

# Detection of quantitative trait loci controlling UV-B resistance in soybean

Hyun-Chul Shim · Bo-Keun Ha · Minhoo Yoo ·  
Sung-Taeg Kang

Received: 30 May 2014 / Accepted: 31 July 2014 / Published online: 15 August 2014  
© Springer Science+Business Media Dordrecht 2014

**Abstract** The depletion of stratospheric ozone has resulted in increased amounts of ultraviolet-B radiation (UV-B: 280–320 nm) reaching the Earth's surface. Even a small increase in the incident UV-B radiation could cause significant biological effects in plants. In this study, we identified putative quantitative trait loci (QTL) associated with the resistance to enhanced UV-B radiation in soybean. A population of 115 recombinant inbred lines (RILs) derived from a cross between Keunolkong (a sensitive soybean variety) and Iksan 10 (a resistance soybean line) was analyzed. A total of 110 SSR markers were used to construct a linkage map. RILs and both parents were grown with supplemental UV-B radiation in a greenhouse. In order to screen for UV-B resistance, the degree of leaf chlorosis (DLC), degree of leaf shape change (DLS), degree of petiole color change (DPC) and degree of total plant damage (DTP) were evaluated. Using composite interval mapping analysis, one major QTL associated with all of the characteristics, DLC, DLS, DPC, and DTP, was detected on soybean

chromosome 19 between Satt495 and Satt238, accounting for 10.76–32.8 % of the phenotypic variance. The 'Iksan 10' allele increased the resistance to UV-B radiation for the DLC, DLS, DPC, and DTP traits. This study is the first trial for identification of QTLs associated with UV-B resistance in soybean. In addition, these results provided basic information not only for the improvement of UV-B resistance through marker-assisted selection, but also for the future identification of putative candidate genes of UV-B resistance.

**Keywords** Mapping · Quantitative trait loci (QTL) · Resistance · Soybean · Ultraviolet-B(UV-B)

## Introduction

Solar ultraviolet (UV) radiation is a form of non-ionizing energy, which is generally divided into three classes: UV-C ( $\leq 280$  nm), UV-B (280–320 nm), and UV-A (320–400 nm). UV-C is highly energetic and extremely damaging to biological systems, while UV-B is the mid to high in the hazardous range, and UV-A is the less energetic and least hazardous part of UV radiation. The stratospheric ozone layer blocks all of the UV-C and approximately 97–99 % of the UV-B radiation (Caldwell et al. 1989). However, depletion of the stratospheric ozone layer has resulted in increased solar UV-B radiation reaching the earth's surface

---

H.C. Shim and B.K. Ha contributed equally to this work.

---

H.-C. Shim · M. Yoo · S.-T. Kang (✉)  
Department of Crop Science and Biotechnology, Dankook  
University, Cheonan 330-714, Republic of Korea  
e-mail: kangst@dankook.ac.kr

B.-K. Ha  
Department of Plant Biotechnology, Chonnam National  
University, Gwangju 500-757, Republic of Korea

(Blumthaler and Ambach 1990). Scenario-based chemistry-climate models show that by the middle of 21st century, UV-B radiation at ground level will be enhanced due to high concentrations of greenhouse gases and halogenated species (Taalas et al. 2000).

High levels of UV-B radiation is known to adverse effects on plants, often reducing leaf area and plant growth (Reed et al. 1992; Teramura and Caldwell 1981), chlorophyll content (Zuk-Golaszewska et al. 2003; Choudhary and Agrawal 2014a), plant biomass and production (Choudhary and Agrawal 2014b; Yuan et al. 2000), photosynthesis (Li et al. 2013), and causing DNA damage (D'Surney et al. 1993). In addition, increased levels of UV-B radiation have been shown to suppress nitrogen fixation of root nodules in pea and tropical mungbean (Choudhary and Agrawal 2014b, a). However, elevated UV-B radiation was found to increase total grain nitrogen content and grain storage protein content in rice (Hidema et al. 2005).

Soybean is a crop sensitive to UV-B radiation. Soybean cultivar Essex showed a 20 % yield reduction under simulated levels of 25 % ozone depletion (Teramura et al. 1990). In addition, elevated UV-B treatment caused approximately 50 % reduction in leaf expansion, leaf area, aerial dry biomass and seed yield in soybean cultivar CNS (Reed et al. 1992). In previous studies, the genetic differences of sensitivity to UV-B among soybean cultivars were also reported. In response to UV-B radiation, plant height (−44 to +21 %), leaf area (−87 to +186 %), total dry weight (−46 to +64 %), seed yield (−41 to +46 %), chlorophyll content (−42 to 8 %), flavonoid content (−30 to 50 %), and membrane permeability (−57 to 80 %) varied among the soybean genotypes (Kakani et al. 2003, in review; Yanqun et al. 2003).

