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Genetic Analysis and Gene Mapping for a Short-Petiole Mutant in Soybean (*Glycine max* (L.) Merr.)

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Abstract: Short petiole is a valuable trait for the improvement of plant canopy of ideotypes with high yield. Here, we identified a soybean mutant line *derived short petiole* (*dsp*) with extremely short petiole in the field, which is obviously different from most short-petiole lines identified previously. Genetic analysis on 941 F_2 individuals and subsequent segregation analysis of 184 $F_{2:3}$ and 172 $F_{3:4}$ families revealed that the *dsp* mutant was controlled by two recessive genes, named as *dsp1* and *dsp2*. Map-based cloning showed that these two recessive genes were located on two nonhomologous regions of chromosome 07 and chromosome 11, of which the *dsp1* locus was mapped at a physical interval of 550.5-Kb on chromosome 07 near to centromere with flanking markers as BARCSOYSSR_07_0787 and BARCSOYSSR_07_0808; whereas, the *dsp2* locus was mapped to a 263.3-Kb region on chromosome 11 with BARCSOYSSR_11_0037 and BARCSOYSSR_11_0043 as flanking markers. A total of 36 and 33 gene models were located within the physical genomic interval of *dsp1* and *dsp2* loci, respectively. In conclusion, the present study identified markers linked with genomic regions responsible for short-petiole phenotype of soybean, which can be effectively used to develop ideal soybean cultivars through marker-assisted breeding.

Keywords: *derived short-petiole* (*dsp*) mutant; soybean (*Glycine max*); map-based cloning; simple sequence repeats (SSR) marker; bulked segregant analysis (BSA)

1. Introduction

Plant canopy architecture is an important agronomic trait for improving the yield potential in soybean and other legumes crops [1,2]. Ideal canopy structure with suitable leaf area index values has advantages of increasing light interception efficiency, that leads to increased photosynthesis as well as accumulation of photosynthetic assimilates, and eventually results in a higher yield [3,4]. It has been suggested that the desirable leaf area index of a population can be achieved by developing new cultivars for dense planting [1]. The interception capacity of crops on both direct and diffuse solar radiation is expected to increase under a horizontal canopy of dense seeding [5]. However, closed canopy profile also results in large within-canopy shading, which subsequently induces shade avoidance response and promotes lodging by excessive vegetative growth [6]. Kokubun (1988) investigated the characteristics of high-yielding soybean cultivars and proposed a high-yield ideotype model with the upper leaves vertically closed. The fraction of light absorption by the lower surface will be increased in

this model [7]. Hence, it is an immediate prerequisite to modify plant canopy in a desirable direction through genetic manipulation for increasing crop yield.

Petiole length is an important trait that influences canopy architecture besides leaf petiole angle and branching capacity [8,9]. The short petiole changes geometric architecture of soybean plants and makes it possible for group canopy closing in a vertical plane when applied in dense seeding. Although the petiole length in soybean varies depending on photoperiod and light quality [9], environmental factors do not have much effect on this trait. Therefore, investigating short-petiole genetic resources is an efficient way to understand petiole development for further plant canopy improvement. Until now, various soybean mutants with short petiole have been identified as well as characterized. For example, D76-1609 and SS98206SP are two short-petiole lines which are controlled by two different single recessive genes, *lps1* and *lps3*, respectively [10,11]. You (1998) described a short-petiole line NJ90L-1SP, which is controlled by two duplicated loci of a recessive gene, *lps1*, that also controls short-petiole trait in D76-1609, and *lps2*, which controls the abnormal pulvinus [12]. These findings suggest that *lps1* and *lps2* might control different stages of petiole development. In addition, Cary and Nickell (1999) described a short petiole LN89-3502TP that is controlled by a single gene with incomplete dominance (*lc*), fitting a 1:2:1 segregation ratio in F_2 population [13]. So far, only *lps3* gene has been mapped on chromosome 13 within the flanking markers Sat_234 and Sct_033 [14]. Hence, identifying short-petiole mutant in soybean as well as the underlying gene will be helpful in promoting ideotype breeding in soybean for increasing yield.

By keeping the above in view, the present study identified a short-petiole mutant named as *dsp*. The objectives of our study were to elucidate the inheritance of the genes controlling short-petiole trait in *dsp* and to map underlying genes by using bulked segregant analysis (BSA) method.

