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目　录

7种箬竹抗寒特性比较研究

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摘 要: 为探明七种箬竹(*Indocalamus*)的抗寒特性，利用叶绿素荧光技术测定了夜间低温胁迫后七种箬竹叶绿素荧光参数的日变化。结果表明:箬竹种PSⅡ实际光化学量子产量日变化（Yield）规律呈近似“W”型；广东箬竹 *Indocalamus. guangdongensis* PSⅡ光化学效率（Fv/Fm）、PSⅡ潜在活性(Fv/Fo)和Yield等参数值均低于其它箬竹种；小叶箬竹 *I.pumilus* 具有最高的初始荧光（Fo）、最大荧光（Fm）值；髯毛箬竹 *I. barbatus* 的 Fv/Fm、Fm/Fo 值均高于其它几种箬竹，说明广东箬竹最容易遭受低温胁迫，抗寒性差，而小叶箬竹和髯毛箬竹抵抗低温能力较强；可见，利用叶绿素荧光分析技术来鉴别箬竹种间抗寒性是可行的。

关键字: 箬竹；叶绿素荧光；低温胁迫

箬竹 *Indocalamus ssp.* 系多年生禾本科竹亚科箬竹属植物，其观赏园艺、食品包装、水土保持等方面的作用越来越受到人们的重视，但相对毛竹、雷竹[1, 2, 3]等竹种的研究而言，关于箬竹的研究甚少，限制了箬竹的进一步开发利用。箬竹分布于热带和亚热带地区，当遇到稍低于其最适生长温度时就会遭受低温胁迫。多数研究表明低温胁迫首先损伤细胞膜半透性导致电解质渗漏率增加，造成脂膜破坏，丙二醛（MDA）含量升高[4]，过氧化作用加强，同时低温胁迫使光合速率下降，引起光能过剩，甚至导致光抑制的发生及光合机构被破坏[5]。

Frachebound 等[6]认为叶绿素荧光参数可作为抗冷性选择指标，叶绿素荧光分析技术也越来越多地被用于研究植物抗寒特性[7, 8, 9]。但迄今为止关于箬竹抗寒性的研究未见报道。为此本研究通过对低温胁迫下七种箬竹叶绿素荧光参数的分析，初探箬竹种抗寒特性，以期为今后箬竹的引种、培育及应用提供一定的理论依据。

1 材料与方法

1.1 实验材料

所采用的实验材料为七种箬竹，即：矮叶箬竹(*I. pedalis*)、广东箬竹(*I. guangdongensis*)、小叶箬竹(*I.pumilus*)、髯毛箬竹(*I. barbatus*)、胜利箬竹(*I. victorialis*)、箬竹(*I. tessellatus*)以及阔叶箬竹(*I. latifolius*)。它们于 2004 年 3 月引种在浙江林学院东湖校区的东侧苗圃，该实验区域有垂直的两条水渠，地表较湿润，光照适宜，地表有一定的草本植被覆盖。

1.2 实验方法

每个箬竹种选择长势健壮的植株进行挂牌标记为标准株，每株选定 3 片受光一致的叶片，且均为主新梢上的功能叶，挂牌标记，以后每次测定都用同样叶片，重复 3 次。荧光参数采用德国 WALZ 生产的便携式叶绿素荧光仪 PAM-2100 测定，选择夜间低温，次日晴朗无风的 2006 年 1 月 7 日进行活体测定，作为受低温胁迫后的数据进行处理。获取的主要荧光参数有:Fo（初始荧光）、Fm（最大荧光）、Fv（Fv=Fm-Fo）（可变荧光）、Fv/Fo 常用于表示植物叶片 PSⅡ潜在活性、Fv/Fm（PSⅡ光化学效率）以及ΦPSⅡ（非环式电子传递的量子效率）等。各参数日变化从 7:00-17:00，每小时测定一次；21:00 测定一次完全自然暗适应下的叶

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绿素荧光参数；另外，于测定当日观测了各箬竹种全株及局部受低温胁迫状况，并进行纪录，其中受低温伤害程度，全株用变黄枯死叶片与全株叶片总数比值表示，叶片用变黄面积与全叶面积比值表示；未受胁迫的叶绿素荧光参数于2006年4月测定新生功能叶所得。

2 结果与分析

2.1 光照强度与温度的日变化

光合有效辐射的日变化(如图1)呈单峰曲线，高峰时期在11:00-14:00之间。10:00-12:00和14:00-16:00是PAR迅速上升和迅速下降的阶段；气温的日间变化与PAR的日变化趋势基本一致，都为近似正态分布如图1、图2，最高峰出现在11:00左右，上午11:00至下午14:00是气温较高的时段，一天中最低气温-3.5 ℃，最高气温10.5 ℃。

