Development of PCR-Based Marker for Resistance to Fusarium wilt Race 2 in Lettuce (*Lactuca Sativa* L.)

kousuke seki (✉ seki-kosuke@pref.nagano.lg.jp)

Nagano vegetable and ornamental crops experiment station <https://orcid.org/0000-0002-5420-838X>

kenji komatsu

Tokyo Nogyo Daigaku - Atsugi Campus

masahiro hiraga

Nagano fruit tree experiment station

keisuke tanaka

Tokyo Nogyo Daigaku - Setagaya Campus: Tokyo Nogyo Daigaku

yuichi uno

Kobe University: Kobe Daigaku

hideo matsumura

Shinshu University - Ueda Campus: Shinshu Daigaku - Ueda Campus

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Abstract

Resistance to multiple races of *Fusarium* wilt is considered a critical trait for lettuce (*Lactuca sativa* L.) cultivars, as it is directly related to grower profitability in Japan. We analyzed F₂ individuals obtained from a cross between 'VI185' (resistance to race 2) and 'ShinanoGreen' (susceptible to race 2), and applied a bioassay to the F₃ population using the race 2 pathogen. ddRAD-seq analysis showed that a single semi-dominant locus on LG1 (*qFOL 1.2*) controls resistance, and that the genotype of a RAD-marker designated as *LG1_v8_117.181Mbp* showed complete co-segregation with the resistance phenotype based on the F₂ population. Fine mapping by PCR-based markers further revealed that *qFOL 1.2* were located in the position of 116.468-117.974Mbp. The 42 cultivars were evaluated for the genotypes and phenotypes using the PCR-based makers designed at this region. As a result of genotyping, all of the susceptible cultivars had the same genotype in this region, but the resistant cultivars had the diversity in the genotype. Notably, the genotype of the PCR-based marker designated as *LG1_v8_116.506Mbp* was consistent across the 25 resistant cultivars. Thus, we reasoned that *LG1_v8_116.506Mbp* was a broadly useful marker for selection of race 2-resistance. Our results provide additional breeding technique for resistance to race 2, and can accelerate pyramiding of resistance loci to multiple races of fusarium wilt.

Introduction

Fusarium wilt (alias Root Rot Disease) of lettuce caused by *Fusarium oxysporum* f. sp. *lactucae* races 1, 2 and 3 presents a serious problem for lettuce production during summer in Japan (Fujinaga et al. 2001, 2003, 2005; Ogiso et al. 2002). As gradually increasing of areas that various races have been found, especially race 1 and 2, the breeding to the resistance cultivar to multiple races is an important issue. For plant breeding programs, molecular markers are powerful tools in marker-assisted selection (MAS) and gene pyramiding to develop new cultivars with the resistance to multiple races (Ashikari and Matsuoka 2006; Kumar et al. 2011). To date, we have successfully identified two loci for resistance to race 1 (Seki et al. 2020a), have attempted to develop molecular marker for race 2 following race 1. It was reported that the major resistance locus of race 2 was single, in addition, race 2 was at present only found in Japan (Shimazu et al. 2009; Aruga et al. 2012). We already developed a marker WF25-42 with random-amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) analysis, and they indicated this marker is only detected the susceptible phenotype (Aruga et al. 2012). The objective of this study, therefore, is to develop a more useful marker tightly linked to resistance gene to race 2 for MAS.

Next generation sequencing (NGS) based genotyping methods are extremely useful for designing molecular marker such as genome wide single nucleotide polymorphisms (SNPs) (Baird et al. 2008; Abe et al. 2012; Matsumura et al. 2014). Double digest restriction site-associated DNA sequencing (ddRAD-seq), high-throughput analysis of multiple samples, allowed for the rapid mapping of the resistance locus because reference genome sequence of lettuce (Reyes-Chin-Wo et al. 2017) was already provided from Lettuce Genome Resource (LGR; <https://lgr.genomecenter.ucdavis.edu>). We applied a ddRAD-seq to a F₂ population obtained from a cross between 'VI185' (resistance to race 2) and 'ShinanoGreen' (susceptible

to race 2), and a bioassay to the F_3 population using pathogen inoculation. We tried to develop a reliable PCR-based marker in the genomic vicinity of race 2-resistance locus for MAS breeding by employing Genomic DNA-seq of parents.

Materials And Methods

Plant materials

Plant materials and pathogen

All plant material was grown at the Nagano Vegetable and Ornamental Crops Experiment Station (Shiojiri City, Nagano prefecture, Japan; 36° 10' N, 137° 93' E). The crisphead types 'VI185', 'ShinanoGreen' are cultivars from the Nagano Vegetable and Ornamental Crops Experiment Station lettuce breeding program. The F_2 population was derived from a cross between 'VI185' and 'ShinanoGreen'. Ninety-six individuals of the F_2 progeny from the 'VI185' by 'ShinanoGreen' cross were used for linkage analysis and to produce selfed F_3 populations, which were used to evaluate genotypes of disease resistance in the F_2 individuals. For the infection assay of *Fusarium oxysporum* f. sp. *lactucae* race 2, the Japanese isolate F9501 was employed.

Infection assay for disease resistance

Infection assay for disease resistance was performed at greenhouse of the Nagano Vegetable and Ornamental Crops Experiment Station. Infested soils using the isolate of F9501 (race 2) were prepared by mixing bran culture medium and sterile soil at a ratio of 1:19 by volume. Seeds were sown directly into artificially inoculated soil, and seedlings were grown in the greenhouse conditions at a temperature between 15°C and 35°C under natural day length. Approximately 1 month after germination, seedlings were inoculated by F9501 and their disease indices were scored as described previously (Seki et al. 2020a). The disease severity was calculated as described by Aruga et al. (2012).

