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A New Gene Conferring the Glabrous Trait in Cucumber Identified Using MutMap

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Abstract

Tubercles and spines on fruit peel are important commercial traits in cucumber (*Cucumis sativus* L.). From an ethyl methane sulfonate cucumber mutant library, we discovered a new glabrous mutant that bears no tubercle or spine on fruit peel and fewer and smaller trichomes on the stem and leaves. The new locus is here designated as *glabrous2* (*gl2*). Genome sequencing of the mutant and linkage analysis revealed that a non-synonymous mutation in the *Csa1G056960* gene rendered the *gl2* phenotype. The mutated gene encodes a C-type lectin receptor-like tyrosine protein kinase. This study provides a novel allele for elucidating the genetic basis of wart and trichome development and a new tool for breeding glabrous cucumber varieties.

Keywords: *Cucumis sativus*; ethyl methane sulfonate; glabrous mutant; MutMap

1. Introduction

Cucumber (*Cucumis sativus*) is an economically important vegetable crop worldwide (Huang et al., 2009). Compared with other Cucurbitaceae fruits, cucumber fruits have a distinguishing wart trait, which is closely related to its economic value. In general, cucumber fruits possess warty and non-warty peel types; Warty peel is present in Chinese cultivated species while non-warty peel is predominant in European and American cultivars (Yang et al., 2009). Non-warty fruits have the advantages of great taste, easy cleaning, resistance to transport, and less pesticide residues, and cucumber varieties with these characters are desirable for producing pollution-free vegetables (Wang et al., 2007). Thus, breeders are working on the warty/non-warty fruit trait to improve the external value of cucumber fruit.

Warts in cucumber are composed of tubercle and spine (Yang et al., 2014). It was reported that the warty fruit trait is dominant to that of the non-warty fruit trait, which is controlled by a single tuberculate fruit (*Tu*) gene (Strong, 1931; Poole, 1944; Andeweg, 1956; Walters et al., 2001). Lately, *Tu* was found to encode a C_2H_2 zinc finger protein on chromosome 5, and its function may be related to cytokinin biosynthesis (Yang et al., 2014). Recently, a class I homeodomain-leucine zipper (HD-Zip) gene was reported to play a critical role in trichome development (Li et al., 2015; Zhao et al., 2015). In this paper, we report the discovery of a new glabrous cucumber mutant

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induced by ethyl methane sulfonate (EMS), which has no warts (tubercles or spines) on the fruit peel and fewer and smaller trichomes on the leaf, stem, and other organs than the wild type.

MutMap is an efficient approach to find important agronomic traits by whole-genome re-sequencing of the bulked DNA from mutant and wide-type plants in segregating populations (Abe et al., 2012). DNA sequencing technology is becoming easier and cheaper; therefore, MutMap can aid the isolation of agronomically important genes by reducing the time and labor involved. In this study, we used the MutMap approach coupled with genetic analysis and found that a non-synonymous mutation in the cucumber gene encoding a C-type lectin receptor-like tyrosine protein kinase rendered the glabrous mutant.

2. Materials and methods

2.1. Plant material

The cucumber inbred line 406 of North China type was used for the mutagenesis. Seeds of line 406 were treated with 1% EMS (Sigma M0880) following Tadmor et al. (2007). M_1 plants were self-pollinated and the glabrous cucumber mutant was identified in an M_2 population. An F_2 segregating population was generated by crossing the glabrous mutant with the wild-type (WT) line 406. All plants were grown in a greenhouse in the Hunan Vegetable Research Institute, Changsha, China.

2.2. Generation and analysis of next-generation sequencing data

Genomic DNA was extracted from fresh leaves using the standardized CTAB method (Murray and Thompson, 1980). Two DNA pools were constructed: the M-pool was constructed from 15 F_2 plants with the mutant phenotype, and the N-pool was constructed from 15 F_2 plants with the WT phenotype. The two pools were re-sequenced at a depth of ~10× using an Illumina HiSeq 2000 sequencer.

Pair-end sequencing libraries (read length 100 bp) with insert sizes of about 500 bp were prepared for sequencing. The short that were obtained reads were aligned against the 9930 (Chinese long cucumber) reference genome (Huang et al., 2009) using Burrows-Wheeler Aligner (Li and Durbin, 2009). The output alignment files were loaded into the SAMtools software package (Li and Durbin, 2009) to identify single nucleotide polymorphisms (SNPs).

The SNPs were filtered using Python scripts according to the following criteria: (1) To reduce the numbers of false positives from sequencing or alignment errors, low-quality SNPs with base quality value < 20, read depth < 3×, and/or > 40× coverage were excluded; (2) The homozygous bases (SNP-index ≥ 0.8) in the M-pool and 406 were retained. In order to reduce the numbers of false positive SNPs, the homozygous SNPs of M-pool same to N-pool were removed. Compared with WT line 406, SNPs of M-pool that exhibit G to A or C to T transitions were extracted for further analysis, which are the most frequent mutations caused by EMS mutagenesis (Abe et al., 2012).

2.3. Development of dCAPS markers

Derived cleaved amplified polymorphic sequences (dCAPS) markers were designed based on selected SNPs using dCAPS Finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html) (Neff et al., 2002). The volume of the PCR reaction system was 16 μL containing 10 ng template DNA, 8 μL 2× GoTaq® Green Master Mix, and 0.25 μmol · L^{-1} each of the forward and reverse dCAPS primers. The PCR conditions consisted of an initial DNA denaturation for 4 min at 94 ℃, followed by 35 cycles of 20 s DNA denaturation at 94 ℃, 20 s annealing at 58 ℃, and 30 s elongation at 72 ℃, then a final 5 min elongation at 72 ℃ before holding at 4 ℃. The PCR products were digested with the corresponding restriction enzymes overnight and analyzed by 8% polyacrylamide gel electrophoresis.

3. Results

3.1. Morphological characterization of the glabrous mutant

The mutant [here named *glabrous2* (*gl2*)] had no warts on the fruit peel and smaller and fewer trichomes on the stem, leaves, and other organs compared with the WT line 406 (Fig. 1, A and B). All the F_1 plants had the same phenotype as the WT. In an F_2 population consisting of 220 plants, we found 23 plants with non-warty fruits and fewer trichomes on the stems and leaves. These data suggested that the *gl2* mutant phenotype was controlled by a recessive gene.

3.2. Identification of the candidate gene for gl2

The HiSeq 2000 re-sequencing generated 45 million reads for the M-pool library with 10× depth and 98% coverage and 41 million reads for the N-pool library with 10× depth and 97% coverage. It was expected that the SNPs responsible for the recessive phenotype was homozygous in the M-pool and heterozygous in the N-pool. In total, 67 homozygous SNPs of M-pool were identified (Table 1). Removing the common SNPs (unrelated SNPs) shared by at least five other mutant lines with different phenotypes (data unpublished), 38 homozygous SNPs were remained (Fig.2, A).18 SNPs of them in M-pool displayed G/C to A/T transitions compared with WT line 406 (Table 2),