2.1 叶绿素荧光参数的日变化

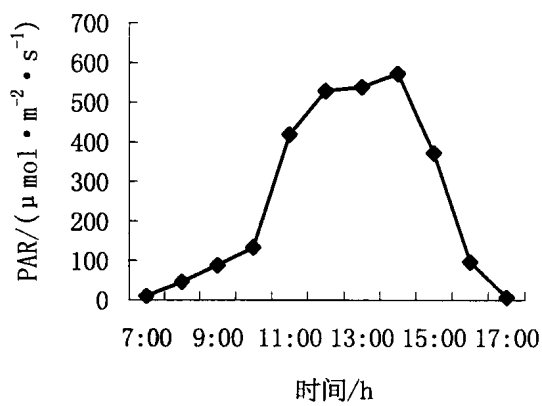

图1 光合有效辐射的日变化
Figure 1 The diurnal changes of PAR

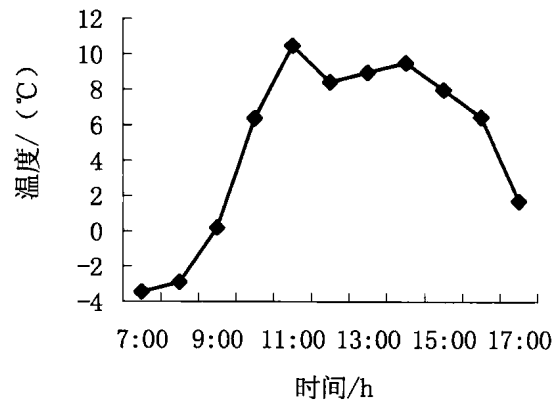

图2 温度的日变化
Figure 2 The diurnal changes of temperature

从图3中可以看出，Yield的日化近似呈“W”型，各竹种Yield值最高值均出现在7:00和17:00左右，最低点出现在8:00-9:00之间，7:00-8:00Yield值迅速下降的原因是由于，7:00时PAR极低，不足以引起PSⅡ中心的激发，Q_A处于氧化状态，PSⅡ反应中心可接受电子处于开放状态，光化学反应几乎为零，叶片吸收的光能主要以热耗散和叶绿素荧光的形式散发，因此7:00时Yield值较高，而随着PAR的升高，PSⅡ反应中心受光激活，Q_A逐渐被还原，光化学反应加强，Yield值迅速下降，并维持在一定水平，10:00Yield值迅速上升，这与PAR的变化趋势是一致的，然而，矮叶箬竹、胜利箬竹、小叶箬竹、髯毛箬竹和广东箬竹Yield值在中午12:00左右又出现下降趋势，15:00又迅速升高，原因可能是因为经过一夜时间直到早晨9:00温度一直很低，竹种遭受不同程度的低温胁迫，叶绿体细胞处于冰晶状态，需要恢复的时间较长，10:00-12:00温度的持续升高，使叶绿体细胞逐渐从冷害中恢复，PSⅡ功能也相应恢复，光化学反应加强，相应叶绿素荧光产量下降；到了15:00时PAR、温度都迅速下降，此时光合能力迅速下降，相应Yield值迅速升高以消耗多余的光能。几竹种相比较，广东箬竹Yield值8:00-16:00一直很低，这说明广东箬竹PSⅡ已经遭受严重破坏。短时间内很难从低温胁迫中恢复过来，相对广东箬竹， 矮叶箬竹、小叶箬竹和胜利箬竹能更快的从低温胁迫中恢复过来，说明这三个竹种PSⅡ受低温胁迫伤害较小。

ΦPSⅡ是PSⅡ非环式电子传递的量子效率，也是PSⅡ功能的指标之一[10, 11]。叶片ΦPSⅡ反映了PSⅡ反应中心在环境胁迫中有部分关闭情况下的实际原初光能捕获效率，可反映实

际的PSⅡ反应中心进行光化学反应的效率[12]。如图4所示，ΦPSⅡ的日变化规律与Yield日变化几乎完全一致，这也进一步说明了广东箬竹PSⅡ反应中心在低温胁迫中实际原初光能捕获效率低，而矮叶箬竹、小叶箬竹和胜利箬竹PSⅡ反应中心进行光化学反应的效率较高。

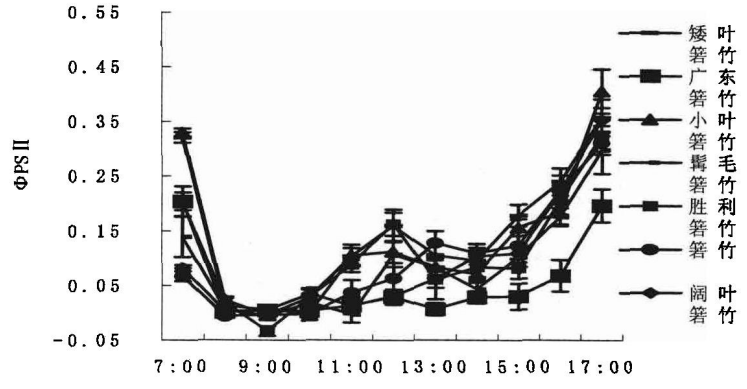

图 4 ΦPSⅡ的日变化

Figure 4 The diurnal changes of ΦPSⅡ

2.3七种箬竹主要叶绿素荧光参数的比较

通过测定七种箬竹全自然暗适应下的叶绿素荧光参数（如表1），小叶箬竹具有最大Fo、Fm值，而箬竹具有较小的Fo、Fm值，Fm值可以反映通过PSⅡ的电子传递情况，说明小叶箬竹PSⅡ的电子传递状况优于箬竹。

表1 七种受胁迫箬竹叶绿素荧光参数比较

Table 1 The compare of chlorophyll fluorescence parameters in seven Indocalamus under stress

竹种	Fo	Fv/Fm	Fm	Fv/Fo	Yield
矮叶箬竹	0.2040±0.007	0.4357±0.013	0.3620±0.004	0.7745±0.042	0.4183±0.242
广东箬竹	0.3473±0.055	0.3013±0.042	0.5050±0.097	0.4549±0.097	0.3253±0.188
小叶箬竹	0.7467±0.575	0.3410±0.046	1.0340±0.767	0.3848±0.767	0.4697±0.271
髯毛箬竹	0.2613±0.082	0.4420±0.081	0.5143±0.189	0.9681±0.189	0.4057±0.234
胜利箬竹	0.2603±0.015	0.4353±0.032	0.4663±0.053	0.7913±0.053	0.3583±0.207
箬　　竹	0.1790±0.093	0.3670±0.019	0.2890±0.148	0.6145±0.148	0.3660±0.211
阔叶箬竹	0.1917±0.096	0.3133±0.089	0.3227±0.171	0.6835±0.171	0.4317±0.249

