Screening of melon genotypes identifies gummy stem blight resistance associated with *Gsb1* resistant loci

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Abstract Gummy stem blight (GSB) is one of the most destructive and economically important, soil borne diseases of melon caused by the ascomycete fungus, *Didymella bryoniae* throughout the world. In Korea, however, no GSB resistant genotype has been reported yet. The study aimed to identify GSB resistant melon germplasm. We screened a total of 60 genotypes including 16 lines and 44 melon cultivars collected from USA and Korea. Among the 16 melon lines, four lines including ‘PI482399’, ‘PI140471’, ‘PI136170’ and ‘PI420145’, and two Korean cultivars viz. ‘Asia Papaya’ and ‘Supra’ showed complete resistance. We were aware that both genotypic and environmental variations could influence the phenotypic screening of resistance and susceptibility. We therefore, further assessed all genotypes using 20 SSR markers. The SSR marker ‘CMCT505’ linked to *Gsb1* in chromosome 1 perfectly grouped resistant and susceptible lines indicating that resistance is probably due to the presence of *Gsb1* gene. Cloning and sequencing of resistant and susceptible *Gsb1* amplicons showed that there were 32-bp deletions in resistant line and 39-bp deletions in resistant cultivar compared to susceptible one. Thus, the resistant melon lines and cultivars identified in

this study could be recommended for the melon breeding program. Furthermore, the SSR marker ‘CMCT505’ which is tightly linked with *Gsb1* could be used for molecular screening of melon germplasm.

Keywords Gummy stem blight, *Didymella bryoniae*, Melon, SSR markers, Screening

Introduction

Gummy stem blight (GSB) is one of the destructive soil borne diseases of melon caused by the ascomycete fungus, *Didymella bryoniae* (Auersw.) Rehm resulting significant economic losses (Crosby et al. 2002; Wako et al. 2002; Frantz and Jahn, 2004). The disease symptoms initially comprise with water-soaked lesions that later spread and produce necrotic lesions on both leaves and hypocotyls resulting formation of stem cankers in cortical tissue that consequently produces brown gummy exudate (Frantz and Jahn, 2004). During severe infections, these lesions continue to expand, finally girdling the stem leading to wilting and death of the plant (Sitterly and Keinath, 1996). The pathogen also rigorously attacks the other genera of the Cucurbitaceae family around the world and causes substantial losses in the yield and quality (StAmand and Wehner, 1991; Zitter and Kyle, 1992; Keinath et al. 1995; Gusmini and Wehner, 2002). Though, chemical control has been used to manage GSB, its frequent use poses adverse effect on the environment (Joseph et al. 2009). Crop rotations were often found partially efficacious, because of airborne inoculum coming in from other areas that initiate the incipient infections (Tullu et al. 2002). Consequently, the utilization of resistant cultivars can be the most economically and environmentally sustainable way of GSB disease management. GSB resistance in melon have been reported by a number

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of sources have been reported GSB resistance in melon, however, the most GSB-resistant melon varieties and breeding lines released to date have been developed from an USDA-developed resistant accession, ‘PI140471’ (McGrath et al. 1993; Norton and Cosper, 1989), and have failed to offer satisfactory levels of resistance (Zhang et al. 1997). But conflicting data exists for that USDA-developed melon accession. For example, plant introduction ‘PI140471’ was reported to be ineffective against a Japanese isolate of *D. bryoniae* (Sakata et al. 2000). The melon accessions ‘PI482398’, ‘PI157082’ and ‘PI511890’ were reported to carry the monogenic dominant gene for GSB resistance, whereas ‘PI482399’ was monogenic recessive (Frantz and Jahn, 2004). Among the three accessions bearing monogenic dominant genes ‘PI482398’ was reported to be a highly resistant against *D. bryoniae* (Zhang et al. 1997 and Wang et al. 2012). On the other hand, Joseph et al. (2007) reported monogenic recessive gene bearing ‘PI482399’ as a susceptible genotype to GSB in their study. However, no *D. bryoniae* race has been reported to date. Therefore, exploration of novel sources GSB resistance is apparent which could be useful in melon-engendered areas where existing sources of resistance are not efficacious (Wolukau et al. 2007).

Screening of resistant germplasm based on phenotypes is not an effective means due to the influence of environmental factors. Hence, the use of molecular markers linked to resistance could be an essential step forward for both marker assisted selection (MAS) and map based cloning (Wolukau et al. 2009). In several genetic applications like genetic diversity, genetic mapping and fingerprinting, micro-satellite or simple sequence repeats (SSR) markers were frequently used (Lv et al. 2012; Li et al. 2013). For reproducibility, co-dominance and multiallelism, SSR markers are expected to continue to be used in various genetic applications such as MAS, genetic diversity and gene mapping (Zhu et al. 2016). Therefore, this study endeavored to screen and evaluate the melon germplasm resistance to GSB with the combination of bioassay as well as SSR markers.