To overcome the reduction in productivity caused by a high UV-B environment, a UV-B resistant germplasm is essential. Genotypes of several crop species have been screened for resistance to UV-B radiation in rice (*Oryza sativa* L.) (Teranishi et al. 2004), wheat (*Triticum aestivum* L.) (Yuan et al. 2000), maize (*Zea mays* L.) (Correia et al. 1998), and soybean (*Glycine max* L.) (Baroniya et al. 2011). Compared to UV-B susceptible plants, UV-B resistant plants usually produce more secondary metabolites (such as flavonoids and anthocyanins) in the epidermal and subepidermal cell layers which effectively absorb the UV-B and prevent it from penetrating into the leaf mesophyll cells (Hidema and Kumagai 2006, in

review). In soybean, UV-B resistant cultivars contained more flavonols than the susceptible cultivars (Reed et al. 1992; Sullivan and Teramura 1990). Xu et al. (2008) showed that a flavonoid-reduced soybean line had greater oxidative stress in response to UV-B radiation than the normal line. However, Teranishi et al. (2004) found the photo-repair ability of cyclobutane pyrimidine dimer (CPD) to be main factor determining the UV-B sensitivity in Japanese lowland rice cultivars. Spontaneously occurring mutations in the CPD photolyase gene enhanced photolyase function, which resulted in the improvement of UV-B resistance in rice (Ueda et al. 2005; Hidema et al. 2007).

In soybean, although many researchers have tried to identify the varietal difference for supplemental UV-B, no reports have been made on the identification of QTLs or genes for UV-B resistance. Therefore, this study was conducted to identify the QTL associated with resistance to UV-B with a population of recombinant inbred lines (RILs) of soybean.

## Materials and methods

### Plant materials

The mapping population used in this study consisted of 115 F<sub>2</sub>-derived F<sub>11</sub> RILs developed from a cross between Keunolkong and Iksan 10. This population has previously been used for QTL analysis of pod dehiscence (Kang et al. 2009) and leaflet type (Kim et al. 2005). Briefly, the RILs were advanced by single seed descent (SSD) from crosses between Keunolkong and Iksan 10. Keunolkong, selected from the Korean landrace with early maturity and large seed size, was susceptible to UV-B (Suh et al. 1992). In contrast, Iksan10, developed from KW552 × Pangsakong, showed resistance to UV-B.

### Evaluation of UV-B tolerance

Evaluation of UV-B tolerance was performed in a greenhouse sheltered from rain. The seeds of RILs and their parents were planted in plastic pots filled with synthetic cultivation soil. After germination, plants were thinned to two per pot. Supplemented UV-B radiation was provided by a UV-B lamp (G40T10E UV-B lamps, Sankyo Denki, Japan). The UV-B lamp

was wrapped with 0.13 mm of cellulose acetate film (Cadillac Plastics Co., Baltimore, MD, USA) to filter the UV radiation to a wavelength below 290 nm. The film was replaced once per week due to aging from the UV-radiation. The distances between the lamps and tops of the plants were adjusted to 20–30 cm to maintain the UV-B dosage by controlling the frame. The mean UV-B intensity at plant level under UV-B lamp was 1.5–2.0  $\text{Wm}^{-2}$ . At the V2 stage, plants were irradiated with supplemental UV-B for 5 h per day (10:00–15:00) for 4 weeks. The dose of UV was calculated with a UV-radiometer (DO 9847, Delta OHM) equipped with LP 471 for UV-B, and LP 471 for a UV-C sensor. After UV-B irradiation for 4 weeks, damage was scored on a scale of 1–9, where 1 = no symptom and 9 = severe damaged, which was slightly modified previous research (Teramura et al. 1980). In detail, leaf shape change was scored by 1 = no damage, 3 = very slight wrinkling, 5 = interveinal wrinkling, 7 = pronounced interveinal wrinkling, and 9 = pronounced interveinal wrinkling and leaf curl evident. Also, the changes of leaf color and petiole color, and total plant damage were investigated and scored on a scale of 1–9, where 1 = no symptom and 9 = severe damaged.

### SSR genotyping

Genomic DNA was isolated from healthy leaves according to the protocol by Keim et al. (1988), and used for SSR marker analysis. After the quality of the DNA samples was confirmed on an agarose gel, samples were diluted to working concentration with TE buffer (pH 8.0) and stored at  $-20\text{ }^{\circ}\text{C}$  until used. A total of 110 primer pairs showing parental polymorphisms were used for SSR genotyping in the RIL population. PCR was performed in a total volume of 10  $\mu\text{l}$ , containing 50 ng of template DNA, 0.15  $\mu\text{M}$  each of the forward and reverse primers, 200  $\mu\text{M}$  of each dNTP, 2 mM  $\text{MgCl}_2$ , 0.1 % Triton X-100, 1 $\times$  reaction buffer (10 mM Tris-HCl pH 8.5, 100 mM KCl), and 0.5 U of *Taq* DNA polymerase (BioBasic Taq Polymerase, Applied Bio Basic, Canada). Template DNA was initially denatured at  $94\text{ }^{\circ}\text{C}$  for 2 min, followed by 40 cycles of PCR amplification using the following conditions: denaturation at  $94\text{ }^{\circ}\text{C}$  for 25 s, annealing at  $47\text{ }^{\circ}\text{C}$  for 25 s, and extension at  $68\text{ }^{\circ}\text{C}$  for 60 s on a 96-Well GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The

segregation pattern for each SSR marker was determined by electrophoresis on polyacrylamide or agarose gels. If the SSR loci showed more than 8 bp size differences, the PCR products were separated on an ethidium bromide-stained 3 % agarose gel made with equal proportions of standard and Metaphor agarose (Camblex BioScience, Rockland, ME, USA). The other SSR-amplified products were resolved on a 4 % denaturing sequencing gel, containing 8 M urea in 0.5 $\times$  TBE buffer, and run at 1,700 V for 1.5–2 h, followed by gel staining with a silver sequencing kit (Promega, Madison, WI, USA).