表2 七种未受胁迫箬竹叶绿素荧光参数比较

Table 2 The compare of chlorophyll fluorescence parameters in seven Indocalamus under no stress

竹种	Fo	Fv/Fm	Fm	Fv/Fo	Yield
矮叶箬竹	0.6600±0.084	0.6610±0.017	1.9270±0.152	1.9710±0.154	0.1526±0.018
广东箬竹	0.7340±0.032	0.6490±0.030	2.1105±0.090	1.9066±0.246	0.1050±0.007
小叶箬竹	0.6615±0.049	0.6380±0.054	1.8615±0.187	1.8898±0.421	0.2011±0.024
髯毛箬竹	0.7070±0.029	0.6165±0.058	1.9905±0.351	1.7968±0.428	0.1283±0.014
胜利箬竹	0.7650±0.081	0.6145±0.084	1.9890±0.393	1.6007±0.350	0.1303±0.019
箬　　竹	0.7455±0.086	0.6325±0.018	2.0580±0.322	1.7312±0.129	0.1087±0.017
阔叶箬竹	0.7360±0.099	0.6350±0.092	2.015±0.433	1.7378±0.399	0.1840±0.041

Fv/Fm代表PSⅡ光化学效率，反映PSⅡ最大光能转换效率，非胁迫条件下基本在0.84左右，对环境变化非常敏感，在胁迫条件下该参数明显下降，七种箬竹的Fv/Fm值均小于0.5，

说明受到较强的低温胁迫，而Fv/Fo通常用来度量PSⅡ的潜在活性，髯毛箬竹的Fv/Fm、Fv/Fo值均高于其它几种箬竹，而广东箬竹Fv/Fm、Fv/Fo值相对较低，说明髯毛箬竹具有较高的PSⅡ原初光能转化效率和PSⅡ潜在活性，而广东箬竹PSⅡ原初光能转化效率以及潜在活性较低。

从表1中可以看出小叶箬竹具有较高Yield值，广东箬竹较低。不难发现广东箬竹Fv/Fm、Fv/Fo、Yield都较其它几种低，这说明广东箬竹PSⅡ可能已经受到破坏或失活，低温胁迫后，受害较严重；而小叶箬竹Fo、Fm、Yield值，髯毛箬竹Fv/Fm、Fv/Fo值较高，说明小叶箬竹和髯毛箬竹低温胁迫后，受害较轻。

相对受胁迫状况下七种箬竹叶绿素荧光参数，未受低温胁迫时各参数值趋于一致（如表2），且各箬竹种PSⅡ光化学效率以及PSⅡ潜在活性等都明显优于受低温胁迫状态。不难看出，广东箬竹Fv/Fm前后变化最大，相差0.3477，而髯毛箬竹变化最小，只有0.1745，从这一侧面可以看出，广东箬竹抵抗低温的能力较差，低温胁迫后，PSⅡ损伤严重，而髯毛箬竹抵抗低温的能力较强，低温胁迫后，PSⅡ损伤较轻。

2.4 七种箬竹受害状况

为了验证试验数据的客观性，于测定当日对七种箬竹进行了低温伤害状况调查（如表3）。调查发现，各竹种间受害差异较大，受低温伤害尤以广东箬竹为甚，全株60%-70%叶片受害死亡，受害叶片的90%-100%受害枯黄；而髯毛箬竹相对受害较轻，全株只有3%-5%的叶片受到低温伤害，受害叶片也只有1%-2%的叶稍部位枯黄，其他竹种受低温伤害具体情况如表3所示。七种箬竹受低温伤害的实际状况与实验数据分析的结果是吻合的，说明利用叶绿素荧光分析技术来研究种间抗寒特性是可行的。

表3 七种箬竹受低温伤害状况

Table 3 The chilling stress situation of seven Indocalamus

竹种	矮叶箬竹	广东箬竹	小叶箬竹	髯毛箬竹	胜利箬竹	箬 竹	阔叶箬竹
全株受损比（%）	10	60-70	20-30	3-5	20	20-30	8-10
叶片受损比（%）	30-50	90-100	50-60	1-2	40-60	40-60	25-30

3 结论与讨论

植物叶绿素荧光动力学是近年来发展的一种新型、快速、简便、准确、无损伤的检测植物光合作用生理状况的新兴技术。因为它包含了十分丰富的光合作用过程变化的信息，被视为植物光合作用与环境关系的内在探针，为植物抗性生理研究提供了方便[13]。人们常常在低温胁迫下测定植物叶片的某种荧光参数的变化以确定它们的抗冷性及受伤害的程度。1984年Smille提出用荧光上升最大速率FR作为鉴别植物抗寒性的指标[14]，此方法在比较不同种类冷敏感植物抗冷性上较为成功，但用于同种植物的品种间鉴别时则难以判断。从本实验中可以看出，利用叶绿素荧光分析技术鉴别种间抗寒性是可行的。

从本实验可以看出，箬竹种间抗寒特性存在很大差异。箬竹种PSⅡ实际光化学量子产量（Yield）日变化规律呈近似“W”型；髯毛箬竹的Fv/Fm、Fv/Fo值均高于其它几箬竹种，具有较好的PSⅡ原初光能转换效率和PSⅡ潜在活性，表现出较好的光合性能，抵抗低温胁迫的能力较强；广东箬竹PSⅡ光化学效率（Fv/Fm）、PSⅡ潜在活性（Fv/Fo）和Yield等参数值均低于其它箬竹种，容易遭受低温胁迫，抗寒性差。

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Comparative Study the Characteristics on Chilling Resistance of Seven Bamboos(*Indocalamus ssp* .)

