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Article

Candidate Gene Identification for a Lethal Chlorophyll-Deficient Mutant in Soybean

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Abstract: Chlorophyll-deficient mutants have been studied persistently to understand genetic mechanisms controlling metabolic pathways. A spontaneous chlorophyll-deficient lethal mutant was observed in self-pollinated progeny of a soybean cultivar "BSR 101". Observed segregation patterns indicated single-gene recessive inheritance for this lethal-yellow mutant. The objectives of this investigation were to develop a genetic linkage map of the region containing the lethal-yellow (*YL_PR350*) gene and identify putative candidate genes for this locus. The *YL_PR350* gene was mapped to chromosome 15 and is flanked by BARCSOYSSR_15_1591 and BARCSOYSSR_15_1597. This region physically spans ~153 kb and there are 14 predicted genes that lie in this region. The predicted gene *Glyma.15g275900* is an excellent candidate for the *YL_PR350* gene as it is homologous to an Arabidopsis gene, *At3g08010*, which codes for a chloroplast-localized protein (ATAB2) involved in the biogenesis of Photosystem I and II. This thylakoid membrane protein is crucial for photosynthesis in Arabidopsis. Future characterization of

the candidate gene may enhance our knowledge about photosynthesis, a complex metabolic process critical for sustainability of plants.

Keywords: lethal-yellow; chlorophyll-deficient; genetic map; soybean

1. Introduction

Variability is a key component that plays an important role in a greater understanding of genetic frameworks and characterization of genomic regions containing important traits. Chlorophyll-deficient mutants have been instrumental in identifying target genes, understanding gene regulation and characterizing metabolic pathways [1–7]. Plant pigments, including chlorophylls, are vital for plant development and yield [8]. Chlorophylls play an indispensable role in photosynthesis; specifically, photosynthetic light-harvesting in antennae systems and energy transfer in reaction centers within the chloroplasts [9]. Photosynthesis is a complex metabolic process involving a number of enzymes and biochemical reactions. Mutations in genes encoding any of these enzymes could have drastic effects on a plant phenotype [10–12]. Understanding genetic control of the chlorophyll-deficient mutants can help in identification of different players involved in the photosynthetic mechanism.

In soybean, more than 25 nuclear genes affecting chlorophyll-deficiency have been identified and some are genetically mapped to various soybean chromosomes [2,3,6,7,13,14]. In summer 2009, a soybean cultivar "BSR 101" was evaluated for flower color, plant color, pubescence color, and phenotypic variation. During evaluation at early stage of development, a spontaneous chlorophyll-deficient lethal mutant was observed in self-pollinated progeny of "BSR 101".

Objective for this investigation were to develop a genetic linkage map of the region containing this lethal-yellow gene and to identify putative candidate genes at this locus.

2. Results and Discussion

In its original background of "BSR 101", the lethal-yellow mutant was inherited as a single-recessive gene. A homozygous recessive mutation in the lethal-yellow gene results in yellow plants that will perish within 15 days from germination. However, both homozygous dominant and heterozygous genotypes result in normal green plants. Heterozygous green plants from the entries segregating for green and lethal-yellow plants were used as male parents and crossed with "Minsoy" as female parents. The F_2 populations segregating for green and lethal-yellow plants were used as mapping populations. We temporarily named the lethal-yellow mutant gene as *YL_PR350* based on the population name. The F_2 populations followed expected 3:1 ratios (Table 1). Evaluation of $F_{2:3}$ population PR350-1 showed the expected 1:2 ratio for non-segregating: segregating lethal-yellow. However, the PR350-6 population differed from the expected segregation ratio with a P value 0.04 (Table 1). The combined data from both populations showed P values of 0.62 and 0.47 for the F_2 and $F_{2:3}$ populations, respectively. This confirmed the expected single-recessive gene inheritance of the mutant phenotype, despite an unexpected segregation ratio of green to lethal-yellow in the PR350-6

population. Deviation in the $F_{2:3}$ population may have occurred due to small sample size and the early death of the lethal-yellow plants.

