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Chlorophyll Content, Chloroplast Ultrastructure and Transcriptome Analysis in Wild-type and Yellow-bud-mutant Hot Peppers

Z. H. Ma[1], G. S. Sun[1], C. W. Zhang[2], Q. Wang[3], Z. L. Dai[1], C. Q. Sun[1], and Y. P. Pan[1*]

ABSTRACT

The yellow-bud mutant hot pepper, 96-140YBM, which exhibits a yellow leaf phenotype in its young leaves but whose matured leaves are green, was isolated from wild type 96-140 in this study. The results of photosynthetic pigment determination and chloroplast ultrastructure observation revealed that the young mutant leaves displayed Chl a+b and Cars content, increased Chl a/b and Car/Chl a+b ratios, and delayed chloroplast development compared with the wild type leaves. Here, we obtained 95,714 transcripts from cultured yellow-bud mutant yellow leaves and cultured wild-type seedling leaves using the Illumina HiSeq-2000 (Illumina Inc., USA) platform. A total of 42,384 unigenes were identified, among which 37,949 were annotated using gene descriptions or gene ontology terms. Based on Differentially Expressed Genes (DEG) analysis, 1,056 of the 1,101 DEGs were annotated in the Nr database, and 302 unigenes were mapped to 130 pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. Finally, we found that 6 pathways were related to chloroplast and chlorophyll biogenesis.

Keywords: Hot pepper; Chloroplast; Transcriptome; Yellow-bud mutant; Wild type.

INTRODUCTION

Hot pepper (*Capsicum annuum* L.), a type of annual or perennial herb that belongs to *Solanaceae Capsicum* (Salari, *et al*., 2012), is an important commercial vegetable, one of the main crops for greenhouse cultivation (Abdel Latef, 2013) and an indispensable ingredient in recipes around the world (Arptia *et al*., 2012). Hot pepper is native to South America and has since spread globally. In the late Ming dynasty (1740), the hot pepper was introduced to China and was subsequently widely planted because of its popular flavor and rich nutrition. Currently, the hot pepper is one of the most widely planted vegetables in China, with over 20 million acres planted, second only to Chinese cabbage (Zou, 2002).

Leaf etiolation mutants have been identified in several plant species such as cotton, maize, rice, and cabbage (Killough and Horlacher, 1993; Liu *et al*., 2007; Roth *et al*., 2001; Tanya and Falbel, 1996; Falbel *et al*., 1996). These mutants typically exhibit yellow-green leaves either during the seedling phase or throughout the entire growth period. These mutants can be used not only as morphological markers in genetic crop breeding but also to examine

[1] Zhenjiang Institute of Agricultural Sciences in Hilly Area of Jiangsu Province, 112# Ninghang Road, Jurong, Jiangsu, 212400, People's Republic China.
[2] College of Horticulture, Nanjing Agricultural University, 1# Weigang, Nanjing, Jiangsu, 210095, People's Republic China.
[3] Department of Horticulture, Jinling Institute of Technology, 130# Heyan Road, Nanjing, Jiangsu, 210038, People's Republic China.
[*] Corresponding author; e-mail: pyp1962@163.com

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chloroplast development, photosynthetic characteristics, and the interaction between the chloroplasts and the nucleus (Killough and Horlacher, 1993; Zhang *et al*., 1996; Jong *et al*., 1998; Ryder *et al*., 1999; Zhao *et al*., 2008).

The mechanism underlying leaf etiolation or modification is the breakdown of chlorophyll or other pigment synthase genes during chloroplast development (Klein *et al*., 1988; Falbel and Staehelin, 1994; Oster *et al*., 2000; Masuda *et al*., 2003). Van der Biezen *et al*. (1996) reported a tomato mutant characterized by yellow to light green cotyledons and leaves that contained half as much chlorophyll as the wild type. The aurea and yellow–green F_2 mutants of tomato were characterized by a severe reduction in protochlorophyllide accumulation (Terry *et al*., 2001). An undifferentiated plastid mutant of maize exhibited disrupted plastid biogenesis in the bundle sheath and in mesophyll cells, resulting in a reduction of chloroplast-encoded proteins (Roth *et al*., 2001). Wu *et al*. (2007) isolated a rice (*Oryza Sativa*) Chl-deficient mutant, yellow-green leaf 1 (ygl1), which displayed sequence similarity to the *Chl synthase* gene according to map-based cloning.

However, leaf coloration mutants have not been reported for hot pepper. In this study, we isolated a yellow-bud mutant of hot pepper, 96-140 YBM, from the wild type, 96-140. The mutant plant exhibits a yellow leaf phenotype in its new leaves, which turn green as they mature. Throughout the entire growth period, the disease resistance, light intensity and temperature tolerance, and biomass of this mutant were no different from the wild type. Genetic analysis has revealed that this mutant is recessive with stable genetics and can be used for hybrid breeding and for purifying experimental F_1 seeds. To explore the mechanism underlying this mutant, we examined the relationship between the leaf color change and both chlorophyll content and chloroplast ultrastructure. Moreover, *de novo* transcriptome sequencing of the leaves was performed to identify the mutant genes.