### QTL analysis

Based on the segregation of the 110 SSR marker data from the RIL population, linkage maps were constructed using the Map Manager QTX version 0.3 software (Manly et al. 2001). Recombination fractions were converted to map distances by applying the Kosambi map function (Kosambi 1944). For identification of candidate QTL regions for UV-B tolerance, QTL mapping was performed with composite interval mapping (CIM), executed by WinQTL Cartographer 2.5 (Wang et al. 2011), and multiple regression with SAS 9.03. QTL detection was performed for each trait separately with CIM and SAS.

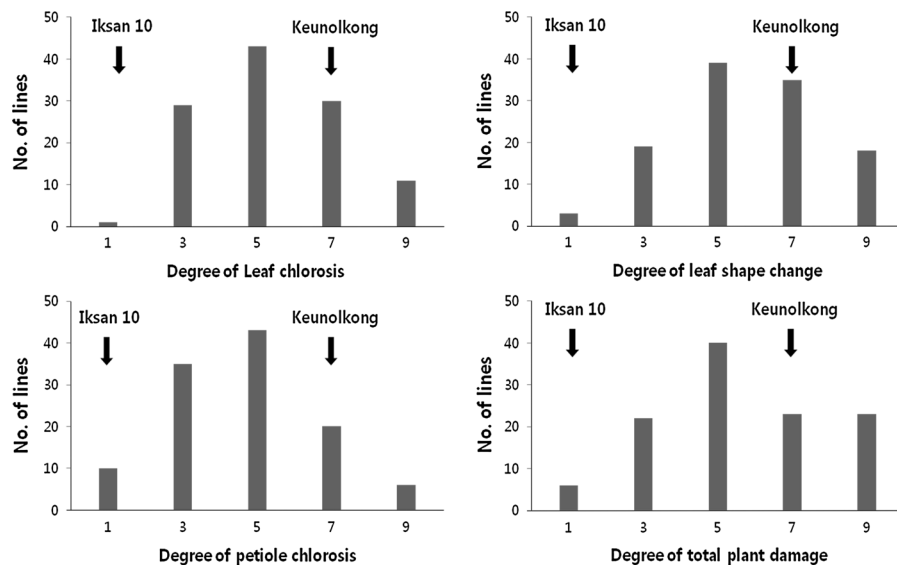
The main effect QTLs and their genetic effects were detected by composite interval mapping using WinQTL Cartographer, version 2.5 (Wang et al. 2011). In each environment, CIM was performed using Model 6 after scanning the genetic map. The empirical significance threshold was determined by 1,000 permutations with a walk speed of 1 cM and a significance level of 0.05.

## Results

### Phenotypic variation under supplemental UV-B radiation

The morphological damage caused by supplemental UV-B radiation was examined in Keunolkong, Iksan 10, and the RIL population in greenhouse conditions. Four categories of plant damage caused by UV-B radiation were investigated: degree of leaf chlorosis (DLC), degree of leaf shape change (DLS), degree of petiole color change (DPC), and degree of total plant

**Fig. 1** Frequency distribution of UV-damage in the 115 RILs derived from Keunolkong  $\times$  Iksan 10. After UV-B irradiation for 4 weeks, UV-B damage was scored on a scale of 1 to 9, where 1 = no symptom and 9 = severe damaged



**Table 1** Correlation coefficient among four traits under supplemental UV-B radiation in 115 RILs derived from Keunolkong  $\times$  Iksan 10

	Leaf chlorosis	Leaf shape change	Petiole color change
Leaf shape change	0.646***		
Petiole color change	0.603***	0.572***	
Total plant damage	0.746***	0.694***	0.692***

\*, \*\*, \*\*\* Highly significant at 0.05, 0.01, 0.001 % level, respectively

damage (DTP). The UV-B susceptible parent, Keunolkong, was assigned a score of 7 in all categories (DLC, DLS, DPC, and DTP). However, Iksan10 did not show any symptoms of damage by UV-B radiation for any of the traits.

Like the big difference between two parents to UV-B radiation, the RILs also showed large phenotypic differences in damages from UV-B radiation in all traits (Fig. 1). Ranges of DLC, DLS, DPC, and DTP under UV-B supplemental condition ranged from 1 (no symptoms) to 9 (severe damaged) in the population, showing continuous and normal distribution. In addition, transgressive segregations were observed in all traits, with some lines showing even more severe damage than Keunolkong. Overall, the distributions of the RIL population suggested that multiple genes may be responsible for regulation of UV-B resistance for DLC, DLS, DPC, and DTP.

Phenotypic correlation coefficients were estimated among DLC, DLS, DPC, and DTP (Table 1). All morphological damages showed positive and highly significant association with each other. Especially, the highest correlation coefficient ( $r = 0.746$ ) was obtained between DLC and DTP. These results suggest that four different morphological traits to UV-B damage might be influenced by common genes.