2. Materials and Methods

2.1. Plant Material

The *dsp* is a derived soybean mutant with extremely short petiole, which is identified during soybean breeding in the field. Two crosses were made by crossing two soybean cultivars, viz., HDS-1 and BW-2, with the common *dsp* short-petiole mutant for genetic analysis and gene mapping of *dsp*. All F_1 seeds were planted and single-plant threshed. The 703 and 238 F_2 seeds were planted for HDS-1 × *dsp* and BW-2 × *dsp* F_2 populations, respectively (Table 1). All the 941 F_2 lines together with three parents were evaluated by visual inspection in the summer of 2014. In the summer of 2015, 50 seeds of 184 random $F_{2:3}$ progenies of HDS-1 × *dsp* were sown and used to determine the genotype of each F_2 plant.

Table 1. Genetic analysis of the short-petiole trait in the F_2 and $F_{3:4}$ populations.

Population	Year	No. of Wild-type Plants	No. of Mutant-type Plants	Total Number	Expected Ratio	χ^2	*p-Value*
F_2(HDS-1 × *dsp*)	2014	681	22	703	15:1	11.16	0.00
F_2(BW-2 × *dsp*)	2014	230	8	238	15:1	2.91	0.09
		No. of Segregating Lines	**No. of Non-segregating Lines**	**Total Number**	**Expected Ratio**	χ^2	*p-Value*
$F_{2:3}$(HDS-1 × *dsp*)	2015	87	97	184	8:7	2.47	0.12
$F_{3:4}$(HDS-1 × *dsp*)-*Dsp1_dsp2dsp2*	2016	54	27	81	2:1	0.01	0.91
$F_{3:4}$(HDS-1 × *dsp*)-*dsp1dsp1 Dsp2_*	2016	59	32	91	2:1	0.07	0.80

To further validate the inheritance of *dsp* controlled by two loci, F_2 plants of HDS-1 × *dsp* with one locus as recessive homozygous and the other locus as heterozygotic were specifically selected by simple sequence repeats (SSR) markers according to gene mapping results. In this process, the flanking markers BARCSOYSSR_07_0787 and BARCSOYSSR_07_0808 for *dsp1* locus and BARCSOYSSR_11_0037 and BARCSOYSSR_11_0049 for *dsp2* locus were used to screen out those F_2 plants of which genotypes as *Dsp1dsp1dsp2dsp2* or *dsp1dsp1Dsp2dsp2*. Then 81 and 91 $F_{2:3}$ dominant individuals derived from the above selected F_2 plants were randomly harvested, and 50 seeds for each $F_{3:4}$ progenies were grown to evaluate the genotype of above $F_{2:3}$ individuals in the summer of 2016 (Table 1). A total of 30 mutant individuals from F_2 populations of HDS-1 × *dsp* and BW-2 × *dsp* were collected for gene mapping. There were 132 $F_{2:3}$ recessive individuals derived from *Dsp1dsp1dsp2dsp2* or *dsp1dsp1Dsp2dsp2* F_2 plants used to verify the mapping results of *dsp1* and *dsp2* loci.

The *dsp* mutant has extremely short petiole, however, a very small, nonsignificant variation exists for petiole length at different leaf positions, and thus were ignored. Therefore, short petiole of *dsp* was regarded as a qualitative trait in our study, and the petiole length was phenotypically evaluated as mutant-type (MT) and wild-type (WT) by visual inspection.

All materials were planted at the research field in Jiangpu Experimental Station of Nanjing Agricultural University (Nanjing, China).

2.2. Genetic Analysis of dsp

A Chi-square (χ^2) test (1) was used to analyze the segregation ratio of alleles with the expected ratio at a significance threshold of p-value>0.05 ($\chi^2 < 3.84$) [15,16]. The formulas used are shown as below

$$\chi 2 = \sum \frac{(|O - E| - 0.5)^2}{E} \quad (1)$$

where O and E represent observed and expected value, respectively, under the expected ratio.