MATERIALS AND METHODS

Plant Materials

Hot pepper (*Capsicum annuum* L.), including yellow-green seedling mutant '96-140 YBM' and Wild Type (WT) '96-140', was cultivated in the greenhouse of the Zhenjiang Institute of Agricultural Sciences in the hilly area of Jiangsu Province. All plants were grown under standard greenhouse conditions, 25-28 ℃, 65-75% relative humidity, and 16 hour light/8 hour dark cycles. Two months later, newly grown 96-140 YBM yellow leaves and newly grown green 96-140 leaves were picked for experimentation.

Prior to this, 96-140 were crossed with 96-140 YBM to obtain F_1 (96-140×96-140 YBM) seeds, F_2 (96-140×96-140 YBM) seeds were obtained by F_1 (96-140×96-140 YBM) selfing; same ways to produce F_1 (96-140 YBM×96-140) and F_2 (96-140 YBM×96-140) seeds. Each F_1 and F_2 performance was genetically analyzed.

Chlorophyll and Carotenoid Levels Determination

The Chl and Car contents were determined using DU 800 UV/Vis spectrophotometers (Beckman Coulter Inc., USA). The newly developed leaves (approximately 30 mg fresh weight) of the wild type and the mutant were cut and homogenized in 5 mL acetone:0.1M NH_4OH (9:1 V/V), followed by centrifugation at 3,000×g for 10 minutes. The supernatants were combined and washed three times with an equal volume of hexane prior to spectrophotometric analysis. All tests were repeated three times.

Transmission Electron Microscopy Analysis

Wild-type '96-140' and mutant '96-140 YBM' leaf samples were harvested from newly cultivated plants in a greenhouse at medium light intensity (approximately 150 μmol photons m^{-2} s^{-1}) and were immediately pre-fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide in the same phosphate buffer. All specimens were dehydrated using a graded alcohol series and were then embedded in Spurr's resin. Ultrathin sections were generated using a Leica ultramicrotome, double-stained with uranyl acetate and lead citrate, and subsequently observed and photographed using a JEM-1230 transmission electron microscope.

De Novo Transcriptome Sequencing

Fifteen healthy strong plants were selected from each genotype, which were selected from the selfing progeny of the original yellow bud mutant and the wild type. Trizol (TAKARA, Japan) was used to extract RNA from each plant, and the RNA concentration was determined using a Nano Drop 2000™ micro-volume spectrophotometer (Thermo, USA). The RNA samples were pooled to generate equal amounts for the production of a cDNA library. Pooling is a cost-effective strategy when the primary research goal is to identify gene expression profiles (Xu *et al*., 2012). This strategy was well justified based on statistical and practical considerations (Peng *et al*., 2003; Liu *et al*., 2010; Everett *et al*., 2011).

The mRNA was condensed and purified using oligo (dT) and magnetic beads followed by incubation in a ribonuclease reagent that fragmented the mRNA into 200-700 nt fragments. The fragmented mRNA was used as a template to synthesize double-stranded cDNA using random hexamer primers. The products were purified using the QiaQuick PCR Kit (QIAGEN Inc., GER) and were washed with EB (Ethidium Bromide) buffer. For end repairing, poly (A) was added, and Solexa sequencing adaptors were ligated onto the fragments. The segments were extended *via* PCR after selecting the appropriate fragments via agarose gel electrophoresis, and the constructed library was sequenced using an Illumina HiSeq™ 2000 sequencing system.

The average read length obtained was 90 bp. After eliminating the low-quality reads (more than 5% unknown nucleotides or more than 50% bases with a Q-value≤ 20), we obtained the clean reads. Then, we performed the Trinity method (Grabherr *et al*., 2011) to *de novo* assemble the reads to form contigs. Based on the paired-end reads, the order of the available contigs was used to assemble the transcripts, and then, all transcripts were clustered into unigenes.

The sequence directions of the resulting unigenes were determined by performing BLASTX searches against protein databases using the priority order of Non-Redundant protein sequences in NCBI (NR), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes database (KEGG), and COG (E-value≤ 1e-5) if conflicting results were obtained. ESTScan software (Iseli *et al*., 1999) was also used to determine the directions of the sequences that were not aligned to those in any of the databases mentioned above.

The expression levels of each unigene were measured as the number of clean reads mapped to its sequence. The number of clean reads mapped to each annotated unigene was calculated and then normalized to the number of Reads Per Kb per Million reads (RPKM) using ERANGE3.1 software (Mortazavi *et al*., 2008), followed by adjustment using a normalization factor (Robinson and Oshlack, 2010).

