微管骨架在苔藓植物适应干旱胁迫应答中的功能研究

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摘　要　植物体通过一系列生理生化反应的改变来适应干旱胁迫。对干旱/复水及秋水仙素处理后再干旱/复水的仙鹤藓(*Atrichum undulatum*)原丝体细胞中微管骨架的动态变化进行了研究,发现干旱处理后细胞内微管骨架从有规律排列的较细的丝状形式转换为无规律排列的较粗的微管束;复水后微管骨架的结构和分布与对照细胞中无明显区别;秋水仙素处理后再干旱/复水的细胞中,微管骨架呈分散的棒状或点状分布,而且原丝体丧失了干旱胁迫后正常复水的能力,进而导致细胞不能恢复正常的生理活动。因此认为,微管骨架在仙鹤藓原丝体适应干旱逆境的过程中起着重要作用。

关键词　微管骨架,干旱/复水,仙鹤藓(*Atrichum undulatum*)
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水是植物体的重要组成部分,它在植物细胞的多项生理生化活动中发挥着重要的作用。然而,在植物的整个生长发育过程中植物体或多或少地会遭遇到干旱胁迫。因此,研究植物忍耐干旱胁迫的机制对于解决植物适应地球上日益缺乏的水资源环境具有至关重要的意义[1,2]。在植物界,苔藓植物被认为是从水生向陆生生活过渡的植物类群[3],由于苔藓植物独特的结构特征以及在严重干旱胁迫后能继续吸收水分保持生长的能力,苔藓植物已经成为研究植物营养器官耐旱胁迫机制的模式材料。

目前人们对苔藓植物耐旱机理的研究主要集中在以下三方面:一是研究干旱条件下的光合作用的变化,二是研究干旱/复水过程中蛋白质代谢的情况,三是从复水前后的材料中寻找差异表达的基因,进而分离耐旱相关基因[4-6]。植物细胞的微管骨架在细胞的多项生理活动中发挥着重要的作用,其中包括对逆境胁迫的应答反应[7]。近年有文献报道,周质微管能针对低温、离子强度、激素、病原物等胁迫发生动态组装变化[7-9]。例如:脱落酸处理后的小麦根细胞中,微管列阵的方向从原来的横向排列转换为纵向排列或倾斜排列,随之发生的还有处理细胞对低温抗性的增强[8]。但是对微管骨架是否参与植物适应干旱胁迫还未见报道。

本文以苔藓植物为试材,对微管骨架在干旱胁迫适应过程中的功能进行了研究。

1　材料与方法

1.1　材料

仙鹤藓(*Atrichum undulatum*)原丝体,生长在改良 MS 培养基上,每 3~4 周继代 1 次,培养温度:23±2℃,光周期:16h / 8h,实验中所选用的材料是继代培养后至少 7d 的原丝体。

1.2　药物处理

秋水仙素新鲜配制,溶于蒸馏水中,工作浓度为 5mmol/L,对照及处理中均为 0.2% DMSO,室温黑暗处理 2 h。

1.3　干旱胁迫处理

将对照和秋水仙素处理后的仙鹤藓原丝体分别转移到滤纸上,置于超净工作台中吹风干燥,直至干燥后的重量为处理前鲜重的 20%。复水时,将材料转至盛有蒸馏水的培养皿中,为了防止微管骨架的恢复,继续加入 5mmol/L 秋水仙素。

1.4　微管骨架的间接免疫荧光定位

仙鹤藓原丝体用含有 1%DMSO,0.15% TritonX-

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100的8%多聚甲醛(PEM buffer：20mmol/L Pipes，5mmol/L EGTA，5mmol/L $MgSO_4$，0.5mmol/L PMSF，pH 6.9配制)固定1h，PEM缓冲液冲洗后，0.2%果胶酶(10mmol/L Mes配制，pH 5.5)处理30min，0.5% TritonX-100抽提30min，PEM缓冲液冲洗后，梯度乙醇脱水以除去大量的叶绿素干扰，复水后，磷酸缓冲液冲洗一次，含1%牛血清白蛋白的一抗(免疫小鼠的anti-β-tubulin，1∶500)室温孵育过夜，FITC标记的二抗(山羊抗小鼠，1∶100)37℃孵育1h，磷酸缓冲液冲洗后，封片。