#### Distribution of QTLs associated with UV-B tolerance

A total of 110 SSR markers were used to identify QTLs conferring resistance to UV-B radiation in the population of 115 RILs. SF-ANOVA analysis between leaf chlorosis and marker genotypes revealed six markers to be associated with DLC on Chr. 7, Chr. 11, Chr. 13, Chr. 14, and Chr. 19, explaining 3.8–9.2 % of the phenotypic variances (Table 2). In Satt197, Satt114, and Satt577, the ‘Keunolkong’ allele was associated with the resistance to UV-B radiation, whereas the ‘Iksan 10’ allele in Satt590, Satt238, and Satt495 increased the resistance to UV-B. Among them, the Satt238 marker on Chr.19 showed a significant effect on DLC, accounting for 11.4 % of the phenotypic variance in multiple regression analysis.

Subsequent SF-ANOVA analysis between leaf shape change and marker genotypes revealed ten markers to be associated with DLS on Chr. 3, Chr. 6,

**Table 2** Molecular markers linked to QTLs for leaf chlorosis caused by supplemental UV-B irradiation in the 115 RILs derived from Keunolkong × Iksan 10

Marker	Chr. <sup>a</sup>	SF-ANOVA <sup>b</sup>		Allelic means <sup>c</sup>		SLG-regr <sup>d</sup>		MLG-regr <sup>e</sup>	
		<i>p</i>	R <sup>2</sup> (%)	K/K	I/I	<i>p</i>	R <sup>2</sup> (%)	<i>p</i>	R <sup>2</sup> (%)
Satt590	7	0.0408	3.8	3.8	3.0	NA			
Satt197	11	0.0297	4.3	3.1	3.9	NA <sup>f</sup>			
Satt114	13	0.0188	5.0	3.0	3.9	NA			
Satt577	14	0.0404	3.8	3.1	3.9	NA			
Satt238	19	0.0012	9.2	3.9	2.3	0.0009	10	0.0004	11.4
Satt495	19	0.0198	4.9	3.8	3.0				
Total			31.0						11.4

<sup>a</sup> Chr chromosome<sup>b</sup> Single factor analysis of variance<sup>c</sup> K/K: homozygous Keunolkong, S/S : homozygous Iksan10<sup>d</sup> Multiple regression with makers on each linkage group<sup>e</sup> Multiple regression with all significant markers from the SLG-Reg model and unlinked markers<sup>f</sup> Not applicable. Not linked to other markers**Table 3** Molecular markers linked to QTLs for leaf shape change induced by supplemental UV-B irradiation in the 115 RILs derived from Keunolkong × Iksan 10

Marker	Chr. <sup>a</sup>	SF-ANOVA <sup>b</sup>		Allelic means <sup>c</sup>		SLG-regr <sup>d</sup>		MLG-regr <sup>e</sup>	
		<i>p</i>	R <sup>2</sup> (%)	K/K	I/I	<i>p</i>	R <sup>2</sup> (%)	<i>p</i>	R <sup>2</sup> (%)
Satt022	3	0.0356	3.9	4.4	3.7	NA			
Satt100	6	0.0404	4.1	3.7	4.5	NA		0.019	5.3
Satt567	7	0.0404	3.8	4.4	3.7				
Satt590	7	0.009	6.1	4.4	3.4	0.006	7.1		
Satt187	8	0.0146	5.3	3.3	3.8	NA <sup>f</sup>		0.041	3.9
Satt114	13	0.0162	5.2	3.5	4.4	NA			
Satt238	19	0.0005	10.4	4.5	3.1	0.004	8.5	0.0009	11.5
Satt462	19	0.0208	4.9	4.3	3.4				
Satt495	19	0.0319	4.2	4.4	3.6				
Satt523	19	0.0242	4.8	3.7	3.0				
Total			52.7						20.7

<sup>a</sup> Chr chromosome<sup>b</sup> Single factor analysis of variance<sup>c</sup> K/K: homozygous Keunolkong, S/S : homozygous Iksan10<sup>d</sup> Multiple regression with makers on each linkage group<sup>e</sup> Multiple regression with all significant markers from the SLG-Reg model and unlinked markers<sup>f</sup> Not applicable. Not linked to other markers

Chr. 7, Chr. 8, Chr. 13, and Chr. 19, explaining 3.8–10.4 % of the phenotypic variances (Table 3). In most markers, excluding three (Satt100, Satt187, and Satt114), the ‘Iksan 10’ allele increased resistance to UV-B radiation. Four markers (Satt100, Satt590,

Satt187, and Satt238) were detected on Chr. 6, Chr. 7, Chr. 8, and Chr. 19 by MLG regression. These accounted for 20.7 % of the total phenotypic variation.