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Abstract: In order to research the chilling resistance of seven *Indocalamus ssp* ., the diurnal changes of the chlorophyll fluorescence were studied by the technique of chlorophyll fluorescence after the whole night low temperature, the result indicated that the diurnal changes of the actual photochemistry quantum yield of the PS Ⅱ (Yield) to assume "W" approximately, the parameter of the photochemistry efficiency of PS Ⅱ (Fv/Fm), latent activeness of PS Ⅱ (Fv/Fo) and Yield of *I. guangdongensis* are lower than other Indocalamuses; *I.pumilus* has the biggest numbers of the initial fluorescence (Fo) and the maximal fluorescence (Fm); the Fv/Fm and Fv/Fo value of *I. barbatus* are higher than other several indocalamuses, all this suggested that *I. guangdongensis* is easiest to suffer the chilling stress, and its characters on Chilling resistance is weak , but the ability on chilling resistance of *I.pumilus* and *I. barbatus* is strong.

Key Words: indocalamus; chlorophyll fluorescence; chilling resistance

Isolation and characterization of TB1 homologs in bamboo

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ABSTRACT: Bamboo (*Bambusoideae*) is by far the largest member of grass family *Poaceae*. The study on mechanism of rhizome branching especially the bamboo shoot development is one of the most important fields in bamboo research. Previous studies reveal some anatomic and physiological mechanism about the complicated process of bamboo shoot development. But little is known about the molecular mechanism of bamboo shoot development. In this paper, two transcript similar to maize *TB1*, called *PpTB1-1* and *PpTB1-2* respectively, were cloned from *Phyllostachys praecox*, a good model to study the mechanism of rhizome branching. In situ hybridization indicated that *PpTB1* was closely related to the apical dominance of bamboo shoot development. Cloning of TB1 homologies from different types of bamboo species and their phylogenetic analysis showed they were probably valuable to bamboo taxonomy.

Keywords: TB1, apical dominance, bamboo taxonomy

竹子 TB1 同源基因的克隆和特点分析

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摘 要: 竹子是禾本科植物中最大的成员，竹林是重要的森林资源。竹子地下茎（竹鞭）的生长和繁殖规律是竹子最重要的特色之一，竹鞭出笋有关调控机理的研究是竹子生长发育研究的热点，现有的仅从营养激素水平进行的研究有相当大的局限性，有必要在此基础上开展竹子发育分子机理特别是基因调控机理的研究，然而目前尚无竹子出笋有关基因的研究报导。本文从早竹中克隆了玉米 TB1 的相似基因，通过 RT-PCR 和原位杂交检测了该基因在竹笋形成过程中的时空表达特点，揭示该基因与竹笋形成过程中的顶端优势紧密相关，由此，推测该基因可能在竹子出笋调控过程中发挥重要作用。另外，该基因对于研究竹种的分类进化也有着潜在的重要价值。

关键词: 早竹；竹笋形成；TB1；竹子分类

Introduction

Bamboo (*Bambusoideae*) is by far the largest member of grass family *Poaceae*, which has been put to over 1,000 practical uses ranging from paper making, food and handicrafts to construction in the tropics and subtropics. Most cultured bamboos are perennial woody evergreens and basically reproduced by rhizome branching, which are quite different from other poaceous plants. Based on forms of rhizome branching, bamboo is usually divided into three types, termed

scattered bamboos with monopodial rhizome, caespitose bamboos with sympodial rhizome, and pluricaespitose bamboos with monopodial and sympodial rhizome (Li et al., 2003). The complexity of rhizome branching is also exhibited in the development of bamboo shoot which generally grows to their full height in a single season, making it the fastest growing plant in the world. Thus, the study on mechanism of rhizome branching especially the bamboo shoot development is one of the most important fields in bamboo research.

Previous studies reveal some anatomic and physiological mechanism about bamboo shoot development. According to the morphological and structural changes, the bamboo shoot development of *Phyllostachys praecox* is divided into six stages: dormancy, germination, development stage I, II, III and shoot stage (Zhang et al. 1996). The high concentrations of GA_3、ZT 和 IAA are detected in the rhizome bud before the formation of bamboo shoot in *Phyllostachys praecox* (Hu et al. 1996). In *Phyllostachys pubescens*, the high concentrations of IAA and GA_3 are found mainly at the apical part of bamboo shoot during the growth of bamboo shoot (Ding 1997). More precise detection by ELISA shows that the high IAA is correlative to the outgrowth of rhizome bud before the formation of new rhizome and bamboo shoot in *Phyllostachys praecox* while high ZT is only corresponding with the formation of bamboo shoot from rhizome bud (Huang et al. 2002). Although they have improved our understandings on the mechanism of bamboo shoot development, the previous studies fail to elucidate whether the phytohormones work efficiently as we now know the phenotype of plant is not only influenced by the phytohormone concentration but also related to the sensitivity of receptor and interference from other signal pathways. So, it is very limited only from phytohormone detection to explain the rhizome development Besides, the previous studies by mixing several samples may counteract the difference of phytohormone concentration in different individuals, and obviously can't reveal the spatial distribution of signal or regulator in single sample, which is profoundly important to organ development.

The anatomic study indicates the bamboo shoot development is a process of shoot branching which has been widely investigated in model plant such as *Arabidopsis*, rice and so on. Some shoot branching related genes have been described, which fall into three classes on the basis of whether they affect meristem initiation (e.g. *REVOLUTA, MOC1*), meristem outgrowth (e.g. *MAX*) or both (e.g. *TB1*) (Ward et al. 2004). But little is known about the gene regulation of shoot branching in bamboo, especially the complicated process of bamboo shoot development.