Materials and Methods

Plant materials and growing condition

A total of 60 melon genotypes, 16 lines, and 44 commercial cultivars, were used in this experiment (Table 1). Among these, 15 melon lines including control resistant melon line ‘PI482398’ and control susceptible line ‘Honeydew Green-

flesh’ were collected from, Department of Plant Breeding and Genetics, Cornell University and USDA, ARS, USA. The resistance reaction of these germplasm was re-assessed in this study to confirm the control resistant and susceptible lines would be consistently resistant or susceptible, respectively, against tested fungal isolate. The melon line SCNU1154 was collected from Department of Horticulture, Sunchon National University (SCNU). Forty-four melon commercial cultivars were collected from Korean Genebank, National Agrobiodiversity Center. Leaves of 12 other melon inbred lines, namely R36, R37, R40, R51, R60, S1, S2, S3, S4, S6, S6 and S50 were collected from Daeyeon Breeding Company, Republic of Korea, which were used for screening 38 SSR markers. Seedlings were developed in 32-celled trays containing artificial soil mix in a controlled plant growth chamber at $24 \pm 2^\circ\text{C}$, 16 h days, and light intensity $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ at bench level. In the entire screening tests, the melon lines ‘PI482398’ and ‘Honeydew Greenflesh’ were used as a resistant and susceptible control, respectively with a view to compare the disease reactions of gummy stem blight disease symptoms on the inoculated leaves.

Fungal isolate and inoculum preparation

D. bryoniae fungal isolate no. 12-003 was collected from National Institute of Horticultural and Herbal Science, Republic of Korea. The fungus was cultured on petri plates having 15 mL potato dextrose agar (potato infusion 4 g L^{-1} , dextrose 20 g L^{-1} , agar 15 g L^{-1}). Inoculated plates were incubated at $24 \pm 2^\circ\text{C}$ under alternating periods of 12 h fluorescent light ($40 \text{ to } 90 \mu\text{mol.m}^{-2}.\text{sec}^{-1}$ PPFD) and 12 h darkness for 2 to 3 weeks until sporulating pycnidia formed. A spore suspension was prepared by inundating the culture plates with 5 to 10 mL of sterile, distilled water having Tween-20 (20 drops L^{-1}) that helps to dislodge the spore from mycelia and pycnidia, and by gently scraping the surface of the agar using an L-shaped rubber spreader. The liquid suspension from each plate was sieved through a four-layered Mira-cloth (EMD Millipore Corporation, USA) to eliminate mycelia, pycnidia and dislodged agar. Spore concentration was adjusted with a hemocytometer and light microscope (Leica DM750, Leica, Switzerland) to a concentration of 5×10^5 spores mL^{-1} by adding deionized water.

Inoculation of plants

Plants were inoculated at the fourth true leaves stage. The third leaf was cut keeping the long petiole where the youngest leaf was the reference. The petri dishes ($90 \times 15 \text{ mm}$, SPL

Table 1 List of melon genotypes used for genotypic and phenotypic assay for resistance to *Didymella bryoniae*

Sl. no.	Genotype	Seed source	Sl. no.	Genotype	Seed source
1	PI482398	USDA	31	JeonnamMelli	KGNAC
2	PI482399	CU, USA	32	Sweet Lady	KGNAC
3	Cornell ZPPM 339 (ZM)	CU, USA	33	Earls Elite	KGNAC
4	Honeydew Greenflesh (HD)	CU, USA	34	Earls Taitan	KGNAC
5	PI140471	USDA, USA	35	Kings Daebak	KGNAC
6	PI157082	USDA, USA	36	Earls Mountain PMR	KGNAC
7	PI136170	USDA, USA	37	Kingdom	KGNAC
8	PI420145	USDA, USA	38	Earls Grand	KGNAC
9	MR1	USDA, USA	39	SS-402	KGNAC
10	PMR5	USDA, USA	40	Muskmelon SS Anup	KGNAC
11	PMR45	USDA, USA	41	Asulan PMR	KGNAC
12	WMR29	USDA, USA	42	Ropey King	KGNAC
13	Edisto47	USDA, USA	43	Napoyon	KGNAC
14	PI414723	USDA, USA	44	Baldo	KGNAC
15	PI124112	USDA, USA	45	VCR-601	KGNAC
16	SCNU1154	SCNU, Korea	46	Earls ok	KGNAC
17	Charantais	KGNAC	47	White Honey	KGNAC
18	Ananas	KGNAC	48	Giant Iryesaback	KGNAC
19	Alpeon	KGNAC	49	Jochun 26	KGNAC
20	Dakkijeogyuk	KGNAC	50	Valencia Early-rochet	KGNAC
21	Earls Red	KGNAC	51	Asia Papaya	KGNAC
22	Earls top one	KGNAC	52	Eunpa 3	KGNAC
23	Ipchumdaegil	KGNAC	53	ME4759	KGNAC
24	Captain	KGNAC	54	Eunpa fall & Winter melon	KGNAC
25	Darle Spring & Fall	KGNAC	55	Happiness	KGNAC
26	Asia Summer	KGNAC	56	Sunflower	KGNAC
27	Raina	KGNAC	57	Vivadi	KGNAC
28	Pestar	KGNAC	58	Snow White Melon	KGNAC
29	Earls Focus	KGNAC	59	Supra	KGNAC
30	Earls Grand Prix	KGNAC	60	Chungja	KGNAC