Unigene Annotations and Differentially Expressed Gene (DEG) Analysis

The unigenes assembled using the Trinity method longer than 200 bp were annotated according to their sequence similarity to previously annotated genes. We used sequence-based and domain alignments to compare these sequences. The sequence-based alignments were performed using three public databases (NR, Swiss-Prot and

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KEGG; significant thresholds of E-value≤ 1e-5). The domain-based alignments were generated using the COG database at NCBI at a threshold *E*-value≤ 1e-5.

The resulting BLAST hits were processed using Blast2GO software (Conesa *et al*., 2005) to retrieve associated Gene Ontology (GO) terms describing the biological processes, molecular functions, and cellular components of these genes (Ashburner *et al*., 2000).

KEGG mapping was performed to identify the metabolic pathways corresponding to these genes (Kanehisa and Goto, 2000; Rismani-Yazdi *et al*., 2011). The sequences with corresponding EC numbers obtained from Blast2GO were mapped to the KEGG metabolic pathway database. To further enrich the pathway annotation and to identify the BRITE functional hierarchies, these sequences were also submitted to the KEGG Automatic Annotation Server (Moriya *et al*., 2007), and the single-directional best-hit information method was selected. KAAS annotates every submitted sequence using KEGG Orthology (KO) identifiers, which represents an orthologous group of genes directly associated with an object in the KEGG pathways and the BRITE functional hierarchy (Moriya *et al*., 2007; Mao *et al*., 2005). Therefore, these methods incorporate different types of relationships that exist in biological systems i.e., genetic and environmental information processing, cellular processes, and organism systems.

The DEGs were indentified using IDE6 software with the general *Chi*-squared method, using a threshold *False Discovery Rate (FDR)*≤ 0.01 for the analysis of the results. The gene expression levels were estimated using *RPKM* values. When the *RPKM* value of one gene in a sample was twice that of another sample, we considered the gene a DEG. The DEGs were annotated using the Nr, KEGG, COG and GO databases as described for the unigene annotation.

RESULTS

Genetic Analysis

Reciprocal crosses between the yellow bud mutant and wild type of hot pepper in F_1 progenies revealed that there was no separation and all the leaves were green. Therefore, it could be inferred that yellow color trait was a recessive character. The self-fertilization F_2 progenies of each reciprocal cross appeared segregation of 3:1, which agreed well with the theoretical ratio. And then, it could be concluded that the yellow trait was controlled by one recessive gene (Table 1).

Table 1. The segregation of F_2 progeny from reciprocal crosses of 96-140 and 96-140 YBM.[a]

Combination	Phenotypes	Green	Yellow	Total
F_1 (96-14×96-140 YBM)	Result	100	0	100
	Excepted result	100	0	100
F_1 (96-140 YBM×96-140)	Result	100	0	100
	Excepted result	100	0	100
F_2 (96-140×96-140 YBM)	Result	1213	435	1592
	Excepted result	1194	398	1592
	χ^2		χ^2=1.64	
F_2 (96-140 YBM×96-140)	Result	987	309	1296
	Excepted result	972	324	1296
	χ^2		χ^2=0.87	

[a] Reciprocal crosses results shows χ^2 (1.64, 0.87 *df* =1)< $\chi^2_{0.05,1}$= 3.84. The results matched the theory ratio 3:1, the yellow trait was cause of one recessive gene.

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Photosynthetic Pigment Determination and Chloroplast Ultrastructure Observation

The '96-140YBM' mutant was a spontaneous mutant isolated from cultivar '96-140' that exhibited a yellow-bud phenotype. At the seedling stage, its newer leaves were yellow, but its older leaves were yellow-green. After the blossom stage, the newly developed mutant leaves were yellow, the leaves in the middle portion of the plant were yellow-green, and the bottom leaves were green (Figurer 1). The mutant plant height was no shorter than wild type throughout the developmental stage. It exhibited increased levels of Chl a+b as well as Car content (Figures 2-A and -B). The contents of Chl a+b and Car were higher in the wild type than in the mutant at various stages, with the most significant differences at the seedling stage. The Chl a/b and Car/Chl a+b ratios of the mutant were higher than those of the wild type at various stages and appeared to be highest at the blossom and young seedling stages. After the blossom stage, the contents of Chl a+b and Car and the Chl a/b and Car/Chl a+b ratios of the mutant were not significantly different from those of the wild type (Figures 2-C and -D).

