Plant Science 266 (2018) 37–45

Contents lists available at ScienceDirect

Plant Science

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

Research Article

Gene mapping and transcriptome profiling of a practical photo-thermo-sensitive rice male sterile line with seedling-specific green-revertible albino leaf

Xin Li, Ying He*, Jie Yang, Yin-Hua Jia, Han-Lai Zeng*

MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, No.1, Shizishan Street, Hongshan District, Wuhan, Hubei Province, 430070, PR China

ARTICLE INFO

Keywords:
Photo-thermo-sensitive male sterile line
Green-revertible albino leaf
Ent-isokaurene C2-hydroxylase
Plant hormone
Gibberellins
Cytochrome P450
Oryza sativa

ABSTRACT

Abnormal environment weather can cause rice photoperiod-thermo-sensitive genic male sterile (PTGMS) lines fertile or partially fertile and thus cause the mixture of true hybrids with selfing seeds. Seedling-specific green-revertible albino leaf color mutant can be used to distinguish the real hybrids. Besides, it can also be used as an ideal material to research the development of chloroplast and biosynthesis of chlorophyll.

The phenotype of leaf color mutants includes light green, yellowing, albino, green-revertible albino. Gene mutations affecting the synthesis and degradation of photosynthetic pigments, lycopene and heme, the differentiation and development of chloroplast, gibberellins (GAs) biosynthesis, can change the leaf color.

We have created a PTGMS line with seedling-specific green-revertible albino leaf named W01S. The leaf phenotype, pollen sterility and fertility, agronomic traits, heredity, gene mapping and RNA-Seq of the differentially expressed genes between albino and green-revertible leaves were investigated. The results showed that W01S is a practical PTGMS line as Pei'ai 64S. The mutation of candidate gene Os03g0594100 (*ent*-isokaurene C2-hydroxylase-like) in W01S can be related to the biosynthesis of GAs, indole acetic acids, ethylene.

1. Introduction

The photo-thermo-sensitive genic male sterility line (PTGMS) of rice exhibit sterile pollen in long-day and high-temperature condition, while have fertile pollen in short-day and low-temperature condition [1]. Therefore, the PTGMS can be used as female parent in hybrid seed breeding during sterile stage and for self-reproducing by self cross during fertile stage. The use of this system in two-line hybrid rice seed breeding is simple, inexpensive and efficient.

However, most PTGMS lines require a specific temperature to retain their sterility. Abnormal natural weather could bring the temperature down to below the critical level that is required to prevent the conversion of PTGMS lines from sterility to fertility, simply termed 'fertility conversion', which makes PTGMS lines fertile or partially fertile in the location where they are supposed to be male sterility in normal years, resulting in the mixture of true hybrids with selfing seeds. Labeling the seeds from PTGMS lines with genetic markers to distinguish and remove the false hybrids from the mixture is practical for the safety of seed production.

Morphological and molecular markers have been investigated as genetic markers to distinguish the real F_1 hybrids from selfing seeds (false hybrids) at the seedlings stage. Leaf color mutant, a common mutant type, has attracted much attention because of its visible and discernible phenotype. The seedling-specific green-revertible albino leaf is a kind of leaf color mutant in rice. The mutation can be used as a phenotypic marker for monitoring seed purity in hybrid rice production, especially in two-line hybrid rice in which seed purity is usually affected by temperature. In addition, it can also be used as an ideal material for research on the development of chloroplast, regulation on biosynthesis of chlorophyll, signal transduction and photosynthesis mechanisms.