Melon lines (1-16) and melon commercial cultivars (17-60), CU = Cornell University, United States of America and USDA = United States Department of Agriculture, SCNU = Sunchon National University, Republic of Korea, KGNAC = Korean Genebank, National Agrobiodiversity Center

Life Sciences Co. Ltd, Korea) were arranged and made a hole at the border region of the cover of each petri dish. About 20 mL of distilled water was added to each petri dish. The spore suspension was applied on the leaf surface near to runoff using a hand-pumped spray bottle. Immediately after inoculation, leaves were kept on the petri dish facing the upper surface of the leaf to air and the lower surface in touch of water so that leaf can take enough moisture from petri dish for disease development. The leaf petioles were inserted through the hole created on the cover of the petri dish. The petri dishes with inoculated

leaves were placed in a plastic tray box and properly sealed to maintain 90 ~ 100% relative humidity inside the box. To spread of the inoculum and encourage uniform disease development the leaves were watered three days consecutively after inoculation.

Disease ratings

Symptoms began to visible on the leaves at seven days after inoculation. The final scoring was done at 14 days after inoculation when symptoms were much prominent.

Fig. 1 Scale of phenotypic categorization according to the reaction of melon leaves infected with *Didymella bryoniae*. Leaves were rated according to the following scales: 1, 0% leaf area affected; 2, 1–10% leaf area affected; 3, 11–30% leaf area affected; 4, 31–50% leaf area affected; 5, 51–100%

The rating system was done following Zhang et al. (1997) with modifications, to distinguish resistant and susceptible plants. During screening, each leaf was given a rating, and that was averaged across three replications within a genotype to determine a mean rating for each genotype. Leaves were rated according to the following scales: 1 = 0% of leaf area affected, 2 = 1–10% leaf areas affected, 3 = 11–30% leaf area affected, 4 = 31–50% leaf area affected, 5 = 51–100% (Fig. 1). Percent infected area was measured by eye estimation as a ratio between infected areas by total leaf area and multiplied by 100.

Analysis of SSR markers

Thirty-eight SSR markers which were initially selected from melon (Liu et al. 2009; Wang et al. 2012) and cucumber genome (Ren et al. 2009) linked to GSB resistance. These 38 markers were amplified using 12 melon lines (Table 2). A total of 20 markers out of 38 amplified genomic complements of melon. The selected 20 markers were used to analyze the status GSB resistance in melon genotypes.

Genomic DNA isolation and marker screening

Young leaf samples were collected for DNA isolation from the individually tagged melon plants grown in a growth chamber at three weeks after germination. Genomic DNA of all melon genotypes was extracted using the DNeasy Plant Mini Kit (QIAGEN, Hidden, Germany) as per manufacturer's instructions. The quality of extracted DNA was determined by a Nanodrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). The genomic DNA of 16 melon lines and 44 cultivars were screened using 20 amplified SSR markers.

PCR amplification and electrophoresis

The PCR reaction was set up to a final volume of 20 μ l containing 1.0 μ l of template DNA of 50 ng concentration, 1.0 μ l each of forward and reverse primers of 10 pmol concentration, 10 μ l of 2X Prime Taq Premix (GENET BIO) and 7 μ l distilled water. The PCR program comprised an initial denaturing at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and at 72°C for 1 minute with a final extension of 72°C for 7 minutes. The PCR products were separated on 1.5 % denaturing agarose gels with a 100 bp ladder prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 μ g/ml) in a gel documentation system.

Cloning and sequencing of marker amplicons

Markers with high adaptability were selected for cloning and sequencing. Resistant and susceptible lines were cloned and sequenced using three amplified markers CMCT505, SSR02460, SSR13251 to find out genetic variants between resistant and susceptible genotypes. The cloned sequences were compared with the published sequences of *C. melo* to confirm whether the primers correctly amplified the target sequences or not. To clone the marker sequence, the PCR protocol as stated above was followed to amplify resistant and susceptible lines. The amplified DNA fragments were purified using Promega DNA Purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cloning was performed using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) following the instructions provided by the manufacturer, but the reaction volumes were halved. The cloned amplicons were sequenced by the universal primers M13F and M13RpUC using the ABI3730XL sequencer (Macrogen Co., Seoul, Republic of Korea). Each forward and reverse sequence of each of the melon resistant and susceptible line was repeated five times to eliminate all ambiguities. The marker sequences between resistant and susceptible lines were also compared using