Table 1. Segregation patterns, Chi-square values and P-values for the PR350-1 and PR350-6 populations.

Population	Gene	No. F_2 plants				No. $F_{2:3}$ families			
		Green	Yellow	χ^2 (3:1)	*P*	All Green	Segregating	χ^2 (1:2)	*P*
PR350-1	*YL_PR350*	137	36	1.62	0.20	38	94	1.23	0.27
PR350-6	*YL_PR350*	153	55	0.23	0.63	59	83	4.32	0.04
Total		290	91	0.25	0.62	97	177	0.53	0.47

Chi-square values calculated to test goodness of fit to a 3:1 or 1:2 ratio; *P* = probability of a greater value of chi-square.

To determine the genetic location of the lethal-yellow (*YL_PR350*) gene, 800 Simple Sequence Repeat (SSR) markers covering all 20 soybean chromosomes were used to find polymorphisms between green and lethal-yellow bulks. SSR marker Satt231 showed polymorphism between bulks, which suggested that the lethal-yellow mutation was located on chromosome Gm15, Molecular Linkage Group (MLG) E. Thirty additional SSR markers near Satt231 were analyzed for polymorphism between the bulks. Of these markers, eight detected polymorphism. A total of nine SSR markers were used on the entire F_2 populations to develop a genetic linkage map of the region. Analysis of the marker data showed that the *YL_PR350* gene was located between BARCSOYSSR_15_1591 and BARCSOYSSR_15_1597 at a distance of 1.4 cM and 1.1 cM, respectively (Figure 1). SSR markers present near the gene were physically mapped using the soybean genome sequence [15,16]. The *YL_PR350* gene was located in a ~153 kb region between the BARCSOYSSR_15_1591 and BARCSOYSSR_15_1597 markers. Fourteen predicted genes were located in this region (Table 2; [16]). *Glyma.15g275900* is the most probable candidate gene for *YL_PR350* as it is homologous to an Arabidopsis gene (*At3g08010*), which codes for a protein (ATAB2) that is involved in the biogenesis of Photosystem I and II [17]. *At3g08010* is expressed within the thylakoid membranes and has a crucial role in chloroplast ultrastructure [17]. Thylakoid membrane development is partially dependent upon ATAB2 in Arabidopsis and results in an albino phenotype in the species if ATAB2 is inactivated. If the candidate gene operates in a similar fashion to its Arabidopsis homolog, then we can conclude that problems with thylakoid membrane development are the cause of this lethal-yellow phenotype. Although we do not know the genetic mechanism, our data suggest that the lethal-yellow phenotype may be due to a problem in the thylakoid membranes, which is essential for photosynthetic processes (Table 2). Future characterization of the candidate gene is warranted to isolate and clone the gene responsible for the lethal-yellow phenotype. This may facilitate deeper understanding of the complex metabolic pathway of photosynthesis.

Figure 1. Physical and genetic linkage maps of soybean chromosome Gm15 (MLG E) showing locations of SSR markers close to the yellow-lethal gene *YL_PR350*. Physical distances are shown in base pairs (bp) and genetic distances are shown in centiMorgans (cM).