Analysis between petiole chlorosis and marker genotypes using SF-ANOVA identified eight markers

**Table 4** Molecular markers linked to QTLs for petiole chlorosis induced by supplemental UV-B irradiation in the 115 RILs derived from Keunolkong × Iksan 10

Marker	Chr. <sup>a</sup>	SF-ANOVA <sup>b</sup>		Allelic means <sup>c</sup>		SLG-regr <sup>d</sup>		MLG-regr <sup>e</sup>	
		<i>p</i>	R <sup>2</sup> (%)	K/K	I/I	<i>p</i>	R <sup>2</sup> (%)	<i>p</i>	R <sup>2</sup> (%)
Satt022	3	0.0291	4.2	3.1	2.4	NA			
Satt590	7	0.0026	8.0	3.2	2.1	NA			
Satt137	9	0.0225	4.7	3.0	2.2	NA		0.04	3.4
Satt114	13	0.0397	3.9	2.3	3.0	NA			
Satt185	15	0.0344	4.1	3.0	2.3	NA <sup>f</sup>			
Satt238	19	<0.0001	14.3	3.2	1.7	<.0001	16.6	<.0001	20
Satt462	19	0.0142	5.5	3.0	2.0				
Satt495	19	0.023	4.7	3.0	2.2				
Total			49.38						23.4

<sup>a</sup> Chr chromosome<sup>b</sup> Single factor analysis of variance<sup>c</sup> K/K: homozygous Keunolkong, S/S : homozygous Iksan10<sup>d</sup> Multiple regression with makers on each linkage group<sup>e</sup> Multiple regression with all significant markers from the SLG-Reg model and unlinked markers<sup>f</sup> Not applicable. Not linked to other markers**Table 5** Molecular markers linked to QTLs for total plant damage caused by supplemental UV-B irradiation in 115 RILs derived from Keunolkong × Iksan 10

Marker	Chr. <sup>a</sup>	SF-ANOVA <sup>b</sup>		Allelic means <sup>c</sup>		SLG-regr <sup>d</sup>		MLG-regr <sup>e</sup>	
		<i>p</i>	R <sup>2</sup> (%)	K/K	I/I	<i>p</i>	R <sup>2</sup> (%)	<i>p</i>	R <sup>2</sup> (%)
satt022	3	0.0362	3.8	5.2	4.5	NA			
satt371	6	0.0241	4.5	4.5	5.2	NA		0.0156	4.3
satt567	7	0.017	5	5.2	4.4				
satt590	7	0.0001	12.5	5.2	4.5	0.0002	12		
satt238	19	<.0001	20.4	5.3	3.6	<.0001	17.9	<.0001	21.5
satt462	19	0.045	3.5	5	4.3				
satt495	19	0.0034	8	5.2	4.2				
satt523	19	0.0081	6.6	5.1	4				
Total			64.3						25.8

<sup>a</sup> Chr chromosome<sup>b</sup> Single factor analysis of variance<sup>c</sup> K/K: homozygous Keunolkong, S/S : homozygous Iksan10<sup>d</sup> Multiple regression with makers on each linkage group<sup>e</sup> Multiple regression with all significant markers from the SLG-Reg model and unlinked markers<sup>f</sup> Not applicable. Not linked to other markers

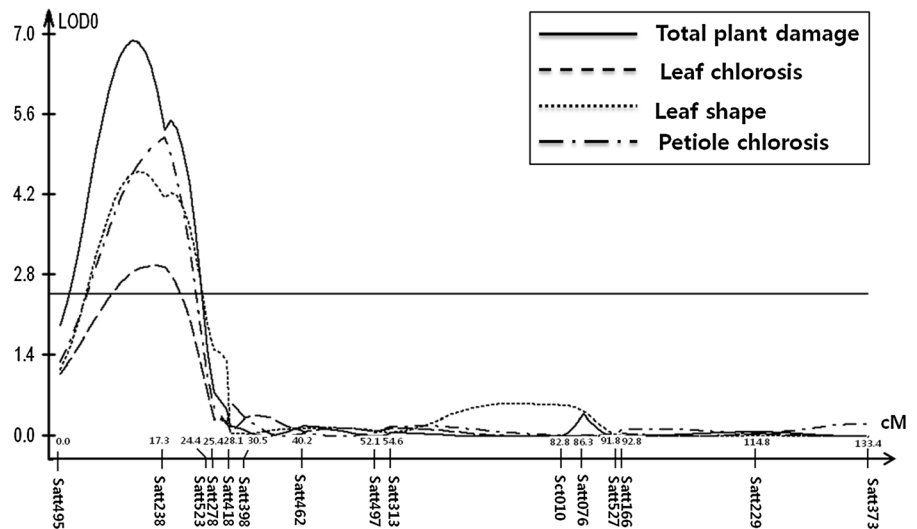
associated with DPC on Chr. 3, Chr. 7, Chr. 9, Chr. 13, Chr. 15, and Chr. 19 (Table 4). They explained 3.9–14.3 % of the phenotypic variances. Except for Satt114 on Ch. 13, the ‘Iksan 10’ allele in most markers increased resistance to UV-B radiation. In

MLG-regression revealed the Satt238 marker on Chr.19 to have a significant effect on DPC, accounting for 20.0 % of the phenotypic variance.