Table 2 List of SSR markers linked to gummy stem blight resistant (GSB) gene and specifications

Sl. No.	SSR name	Forward sequence	Reverse sequence	Length (bp)
1	CMCT505	GACAGTAATCACCTCATCAAC	GGGAATGTAAATTGGATATG	210
2	CMGA104	TTCTGGGTTTTGCCGATTT	AATTCCGTATTCAACTCTCC	120-250
3	CMAT141	AAGCACACCACCACCCGTAA	GTGAATGGTATGTTATCCTTG	120-250
4	CMTA170a	TTAAATCCCAAAGACATGGCG	AGACGAAGGACGGTAGCTTT	120-250
5	CMCT160a + b	GTCTCTCTCCCTTATCTTCCA	GATGGTGCCTTAGTTGTTCCG	120-250
6	SSR02697	TGCTAACCCAACCAACAAA	CTGCCATTTCAAGCTATGGG	219
7	SSR26165	TCTTTTGTGGTGAAAATTGAAA	CCTTCTCATGTGTATTGTCTTTTG	148
8	SSR06347	TATGGGCCCAGTCCATCATA	CCCAAATCTGACCTTCCCTT	212
9	SSR04534	GGTTCAAACCCCCTCAACTT	GGGGGTTGGGAGACAGTTAT	151
10	SSR17406	GAGCCATCCATCAGAGAGAGA	ACCCACAAGCTTCAGAGGTC	174
11	SSR04649	TTTGAAATTGATGACATCCCA	ACATGGAGGAAGACAGGCAC	195
12	SSR15203	AGTGACCTGAGGTTGCTGCT	AACGTTGTCCATCCAAATGC	179
13	SSR28327	TGTTTTTATGTGAAATGGGGC	CGCATCCCTACCCATTTTTA	203
14	SSR30478	AATTCACCACGCAATATC	AAATTACTTTTAAAACACAACCCCTT	194
15	SSR17459	TTGCCTTCCCTCATATTTGC	TGGTGGGTATTTGTGGTAGGA	202
16	SSR04454	GGAAAGTGTGTGTTGCTCTTG	GATCCATTTGATGCATTGCT	218
17	SSR07747	GATGCACTGTTGGGATTCAA	TTTTTGCCAATTCACAGAGAT	207
18	SSR02460	CTCAGAAACCCCTCCACCAA	CTGTACCGCGAGGACAGTTT	160
19	SSR06632	TCAGATGTTGATTGGCTCTCA	AGGGCCAACATTAAAGGGTC	206
20	SSR21936	TTGGTTGAAAAAGGAAGGTT	GGGCAGAGGCTTTTCAATA	219
21	SSR23856	GAAACGAACAGATGATGGGAA	TTGCTATAGATTCTTCTCGGTC	192
22	SSR12833	TCCCAGCTCTTCACGTAAC	GGAAGGCTCATAAGTGGGA	130
23	SSR15316	TCCAATTTCTGGCGAAGAC	CAACCGAAACAACGTCTCCT	189
24	SSR02906	GAAAAGTGCACTCGGTCCAT	CGCATAAAATCCTCCACGTT	189
25	SSR13251	GGTCAATCCAAAAGAGAAAGCA	ATCAACACCATTGACGACCA	162
26	SSR03147	GCAAAGCTTTAAGGTTGTTTTG	GCAAAATGATGTGTGCTTTACC	220
27	SSR12944	AAGTTTATATTACAGTTGCAACAACCA	TGCACATGTTAATGGTCCAA	193
28	SSR15606	GTGGATCCATGGGGATACTG	CCCCCACTTCTCTCTCCTCT	218
29	SSR20599	CATGGTAACTGGTTAGCTCATTTG	TTTATATGGTGTGAATTGAATTTTA	216
30	SSR20218	CTGGTGGGTTTTCTGAAACG	TCGCCCACGTCTCTATATC	210
31	SSR21115	GCAGCAGTCACCCAACCTAT	CACTGTGAACATATGGGAAGGA	179
32	SSR19755	TATCAGCGAGGGAAGGAAGA	TAATTGCTGCATCGAAGACG	215
33	SSR16683	TATTCTCATCGGAAAACGCC	TTTGCCCTACTACCCCTCCT	135
34	SSR12510	TCCTGTAAGCAATCTCCGGT	GGTGGCGACGATATGTCTTT	178
35	SSR07477	CGTTTCATTTCATTGCTGCAC	GCCATCACTAACTGACGCCT	193
36	SSR20704	ATTGGCCGACCTTATCCTCT	GCCCAGATTTGGTCATTCTT	159
37	SSR03150	TTGATTGAATGAATGGTTTGA	GCCTCTCCTTTTCTCTCAACC	193
38	SSR19755	TATCAGCGAGGGAAGGAAGA	TCAAAATTGATTGGTAAGAAGAAGC	215

SSR marker Sl. No. 1-5 from melon genome and 6-38 from cucumber genome

ClustalW software (<http://www.genome.jp/tools/clustalw/>) to detect any sequence variation.
Statistical analysis

Analysis of variance on disease score ratings was done using Minitab18 statistical software (State College, Pennsylvania, USA). Mean disease scores were compared after Tukey's pairwise comparisons.