2.3. DNA Extraction and SSR Markers Analysis

A plant tissue kit from Tiangen Biotech (Beijing, China) was used to extract DNA from the young and healthy fresh leaves of three parents, F_2 generations, and 132 $F_{2:3}$ individuals derived from the crosses HDS-1 × *dsp* and BW-2 × *dsp*. PCR amplifications were performed in 10 μL reactions containing 50–100 ng of template DNA, 1 × PCR buffer, 2.0 mM $MgCl_2$, 75 μM of each dNTP, 0.2 μM each of the forward and reverse primers, and 0.1 U of Taq DNA polymerase. DNA polymerase, deoxy-ribonucleoside triphosphate (dNTP) mix, and DNA Ladder (50 base pairs) were purchased from Tiangen Biotech (Beijing, China). Primer sequences were obtained from SoyBase website (http://www.soybase.org) and were synthesized by Invitrogen Biological Technology (Shanghai, China). The PCR reaction was performed under the following condition: Initial denaturation at 95 °C for 5 min, followed by 29 cycles with 30 seconds of denaturation at 94 °C, 30 seconds of annealing at 43–56 °C, depending on the optimum annealing temperature for each primer pair, and 30 seconds of extension at 72 °C, with a final 10 min extension at 72 °C on a Peltier thermal cycler (PTC-225, MJ Research, Quebec, QC, Canada). The PCR products were separated by electrophoresis through 8% non-denaturing polyacrylamide gels, and then the gels were stained with 1 g L^{-1} $AgNO_3$ for 15 min, followed by a 1% NaOH and 1% CH_3OH solution for 10 min before visualizing under LED light box.

2.4. Bulked Segregant Analysis (BSA) and Target Gene Mapping of the dsp Mutant

Bulked segregant analysis (BSA) was performed to identify SSR markers potentially linked to the genes responsible for short-petiole trait [17]. A total of 1015 pairs of SSR (simple sequence repeat) primers covering all the 20 chromosomes were included in this process. The normal bulk was formed by pooling DNA of six individuals with normal petiole from the F_2 populations. Similarly, the mutant bulk was created with the DNA of six individuals with short petiole. The normal and mutant DNA bulks as well as the DNA of two parents for the F_2 population of HDS-1 × *dsp* cross were screened with 1015

SSR markers to identify polymorphic markers that are potentially linked to the short-petiole trait of *dsp*. Linkage relationship of the locus and SSR markers were calculated with the program Mapmaker 3.0 [18], using a minimum LOD (logarithum of the odds) score of 3.0 and a maximum recombination value of 0.4 as a threshold. Linkage calculations were completed using the Kosambi mapping function [19]. SSR markers identified to be linked were consequently screened against the entire mapping population. Then subsequent mapping processes were conducted according to Song (2004) [20]. Searching of the physical position of the primer sequence was performed via BLASTN engine on National Center for Biotechnology Information database (NCBI) (http//www.ncbi.nlm.nih.gov/) and converting the genetic map to a physical map based on the physical position of SSR markers.

2.5. Synteny Information and Homologous Protein Information Retrieval

For every locus, the synteny information on genome was retrieved from the SoyBase website (https://soybase.org/) through "Genome Browser" [21]. The duplicated region of this locus (if it exists) would be displayed under the precondition of checking "old duplicated blocks" or "recent duplicated blocks" options in select track. Phytozome website (http://www.phytozome.net) [22] was used to retrieve the corresponding homologous proteins information of every candidate protein.

3. Results and Discussion

3.1. Characteristics of Short-Petiole Trait in the dsp Mutant

Soybean short-petiole mutant *dsp* has a compact stature in the natural field conditions (Figure 1), and also revealed different levels of dwarfing under different genetic background and environments (plant height variation data not shown). The petiole length of *dsp* was less than 2 cm, showing a difference from previously described short-petiole mutants, for instance D76-1609 (8.59 ± 0.93 cm) [10], NJ90L-1SP (7–13 cm) [12], and LN89-3502TP (5–12 cm) [13]. Moreover, *dsp* was relatively more valuable for progenies selection compared with NJ90-1SP and LN89-3502TP, which have inferior agronomic characters including abnormal leaf and pulvinus trait. Considering the ideal soybean architecture model proposed as compact plants with a small stature as well one or two branches [1], *dsp* mutant provides a valuable genetic resource for the development of a soybean ideal plant-type.