The leaf phenotype of these mutants includes light green, yellowing, albino, purple, spotted, zebra, green-revertible albino, bright-green. Gene mutations affecting the synthesis and degradation of photosynthetic pigments [2], anthocyanin, lycopene and heme [3], the differentiation and development of chloroplast [4] and other factors, can

Abbreviations: CMS, Cytoplasmic-genetic Male Sterile; PGMS, Photosensitive Genic Male Sterile; PTGMS, Photoperiod-Thermo-sensitive Genic Male Sterile; SSR, Simple Sequence Repeats; SNP, Single Nucleotide Polymorphism; InDels, small Insertion/Deletion; PCR, Polymerase Chain Reaction; PA64S, Pei'ai 64S (Sterile line); W01S, White Sterile line No.01; ROS, Reactive Oxygen Species; RNAi, RNA interference; GA3, gibberellins 3; IAA, indole 3-acetic acid; TIBA, 2, 3, 5-Triiodobenzoic acid

* Corresponding authors.
E-mail addresses: yinghe@mail.hzau.edu.cn (Y. He), zenghl@mail.hzau.edu.cn (H.-L. Zeng).

http://dx.doi.org/10.1016/j.plantsci.2017.10.010
Received 27 May 2017; Received in revised form 20 September 2017; Accepted 20 October 2017
Available online 31 October 2017
0168-9452/

cause changes in the leaf color.

The farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) in the biosynthesis process of gibberellins (GAs) can produce other terpenoids including sesquiterpene (abscisic acid), diterpene (GAs, phytol), triterpene and tetraterpene (cartenoid) [5]. Dysfunction mutation of GAs synthesis genes can also lead to leaf color change [6]. *OsKS1* encodes *ent*-kaurene synthase (KS), which catalyzes the second step of the biosynthesis of GAs, regulates the growth and development of the above ground part of rice and is sensitive to exogenous GAs. In the osks1 mutant, its stem elongation in seedling was retained and the leaf color was dark green [7]. *OsKS2*, another *ent*-kaurene synthase gene, also plays a role in the early step of GAs synthesis; the leaf of *osks2* mutants was short and dark green [8].

Our lab has created a new PTGMS line with seedling-specific green-revertible albino leaf. This PTGMS line was termed W01S (White Sterile line No.01). The first three leaves of W01S are albino and convert to green when the fourth leave come out. This trait of green-revertible albino leaf had no effect on the agronomic traits of the sterile line and its F_1 hybrids. The sterility and fertility performance of W01S was similar to that of Pei'ai 64S (PA64S). Showing an up-regulated process where the chloroplasts gradually developed normally, the chlorophyll content increased and the leaf turned green. Considering that the late growth period of the flag leaf is a process where the chloroplast stability is declined, its structure is damaged and its content of chlorophyll is decreased, we can speculate that the genes that control green-revertible albino leaf phenotype may also play a role in the regulation of the function of recession of the rice flag leaf during the late-growth period.

In this study, the leaf phenotype, ultrastructures of leaf chloroplast, pollen sterility and fertility performance, agronomic traits, heredity, gene mapping and RNA-Seq of differentially expressed genes between albino leaf and green-revertible leaf have been investigated. The results showed that W01S with seedling-specific green-revertible albino leaf phenotype is a practical photo-thermo-sensitive rice male sterile line. Moreover, the mutation of candidate gene (Os03g0594100) in W01S can be related to the biosynthesis of GAs, indole acetic acids, ethylene or cleavage of reactive oxygen species, thus leading to dysfunction of the chloroplast structure/function and the decrease of chlorophyll content. This research can be useful for future research on the development of chloroplast and biosynthesis of chlorophyll.

2. Materials and methods

2.1. Plant materials and growth conditions

A new sterile line designated W01S (White sterile line No.01), exhibiting seedling-specific green-revertible albino leaf, was produced following the pedigree chart depicted in Supplementary Fig. S1. One mutant plant exhibiting seedling-specific green-revertible albino leaf at 16–28 °C and male sterile phenotype was generated in the anther-culture population. In this mutant, the first three leaves, including the first incomplete leaf and the other two complete leaves, are almost albino, but the forth leaf and the subsequent leaves are all normal green. After the appearance of the fourth leaves, the earlier leaves also become green if they are still alive. The perfect candidate was eventually obtained and named WS (White-Sterile line). WS was backcrossed for three generations with PA64S. PA64S is one of the male sterile lines that had the most cross combinations and biggest application area in China (http://www.ricedata.cn/variety/index.html). Eventually, W01S was generated. In addition, reciprocal crosses of W01S with Gui 99 or Mian Hui 725 were also performed to produce the first and second generation seeds (F_1 and F_2). The breeding process was conducted in Wu'han (30°47′N, 114°35′E), China and Ling'shui (110°1′E, 18°29′N), China.