1.5　显微镜观察

仙鹤藓原丝体的整体形态用体视镜观察，研究用光学显微镜观察细胞形态，体视镜和研究用光学显微镜均与冷CCD照相系统相连接。微管骨架的观察用Bio-Rad MRC1024型激光共聚焦显微镜，激发光波长为488nm，连续采集光切片10～20祯，用伯乐公司专用软件进行图像重组。

2　结　果

2.1　干旱和复水过程中原丝体形态和细胞形态的变化

原丝体是从孢子萌发形成的类似于藻类的绿色丝状结构。从外观上观察，仙鹤藓原丝体呈鲜艳的翠绿色，表面湿润(图版Ⅰ-Aa)。在光学显微镜下观察发现，单根原丝体是由一列细胞组成，每个细胞中都有许多整齐排列的叶绿体(图版Ⅰ-Ad)。当原丝体受到干旱胁迫时，大部分叶绿素消失，颜色由原来的绿色变为黄色，表面干燥(图版Ⅰ-Ab)。细胞水平观察结果表明，细胞发生了质壁分离，并且在大多数细胞中，原生质体与细胞壁并未完全脱离，而是存在细胞壁与质膜相连的一小部分区域，这一区域主要集中在细胞的一端(图版Ⅰ-Ae)。将其置于复水条件之下，观察形态恢复情况。从图版Ⅰ-Ac和Af可以看出，复水6h后，原丝体细胞的质壁分离复原，叶绿素的颜色也重新恢复，整体外观与胁迫前比较几乎无差别。以上结果表明，在极端干旱条件下仙鹤藓原丝体具有很强的生存能力。

2.2　干旱和复水过程中原丝体细胞微管骨架的组装

根据已有文献报道，植物细胞的微管骨架在细胞的多项生理活动中发挥着重要作用，其中包括对低温、光线等逆境胁迫的反应[7]。至于微管骨架在植物干旱适应过程中是否具有重要作用目前还未见报道。我们以模式植物仙鹤藓为实验材料，首先研究了微管骨架在干旱及复水恢复过程中的动态变化。对胁迫之前的原丝体细胞进行了微管骨架的免疫荧光定位，共聚焦显微镜观察表明，微管骨架主要分布在靠近质膜的细胞质中，方向与细胞的长轴方向一致，呈细密的丝状列阵分布(图版Ⅰ-Ba)，经过干旱处理之后，细胞内微管骨架变成较粗的束状，排列没有明显的规律，而且随着原生质体的收缩发生移动，远离细胞壁(图版Ⅰ-Bb)。复水后的细胞中，不仅细密的微管列阵重新恢复(图版Ⅰ-Bc)，而且还观察到分裂相的微管列阵，如成膜体微管列阵(图版Ⅰ-Bd)，表明细胞的生长分裂能力完全恢复。微管骨架的这些动态变化结果表明，微管骨架在仙鹤藓适应干旱胁迫过程中具有重要作用。

2.3　微管骨架对干旱胁迫后原丝体生理功能恢复的影响

为了进一步探讨微管骨架在仙鹤藓干旱适应中的功能，首先用微管骨架阻断剂秋水仙素处理原丝体，然后进行干旱胁迫，再给以复水条件，为了防止微管骨架在复水条件下恢复其正常结构，在复水过程中仍然保留了秋水仙素。如图版Ⅰ-Cc所示，处理后的原丝体细胞内微管骨架以棒状或点状分散分布，由于细胞壁没有荧光，点状分散的周边位置便是细胞质膜的区域。与未用微管骨架阻断剂处理，只是干旱胁迫的细胞相比较，说明微管骨架被破坏之后，细胞丧失了恢复正常生理活动的能力。原丝体的整体形态与干旱处理后的很相似，质膜与细胞壁分离，大量叶绿素消失(图版Ⅰ-Ca，Cb)。以上实验结果表明，微管骨架结构的完整性对于干旱胁迫后的植物体恢复正常生理状态起着非常重要的作用，微管骨架参与了植物细胞对干旱这一胁迫的适应反应。