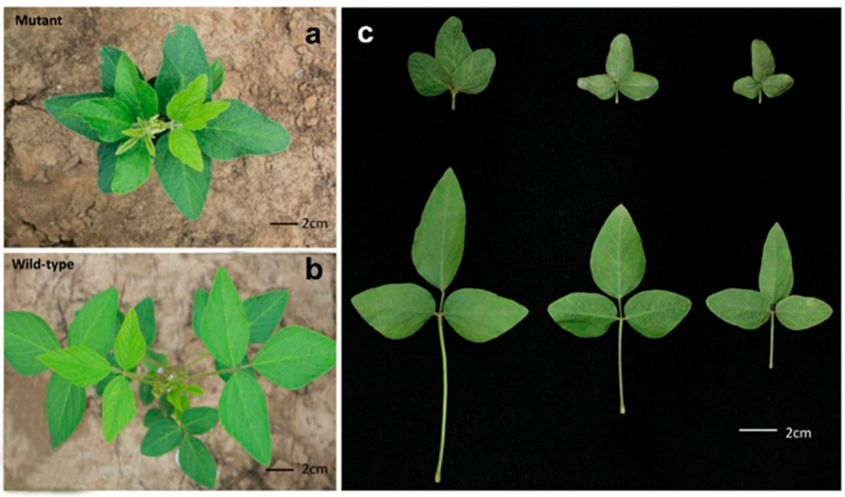

Figure 1. Canopy architecture and petiole morphology of the *dsp* mutant (**a**–**b**). Aerial view of the *dsp* mutant (**a**) and BW-2 wild-type plant (**b**). (**c**) The morphology and petiole length of wild type and mutant. Scale bars represent 2cm in all pictures.

3.2. Genetic Analysis of Petiole Length in the dsp Mutant

Two crosses were made between the common short-petiole *dsp* mutant and two soybean cultivars with normal petiole, HDS-1 and BW-2, respectively. All the F_1 plants obtained from the two populations (HDS-1 × *dsp* and BW-2 × *dsp*) had normal petioles, indicating that the short petiole of *dsp* mutant was recessive to the normal petiole. This result was consistent with that of previous genetic studies based on short-petiole lines, which also revealed the recessive nature of short-petiole length in soybean [10–12]. However, the results of Cary and Nickell (1999) [13] were in contrast. They reported that a short petiole was controlled by a single gene with incomplete dominance.

In the F_2 population from the cross BW-2 × *dsp*, eight out of 238 F_2 individuals had the mutant-type phenotype the same as the *dsp* parent (MT plants). However, the ratio of wild-type plants (WT plants) relative to mutant-type plants (MT plants) was not significantly different from a 15:1 ratio ($\chi^2 = 2.91$, $p = 0.09$) (Table 1). In the case of HDS-1 × *dsp* cross, segregation for petiole-length trait was observed in 87 of the 184 $F_{2:3}$ rows derived from 184 F_2 WT plants, which was not significantly different from a 8:7 ratio for segregating and nonsegregating in long-petiole rows ($\chi^2 = 2.47$, $p = 0.12$) (Table 1). According to subsequent mapping results of genes responsible for *dsp*, F_2 WT plants with genotype as *Dsp1dsp1dsp2dsp2* or *dsp1dsp1Dsp2dsp2* from HDS-1 × *dsp* cross were screened out based on flanking markers of two mapping regions (Figures 2 and 3), and random 81 and 91 $F_{2:3}$ WT plants were grouped to validate the inheritance of *dsp* mutant. As a result, both ratios between those segregating and homozygous (nonsegregating) F_3 individuals fit a 2:1 ratio based on $F_{3:4}$ families ($\chi^2 = 0.01$, $p = 0.91$; $\chi^2 = 0.07$, $p = 0.80$) (Table 1).