To investigate the relationship between leaf etiolation and the development of chloroplast, the ultrastructures of the chloroplasts in the mutant and wild-type plants at the seedling stage were compared by transmission electron microscopy. Compared with the wild type plant, there were fewer thylakoid grana in the mesophyll cells of the mutant plant, and the thylakoid grana were irregular and unclear. Besides, there were starch grains of different sizes and more osmiophilic globules in the mesophyll cells. In the wild type, the chloroplast ultrastructure was normal (Figure 3-B), there were more clear thylakoid grana, and less osmiophilic globules and regular starch grains in the mesophyll cells.

Figure 1. Phenotypic characterization of the pepper '96-40YBM 'mutant and its wild type. (A) Young seedling stage; (B) Seedling stage; (C) Blossom stage, (D) Fruit stage.

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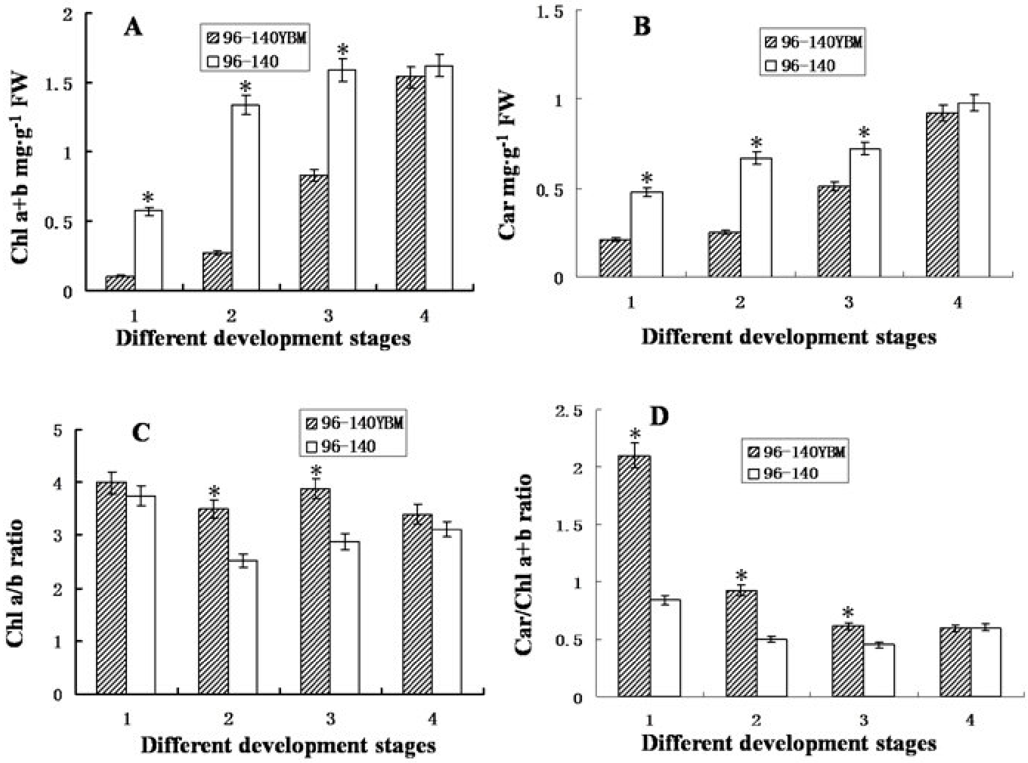

Figure 2. Comparison of pigment contents in leaves of WT and '96-140 YBM' mutant during different development stages**.** (A) Chl a+b content; (B) Car content; (C) Chl a/b ratio; (D) Car/Chl a+b ratio. 1: Young seedling stage; 2: Seedling stage; 3: Blossom stage; 4: Fruit stage. Differences between the 96-140 and 96-140 YBM data were tested with a *T* test. * Indicates a significant difference between the two data (< 0.05).

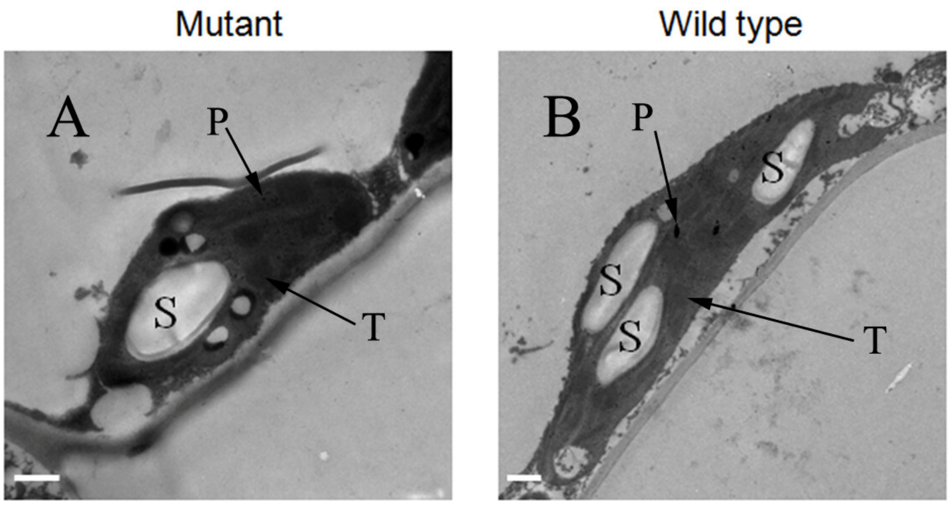

Figure 3. Chloroplast ultrastructures of '96-140 YBM' mutant and its wild type. (S) Starch grains; (T) Thylakoid grana; (P) Osmiophile globule, Bar= 1μm.