Table 3 Foliar disease indices for melon (*Cucumis melo* L) Genotypes in response to *D. bryoniae* inoculation from growth chamber screening

Sl. No.	Genotype	Horticultural group	Leaf disease rating
1	PI482398	Melo	1.0 h
2	PI482399	Melo	1.0 h
3	Cornell ZPPM 339 (ZM)	Melo	3.9 abcdef
4	Honeydew Greenflesh (HD)	Melo	4.8 a
5	PI140471	Melo	1.0 h
6	PI157082	Melo	3.6 abcdef
7	PI136170	Melo	1.0 h
8	PI420145	Melo	1.0 h
9	MR1	Melo	4.4 abc
10	PMR5	Melo	3.8 abcdef
11	PMR45	Melo	3.7 abcdef
12	WMR29	Melo	3.7 abcdef
13	Edisto47	Melo	3.6 abcdef
14	PI414723	Melo	4.0 abcde
15	PI124112	Melo	4.6 ab
16	SCNU1154	Melo	3.6 abcde
17	Charantais	Melo	2.6 defg
18	Ananas	Melo	2.9 cdef
19	Alpeon	Melo	4.1 abcde
20	Dakkijeogyuk	Melo	3.4 abcdef
21	Earls Red	Melo	2.7 defg
22	Earls top one	Melo	3.5 abcdef
23	Ipchumdaegil	Melo	2.3 abcdef
24	Captain	Melo	3.3 abcdef
25	Darle Spring & Fall	Melo	3.2 bcdef
26	Asia Summer	Melo	3.5 abcdef
27	Raina	Melo	3.9 abcdef
28	Pestar	Melo	3.7 abcdef
29	Earls Focus	Melo	2.9 cdef
30	Earls Grand Prix	Melo	4.0 abcde
31	JeonnamMelli	Melo	3.1 cdef
32	Sweet Lady	Melo	2.6 defg
33	Earls Elite	Melo	3.7 defg
34	Earls Taitan	Melo	3.2 bcdef
35	Kings Daebak	Melo	3.5 abcdef
36	Earls Mountain PMR	Melo	3.9 abcdef
37	Kingdom	Melo	3.2 bcdef
38	Earls Grand	Melo	3.0 cdef
39	SS-402	Melo	4.1 abcd
40	Muskmelon SS Anup	Melo	3.7 abcdef
41	Asulan PMR	Melo	3.6 abcdef
42	Ropey King	Melo	3.3 abcdef
43	Napoyon	Melo	3.3 abcdef
44	Baldo	Melo	3.1 bcdef
45	VCR-601	Melo	2.8 def
46	Earls ok	Melo	2.7 defg
47	White Honey	Melo	3.2 bcdef
48	Giant Iryesaback	Melo	2.6 defg
49	Jochun 26	Melo	3.5 abcdef
50	Valencia Early-rochet	Melo	2.5 efgh
51	Asia Papaya	Melo	1.0 h
52	Eunpa 3	Melo	3.4 abcdef
53	ME4759	Melo	2.7 defg

Table 3 Foliar disease indices for melon (*Cucumis melo* L) Genotypes in response to *D. bryoniae* inoculation from growth chamber screening (Continued)

Sl. No.	Genotype	Horticultural group	Leaf disease rating
54	Eunpa fall & Winter melon	Melo	3.0 cdef
55	Happiness	Melo	3.0 cdef
56	Sunflower	Melo	2.6 defg
57	Vivadi	Melo	2.6 fgh
58	Snow White Melon	Melo	2.4 defg
59	Supra	Melo	1.0 h
60	Chungja	Melo	2.5 efgh
Minimum			1.0
Maximum			4.8
CV (%)			0.21

Melon lines (1-16) and melon commercial cultivars (17-60)

Fig. 2 The disease score of 60 melon genotypes under *Didymella bryoniae* infection