Finally, SF-ANOVA analysis between total plant damage and marker genotypes identified eight

**Table 6** The major QTLs for UV-B resistance identified by Composite interval mapping in the 115 RILs derived from Keunolkong × Iksan 10

Trait	Interval	Chr. <sup>a</sup>	Cloest marker	Position <sup>b</sup> (cM)	Additive effect (%) <sup>c</sup>	R <sup>2</sup> value (%)	LOD score <sup>d</sup>
Leaf chlorosis	Satt495-Satt238	19	Satt238	1.3	−0.65	10.76	2.98
Leaf shape	Satt495-Satt238	19	Satt238	4.29	−0.98	21.48	4.61
Petiole chlorosis	Satt495-Satt238	19	Satt238	0.02	−0.87	17.6	5.22
Total plant damage	Satt495-Satt238	19	Satt238	5.29	−1.07	32.8	6.90

<sup>a</sup> Chr chromosome<sup>b</sup> QTL position from the closest marker<sup>c</sup> Additive effect: positive values indicate that Keunolkong carried the resistant allele for UV-B, while negative values indicate that Sinpaldalkong contributed the resistant allele for UV-B<sup>d</sup> Maximum-likelihood LOD score for the individual QTL**Fig. 2** Composite interval mapping of the major QTL associated with UV-B resistance on soybean chromosome 19 in the 115 RILs derived from Keunolkong × Iksan 10

markers associated with DTP on Chr. 3, Chr. 6, Chr. 7, and Chr. 19 (Table 5). These explained 3.5–20.4 % of the phenotypic variances. Excluding Satt371 on Chr. 6, the ‘Iksan 10’ allele in most markers increased the resistance to UV-B radiation. Satt371 on Chr. 6 and Satt238 on Chr. 19 were detected by MLG-regression, accounting for 25.8 % of the total phenotypic variation.

#### Composite interval mapping of QTLs associated with UV-B tolerance

For identification of candidate QTL regions conferring UV-B resistance, composite interval mapping was performed using the four categories of UV-B damage:

DLC, DLS, DPC, and DTP (Table 6, Fig. 2). Based on CIM analysis, a single QTL located near the marker Satt238 on Chr. 19 was identified for all of the traits. In addition, the ‘Iksan 10’ allele increased the resistance to UV-B radiation in DLC, DLS, DPC, and DTP.

For DLC, the QTL peak was 1.3 cM from Satt238, accounting for 10.76 % of the phenotypic variation. For DLS, the putative QTL was located approximately 4.3 cM from Satt238 and explained 21.5 % of the phenotypic variation. For DPC, the QTL peak was closely located on Satt238, accounting for 17.6 % of the phenotypic variation. For DTP, the QTL peak was 5.3 cM from Satt238. The putative QTL for DTP showed the highest phenotypic variation (32.8 %). Based on these results, a major QTL on Chr. 19

appears to play a key role for increasing the resistance to UV-B radiation in the Iksan 10 soybean cultivar.

## Discussion

Supplementary exposure to UV-B radiation causes morphological, physiological and biochemical damage in plants, such as reduction of biomass and yield, inhibition of photosynthesis, and damage to DNA (Hollósy 2002; Kakani et al. 2003; Hidema and Kumagai 2006). Soybean is a crop which is sensitive to UV-B radiation. Increased UV-B radiation caused more than a 20 % yield reduction in UV-B susceptible soybean cultivars (Reed et al. 1992; Teramura et al. 1990). In this study, “Iksan10” showed highly resistant responses to UV-B radiation. Iksan10 was developed from KW552 × Pangsakong. Through the UV-B screening of the ancestors, Pangsakong also showed resistance to UV-B radiation (data not shown). This result suggests that Pangsakong may be the original source of UV-B resistance in Iksan10.

Due to the rapid development of genomic and molecular technology, identification of new genes has become easier to achieve. In addition, marker-assisted selection and genomic selection may be introduced in plant breeding. For the development of resistant varieties to abiotic or biotic stress, the identification of resistance gene sources is an essential process. In this research, the QTLs associated with resistance to supplemental UV-B radiation in soybean were identified for the first time. A total of 115 RILs developed from a cross between Keunolkong and Iksan 10 were genotyped with 110 SSR markers. In SF-ANOVA analysis based on the integration of genotype data and morphological damage from UV-B radiation, five, six, six, and four QTLs were detected across the soybean chromosomes to analyze the degree of leaf chlorosis (DLC), degree leaf shape change (DLS), degree of petiole color change (DPC) and degree of total plant damage (DTP), respectively. One major QTL, located at Satt238 on Chr. 19, was found to explain 9.2–20.4 % of the phenotypic variance, while one minor QTL located at Satt590 on Chr. 7 explained 3.8–12.5 % of the phenotypic variance. These two QTLs were identified in all four of the categories of UV-B damage tested. In addition, two minor QTLs located at Satt022 on Chr. 3 and Satt114 on Chr. 13, respectively, were identified in three of the categories.

Excluding Satt114, the alleles of Iksan 10 at QTLs located on Chr. 3, 7, and 19 contributed to the resistance to UV-B radiation. However, only one major QTL contributing to resistance in all four categories was detected on Chr. 19, between Satt495 and Satt238, in CIM analysis. This QTL accounted for 10.76–32.8 % of the phenotypic variance.