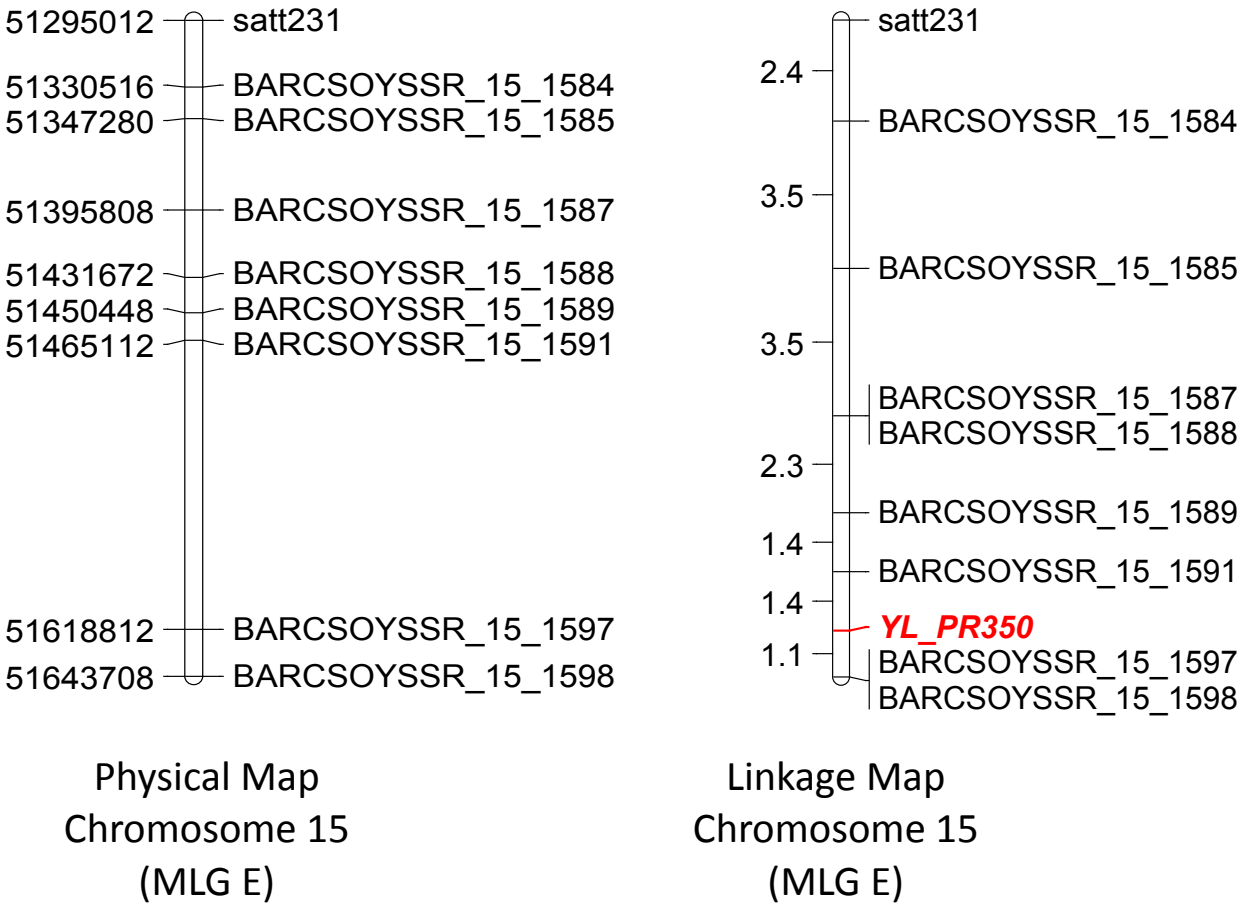

Table 2. Predicted genes present in the genomic region containing the lethal-yellow gene; names and predicted functions of the putative proteins encoded by 14 genes that are flanked by BARCSOYSSR_15_1591 and BARCSOYSSR_15_1597 on Gm15 (MLG E) are shown.

Gene	Start Position (bp)	End Position (bp)	Predicted Protein/Function
Glyma.15g275700	51472288	51473210	No functional annotation
Glyma.15g275800	51473359	51476124	Unknown
Glyma.15g275900	51486036	51491942	Biogenesis of Photosystem I and II and chloroplast ultrastructure
Glyma.15g276000	51495263	51502516	DNA Gyrase/Topoisomerase IV, DNA binding, ATP binding
Glyma.15g276100	51510448	51513137	Unknown (DUF642)
Glyma.15g276200	51533618	51534670	Unknown
Glyma.15g276300	51541078	51546842	Sterol methyltransferase C-terminal, steroid biosynthetic process

