Plant Physiology and Biochemistry 97 (2015) 156–164

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Identification of genes involved in color variation of bamboo culms by suppression subtractive hybridization

Xiangwan Xia [1], Renyi Gui [1], Haiyun Yang, Ying Fu, Fang Wei, Mingbing Zhou [*]

[a] The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang A & F University, Lin'an, Zhejiang Province, PR China

ARTICLE INFO

Article history:
Received 14 February 2015
Received in revised form
17 September 2015
Accepted 4 October 2015
Available online 9 October 2015

Keywords:
Phyllostachys vivax cv. aureocaulis
Stripy culms
Color variation
Photosynthetic membrane
Light harvesting complexes

ABSTRACT

Phyllostachys vivax cv. aureocaulis is a widely planted ornamental bamboo with evergreen leaves. This plant's culm exhibits a golden-yellow background color marked randomly with narrow and broad green stripes but is occasionally light green with yellow stripes. In this study, we attempt to identify the molecular mechanism underlying the color variation in striped culms. We found that neither stroma nor grana lamellas were observed in plastids in yellow tissue cells, while complete chloroplasts were observed in green tissue. In addition, chlorophyll a and b were mainly distributed in ground tissue under the epiderm and in the cells surrounding the bundle sheath in the green portion of internodes. The amount of chlorophyll contained in cross-sections of the green portion of culms is significantly higher than in the yellow portion. However, carotenoid was nearly undetectable in both samples. In addition, we found that the expression levels of 7 ESTs, including PvESTs-F641 (JZ893845), PvESTs-F681 (JZ893885) and PvESTs-F798 (JZ894002), were significantly higher in green samples than that in yellow samples, while PvESTs-R200 (JZ894906), PvESTs-R541 (JZ895247), PvESTs-R333 (JZ895039) and PvESTs-R266 (JZ894972) were found at a higher level in yellow samples. These ESTs are thought to play a role in this color variation in plants. Our current results indicate that insufficient photosynthetic membrane proteins and lipids in yellow tissue could lead to chloroplast dysfunction and could result in the yellow appearance on certain *P. vivax* cv. aureocaulis culms.

1. Introduction

Phyllostachys vivax cv. aureocaulis is a typical ornamental bamboo that has been cultivated over the last thirty years in mainland China to great economic, social and ecological benefit (Peixin Zhang and Zhang, 2013). The evergreen leaves and the glossy culm of *P. vivax* cv. aureocaulis are used ornamentally and display special alternating green and yellow stripes. Based on our observations, the stripe width, position, and number of culms are random and exhibit polymorphism. These striped culms represent a special type of variation in the plant world; there exist yellow bamboo internodes randomly marked with green stripes, and even green internodes with yellow stripes. There are also different striping types between different internodes on the same culm. Although this type of variation is common among bamboo species such as *Phyllostarchys heterocycla* cv. Huamozhu and *Bambusa oldhami* f. striata, the mechanism underlying striped culm variation in bamboo remains unknown. Plant pigmentation is generated through rather complex mechanisms, and color variation does not correspond to the mutation of any one gene or group of genes.

A functional abnormality of one or several genes could influence the metabolism, transportation, and accumulation of pigment, directly or indirectly leading to phenotypic variation in plant color (Eckhardt et al., 2004). Three main physiological processes are involved in the pigmentation of plant tissues: (1) The metabolism and accumulation of pigments (i.e., chlorophyll, carotenoid, and flavanoid). The metabolism of these pigments is complex, and functional mutations in any catalytic enzymes will result in a failure to synthesize pigments, resulting in a change in the color phenotype of plant tissue (Kim et al., 2005; XiaoQiu et al., 2011). Kim used VIGS (virus-induced gene silencing) to disable the NbERS gene, which encodes the glutamine tRNA synthetase involved in the synthesis of chlorophyll, resulting in a block in the chlorophyll synthesis pathway and a yellow leaf appearance (Kim et al., 2005).

* Corresponding author.
E-mail addresses: kasyouen@163.com (X. Xia), gry@zafu.edu.cn (R. Gui), yhy2006@zafu.edu.cn (H. Yang), fy97@163.com (Y. Fu), fwl@zafu.edu.cn (F. Wei), zhoumingbing@zafu.edu.cn (M. Zhou).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plaphy.2015.10.004
0981-9428/

In the chlorophyll-deficient mutant *ygl9*, a decrease in chlorophyll resulted in yellow-green leaves and a finely striped pattern (XiaoQiu et al.,2011). The transcriptional down-regulation of carotenogenic geneβ-ring hydroxylase led to a whitish loquat phenotype in *Eriobotrya japonica* Lindl (SUN et al., 2013). Furthermore, disabling the production of any of the many other pigments (i.e anthocyanidin, betalains) responsible for the different colors of plants would induce a phenotypic change (Eckhardt et al., 2004). (2) The development and degradation of chloroplast. The synthesis and degradation of chloroplasts require the combined effects of both chloroplast-encoded and nuclear genes (Waters and Langdale, 2009). The functional mutation of these genes may lead to defective chloroplasts with disorganized thylakoid systems or a lack of photosynthetic components. These mutations would in turn affect the stability of chlorophyll and other photosynthetic pigments, finally altering the content and distribution of the pigments within chloroplasts. For example, in *egy1*, a yellow leaf mutant, damage to the lamellar network leads to a decrease in the accumulation of the chlorophyll a/b binding proteins Lhca[2] and Lhcb[3] (Chen et al., 2005). In addition, signal transduction between chloroplast and nucleus may also influence the creation of a chloroplast (Koussevitzky et al., 2007). (3) Pigmentation can also be affected by the shape of cells (Noda et al., 1994), the pH (i.e., hydrogen ion concentration) (Brouillard, 1988), and many other factors.