Results

Screening of melon lines against *D. bryoniae*

We screened a total of 60 genotypes of melon including resistant and susceptible control against *D. bryoniae* of which 16 were lines and 44 cultivars. Most of the lines were susceptible except ‘PI482399’, ‘PI140471’, ‘PI136170’ and ‘PI420145’, those exhibited high level of resistance as like as control resistant melon line ‘PI48239’8 with a disease rating of 1.0 (Table 3 and Figure S1). Remaining lines viz. ‘Cornell ZPPM 339’, ‘PI157082’, ‘MR1’, ‘PMR5’, ‘PMR45’, ‘WMR29’, ‘Edisto47’, ‘PI414723’, ‘PI124112’ and ‘SCNU1154’ showed a similar disease reaction (susceptible) to ‘Honeydew Greenflesh’, a control susceptible line (Table 3 and Fig. 2). Large differences were observed between the most resistant and susceptible lines (Fig. 2). Among these lines, high level

of susceptibility was found in control line ‘Honeydew Greenflesh’ with disease rating 4.8 which was followed by ‘PI124112’ (4.6), ‘MR1’ (4.4), ‘PI414723’ (4.0), ‘Cornell ZPPM 339’ (3.9), ‘PMR5’ (3.8), ‘PMR45’ (3.7), ‘WMR29’ (3.7), ‘PI157082’ (3.6) and ‘SCNU1154’ (3.6) (Table 3 and Fig. 2).

Screening of Korean melon commercial cultivars against *D. bryoniae*

Most of the commercial cultivar showed susceptibility in the disease reaction in response of *D. bryoniae* except ‘Asia Papaya’ and ‘Supra’ (Fig. S1). ‘Asia Papaya’ and ‘Supra’ were found to be highly resistant as like as control resistant line ‘PI482398’ with disease score rating 1.0 (Table 3 and Fig. 2). In contrast, ‘Alpeon’ and ‘SS-402’ were highly susceptible with rating 4.1 and which was near to control susceptible line Honeydew Greenflesh (Table 3 and Fig. 2).

Screening of melon genotypes by SSR markers

We analyzed a total of 38 previously reported simple sequence repeat (SSR) markers flanking to the resistant genes located in chromosome 1 in melon (Liu et al. 2009), and 4 and 6 in cucumber (Wang et al. 2012 and Ren et al. 2009) (Fig. 3). Twenty SSR markers were shown to be amplified from 12 melon lines, of these eleven were polymorphic between resistant and susceptible line (Fig. 4).

Genotyping of sixteen melon lines

Twenty amplified SSR markers were further used for

Fig. 3 The map shows the position of SSR markers linked to gummy stem blight resistance in melon and cucumber (Based on Liu et al., 2009 and Lou et al., 2013). Chr1 belongs to melon genome, Chr4, and Chr6 from cucumber genome

genotyping 16 melon lines. The result showed that only nine SSR markers were found to be polymorphic (Fig. 5). Among the nine polymorphic markers, ‘CMCT505’, which was linked to *Gsb1* perfectly, grouped resistant and susceptible lines as like as phenotypic data (Table S1). In

Fig. 4 Banding profile of 20 amplified SSR markers where number in the left and upper side of the gel indicating markers and genotypes, respectively

Fig. 5 PCR product for molecular screening of 16 melon lines with 20 amplified SSR markers linked to Gummy Stem Blight and electrophoresed in a 1.5% agarose gel. L: 100-bp ladder. Number in the left and upper side of the gel indicating markers and genotypes, respectively. CR indicates control resistant line PI482398 and CS indicates control susceptible line Honeydew Greenflesh. R and S indicates resistant and susceptible lines, respectively

Fig. 6 PCR product for molecular screening of 44 melon commercial cultivars with SSR marker CMCT505 linked to Gummy Stem Blight and electrophoresed in a 1.5% agarose gel. L: 100-bp ladder. CR and CS indicate control resistant (PI482398) and control susceptible (Honeydew Greenflesh) lines, respectively

addition, the markers 2, 4, 5, 18, 23, 25, 28 and 34 showed 68.8%, 43.8%, 25.0%, 68.8%, 37.5%, 75.0%, 68.8% and 50.0% adaptability, respectively.

Genotyping melon commercial cultivars

Forty-four melon commercial cultivars were also screened by using the polymorphic SSR marker ‘CMCT505’. Among 44 cultivars, two cultivars like ‘Asia Papaya’ and ‘Supra’ produced identical amplicons as resistant line ‘PI482398’ (Fig. 6 and Table S2).

Sequencing and comparison of marker amplicons

Cloning and sequencing identified notable variations in the nucleotide sequences between resistant and susceptible lines (Fig. S2). These variations include insertion/deletion (InDel) and single nucleotide polymorphism (SNP) between resistant and susceptible lines and cultivars. The sequence similarity of polymorphic resistant and susceptible lines and cultivars were accounted from 81.4 to 98.3% (Table S3).

Discussion

The rate of development in any breeding program can intensely be increased by a reliable screening test that exposes clear differences among individuals or progenies (Abad and Wehner, 1992). In this study, melon leaves were inoculated with *D. bryoniae* to evaluate GSB resistance. Information on the genetics of resistance to the GSB in most melon cultigens are not well elucidated yet (Pitrat et al. 1998). Two genetic systems have been proposed for GSB resistance including monogenic dominant and monogenic recessive (Prasad and Norton, 1967; Frantz and Jahn, 2004).