In our bamboo research project, we consider that *Phyllostachys praecox* is an excellent model to study rhizome development. *Phyllostachys praecox*, a typical scattered bamboo with high economical value in east China, is named after the characteristic that it produces edible bamboo shoots earlier than any other bamboo in spring. It is also a fit system to explore the molecular mechanism of rhizome development since numerous physiological studies has been carried out in China in recent ten years. In this paper a TB1 homolog was cloned from *Phyllostachys praecox*. In situ hybridization indicated that the gene was highly related to the apical dominance of bamboo shoot development. Cloning of TB1 homologs from two other bamboo species and phylogenetic analysis showed they were probably very important to bamboo taxonomy.

Materials and Methods

Materials

All samples of bamboo were collected from Bamboo Botanical Garden of Zhejiang Forestry Academy.

RNA isolation

Tissues were ground in liquid nitrogen and RNA was extracted with TRIZOL Reagent (BBI) according to the manufacturer's instructions and was then treated with proportional DNase I (Promega) at 37°C for 30 minutes. The quality of total RNA was measured by both electrophoresis and optical absorbency. Only RNA samples with the A260/280 >2.0 were used for RT-PCR.

Histological analysis and *in situ* hybridization

The apical parts of bamboo shoots were fixed in 4% paraformaldehyde in phosphate buffer, pH 7.0, overnight at 4°C. The fixed tissue was dehydrated in graded ethanol series, replaced with xylene, embedded in paraffin, and sectioned at 10μm on rotary microtome (Leica RM2135). After being checked in microscope, the selected slides were treated as described (Braissant and Wahli 1998). The template for riboprobe synthesis was constructed by cloning the CDS of the *PpTB1* cDNA into pBluescript (Invitrogen). The antisense and sense RNA probes were generated by T3 and T7 RNA polymerase separately after the linearization of plasmid. Some sections were stained with Ehrlich's hematoxylin for histological analysis.

Gene cloning and sequence analyzing

The 3' RACE was fulfilled with gene specific primer tb1f1: 5'GGAGTCCCATCAGTAAAGC3' using BD SMART™ RACE cDNA Amplification Kit (Clontech) according to the manufacturer's instructions. The amplified fragment was ligated into pBluescript and three clones were then sequenced by ABI 377. The cloning of TB1 homologs from bamboo genomic DNA was carried out by PCR with primers tb1f1 and tb1r1: 5'CGCATCCGGTTCTTCTCCTTGGT3'. The amplification was performed 4min at 94℃; for 30 cycles 30s 94℃, 60s 58℃, 72℃ 1min; and 5min at 72℃. The fragments were also cloned and sequenced as the above. Sequence alignments were conducted using AlignX of Vector NTI suite9.0 with the multiple alignment parameters gap opening penalty 4, gap extension penalty 0.2 and PAM protein weight matrix and the Dayhoff amino acids distance matrix. The phylogenetic tree of homologous genes was constructed employing the Minimum Evolution of MEGA3.1.

Result

Gene cloning and sequence analysis

Two cDNAs, 1296bp and 1185bp respectively, were cloned by 3'RACE with upstream gene specific primer designed according to the identity of known TB1 homologs in 5' untranslated region (5'UTR). They almost shared the same cds except two mismatches leading to minus difference in their putative amino acids. Besides all 3'UTR of the shorter one, the longer cDNA increased over 100bp before the poly (A) sequence of 3'UTR which might be related to the translation of transcript. BLAST search showed that they were similar to *TB1* gene from maize and thus named as *PpTB1-1* (1296bp) and *PpTB1-2* (1185bp) respectively. *PpTB1* was a member of TCP gene family, encoding 349 amino acids with SP, TCP and R conserved domain (Figure 1). PpTB1-1 shared the highest identity in Genbank with DdTB1 (71.7%) from *Danthoniopsis dinteri*, and only 64.7% and 62.6% identity with maize TB1 and rice OsTB1 respectively based on the alignment of SP, TCP, R domain. The sequence of *PpTB1* was also cloned from genomic DNA with gene specific primers , which showed it contained no introns just like any other known TB1 homologous genes.