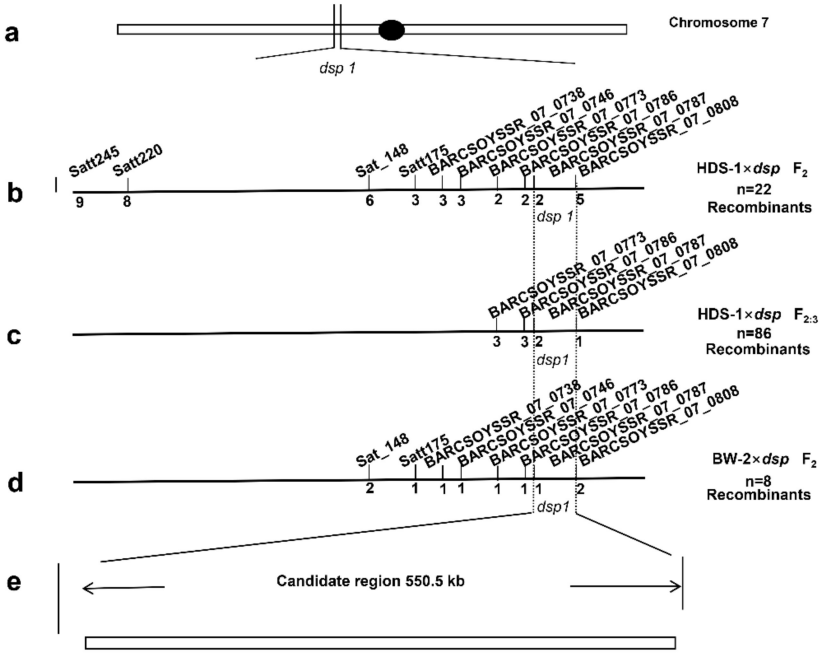

Figure 2. Mapping of the *dsp1* locus. (**a**–**d**) Two F_2 populations of HDS-1 × *dsp* (**b**) and BW-2 × *dsp* (**d**) were used here. One $F_{2:3}$ population derived from *Dsp1dsp1dsp2dsp2* F_2 plants of HDS-1 × *dsp* cross was used to verify the mapping result of *dsp1* (**c**). The *dsp1* locus was mapped to a 550.5-kb region nearby centromere on chromosome 07 with BARCSOYSSR_07_0787 and BARCSOYSSR_07_0808 as flanking markers (**e**). Black spot represents centromere. Number below every SSR marker means recombinant individual number.

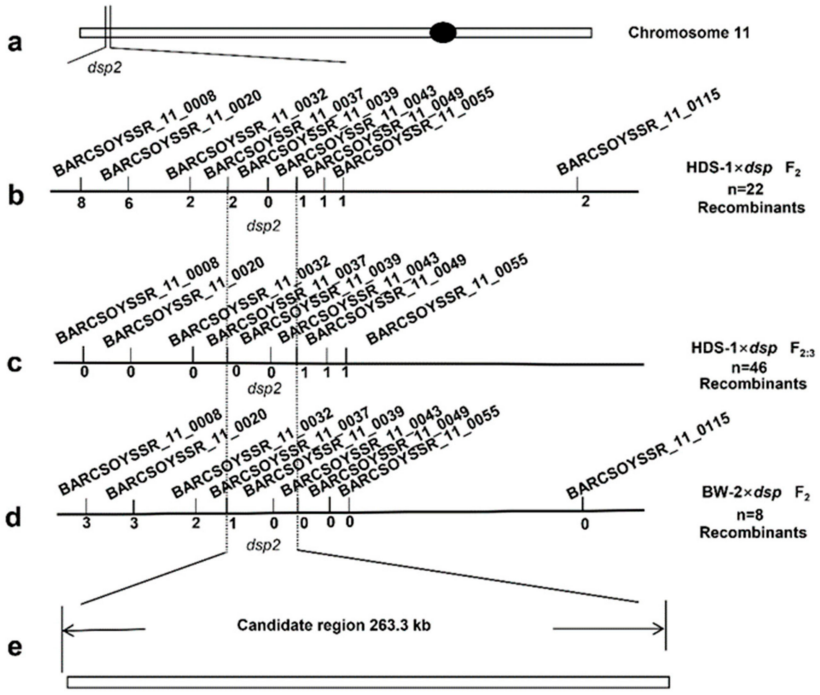

Figure 3. Mapping of the *dsp2* locus. (**a**–**d**) Two F_2 populations of HDS-1 × *dsp* (**b**) and BW-2 × *dsp* (**d**) were used here. One $F_{2:3}$ population derived from *dsp1dsp1Dsp2dsp2* F_2 plants of HDS-1 × *dsp* was used to verify the mapping result of *dsp2* (**c**). The *dsp2* locus was mapped between BARCSOYSSR_11_0037 and BARCSOYSSR_11_0043, a 263.3-kb region in the front end of chromosome 11 (**e**). Black spot represents centromere. Number below every SSR marker means recombinant individual number.