3　讨　论

植物体在干旱胁迫过程中发生一系列复杂的生理生化活动。尽管目前有许多关于植物耐旱的生理和分子机制的研究报道[10－12]，但对细胞水平上尤其是微管骨架是否参与了干旱胁迫还不清楚。本文对仙鹤藓原丝体干旱及复水后微管骨架的结构进行了研究，发现干旱及复水过程中微管骨架的结构发生了明显的动态变化。干旱处理后，微管骨架组装成较粗的束，而复水后，微管骨架又组装成类似于对照细胞的细密微管列阵。

微管骨架的动态结构变化在耐低温胁迫的小麦根细胞和耐渗透胁迫的水绵细胞中均有报道[9，13]。

在小麦根的伸长区表皮细胞中,-7℃低温导致细胞内微管骨架的解聚,转移至 25℃后,微管骨架又恢复正常。水绵细胞首先用微管骨架阻断剂 APM 处理,之后在恢复的过程中去掉 APM,但给予高渗透压条件,结果微管骨架形成的列阵不是原来的横向排列方式,而是呈纵向或斜向排列。由以上结果推测 :对不同的外界刺激来说,微管骨架的动态组装所发生反应的机理有所差异。

对原丝体细胞干旱/复水过程中微管骨架的分析表明,丝状的微管结构被破坏后,原丝体丧失了干旱胁迫后继续生存的能力,仙鹤藓对干旱胁迫的适应依赖于完整微管列阵的存在。近年来,有人提出微管参与了质膜与细胞壁之间的连接[14],而且有数据表明,微管骨架动态结构的改变明显抑制了拟南芥细胞的内向钾离子电流,与离子进出细胞密切相关[15]。因此我们推测,干旱胁迫后又复水的苔藓原丝体细胞中,微管骨架恢复正常,尽管这一动态转换的调控机制目前还不清楚,但是其排列方式的恢复可能使得细胞内外的离子运输恢复正常、细胞内膨压增加,而且在这一过程中微管骨架能够随着细胞质体积的增加介导质膜与细胞壁的连接。另有报道表明,在感受外界环境刺激的气孔保卫细胞中,只要微管骨架的完整结构能够恢复,细胞的生理活动也能恢复正常[16-17]。如 :蚕豆保卫细胞被脱落酸处理后,辐射状微管列阵消失,同时伴随着气孔的关闭,然而这种变化是可逆的,一旦去掉脱落酸,辐射状微管列阵重新形成,气孔也随之开放。综合以上结果,我们认为,完整的微管骨架在植物参与外界信号刺激过程中发挥着重要的作用,微管骨架的动态结构变化可能有助于植物适应逆境胁迫。

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The Role of Cortical Microtubules in Moss Protonemal Cells During Dehydration/rehydration Cycle