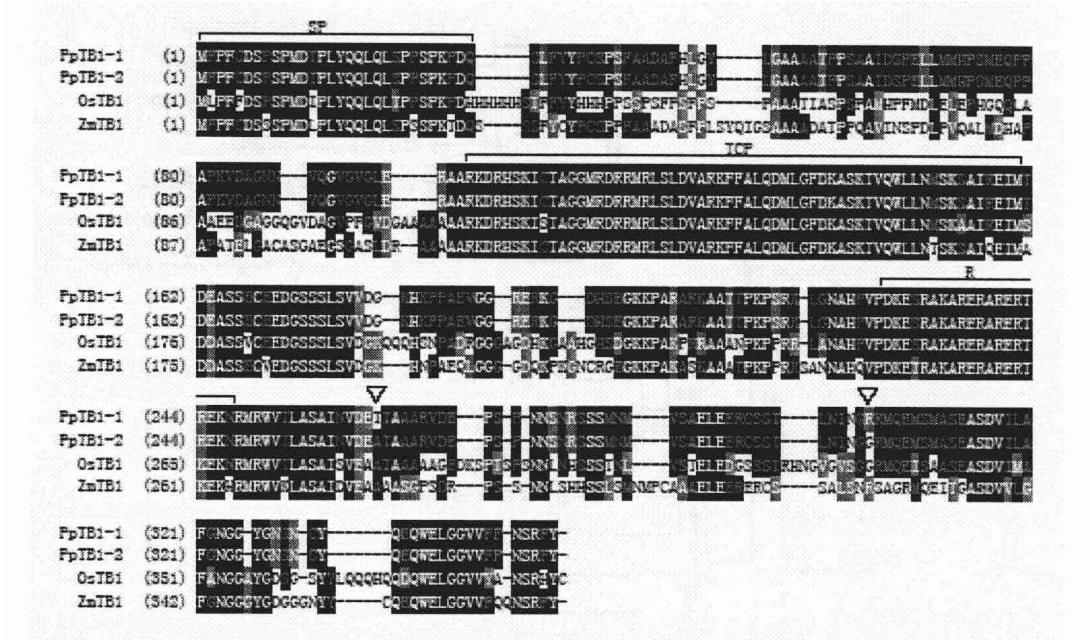

Figure 1. The alignment of some known TB1(ZmTB1) homologs. Sequence alignment of the deduced amino acids of *Phyllostachys praecox PpTB1-1*(DQ842222), *PpTB1-2*(DQ842223), rice *OsTB1* (AY286002), *ZmTB1*(U94494) was conducted with AlignX. Regions of identity (white letter with black background), conservation (gray letter with black background), similarity (white letter with gray background) and differences (white) are indicated. The SP, TCP and R domains were predicted according to the ZmTB1.Two mismatches between PpTB1-1 and PpTB1-2 were indicated with triangle.

Gene expression analysis

RT-PCR showed that PpTB1 was not only expressed in all kinds of bud but also expressed weakly in young floral organs. Bamboo shoot contains plenty of lateral bud, which is like a microminiature of bamboo (Figure 2 A, B). In situ hybridization indicated that PpTB1 was expressed strongly in the tip of bamboo shoot and rarely in other regions, which was consistent to that of rice, maize and sorghum (Figure 2 C, D).

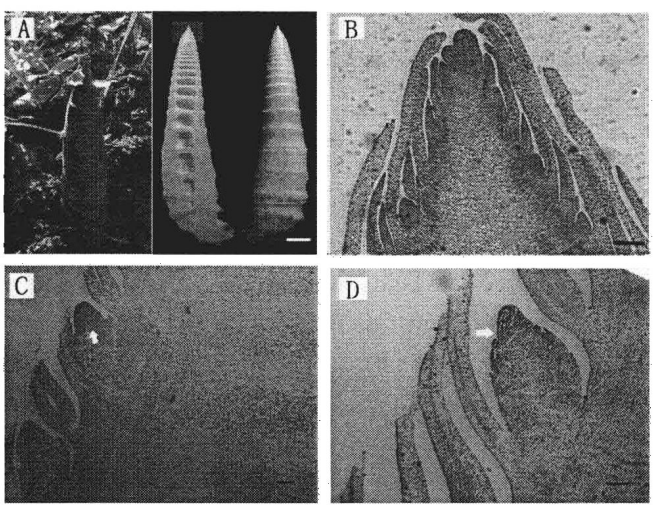

Figure 2. The anatomical analysis of bamboo shoot development and in situ *PpTB1* expression in *Phyllostachys praecox*. a. bamboo shoot, bar=2cm; b. the microsection of apical part of bamboo shoot; c, d. the expression of PpTB1 in bamboo shoot (indicated by white arrow). bar = 100 μm.