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Transcript and Unigene Assembly

From the data obtained from transcriptome sequencing of YBM and WT (2.4 G and 2.8 G, with a GC content of 44.68 and 44.48%, respectively), 91.28 and 92.31% of the transcripts had a score of Q20 (Error probability≤ 1%), with an average length of 90 bp. Trinity assembled 77,343 and 95,714 transcripts with an N50 length of 1,637 and 1,653 bp, with average lengths of 1,003 and 1,019 bp, respectively.

DEGs

Using IDE6 software, 1,101 genes matching the criteria of DEGs, and 1,056 of these DEGs were annotated in the Nr database. We selected the genes displaying values of *log2 [RPKM(YBM)/RPKM(WT)]≥* |10| to reduce the number of dubious DEGs; 127 of the 1,101 DEGs were eliminated, all of which were annotated in the Nr database (Figure 4).

GO and COG Classification of the DEGs

The unigenes homologous to known sequences in NR, Swiss-Prot, and KEGG were further annotated with GO terms using Blast2GO (Conesa *et al*., 2005). A total of 865 (78.56%) DEGs were assigned 1,101 GO term annotations, which could be classified into three categories: biological process, molecular function, and cellular component (Figure 5).

All assembled unigenes were further annotated based on COG category (Tatusov *et al*., 2001). A total of 417 DEGs were assigned 1,101 functional annotations, which could be grouped into 25 functional categories (Figure 6). The largest category was “General function prediction only”. The following COG categories were associated with chloroplast and chlorophyll: chromatin structure and dynamics, cell cycle control,

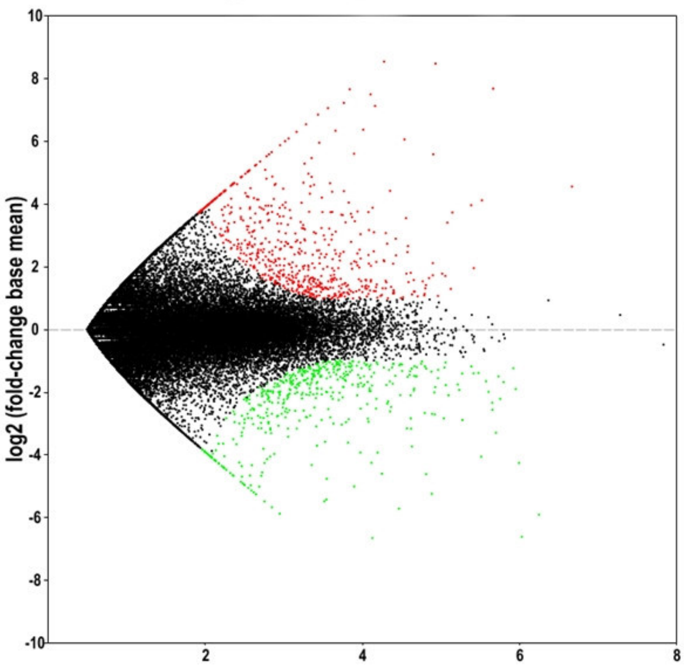

Figure 4. WT and YBM expression plot. In this figure, grey dots above zero line in *y*-axis indicate genes had a higher expression level in WT, while grey dots below zero line in *y*-axis indicate genes had a higher expression level in YBM, and black dots indicate that genes were similar in both libraries. *FDR*<0.001 and *log2Ratio*≥ 1 were used as the thresholds to judge the significance of gene expression difference.