The major QTLs detected in this study seemed to have similar chromosomal locations as the previously reported mapping study (Kim et al. 2005). With the same mapping population of Keunolkong × Iksan 10, Kim et al. (2005) identified QTLs near Satt238 on Chr. 19 controlling the length and width of the terminal leaflet. Keunolkong has an ovate leaflet type with a terminal leaflet length of 11.23 cm and a terminal leaflet width of 7.59 cm. In contrast, Iksan 10 has an intermediate leaflet type with a terminal leaflet length of 8.53 cm and a terminal leaflet width of 8.53 cm (Kim et al. 2005). Under enhanced UV-B radiation conditions, morphogenetic changes to the leaf such as reduction of size, increase of thickness and leaf mass per unit area, and accumulation of leaf surface waxes and ‘sunscreening’ compounds may be considered as protective mechanisms to UV-B radiation in plants (Wargent and Jordan 2013; Kakani et al. 2003). In this study, Iksan 10, the new source of resistance to UV-B radiation, has a smaller sized leaflet. In addition, there was a positive correlation ( $R = 0.3^{**}$ ) between the terminal leaflet length and degree of total plant damage caused by UV-B (data not shown). These results suggest that leaflet morphology may be one of the main factors increasing the UV-B resistance in the Iksan 10 soybean cultivar. However, the contents of waxes and flavonoid compounds in the leaf of Iksan 10 have not yet been evaluated. Therefore, further studies on the physiological responses of soybean leaves in Iksan 10 and fine-mapping of the major QTL region should be conducted to identify the putative candidate genes for UV-B resistance in the future.

In summary, we identified a new source of UV-B resistant soybean, Iksan 10, showing high resistant responses to UV-B radiation in DLC, DLS, DPC, and DTP. In addition, we positioned a major QTL for UV-B resistance on Chr. 19 in the soybean. Therefore, the positioning of the UV-B resistant QTL and identification of flanking DNA markers will be useful for the introduction of the Iksan 10 allele into soybean elite cultivars with marker-assisted selection to improve UV-B resistance in soybean.



