyma spaces are filled with liquid at early developmental stages of aerenchyma formation of *S. trifolia* petiole, and low Ca and high Zn or/and Cu concentrations are needed for cell expansion. In addition, Zn and Cu deposited on cell wall with a high concentration and showed significant positive linear correlation with each other, suggesting that Zn and Cu may share the same or similar uptake and transport mechanisms in plants.

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细胞壁矿质元素含量在细胞生长中的动态变化 *

梁 峰[1,2] 王 忠[1] 郭建林[1] 沈丽珍[1] 杨 清[1]**
([1]南京农业大学生命科学学院，南京 210095；[2]商丘师范学院生命科学系，商丘 476000)

摘要 在组织水平上已经描述了许多植物通气组织的形成过程，但对其发育过程的调控仍然知道得很少．利用 CSEM-EDX 微量分析技术，定点测量慈姑叶柄通气组织不同发育时期的细胞壁矿质元素的组成．这些元素除了 C，O 以外，还包括 Mg，Ca，Cu，Zn，P 等必需的矿质元素．结果发现，在叶柄发育的早期，通气组织细胞壁的 K 和 Cl 含量很高，分别高达 36%和 4.3%细胞壁干重．Mg 的含量在第二阶段最高，达到细胞壁干重的 0.86%．只有在第三和第四阶段监测到 Cu 和 Zn 元素，最高含量分别为 2.5%和 1.5%细胞干重．仅在第四和第五阶段才能检测到 Ca，其最高含量为 1.3%细胞壁干重．通气组织横膈膜细胞和圆柱体腔壁细胞的元素构成变化有相似的趋势，说明这种变化与组织的发育阶段关系密切．细胞壁的一些元素间呈现较高的相关性，其中 K 和 Cl 及 Cu 和 Zn 之间成较高的正相关．在不同发育阶段，细胞壁的元素含量呈现动态变化，说明细胞壁(质外体物质)的元素构成有很大的变动范围．早期的通气组织细胞壁大量积累 K 和 Cl，暗示早期的气体空间充满液体(组织液)；Mg 可能参与细胞伸展的调控；伸展中细胞的细胞壁积累高浓度的 Cu 和 Zn，并不影响细胞的正常功能；而 Ca 的出现使细胞比硬度增加，将终止细胞伸展．Cu 和 Zn 在细胞壁中的积累呈高度的直线关系，回归分析显示，二者呈现定量关系，推测它们可能有共同的或者类似的转运和吸收机制．

关键词 通气组织，细胞伸展，矿质元素，冰冻扫描电镜，X- 射线能谱
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** 通讯联系人．Tel: 025-84395221，E-mail: gyang19@njau.edu.cn
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