These results demonstrated that the short-petiole trait of *dsp* mutant is controlled by two recessive genes even though the segregation ratio in F_2 population of HDS-1 × *dsp* was significantly different from a 15:1 ratio ($\chi^2 = 11.16$, $p = 0.00$) in the 2014 field experiment. That is probably attributed to poor emergence by poor seed quality of mutant plants (Table 1). This is somewhat similar to the case of genetic analysis for SS98206SP, a short-petiole line controlled by a single recessive gene and fit a 15:1 ratio of F_2 progenies between long and short petioles based on bad seed quality in 2006 [11].

3.3. Mapping Genes dsp1 and dsp2 with SSR Markers

Out of a total 1015 SSR markers screened for polymorphism, only 67 markers distributed to 12 chromosomes were found to be polymorphic between WT and MT DNA pools derived from F_2 population of HDS-1 × *dsp* cross. Finally, polymorphic markers on chromosome 07 (linkage group M) and chromosome 11 (linkage group B1) were detected to be linked with the short-petiole mutant phenotype of *dsp*. These genomic regions governing the mutant phenotype of *dsp* was named as *dsp1* and *dsp2*, respectively.

A total of 22 and 8 F_2 MT plants from HDS-1 × *dsp* and BW-2 × *dsp*, respectively, were genotyped using linked markers screened from chromosome 07 and chromosome 11. New SSR markers from Song (2010) [23] were synthesized to narrow the mapping regions of *dsp1* and *dsp2* loci. Eventually, the *dsp1* locus was mapped to a 550.5-Kb region on chromosome 07 with flanking markers as BARCSOYSSR_07_0787 and BARCSOYSSR_07_0808 (Figure 2). The mapping region is near to centromere according to SoyBase database [21]. A total of 36 gene models were present within this region (Glyma.Wm82.a1.v1.1) (Table 2). The *dsp2* locus was mapped within a 263.3-Kb region between BARCSOYSSR_11_0037 and BARCSOYSSR_11_0049 markers on the front of chromosome 11, harboring 33 gene models (Figure 3, Table 3). Furthermore, these $F_{2:3}$ MT plants from *Dsp1dsp1dsp2dsp2* or *dsp1dsp1Dsp2dsp2* F_2 individuals were used to confirm the mapping regions. Among them, 86 $F_{2:3}$ MT plants from *Dsp1dsp1 dsp2dsp2* F_2 plants were utilized to validate the mapping result of *dsp1*.

Then *dsp1* was mapped to the same genomic region as identified earlier using F_2 populations (Figure 2). Similarly, 46 $F_{2:3}$ MT plants from *dsp1dsp1 Dsp2dsp2* F_2 plants confirmed the locus *dsp2*. The right boundary of *dsp2* is definite with BARCSOYSSR_11_0049, which is also consistent with the mapping result of *dsp2* in F_2 populations (Figure 3). The petiole length of *dsp* mutant is very similar to another SS98206SP line, while the *lps3* locus underlying short petiole of SS98206SP was reported to be mapped on chromosome 13 [14]. Hence, *dsp* is a novel short-petiole line different from SS98206SP.

Table 2. List of gene models in the 550.5-Kb physical interval of the *derived short-petiole1* (*dsp1*) locus.