SSH,[4] a PCR-based cDNA suppression subtractive hybridization method, has already been successfully utilized in bamboo to identify the differentially expressed genes involved in programmed cell death (Rai and Dey, 2012). In this study, SSH and bioinformatics methods were used to investigate differences in gene expression between *P. vivax* cv. aureocaulis bamboo shoots with yellow and green culms. This study contributes to our understanding of the mechanisms underlying culm color variation.

2. Materials and methods

2.1. Materials

P. vivax cv. aureocaulis grown under natural conditions in the Bamboo Garden of Zhejiang A & F University, Zhejiang Province, China were used for phenotypic observation and for other experimental purposes. *P. vivax* cv. aureocaulis has a yellow (or green) culm marked with green (or yellow) stripes of random width, number and location (Fig. 1A and B). Samples with almost entirely yellow culms were referred to as PVY (***P. V****ivax* cv. aureocaulis with **Y**ellow culms), and samples with almost entirely green culms were referred to as PVG (***P. V****ivax* cv. aureocaulis with **G**reen culms).

All *P. vivax* cv. aureocaulis bamboo were grown under identical conditions in the same bamboo forest, and both shoots came from one bamboo rhizome. PVG and PVY shoots of *P. vivax* cv. aureocaulis were collected during the shooting period from April to June in 2012. PVG and PVY shoots at the same development stage and the same height were chosen as a pair order to avoid differences in genetic background between two individual experimental samples.

One pair of PVG and PVY bamboo shoot samples, collected at the same time of the day on 05/21/2012, was chosen as starting material for SSH library construction. The meristematic tissues of this pair of bamboo shoots were chosen as experimental materials for subsequent RNA extraction, and were dissected from the shoots, shelled and stored at −80 centigrade (Fig. 2A and B) until SSH library construction and qPCR. These two samples were used as "tester" and "driver" samples in the construction of the SSH library.

In parallel, the closest internodes to the ground were collected from another pair of green and yellow shoots at the same time of the day on 05/09/2012 and were dissected and used as the materials of the observation of distribution of chlorophyll (Fig. 2C). One stripy internode of new bamboo culm, collected on 04/07/2012, was dissected and used for the observation of chloroplast ultrastructure (Fig. 2D and E). Different color strips from this internode were cut into cubes with and stored at −80 centigrade.

2.2. Confocal imaging of chlorophyll and carotenoid distribution

The green and yellow parts of striped *P. vivax* cv. aureocaulis culms (Fig. 2C) were sectioned at a 12 μm thickness on a cryostat microtome (CM 1860, Leica, Germany). LSCM[5] was used to study the distribution pattern of chlorophyll and carotenoid in the green and yellow parts of striped culms (LSM 710, Carl Zeiss AG. Oberkochen, Germany), and the results were recorded with photographs.

2.3. TEM study of chloroplast ultrastructure

The micro-ultrastructure of chloroplasts in both green and yellow parts of one internode (Fig. 2D and E) was observed under a transmission electron microscope (TEM)[6] (H-7650, HITACHI, Japan). Internodes were cut into 2.0 × 2.0 mm^2 yellow and green blocks. These tissues were immediately fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer, pH 7.0, in the dark for 24 h. Post-fixation dehydration and resin embedding were performed as described previously (Bourett et al., 1999), and sections were stained with lead citrate and uranyl acetate then observed by TEM.

2.4. Total RNA extraction and construction of an SSH cDNA library

Total RNA was isolated from the apical meristem of PVY and PVG shoots (Fig. 2B) using an RNeasy plant Mini Kit (Qiagen, Germany). Forward SSH library was constructed from PVG and PVY mRNA samples following the protocol supplied with the Smart PCR-Select cDNA subtraction kit (Clontech, Canada and USA), using PVG mRNA as the "tester" sample and PVY mRNA as the "driver" sample, while the reverse SSH library was synthesized using PVY mRNA as the "tester" sample and PVG mRNA as the "driver". After two rounds of subtractive hybridization, two rounds of suppression PCR amplification were successively carried out. The efficiency of SSH was determined by gel electrophoresis. The purified PCR products were transformed into Escherichia coli DH5α (Invitrogen, USA), and the recombinant clones were plated onto LB medium containing ampicillin, X-Gal, and IPTG then were incubated overnight at 37 °C. All of the white clones were picked for further analyses and sequencing by the Sanger method.

2.5. Bioinformatics analysis

2.5.1. Pre-procession of the ESTs

Raw sequences were trimmed of vector and adapter by Seqtrim (http://www.scbi.uma.es/seqtrim) and assembled using the CAP3 program (Huang and Madan, 1999). All passing singles and contigs were renamed as PvESTs-F# or PvESTs-R#, where # indicates the number in the forward and reverse pool).

[2] Light-harvesting complexes I.
[3] Light-harvesting complexes II.
[4] Suppression subtractive hybridization.
[5] Laser scanning confocal microscope.
[6] Transmission electron microscope.