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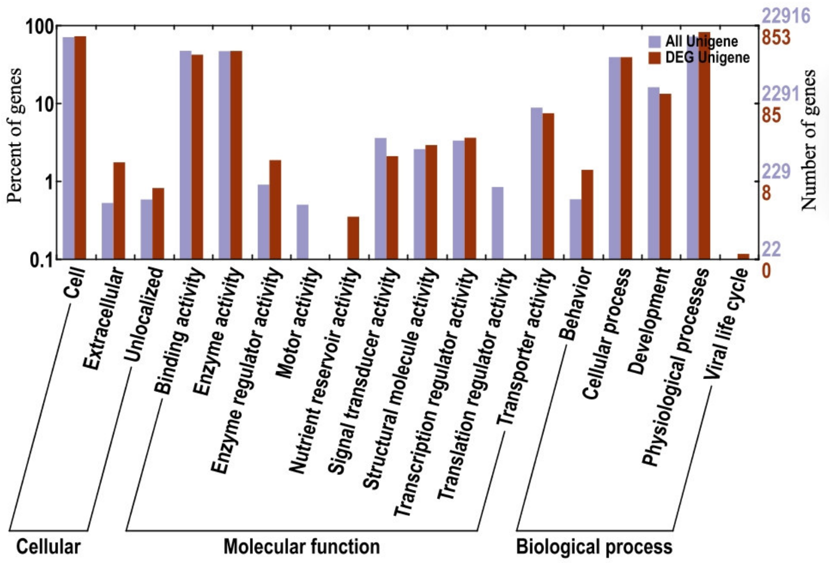

Figure 5. Functional annotation of DEGs and unigenes based on gene ontology (GO) categorization of hot pepper. GO classification of all annotated unigenes and DEG unigenes. All terms belonged to the three main GO categories: Biological process, cellular component and molecular function. The *x*-axis indicates the subcategories; the right *y*-axis indicates the number of genes in each category, the left *y*-axis indicates the percentage of a specific category of genes in the corresponding GO category.

cell division, chromosome partitioning, carbohydrate transport and metabolism, translation, ribosomal structure and biogenesis, and signal transduction mechanisms. The unigenes annotated to the COG categories will be deeply studied in the future.

KEGG Pathway Mapping of DEGs

To identify the biological pathways that were differentially activated in the yellow-bud mutant, the assembled DEGs were annotated using the EC numbers from the BLASTX alignments and the KEGG database (E-value≤ 1e-5). The assigned EC numbers were mapped to the reference canonical pathways. As a result, carbon fixation in photosynthetic organisms, porphyrin and chlorophyll metabolism, photosynthesis - antenna proteins, carotenoid biosynthesis, photosynthesis, and photosynthesis proteins were associated with leaf color mutant. The six pathways related to the molecular function are listed in Table 2.

DISCUSSION

Until now, many yellow bud mutants have been identified, such as tobacco (Archer and Bonnett, 1987), peanut (Benedict and Ketringd, 1972), cotton (Benedict and Kohel, 1968; Benedict and Kohel, 1970; Song *et al*., 2012), rice (Archer and Bonnett, 1987; Dong *et al*., 2013) and so on. All these were of nuclear inheritance and controlled by a pair of allogene (Ma *et al*., 2013). However, the related research has not been reported on hot pepper.

The etiolation mechanisms of leaf mutants, including their genetic characteristics, microstructures, absorption spectra, fluorescence, and physiological properties, have recently been systematically evaluated (Falbel *et al*., 1996; Runge *et al*., 1995; Havaux and Tardy, 1997). Cultivars exhibiting green leaves display higher

[DOR: 20.1001.1.16807073.2016.18.4.2.6]

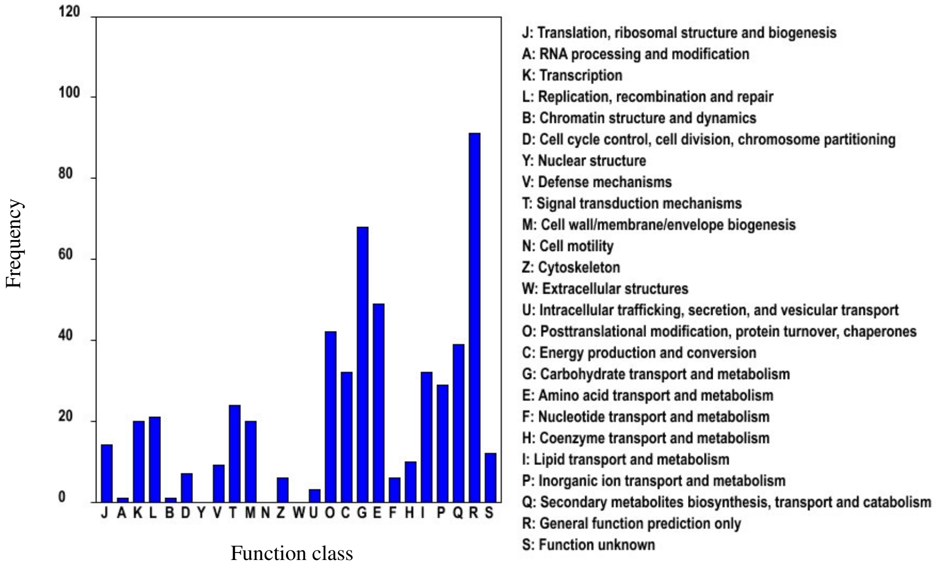

Figure 6. Clusters of DEGS COG classification of hot pepper. All putative proteins were aligned to the COG database and were functionally classified into at least 25 molecular families. The capital letters in *x*-axis indicates the COG categories as listed on the right of the histogram, and the *y*-axis indicates the frequencies of a specific category of DEGs in the corresponding COG category.