Glyma.Wm82. a1.v1.1 Gene Models	Glyma.Wm82. a2.v1 Gene Models	Description
Glyma07g16731	*Glyma.07g139000*	Mitochondrial carrier protein
Glyma07g16740	*Glyma.07g139100*	Major Facilitator Superfamily
Glyma07g16750	*Glyma.07g139200*	Uncharacterized protein
Glyma07g16760	*Glyma.07g139300*	Uncharacterized protein
Glyma07g16770	*Glyma.07g139400*	Probable lipid transfer
Glyma07g16790	*Glyma.07g139500*	PRONE (Plant-specific Rop nucleotide exchanger)
Glyma07g16800	*Glyma.07g139600*	Glutathione S-transferase, C-terminal domain
Glyma07g16810	*Glyma.07g139700*	Glutathione S-transferase, C-terminal domain
Glyma07g16830	*Glyma.07g139800*	Glutathione S-transferase, C-terminal domain
Glyma07g16840	*Glyma.07g139900*	Glutathione S-transferase, C-terminal domain
Glyma07g16850	*Glyma.07g140000*	Glutathione S-transferase, C-terminal domain
Glyma07g16860	*Glyma.07g140200*	Glutathione S-transferase, C-terminal domain
Glyma07g16876	*Glyma.07g140300*	Glutathione S-transferase, C-terminal domain
Glyma07g16893	-	Cytochrome P450 CYP4/CYP19/CYP26 subfamilies
Glyma07g16910	*Glyma.07g140400*	Glutathione S-transferase, C-terminal domain
Glyma07g16925	*Glyma.07g140500*	Glutathione S-transferase, C-terminal domain
Glyma07g16940	*Glyma.07g140700*	Glutathione S-transferase, C-terminal domain
Glyma07g16950	*Glyma.07g140800*	Universal stress protein family
Glyma07g16970	*Glyma.07g141000*	Uncharacterized protein
Glyma07g16980	*Glyma.07g141100*	Myb-like DNA-binding domain
Glyma07g16990	*Glyma.07g141200*	Ctr copper transporter family
Glyma07g17000	*Glyma.07g141300*	Probable lipid transfer
Glyma07g17010	*Glyma.07g141400*	Non-specific serine/threonine protein kinase.
Glyma07g17030	*Glyma.07g141500*	Probable lipid transfer
Glyma07g17060	*Glyma.07g141600*	Ctr copper transporter family
Glyma07g17080	*Glyma.07g141700*	Hsp20/alpha crystallin family
Glyma07g17090	*Glyma.07g141800*	Uncharacterized protein
Glyma07g17101	*Glyma.07g141900*	Domain of unknown function (DUF3527)
Glyma07g17110	*Glyma.07g142000*	PAP2 superfamily C-terminal
Glyma07g17116	*Glyma.07g142100*	Ubiquitinyl hydrolase 1.
Glyma07g17130	*Glyma.07g142300*	RING-variant domain
Glyma07g17140	*Glyma.07g142400*	Multicopper oxidase
Glyma07g17150	*Glyma.07g142500*	Multicopper oxidase
Glyma07g17170	*Glyma.07g142600*	Multicopper oxidase
Glyma07g17180	*Glyma.07g142700*	Fructose-1-6-bisphosphatase
Glyma07g17190	*Glyma.07g142800*	Uncharacterized protein

Table 3. List of gene models in the 263.3-Kb physical interval of the *dsp2* locus.

Glyma.Wm82. a1.v1.1 Gene Models	Glyma.Wm82. a2.v1 Gene Models	Description
Glyma11g01300	*Glyma.11G011000*	RNA-binding proteins
Glyma11g01310	*Glyma.11G011100*	Uncharacterized protein
Glyma11g01320	*Glyma.11G011200*	NADH: ubiquinone oxidoreductase, B17.2 subunit
Glyma11g01330	*Glyma.11G011300*	E3 ubiquitin ligase
Glyma11g01340	*Glyma.11G011400*	Translin-associated protein X
Glyma11g01350	*Glyma.11G011500*	Chalcone and stilbene synthases
Glyma11g01360	*Glyma.11G011600*	PPR repeat
Glyma11g01370	*Glyma.11G011700*	Nuclear transport receptor CRM1/MSN5 (importin beta superfamily)
Glyma11g01380	*Glyma.11G011800*	GTP-binding ADP-ribosylation factor Arf1
Glyma11g01390	*Glyma.11G011900*	Plant protein of unknown function (DUF946)
Glyma11g01405	*Glyma.11G012000*	Guanosine-3′,5′-bis(diphosphate)3′-pyrophosphohydrolase
Glyma11g01420	*Glyma.11G012100*	Ribonuclease P
Glyma11g01430	*Glyma.11G012200*	DEAD/DEAH box helicase/Helicase conserved C-terminal domain
Glyma11g01441	*Glyma.11G012300*	Pwwp domain-containing protein
Glyma11g01450	*Glyma.11G012400*	Cell division cycle 20 (CDC20) (Fizzy)-related
Glyma11g01460	*Glyma.11G012500*	Putative u4/u6 small nuclear ribonucleoprotein
Glyma11g01470	*Glyma.11G012700*	Mitochondrial outer membrane protein
Glyma11g01480	*Glyma.11G012800*	Galactosyltransferases
Glyma11g01491	*Glyma.11G012900*	Aspartyl proteases
Glyma11g01501	*Glyma.11G013000*	Aspartyl proteases
Glyma11g01510	*Glyma.11G013100*	Aspartyl proteases
Glyma11g01520	*Glyma.11G013200*	Uncharacterized protein
Glyma11g01530	*Glyma.11G013300*	PLAC8 family
Glyma11g01536	*Glyma.11G013400*	DYW family of nucleic acid deaminases (DYW_deaminase)
Glyma11g01543	*Glyma.11G013500*	PPR repeat (PPR)
Glyma11g01550	*Glyma.11G013600*	PPR repeat (PPR)
Glyma11g01570	*Glyma.11G013700*	leucine-rich PPR motif-containing protein, mitochondrial (LRPPRC)
Glyma11g01580	*Glyma.11G013800*	Complex 1 protein (LYR family)
Glyma11g01595	*Glyma.11G013900*	KH domain containing RNA binding protein
Glyma11g01610	*Glyma.11G014000*	Protein Phosphatase methylesterase-1 related
Glyma11g01620	*Glyma.11G014100*	Cytochrome c
Glyma11g01640	*Glyma.11G014200*	Ethylene-responsive transcription factor ERF021
Glyma11g01650	*Glyma.11G014300*	Nuclear transport factor 2 (NTF2) domain