2.3 phylogeny analysis

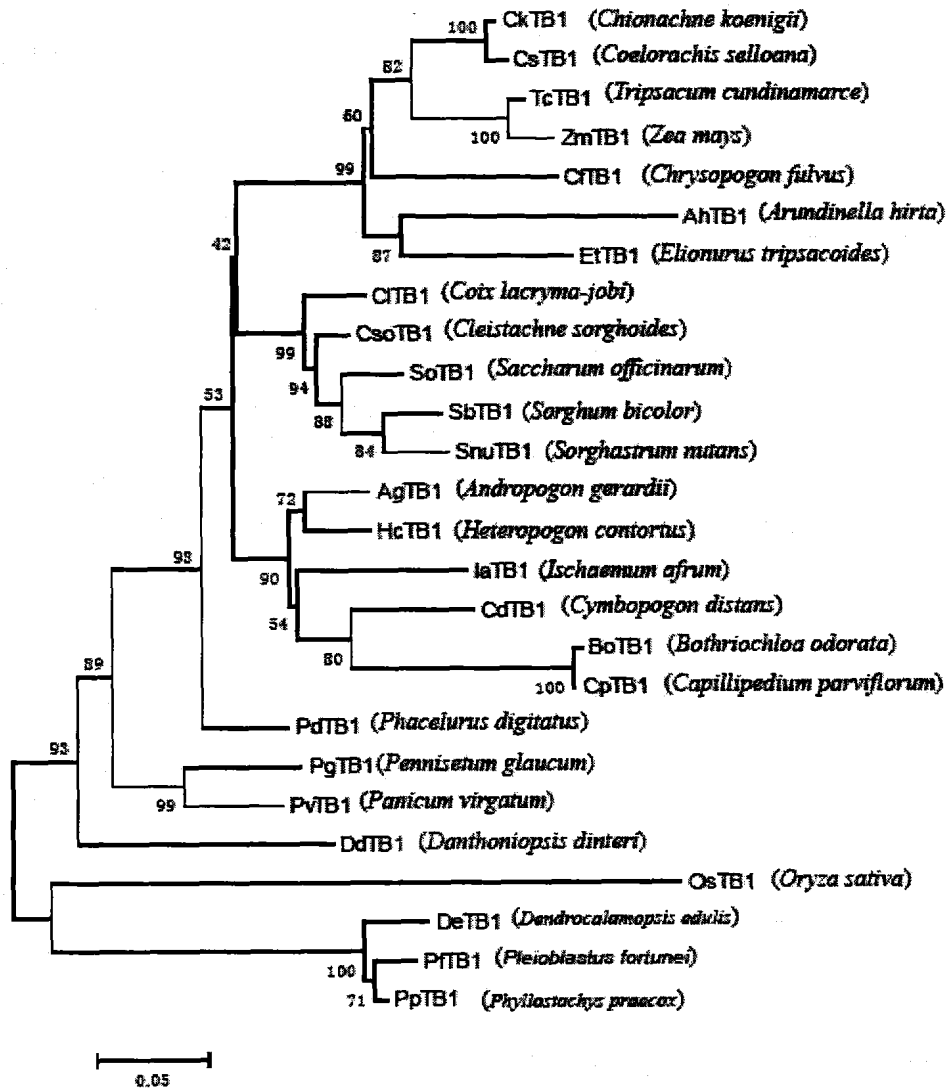

Figure 3. The phylogeny of TB1-like sequences from different genus of *Poaceae*. Phylogeny was reconstructed using SP, TCP and R domain by Minimum Evolution method with Dayhoff Matrix Model and 500 bootstrap replicates. DeTB1 and PfTB1 had been deposited in GenBank (Accession numbers DQ842224, DQ842225). Other sequences referred to Lukens L and Doebley (2001).

Using gene specific primers from *Phyllostachys praecox*, TB1 homologs were further cloned from genomic DNA of *Dendrocalamopsis edulis*, a typical caespitose bamboo and *Pleioblastus fortunei*, a typical pluricaespitose bamboo, respectively. The phylogeny of updated TB1 homologs from different genus was reconstructed (Figure 3), which showed the comparatively high confidence rate with only one lower than 50%. Although OsTB1 and PpTB1 didn't share the highest identity, the phylogeny showed they probably evolved from the nearest common ancestor. The TB1 phylogeny was also used to explain the evolution of bamboo since it had been applied to the evolution analysis of maize and related grasses successfully. According to it, bamboo was comparatively primitive in *Poaceae* and caespitose phenotype might appear early than scattered one, which were consistent with classical taxonomy. But it also suggested that *Phyllostachys praecox* was more primitive than *Dendrocalamopsis edulis*, which seemed contrary to the classical bamboo taxonomy. In classical bamboo taxonomy, caespitose bamboos are thought to be more primitive than the scattered ones. However, no molecular evidence has ever been reported yet to

support this theory. Contrarily, the molecular phylogeny here suggested the scattered *Phyllostachys praecox* might be more primitive although the phenotype of monopodial rhizome probably evolved later according to the topology of phylogeny tree. But more evidence should be collected to support this conclusion.

Discussion

The function of PpTB1 and the apical dominance of bamboo

TB1 is a member of TCP transcription factor family which usually shows important roles in meristem growth: snapdragon CYC controls the growth of the floral meristems and primordia; maize TB1 affects axillary meristem growth; rice PCF1 and PCF2 bind to the promoter of a gene (PCNA) involved in meristematic cell division (Cubas et al., 1999; Kosugi et al., 2002). Researches on the maize mutant *teosinte branched1* (*tb1*) have identified the *TB1* gene as a major contributor to the evolutionary change of maize from teosinte （Doebley et al., 1995; Doebley et al., 1997）. RNA in situ hybridization showed that *TB1* was expressed in maize axillary meristems and in stamens of ear primordia, consistent with a function of suppressing growth of these tissues （Hubbard et al., 2002）. The analysis of genetic locus and genome synteny suggests that *OsTB1* is a real counterpart of maize *TB1*. Transgenic rice plants overexpressing *OsTB1* exhibit markedly reduced lateral branching without the propagation of axillary buds being affected. Expression of *OsTB1*, as examined with a putative promoter–glucuronidase (GUS) gene fusion, is observed throughout the axillary bud, as well as the basal part of the shoot apical meristem, vascular tissues in the pith and the lamina joint (Takeda et al., 2003). The expression and function of *SbTB1* in sorghum are very close to those of the homologs and further proved to be regulated by Phytochrome B (Kebrom et al. 2006).

Although it is much more complex, the shoot branching of bamboo is somewhat like tillering of poaceous plant, e.g., rhizome development of caespitose bamboo and pluricaespitose bamboo. The preliminary research on bamboo chromosome has revealed that the chromosome size and complexity of bamboo genome are comparable to those of rice with the restriction that bamboos are polyploids（Gielis et al. 1997）. According to the recent analysis on eight full length mRNA sequences of bamboos (*Dendrocalamus latiflorus*, *Dendrocalamopsis edulis*, *Phyllostachys pubescens*) and cereal (rice, maize, wheat and barley) in the public nucleotides databases, bamboo probably has more shorter phylogenetic distance and similar sequence composition (GC content and codon usage) with rice than with other cereals (Fan et al. 2006). The molecular phylogeny in this paper showed that the TB1 homologs of bamboo tended to be grouped with OsTB1 instead of others even if they didn't share the highest sequence identity. Although plenty of axillary bud forms simultaneously during the formation of bamboo shoot, the buds don't outgrow until the bamboo shoot almost grows to its full length in a short time, indicating a strong apical dominance in the process. The expression of *PpTB1* in the tip of axillary bud coincided with the stages of bamboo shoot and was consistent with other homologs, suggesting that *PpTB1* probably played an important role in the suppression of bud outgrowth. In fact, the outgrowth of bamboo shoot from rhizome bud is also influenced by apical dominance, which determine the output of bamboo shoot and bamboo culm. Furthermore, the high concentration of IAA found in the rhizome bud and bamboo shoot is also an important signal of apical dominance in bamboo shoot (Huang et al., 2002) since apical dominance is thought to be caused by the apical bud producing IAA (auxin) in abundance. In addition, it should be further verified whether the aforementioned two transcripts of *PpTB1* have same expression patterns, translational efficiency and functions. Studies on maize

TB1 indicated that the change of TB1 transcriptional element instead of TB1 itself contributes greatly to the evolution of maize (Doebley et al. 1997; Wang et al. 1999; Clark et al. 2006). Since longer 3'UTR has been found to decrease markedly the translation of transcript (Mbongolo Mbella et al. 2000),the different transcripts found here might suggest another way to regulate the function of TB1 homologs, i.e., regulation of translational efficiency. Thus, further studies on the expression and functions of different *PpTB1* transcripts, and their relation to auxin in bamboo are helpful to elucidate molecular mechanism of bamboo shoot development, which are extremely significant to the production of bamboo.