GSB resistant melon genotypes are essential for further advancement of the melon breeding program as this disease causes serious economic losses. In the present study, we reported some melon lines with a high level of resistance, namely ‘PI482399’, ‘PI140471’, ‘PI136170’ and ‘PI420145’.

Similar results were reported by Zhang et al. (1997). Nonetheless, they reported ‘PI140471’ as moderately resistant line. Moreover, we identified ‘PI157082’ as a susceptible line which was previously reported as resistant line by Zhang et al. (1997) and Frantz and Jahn (2004) (Table 3 and Fig. 2). In addition, Joseph et al. (2007) reported ‘PI482399’ as a susceptible but we found as highly resistant with disease score rating 1.0. Furthermore, Sakata et al. (2000) reported ‘PI140471’ as susceptible against a Japanese isolate of *D. bryoniae* while we found it as highly resistant (Table 3 and Fig. 2). These observed disparities might explain isolate-specific and isolate × environment-specific resistance response of melon genotypes (Song et al. 2004). Accordingly, the variations of resistance to GSB between the present study and previous report reveals that there might be differences between USA, Chinese, Japanese and Korean *D. bryoniae* isolates (Table S4). Therefore, further investigation on *D. bryoniae* race identifications is crucial. We also reported two new melon resistant commercial cultivars, including ‘Asia Papaya’ and ‘Supra’ (Table 3 and Fig. 2).

Prasad and Norton (1967) reported that disease resistance is due to presence of resistant gene but its expression must be dependent on genetic background. On the other hand, due to environmental variations, only phenotypic selections are not always been effective as well as sensitive to environment. Therefore, identification of marker linked to the disease is an indispensable step towards both marker assisted selection (MAS) and map based cloning (Wang and Roberts, 2006). Keeping these in mind, we screened our genotypes by previously reported SSR markers (Liu et al. 2009). The polymorphism detected by the marker ‘CMCT505’, ‘SSR02460’ and ‘SSR13251’ revealed that GSB resistant gene might be present in resistant lines and cultivars. Among these three markers, since *Gsb1* in melon chromosome 1 perfectly separated resistant and susceptible melon genotypes which were confirmed by bioassay screening, therefore, it could be speculated that *Gsb1* resistant locus might be existed in Korean resistant melon cultivars. The sequence variations in the flanking SSR markers between

resistant and susceptible in melon lines and cultivars suggesting that mutation might be occurred during evolution.

Our marker based screening speculates that resistance observed in some selected melon genotypes might be associated with presence of *Gsb-1* resistance locus. However, this speculation is a subject of molecular mapping and detection of resistance loci and resistance genes in our melon genotypes.