Table 2. The unigenes met with the related KEGG and their main function annotated in GO database.

Unigene Code [a]	Function	GO [e] Number	KEGG [f]
21794	C and C [b]: Chloroplast envelope	GO:0009941	Carbon fixation in photosynthetic organisms
8462	C and C: Chloroplast thylakoid	GO:0009534	
26723	C and C: Chloroplast envelope B and P [c]: Chlorophyll biosynthetic process	GO:0009941 GO:0015995	Porphyrin and chlorophyll metabolism
39640	C and C: Chloroplast B and P: Photosynthesis, light harvesting M and F [d]: Chlorophyll binding	GO:0009507 GO:0009765 GO:0016168	Photosynthesis - antenna proteins
33067	B and P: Anthocyanin-containing compound biosynthetic process	GO:0009718	Carotenoid biosynthesis
8197	C and C: Chloroplast thylakoid membrane B and P: Photosystem II assembly	GO:0009535 GO:0010207	Photosynthesis
1079	C and C: photosystem II M and F: Chlorophyll binding	GO:0009523 GO:0016168	Photosynthesis proteins

[a] The unigenes codes we got from the biological sequencing company, followed from the DEGs analysis, the listed unigenes were exposed utilizing the KEGG map. The main function related to chloroplast and chlorophyll annotated with GO database was filled in. [b] Cellular Component; [c] Biological Process, [d] Molecular Function. [e] Gene Ontology, [f] Kyoto Encyclopedia of Genes and Genomes.

chlorophyll concentrations and lower Chl a/Chl b ratios, and their chloroplasts contain thicker and denser grana and stroma than cultivars exhibiting light green leaves (Fu *et al*., 2013). In this study, the mutant seedling leaves exhibited a yellow-green phenotype, and older leaves became green as Chl accumulated in the leaves during the mature stage of hot pepper. The Car content was significantly lower in the mutant plants than in the wild type plants, but the Car content in the mutant plants was the same as that in the wild type plants after the blossom stage. The Chl a/b ratio of the mutant appeared to be highest at the seedling stage, most likely because of the potential for Chl b synthesis to suffer a more severe decline than that for Chl a synthesis. Then, the Chl a/b ratio of the mutant declined, ultimately reaching the wild-type level. These results suggest that the newly developed leaves of the mutant exhibited delayed greening due to slow rates of Chl accumulation and delayed chloroplast development.

Chl a is required for the formation of photosynthetic reaction centers and light-harvesting complexes, and Chl b is exclusively localized to the light-harvesting pigment protein complexes of PSI and PSII (Klein *et al*., 1988; Havaux and Tardy, 1997; Jansson, 1994). An appropriate ratio of Chl a/b is critical for the regulation of photosynthetic antenna size (Oster *et al*., 2000; Masuda *et al*., 2003; Jansson, 1994). In this study, a partial reduction in Chl b biosynthesis caused a decrease in the Chl content and an increase in the Chl a/b ratio in the young leaves of the mutant, indicating that the total number of photosystems decreased and that the number of light-harvesting antenna complexes might be less than that of the wild type; however, its high Car content ratio may help the mutant plant eliminate reactive oxygen. Therefore, the yellow leaf tissue increased heat dissipation, especially heat excitation involved in photoinhibitory quenching, to prevent damage caused by excessive light energy in the photosynthetic apparatus. More importantly, after the seedling stage, the upper leaves of the mutant were yellow, protecting the plant in bright sunlight, whereas the lower leaves were green, which provided high photosynthetic efficiency.

Chloroplast ultrastructure analysis demonstrated that the development of chloroplast was delayed in the mutant. The thylakoid structures of the 96-140 YBM were irregular which might lead to the development retardation of chloroplasts in the mutant. The leaves of the mutant had less chlorophyll content, fewer thylakoid grana and starch grains, and more osmiophilic globules in mutant chloroplast. Therefore, the photosynthesis ability decreased in the mutant. The phenomenon caused by irregular structures has been reported in peanut and cotton (Benedict and Kohel 1970; Benedict and Ketringd, 1972).

Leaf coloration mutants play an important role as morphological markers in hybridizable breeding. However, the mutant mechanism has not been reported for hot pepper. Transcriptome sequencing provided vast genomic information for hot pepper, which can reveal the gene expression profiles after experimental treatment or infection, and analyses of conserved orthologous genes can be used for phylogenomic purposes (Surget-Groba and Montoya-Burgos, 2010).