Table 2. *Cont.*

Gene	Start Position (bp)	End Position (bp)	Predicted Protein/Function
Glyma.15g276400	51549706	51555332	Copper Amine oxidase
Glyma.15g276500	51564250	51573306	Flavin containing amine oxidoreductase
Glyma.15g276600	51574389	51574676	Wound induced protein
Glyma.15g276700	51578950	51593999	Uncharacterized protein family, UPF0114
Glyma.15g276800	51600225	51651653	Mandelate racemase / muconate lactonizing enzyme, Thiamine pyrophosphate enzyme
Glyma.15g275600	51456041	51457063	Unknown

3. Experimental Section

3.1. Plant Material

Two mapping populations (PR350-1 and PR350-6) were generated by crossing "Minsoy" (PI 548389) (*YL YL)* as female parent with the lethal-yellow plant (BSR 101) (*YL yl*) as male parent. Two F_1 seeds were planted at Iowa State University in 2011. The F_1 plants were single-plant threshed and 173 and 208 F_2 seeds were planted at the Bruner Farm near Ames, IA, respectively, for PR350-1 and PR350-6 populations. Fifty seeds for each of the $F_{2:3}$ progenies were grown and evaluated to determine the genotype of each F_2 plant by checking leaf color phenotype.

3.2. DNA Isolation and Bulked Segregant Analysis (BSA)

Genomic DNA for parents and mapping populations was extracted by utilizing the Cetyl Trimethyl Ammonium Bromide (CTAB) extraction process [18]. Molecular markers closely linked to the lethal-yellow gene were identified through BSA [19]. Two bulks were generated based on $F_{2:3}$ phenotypic data. One bulk was created by pooling DNA from 10 F_2 individuals identified as homozygous dominant (green phenotype), and the second was created by pooling DNA from 10 F_2 individuals identified as homozygous recessive (lethal-yellow phenotype). Both green and yellow bulks were diluted to a final concentration of 50 ng/µl.

3.3. Molecular Analysis

SSR markers were developed using information from Soybase [20,21]. SSR markers were amplified by polymerase chain reaction (PCR) in a 10 µl reaction mix that contained 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH8.3), 2.0 mM $MgCl_2$, 0.25 µM primer, 200 µM of each dNTP, 50 ng of genomic DNA, and 0.25 units of Biolase DNA polymerase (Bioline USA Inc, Taunton, MA). The PCR program cycled through four steps: an initial cycle of two minutes at 94 °C, 35 cycles lasting 30 seconds at 94 °C, 30 seconds at 58 °C, followed by one minute at 72 °C. The PCR products were separated on 4% agarose gel at 150 V for two to four hours in 0.5× TBE buffer.

Map positions of the locus and SSR markers in the final map were calculated with the program Mapmaker 2.0 [22], using a minimum LOD score of 3.0 and a maximum recombination value of 0.4 as thresholds. Linkage calculations were completed using the Kosambi mapping function [23].

4. Conclusions

We have identified a chlorophyll-deficient mutant that is completely lethal. The trait displayed monogenic inheritance. We developed a genetic linkage map of a gene and placed it within a ~153 kb region on chromosome 15. There are 14 genes present in the region. *Glyma.15g275900* is a strong candidate for *YL_PR350* because of the likelihood of its involvement in biogenesis of Photosystems and chloroplast ultrastructure [17]. Its association with thylakoid development strongly suggests that a mutation within this gene could cause the lethal-yellow phenotype [17].Thylakoid membranes are vital components for photosynthesis. Further characterization of this candidate gene may lead to a better understanding of different players involved in the photosynthesis process and better utilization of those for increased crop productivity.

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Author Contribution

Devinder Sandhu, Katherine Espinosa and Reid G. Palmer conceived and designed the experiments; Katherine Espinosa, Sam Reed, Taylor Atkinson, Carly Gorecki, and Sarah Przybylski performed the experiments; Sam Reed, Taylor Atkinson, Devinder Sandhu, Carly Gorecki, and Sarah Przybylski analyzed the data; Katherine Espinosa, Alcira Susana Goggi, Reid G. Palmer, and Devinder Sandhu contributed reagents/materials/analysis tools; Sam Reed, Taylor Atkinson, Devinder Sandhu, Katherine Espinosa and Alcira Susana Goggi.

Conflict of Interest

The authors declare no conflict of interest

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