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Abstract Plant cells response to water deficit through a variety of physiological processes. In this work, we studied the function of microtubule cytoskeleton during dehydration/rehydration cycle in moss (*Atrichum undulatum*) protonemal cells as a model system. The morphological and cytological change of protonemal cells during dehydration and rehydration cycle were first investigated. Under normal conditions, protonemal cells showed bright green colour and appeared wet and fresh. Numerous chloroplasts distributed regularly throughout the cytoplasm in each cell. After dehydration treatment, protonemal cells lost most of their chlorophylls and turned to look yellow and dry. In addition, dehydration caused plasmolysis in these cells. Upon rehydration, the cells could recover completely from the dehydrated state. These results indicated that moss had a remarkable intrinsic ability to survive from the extreme drought stress. Microtubule, an important component of cytoskeleton, is considered to play crucial roles in the responses to some environmental stresses such as cold and light. To see if it is also involved in the drought tolerance, dynamic organization of microtubules in protonemal cells of *Atrichum undulatum* subjected to drought and rehydration were examined by indirect immunofluorescence combined with confocal lasersharp scanning microscopy. The cortical microtubules were arranged into a fine structure with a predominant orientation parallel to the long axis of the cells in the control cells. After dehydration, the microtubule organization was remarkablly altered and the fine microtubule structure disappeared whereas some thicker cables formed. When the cells were grown under rehydration conditions, the fine microtubule arrays reappeared. These results provided a piece of evidence that microtubules play a role in the cellular responses to drought stress in moss. Furthermore, we analyzed the effects of the microtubule-disrupting agent colchicine on the morphology recovery of the protonemal cells during rehydration process. The cells were incubated with colchicine, followed by drought stress treatment and rehydration in the presence of colchicine to prevent recovery of microtubule organization. Results from immunofluorescence showed that microtubule arrays were broken down into smaller fragments. Compared to the cells treated with drought stress alone, the cells treated with drought stress in the presence of colchicine could not recover after rehydration treatment. The morphology resembled those of the drought treated cells, with obvious plasmolysis phenomena and loss of chlorophyll content. These results support the notion that microtubules were involved in the deccication tolerance mechanism in *Atrichum undulatum*.

Key words microtubules, dehydration/rehydration, *Atrichum undulatum*

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图版说明(Explain of Plate)

图版Ⅰ-A 干旱和复水过程中原丝体的形态和细胞形态

Plate Ⅰ-A Morphology and cytology of protonemata during dehydration/rehydration cycle

a. 对照原丝体的形态:绿色、表面湿润;b. 干旱原丝体的形态:黄绿色、表面干燥;c. 复水后的原丝体形态恢复正常;d. 对照原丝体细胞的形态:一列细胞组成一根原丝体,细胞内整齐排布着叶绿体;e. 干旱处理后的原丝体细胞发生质壁分离;f. 复水后原丝体细胞质壁分离复原,叶绿体分布恢复

a. Light micrography showing protonamata with an appearance of green and fresh (control); b. Morphology of dehydrated protonemata: yellow-and dry-looking (dehydrated treatment); c. Protonemata morphology restored to normal state (rehydrated treatment); d. Light micrography of control protonamata cytology showing filamentous protonema is comprised of multiple cells and numerous disc shaped chloroplasts distributed regularly in each cell; e. Cells showing plasmolysis in dehydrated protonamata; f. Cells recovered from plasmolysis and many chloroplasts distributed regularly in each cell again (rehydrated treatment)

图版Ⅰ-B 干旱/复水过程中原丝体细胞微管骨架的变化

Plate Ⅰ-B Microtubule organization in moss protonemal cells during dehydration/rehydration cycle

a. 正常原丝体细胞内纵向排列的细密微管骨架列阵;b. 干旱后无明显方向性的较粗微管束;c. 恢复后的原丝体细胞内纵向排列的细密微管骨架列阵;d. 恢复后的原丝体细胞处于分裂相的微管骨架列阵:成膜体

a. Confocal images showing fine cortical microtubules array in control protonemal cells; b. Disorganized microtubules array under dehydration: randomly orientated large cable replaced fine orderly arrays; c, d. Microtubules reorganization in protonemal cells during rehydration: fine cortical microtubules array reoccurred in c; phragmoplast microtubules array also reorganized in d

图版Ⅰ-C 秋水仙素处理的原丝体复水后的形态和细胞形态

Plate Ⅰ-C Morphology, cytology and microtubules array after rehydration of protonemata treated by colchicine and dehydration

a. 原丝体的整体外观:黄绿色、表面干燥;b. 细胞内质壁分离未恢复;c. 细胞内微管骨架呈片段状分散分布

a. Unrecovered protonemata showing yellow and dry appearance; b. Unrecovered cells showing plasmolysis; c. Disorganized microtubules showed diffused dots