Paddy field experiments were carried out at Huazhong Agricultural University in Wuhan (30°47′N, 114°35′E) from May to October. The germinated seeds of W01S and PA64S, were sown directly in a paddy field on May 15. The 25-day-old seedlings were transplanted at a spacing of 20 × 20 cm^2 in the paddy field or three seedlings per pot (16 cm in diameter, 20 cm in depth). The total amount of N fertilizer applied was 12.5 kg per 667 m^2, with a N-P-K ratio of 1:0.6:0.9. The first batch of sterile panicles of W01S and PA64S began to appear at about August 5. The second batch of fertile panicles which are stem from the dormant buds began to appear at about October 1.

Growth chamber experiments were carried out in Huazhong Agricultural University, with the average daily air temperature and photoperiod were set at 24 °C/12 h (Supplementary Fig. S2) for seedlings used in RNA-seq and plant hormone treatment. The illumination intensity was 3000 LX and the relative humidity in the growth chamber was 60%. The leaves were sampled, quick-frozen in liquid nitrogen, and stored at −80 °C until further testing. The panicles were collected for pollen fertility examination.

2.2. Transmission electron microscopy (TEM) analyses

For TEM analysis, a transmission electron microscope (H7650, Hitachi, Tokyo, Japan) was used, as previously described [9]. The third and fourth leaves of W01S and PA64S were examined.

2.3. Pollen fertility examination

Around the end of July, the length of young spikelets was 1-1.5 cm, which indicated the third and fourth stage of spikelet differentiation. Meanwhile, the pots with rice plants were moved into the growth chamber maintained at an average daily air temperature of 23.5 °C, 14.5 h of illumination (long-day treatment) and average air temperature of 21.0 °C, 12.5 h of illumination (low-temperature treatment). After 12 days of incubation, the plants were moved out of the growth chamber and continued to grow under natural temperature and illumination conditions. These rice plants would be heading and flowering after about seven days. To analyze the pollen fertility, we chose the panicles that appeared between the seven to 15 days after treatments in the growth chamber. Anthers of five mature flowers from each plant at heading time were collected. Pollen grains were then stained with 1% potassium iodide solution (I_2-KI) and photographed under a microscope [10].

2.4. Agronomic traits statistics

The statistics of agronomic traits, including plant height, flag leaf length, panicle length, neck-panicle node length, grain number, seed setting rate, were collected from 15 individual main stem. The 1000-grain weight and the yield were determined in three replicates. The agronomic traits of LYP9 (Liang you pei 9, 65002) were obtained from the website of the National Rice Data Center of China (http://www.ricedata.cn/variety/varis/600132.html).

2.5. Gene mapping

Genomic DNA was extracted from fresh-frozen leaves of W01S and PA64S using the cetyltrimethylammonium bromide (CTAB) method [11]. The extracted DNA was dissolved in Tris-EDTA (TE) buffer. A total of 325 Simple Sequence Repeats (SSR) markers, with an interval of about 10 cM on the chromosomes, were used in this study (Supplementary Table S1), their information was downloaded from the website (http://www.gramene.org). The SSR primers were synthesized by SBS Gene Technology Ltd. (Shanghai, China). DNA amplification was performed by PCR using the following parameters: an initial cycle of 3 min at 94 °C; 35 cycles of 60 s at 94 °C, 60 s at 55–62 °C, 60 s at 72 °C; and a final cycle of 10 min at 72 °C. Reactions were carried out in 10 uL containing 0.2 uL of each primer (10 uM), 1 uL of dNTPs (2 mM), 20 ng of DNA template, 0.8 uL $MgCl_2$ (25 mM), 1 uL of 10 × PCR buffer and 0.5 U of Tag polymerase.