We performed transcriptome sequencing on cultivated yellow-bud mutant yellow leaves and cultivated wild type seedling leaves by isolating RNA and pooling each RNA sample into an equal amount (Peng *et al*., 2003; Liu *et al*., 2010; Everett *et al*., 2011; Chen *et al*., 2010) to normalize these samples according to practical and statistical considerations. The Illumina HiSeq™ 2000 sequencing system guaranteed the generation of high quality reads (Feldmeyer *et al*., 2011) at a reduced cost and time. Using the paired-end transcriptome sequencing method combined with an in-depth sequencing strategy and an effective assembly tool, transcriptome profiling was possible even though the Illumina system can only produce reads averaging 90 bp (Xu *et al*., 2012).

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In rice, three virescent mutants have been identified, of which leaves were yellow during early stages and then turned green later. The related genes have been cloned. One yellow/pale-green center was detected in the mutant (Archer and Bonnett, 1987). A vyl mutant of rice was identified which belonged to the virescent mutant. Furtherly, a vyl gene was cloned, and the encoded protein was homologous to the Arabidopsis ClpP6, which targeted to chloroplast (Dong, *et al*., 2013). And it has been reported that the rice virescent mutants were caused by the chlorophyll related genes or the pathway. In our research, 5 pathways about chlorophyll and chloroplast, such as porphyrin and chlorophyll metabolism, photosynthesis-antenna proteins, carotenoid biosynthesis, photosynthesis and photosynthesis proteins, were listed out, and the related genes participated in chloroplast envelope, chloroplast thylakoid, chlorophyll biosynthetic process and chlorophyll binding, although the main genes responsible for the mutant were not obtained. Next, we will focus on these pathways and genes to further research on the mutant.

In conclusion, in addition to transcriptome sequencing analysis, this experiment primarily evaluated the pigment content, chloroplast ultrastructure, and expression profile of Chl biosynthesis-related genes in the yellow-bud mutant of hot pepper. This study includes the first comparative *de novo* transcriptome sequencing analysis of the yellow-bud mutant and wild type of hot peppers. In this study, we obtained 42,348 unigenes, of which 37,949 were annotated. *Via* DEG analysis, we narrowed the research scope of the mutation mechanism down to fewer than 200 candidate genes. The transcriptome sequencing data can be used not only to identify mutated genes but also to perform other breeding investigations or genetic and genomic studies. In the future, based on this research, we will identify the individual genes involved in the yellow-bud mutant.

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مقدار کلروفیل، فوق ساختار کلروپلاست، و تجزیه مجموعه آر. ان. ای های پیک تیپ وحشی و تیپ جوانه-زرد جهش یافته در فلفل های تند

ز. ه. ما، گ. س. سان، چ. و. ژانگ، ق. وانگ، ز. ل. دایی، س. ق. سان، و ی. پ. پان

چکیده

در این پژوهش، فلفل تند تیپ جوانه-زرد جهش یافته (با نماد 96-140YBM) که برگ های جوان آن فنوتیپ برگ زرد را نشان می دهد ولی برگ های آن در رشد کامل سبز رنگ هستند از تیپ وحشی 96-140 جدا شد. در ادامه، نتایج تعیین رنگدانه های فتوسنتزی و مشاهده فوق ساختار کلروپلاست آشکار ساخت که Chl a+b و کاروتنوئید (Car) برگ های جوان جهش یافته، باعث افزایش نسبت Chl a/b و Car/Chl a+b شده و نمو کلروپلاست را در مقایسه با برگ تیپ وحشی این گیاه به تاخیر انداختند. در این بررسی، ما با استفاده ازپلتفرم (platform) نوع Illumina HiSeq-2000 (Illumina Inc., USA) از برگ های زرد گیاهچه های تیپ جوانه-زرد جهش یافته و گیاهچه های تیپ وحشی که کاشته شده بودند به تعداد ۹۵،۷۱۴ رونوشت (transcript) به دست آوردیم. در ادامه، تعداد ۴۲،۳۸۴ تکژن (unigene) شناسایی شد که در میان آن ها ۳۹،۹۴۹ مورد با استفاده از تشریح ژنی یا با واژه های هستی شناسی (ontology)تفسیر شده (annotated) بود. بر مبنای تجزیه ژن های بیان شده افتراقی (DEG)، تعداد DEG۱۰۵۶ از ۱۱۰۱ مورد آنها در پایگاه Nr تفسیر شده بودند و نیز با استفاده از پایگاه داد ه های دانشنامه ژن ها وژنوم های کیوتو(KEGG)، تعداد ۳۰۲ تکژن در ۱۳۰ مسیر(pathway) جا نمایی و ترسیم شده بود. بالاخره، دریافتیم که ۶ مسیر مربوط به زیست سازی(biogenesis) کلروپلاست و کلروفیل بود.