Based on the synteny information obtained from SoyBase [21], the mapping region of *dsp1* and *dsp2* belongs to two nonhomologous fragments on chromosome 07 and chromosome 11, respectively (Figure 4). Furthermore, homologous protein information retrieved from Phytozome [22] revealed that none of the encoding proteins within both regions displayed homology (Tables 2 and 3). Hence, these two above loci identified to be responsible for short-petiole phenotype of the *dsp* mutant did not function as duplicated genes.

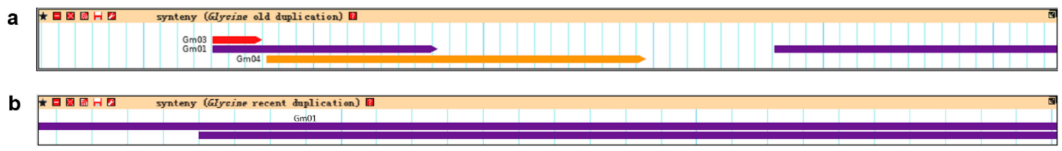

Figure 4. The duplicated regions of *dsp1* and *dsp2* locus from SoyBase. (**a**) The duplicated regions of the *dsp1* locus. (**b**) The duplicated regions of the *dsp2* locus.

As a paleopolyploid, the genome of soybean contains 70.3% duplicate regions due to two whole genome duplication (WGD) events [24–27]. Duplicated genes may undergo pseudogenization, sub-functionalization, or neo-functionalization [28], and the divergence of duplicated genes is thought to provide the basis for adaptive evolution [29]. For example, among the four homologous genes of the Arabidopsis *terminal flower* gene (*TFL1*) in soybean, only one has been found to control growth habit; the other copies may have additional functions because they have been reported to show different transcriptional patterns [30]. A disease-like rugose leaf phenotype in soybean was attributed to two recessive duplicated loci of *rl1* and *rl2* [31]. In our study, all genes located within the mapping regions of *dsp1* and *dsp2* loci have one or more duplicated copies in other chromosomes. The duplicated effect of *dsp1* and *dsp2* candidate genes on the short petiole of *dsp* mutant indicated the possible functional differentiation or genetic interaction during petiole development. Therefore, the short petiole of *dsp* mutant is a complex trait, and the identification of candidate genes underlying *dsp1* and *dsp2* loci will greatly help to clarify the mechanism of petiole development.

4. Conclusions

In summary, we identified a novel short-petiole mutant line "*dsp*" that was demonstrated to be controlled by two recessive gene designated as *dsp1* and *dsp2*. The mapping of *dsp1* and *dsp2* revealed a redundant function between two nonhomologous loci on the formation of short petiole. Hence, the present study provides potential genetic resources, linked markers as well as genes governing the short petiole in *dsp* mutant, and these valuable genes will be in turn used for rapid introgression into elite soybean backgrounds for developing cultivars with short petiole and high yield via marker-assisted breeding. Therefore, the availability of these resources could greatly facilitate the dream of developing soybean ideotype.

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