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References

- Abad ZH, Wehner TC (1992) Development of Seedling test for resistance to gummy stem blight in cucumber. Cucurbit Genetics. Cooperative Report 15:23-27
- Crosby KM., Miller ME, Wolff DW (2002) Screening plant introductions of *Cucumis melo* for resistance to *Monosporascus cannonballus*. In: Maynard D.N. (ed.). Cucurbitaceae 2002, American Society of Horticultural Science Press, Alexandria, Va. p. 188-191
- Frantz JD, Jahn MM (2004) Five independent loci each control monogenic resistance to gummy stem blight in melon (*Cucumis melo* L.). Theoretical and Applied Genetics 108: 1033-1038
- Gusmini G, Wehner TC (2002) Correlation of ratings for resistance to gummy stem blight in watermelon. In: D.N. Maynard (ed.). American Society of Horticultural Science Press, Alexandria, Va. p. 199-205
- Joseph N, Zhou, WXH, Li, Zhang, YB, Chen J F (2007) Resistance to Gummy Stem Blight in Melon (*Cucumis melo* L.) Germplasm and Inheritance of Resistance from Plant Introductions 157076, 420145, and 323498. Horticultural Science 42:215-221
- Joseph N, Zhou, WXH, Chen, JF (2009) Identification of amplified Fragment Length Polymorphism Markers Linked to Gummy Stem Blight (*Didymella bryoniae*) Resistance in Melon (*Cucumis melo* L.) PI 420145. Horticulture Science 44:32-34
- Keinath AP, Farnham, FW, Zitter TA (1995) Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma* spp. Isolated from cucurbits. Phytopathology 85:364-369
- Liu WR, Hing ZY, Hui ZX, Feng CJ (2009) SSR Marker Linked to Gummy Stem Blight Resistance Gene *Gsb-1* in Melon and Its Allelism with Resistance Gene from PI420145 Nanjing Agricultural University Journal 5:1-4
- Lv D, Cuevas H, Yang Li, Garcia MJ, Zalapa J, Staub J, Luan F, Reddy U, He, X., Gong Z, Weng Y (2012) Syntenic relationships between cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) chromosomes as revealed by comparative genetic mapping. Molecular Biology Genomics 12:396
- Li Y, Wen C, Weng Y (2013) Finemapping of the pleiotropic locus B for black spine and orange mature fruit color in cucumber identifies a 50 kb region containing a R2R3-MYB transcription factor. Theoretical and Applied Genetics 126:2187-2196
- McGrath DJ, Vawdrey L, Walker O (1993) Resistance to gummy stem blight in muskmelon. Horticultural Science 28:930-931
- Norton JD, Cosper RD, Smith DA, Rymal K S (1985) Aurora-Ahigh quality disease resistant cantaloupe. Auburn University Alabama Agricultural Experiment Station 278
- Norton JD, Cosper RD (1989) Gummy stem blight resistant muskmelon breeding line. Horticultural Science 24:709-711
- Prasad k, Norton JD (1967) Resistance of *Mycosphaerella cytrullina* in muskmelon. Proceedings for the American Society for Horticultural Science 91:396-400
- Pitrat M, Dogimont C, Bardin M (1998) Resistance to fungal diseases of foliage in melon. American Society of Horticultural Science 98:167-173
- Ren Y, Zhonghua Z, Jinhua L, Jack ES, Yonghua H, Zhouchao C, Xuefeng L, Hingyuan L, Han M, Houxiang K, Bingyan X, Xianfang G, Xiaowu W, Yongchen D, Weiwei J, Sanwen H (2009) An Integrated Genetic and Cytogenetic Map of the Cucumber Genome. Plos One 4, e5797
- StAmand PC, Wehner TC (1991) Crop lossto 14 diseases of cucumber in North Carolina from 1983 to 1988. Cucurbit Genetics. Cooperative Report 14:15-17
- StAmand PC, Wehner TC (1995) Greenhouse detached-leaf, and field testing methods to determine cucumber resistance to gummy stem blight. American Society of Horticultural Science 120:673-680
- Sitterly RW, Keinath AP (1996) Gummy stem blight. In: Zitter TA, Hopkins DL, Thomas C (eds) Compendium of cucurbit diseases. American Phytopathology Society, APS Press 27-28
- Sakata Y, Wako T, Sugiyama M, Morishita M (2000) Screening melons for resistance to gummy stem blight. Acta Horticulturae 510:171-177
- Song R, Gusmini G, and Wehner TC (2004) Asummary of eleven preliminary studies of greenhouse and field testing methods for resistance to gummy stem blight in melon. Progress in Cucurbit Genetics and Breeding Research 301-305
- Tullu A, Bachwaldt L, Warkentin T, Taran B, Vandenberg A (2002) Genetics of resistance to anthracnose and identification

- of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theoretical and Applied Genetics 106:428–434
- Wako T, Sakata Y, Sugiyama Ohara, M, Ishiiji TD, Kojima A, Nishimura S (2002) Identification of melon accessions resistant to gummy stem blight and genetic analysis of the resistance using an efficient technique for seedling test. Acta Horticulturae 588:161–164
- Wang CL, Roberts PA (2006) Development of AFLP and derived CAPS markers for rootknot nematode resistance in cotton. Euphytica 152:185–196
- Wang HY, Chuntao Q, lina L, Qunfeng L, Yongbing Z, Hongping Y, Mingzhu WU, Jinfeng C (2012) A SSR Marker linked to *Gsb-4* loci Resistance to Gummy Stem Blight in Melon. Acta Horticulturae Sinica 39:574–580
- Wolukau JN, Zhou XH, Zhang YB, Chen JF (2007) “Resistance to gummy stem blight in melon (*Cucumis melo* L.) germplasm and inheritance of resistance from plant introductions 157076, 420145, and 323498.” Horticulture Science 42:215–221
- Wolukau JN, Zhou XH, Chen JF (2009) “Identification of amplified fragment length polymorphism markers linked to gummy stem blight (*Didymella bryoniae*) resistance in melon (*Cucumis melo* L.) PI 420145.” Horticulture Science 44:32–34
- Zhang Y, Kyle MM, Anagnostou K, Zitter TA (1997) Screening melon (*Cucumis melo*) for resistance to gummy stem blight in the greenhouse and field. Horticulturae Science 32:117–121
- Zitter TA, Kyle MM (1992) Impact of powdery mildew and gummy stem blight on collapse of pumpkin (*Cucurbita pepo* L.). Cucurbit Genetics. Cooperative Report 15:93–96
- Zhu H, Guo L, Song P, Luan F, Hu J, Sun X, Yung L (2016) “Development of genome-wide SSR markers in melon with their cross-species transferability analysis and utilization in genetic diversity study.” Molecular Breeding 36:153