**Acknowledgments** The present research was supported by the research fund of Dankook University in 2012.

## References

- Baroniya SS, Kataria S, Pandey GP, Guruprasad KN (2011) Intraspecific variation in sensitivity to ambient ultraviolet-B radiation in growth and yield characteristics of eight soybean cultivars grown under field conditions. *Brazilian J Plant Physiol* 23:197–202
- Blumthaler M, Ambach W (1990) Indication of increasing solar ultraviolet-B radiation flux in alpine regions. *Science* 248(4952):206–208. doi:10.2307/2873934
- Caldwell MM, Teramura AH, Tevini M (1989) The changing solar ultraviolet climate and the ecological consequences for higher plants. *Trends Ecol Evol* 4(12):363–367. doi:10.1016/0169-5347(89)90100-6
- Choudhary KK, Agrawal SB (2014a) Cultivar specificity of tropical mung bean (*Vigna radiata* L.) to elevated ultraviolet-B: Changes in antioxidative defense system, nitrogen metabolism and accumulation of jasmonic and salicylic acids. *Environ Exp Botany* 99:122–132. doi:10.1016/j.envexpbot.2013.11.006
- Choudhary KK, Agrawal SB (2014b) Ultraviolet-B induced changes in morphological, physiological and biochemical parameters of two cultivars of pea (*Pisum sativum* L.). *Ecotoxicol Environ Safety* 100:178–187. doi:10.1016/j.ecoenv.2013.10.032
- Correia CM, Areal ELV, Torres-Pereira MS, Torres-Pereira JMG (1998) Intraspecific variation in sensitivity to ultraviolet-B radiation in maize grown under field conditions. I. Growth and morphological aspects. *Field Crops Res* 59(2):81–89. doi:10.1016/S0378-4290(98)00102-6
- D’Surney SJ, Tschaplinski TJ, Edwards NT, Shugart LR (1993) Biological responses of two soybean cultivars exposed to enhanced UVB radiation. *Environ Exp Botany* 33(3):347–356. doi:10.1016/0098-8472(93)90036-F
- Hidema J, Kumagai T (2006) Sensitivity of Rice to Ultraviolet-B Radiation. *Ann Bot* 97(6):933–942. doi:10.1093/aob/mc1044
- Hidema J, Zhang W, Yamamoto M, Sato T, Kumagai T (2005) Changes in grain size and grain storage protein of rice (*Oryza Sativa* L.) in response to elevated UV-B radiation under outdoor conditions. *J Radiat Res* 46(2):143–149. doi:10.1269/jrr.46.143
- Hidema J, Taguchi T, Ono T, Teranishi M, Yamamoto K, Kumagai T (2007) Increase in CPD photolyase activity functions effectively to prevent growth inhibition caused by UVB radiation. *Plant J* 50(1):70–79. doi:10.1111/j.1365-3113X.2007.03041.x
- Hollósy F (2002) Effects of ultraviolet radiation on plant cells. *Micron* 33(2):179–197. doi:10.1016/S0968-4328(01)00011-7
- Kakani VG, Reddy KR, Zhao D, Sailaja K (2003) Field crop responses to ultraviolet-B radiation: a review. *Agric Forest Meteorol* 120(1–4):191–218. doi:10.1016/j.agrformet.2003.08.015
- Kang S-T, Kwak M, Kim H-K, Choung M-G, Han W-Y, Baek I-Y, Kim M, Van K, Lee S-H (2009) Population-specific QTLs and their different epistatic interactions for pod dehiscence in soybean [*Glycine max* (L.) Merr.]. *Euphytica* 166(1):15–24. doi:10.1007/s10681-008-9810-6
- Keim P, Olson TC, Shoemaker RC (1988) A rapid protocol for isolating soybean DNA. *Soybean Genet Newsl* 15:150–154
- Kim HK, Kang ST, Suh DY (2005) Analysis of quantitative trait loci associated with leaflet types in two recombinant inbred lines of soybean. *Plant Breed* 124(6):582–589. doi:10.1111/j.1439-0523.2005.01152.x
- Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugen* 12:172–175
- Li L, Huang Q, Zhang S, Zhao S (2013) Effect of enhanced UV-B radiation and low-energy N + Ion beam radiation on the response of photosynthesis, antioxidant enzymes, and lipid peroxidation in rice (*Oryza sativa*) seedlings. *Appl Biochem Biotechnol* 171(4):1072–1083. doi:10.1007/s12010-013-0361-5
- Manly KF, Cudmore JRH, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. *Mamm Genome* 12(12):930–932. doi:10.1007/s00335-001-1016-3
- Reed HE, Teramura AH, Kenworthy WJ (1992) Ancestral U.S. soybean cultivars characterized for tolerance to ultraviolet-B radiation. *Crop Sci* 32(5):1214–1219. doi:10.2135/cropsci1992.0011183X003200050031x
- Suh HS, Shin IY, Baek IY, Park CK, Kim YC, Lee JM, Lee SK (1992) A new high yielding summer soybean variety “Keunolkong”. *RDA J Agric Sci* 34:16–19
- Sullivan JH, Teramura AH (1990) Field study of the interaction between solar ultraviolet-B radiation and drought on photosynthesis and growth in soybean. *Plant Physiol* 92(1):141–146. doi:10.1104/pp.92.1.141
- Taalas P, Kaurola J, Kylling A, Shindell D, Sausen R, Dameris M, Grewe V, Herman J, Damski J, Steil B (2000) The impact of greenhouse gases and halogenated species on future solar UV radiation doses. *Geophys Res Lett* 27(8):1127–1130. doi:10.1029/1999gl010886
- Teramura AH, Caldwell MM (1981) Effects of ultraviolet-B irradiances on soybean. IV. Leaf ontogeny as a factor in evaluating ultraviolet-B irradiance effects on net photosynthesis. *Am J Bot* 68(7):934–941. doi:10.2307/2443224
- Teramura AH, Biggs RH, Kossuth S (1980) Effects of ultraviolet-B irradiances on soybean. II. Interaction between ultraviolet-B and photosynthetically active radiation on net photosynthesis dark respiration, and transpiration. *Plant Physiol* 65:483–488. doi:10.1104/pp.65.3.483
- Teramura AH, Sullivan JH, Lydon J (1990) Effects of UV-B radiation on soybean yield and seed quality: a 6 year field study. *Physiol Plant* 80(1):5–11. doi:10.1111/1399-3054.ep12724768
- Teranishi M, Iwamatsu Y, Hidema J, Kumagai T (2004) Ultraviolet-B sensitivities in Japanese lowland rice cultivars: cyclobutane pyrimidine dimer photolyase activity and gene mutation. *Plant Cell Physiol* 45(12):1848–1856. doi:10.1093/pcp/pch215
- Ueda T, Sato T, Hidema J, Hirouchi T, Yamamoto K, Kumagai T, Yano M (2005) qUVR-10, a major quantitative trait locus for ultraviolet-B resistance in rice Encodes cyclobutane pyrimidine dimer photolyase. *Genetics* 171(4):1941–1950. doi:10.1534/genetics.105.044735

- Wang S, Basten CJ, Zeng ZB (2011) Windows QTL Cartographer 2.5. Department of statistics. North Carolina State University, Raleigh
- Wargent JJ, Jordan BR (2013) From ozone depletion to agriculture: understanding the role of UV radiation in sustainable crop production. *New Phytol* 197(4):1058–1076. doi:[10.1111/nph.12132](https://doi.org/10.1111/nph.12132)
- Xu C, Sullivan JH, Garrett WM, Caperna TJ, Natarajan S (2008) Impact of solar ultraviolet-B on the proteome in soybean lines differing in flavonoid contents. *Phytochemistry* 69(1):38–48. doi:[10.1016/j.phytochem.2007.06.010](https://doi.org/10.1016/j.phytochem.2007.06.010)
- Yanqun Z, Yuan L, Haiyan C, Jianjun C (2003) Intraspecific differences in physiological response of 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. *Environ Exp Botany* 50(1):87–97. doi:[10.1016/S0098-8472\(03\)00004-2](https://doi.org/10.1016/S0098-8472(03)00004-2)
- Yuan L, Yanqun Z, Haiyan C, Jianjun C, Jilong Y, Zhide H (2000) Intraspecific responses in crop growth and yield of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. *Field Crops Res* 67(1):25–33. doi:[10.1016/S0378-4290\(00\)00080-0](https://doi.org/10.1016/S0378-4290(00)00080-0)
- Zuk-Golaszewska K, Upadhyaya MK, Golaszewski J (2003) The effect of UV-B radiation on plant growth and development. *Plant Soil Environ* 49(3):135–140