Bamboo taxonomy

Another problem in bamboo research is the taxonomy of bamboo species. As we know, flower and fruit are the basis of generic taxonomy in most plants including *Bambuseae*. But most bamboo seldom flowers and when they does, they often flower without breeding. So the vegetative organs especially the structure of rhizome are often used in bamboo taxonomy, which are easily changed by environment and probably lead to misclassification. For example, the positions of *Sinobambusa*, *Indosasa*, *Acidosasa*, *Arundinaria*, *Pseudosasa*, *Oligostachyum* and *Pleioblastus* in bamboo taxonomy are still elusive since they have close branching type but are different in many other structures (Li et al. 2002). With the development of molecular biology in bamboo, molecular markers have been used to differentiate the bamboo genus. The molecular evolution of conserved genes is one of the most effective ways to elucidate the phylogeny. But few homologs genes have been applied to the taxonomy of genus since little is known about the genetic background of bamboo. TB1 homologs were used in this paper to analyze the evolution of three distant bamboo species representing scattered bamboo, caespitose bamboo and pluricaespitose bamboo respectively. It showed the phenotype of sympodial rhizome probably was more primitive than that of monopodial rhizome, which was consistent with the so-called conclusion that the development of bamboo individual from seed replayed the systematic evolution. However, the phylogeny also indicated *Phyllostachys praecox* might be more primitive than *Dendrocalamopsis edulis*, which was contrary to classical taxonomy. In our bamboo research program, the molecular phylogeny was also investigated in rice *MOC1* homologs from 12 bamboo species belonging to *Phyllostachys*, *Pleioblastus*, *Indocalamus* and *Bambusa*, which basically supported the aforementioned viewpoint (unpublished). Thus, the molecular evidence here showed that the traditional taxonomy only based on the difference of phenotype couldn't reflect the real evolution progresses. Like many other genus in bamboo, *Phyllostachys* and *Dendrocalamopsis* are separated based on rhizome branching, which means shoot branching related genes are very significant to explain the bamboo taxonomy. But due to the unbalance of evolution, it should be further proved whether the molecular evidences from floral development are consistent with those from shoot branching.

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不同光照强度对盆栽观赏竹生长的影响

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摘 要：以金镶玉竹和菲白竹为研究材料，对不同光照强度下盆栽观赏竹的生长进行了初步研究，认为70%的自然光照强度对盆栽观赏竹的生长比较有利的，不但能够提高盆栽观赏竹的成活率，还能增加新竹数量，对金镶玉竹的新竹高度、菲白竹的新鞭长度和芽数数量也有一定的促进作用。

关键词：光强；观赏竹；金镶玉竹；菲白竹

光照强度对竹类植物生长影响较大，已有学者进行过不同光照强度对一些观赏植物的生长影响研究，如对风信子(*Hyacinthus orientulis* L. blue daft)[1]，常春藤（*Hedera helix*）[2]，地被菊[3]，杜鹃花[4]等植物都有过研究，对一些竹类植物生长的影响也有过研究[5,6]，但对观赏竹的研究尚未有开展，对盆栽竹类植物的研究也未见报道，而作为观赏竹种，进行盆栽后一般都进入室内培养观赏，因此更需对其进行光强试验研究，所以我们对不同光照强度下盆栽观赏竹生长的变化进行了研究。

1 试验地概况和试验方法

1.1 试验地概况

试验地点位于浙江省林业科学研究院的竹类植物园内。30°16′ N；120°12′ E；50m.a.s.l，年均温为 16.2℃，≧10℃的平均年积温 5119.4℃，年降水量为 1320mm，无霜期 246d。

1.2 试验材料与方法

试验材料选取了 2 个竹种，1 个是散生类的金镶玉竹（*Phyllostachys aureosulcata* f. *spectabilis* C.D.），1 个是混生类的菲白竹（*Sasa fortunei* (Van Houtte) Fiori），金镶玉竹由于秆型较高，采用的是通过埋鞭繁殖后的竹苗，菲白竹则直接上盆栽培。于 2004 年 10 月 21 日上盆栽培，盆内基质为蛭石、泥炭、珍珠岩和黄泥的混合物，体积比为 40：40：10：10，再加总体积 5%的菜饼，用遮荫网对盆栽竹子进行不同程度的遮荫处理，使光照强度分别为自然光强的 70%、50% 、30% ，连同对照共形成 4 个光梯度。每个竹种每种处理各为 10 盆，遇久晴不雨时适当浇水。2005 年 11 月 28 日调查金镶玉竹的成活率、新竹数和新竹高度，菲白竹的成活率、新竹数、新鞭长度以及鞭芽数量。光照强度用 TES-1332 数位式照度计测定。

2 结果与分析

2.1 不同光照强度对成活率的影响

表1 不同光强处理的成活数 单位：盆

	对照	70%光强	50%光强	30%光强
金镶玉竹	3	7	4	1
菲白竹	4	5	2	1

不同竹种不同光强的盆栽观赏竹成活数量见表1。从表1可以看出，70%的光照强度的盆

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