DNA extraction and libraries construction

Genomic DNAs were purified using the NucleoSpin Plant II (Machery-Nagel, Duren, Germany). Libraries of the ddRAD-seq and Genomic DNA-seq were prepared as described method in the previous publication (Seki et al. 2020b). Previously analyzed ddRAD-seq and whole genome sequencing data were used (Seki et al. 2020a).

QTL mapping of disease resistance

The disease severity of the F_3 population derived from ninety-six individuals of the F_2 progeny were evaluated according to the results of infection assay. Genetic mapping of the *F. oxysporum* f. sp. *lactucae* race 2 resistance locus in 'VI185' was performed using previously analyzed ddRAD-seq data from an F_2 population derived from a cross between 'VI185' and 'ShinanoGreen'. Composite interval mapping (CIM)

was conducted using the Haley-Knott regression in R using R/qtl package (Broman et al. 2003). The genome wide LOD threshold at 1% significance level was determined using a 10,000 permutation test. The proportion of phenotypic variance was calculated from the value at the peak indicated by CIM. A detailed script is described in CIM_script.R (https://github.com/KousukeSEKI/RAD-seq_scripts). The resistance phenotype was visualized graphically with the plotPXM function (Broman et al. 2003). MapChart ver. 2.3 was used to depict the linkage map and QTL (Voorrips 2002).

Designing PCR-based markers and their amplification

Polymorphisms between parental lines around the QTL, including insertion, deletion and SNP, were surveyed to identify the marker sites using the IGV software. Primers for amplifying the markers were designed using the Primer3 website (<http://bioinfo.ut.ee/primer3-0.4.0/>), and their IDs (names) was defined as (Linkage group) _ (genome version) _ (genome position). PCR was performed using 0.5 µL DNA template, 0.4 µL of each primer (50 µM), 2 µL dNTP (2 mM), 5 µL 2× PCR Buffer, 0.2 µL KOD FX (1 U/µL, TOYOBO, Japan), and distilled water (dH₂O) to a final volume of 10 µL. PCR conditions were as follows: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, and 61 °C for 30 s, followed by one cycle at 72 °C for 4 min. The amplification products were electrophoresed using capillary electrophoresis apparatus, MCE202 MultiNA (Shimadzu, Kyoto, Japan) and visualized by control and MultiNA Viewer software.

Results

Inheritance of resistance to *F. oxysporum* f. sp. *lactucae* race 2

Disease-resistance genotypes of the F₂ individuals from a cross between resistant 'VI185' and susceptible 'ShinanoGreen' were determined by the infection assay of the subsequent F₃ generation by *F. oxysporum* f. sp. *lactucae* race 2. Since the correlation between their disease severity of shoot and root has already been reported by Aruga et al. (2012) and Seki et al. (2020a), we focused on disease resistance in shoot tissue. Resistance to race 2 derived from 'VI185' was suggested to be controlled by a single semi-dominant gene, according to segregation of putative genotype of resistance gene showing 1:2:1 ratio in the F₂ population (Table 1). This result coincides to the previous studies (Shimazu et al. 2009; Aruga et al. 2012).

ddRAD-seq analysis and fine mapping of resistance to race 2

Genetic mapping of the *F. oxysporum* f. sp. *lactucae* race 2 resistance derived from 'VI185' was performed using previously analyzed RAD-seq data and linkage map of F₂ population derived from a cross between 'VI185' and 'ShinanoGreen' (Seki et al. 2020b). CIM analysis was performed using the disease severity scores determined by race 2 pathogen infection assay and marker genotype data from the ddRAD-seq in 96 F₂ individuals. From the CIM analysis, a single locus for the resistance to the race 2 was mapped in LG1 between two markers (*LG1_v8_113.602Mbp* and *LG1_v8_130.852Mbp*) at a 9.55 cM interval, and designated as *qFOL1.2*. Genotype of the *LG1_v8_117.181Mbp* marker, located in the

qFOL 1.2 locus, showed complete co-segregation with resistance to race 2 in the F₂ population (Table 2, Fig. 1, Fig. 2), and *qFOL 1.2* accounted for approximately 96.47% of the phenotypic variance (Table 2). According to the genotypes of biallelic RAD tags around *qFOL 1.2* and segregation of the deduced resistant locus genotype deduced in F₂ plants, *qFOL 1.2* was located between 116.225 to 118.058 Mbp in LG1 (Fig. 2). Furtherly, its fine mapping using PCR-based markers (Table 3) demonstrated that location of *qFOL 1.2* was narrowed down between *LG1_v8_116.468Mbp* and *LG1_v8_117.974Mbp* markers in LG1 (Table 3, Fig. 2). Genetic distance between these two marker loci was 1.028 cM, corresponding to 1.505Mbp as physical distance. To develop practical DNA markers for *qFOL 1.2* applicable to the marker assisted selection (MAS) in the breeding program, polymorphisms between parental lines were surveyed in the genome sequences within the range between 116.468–117.974Mbp in LG1. The eight PCR-based markers were identified by employing the selected polymorphisms. The marker types of the eight PCR-based markers were one ARMS marker designed using SNPs, two SSR markers, and five INDEL markers (Table 3). These selected eight PCR-based markers were applied to genotyping analysis of 25 resistant and 17 susceptible lettuce cultivars to race 2 (Table 3, Table 4, Fig. S1). In all the analyzed cultivars, all the marker genotypes represented homozygous genotypes. Genotypes of these markers demonstrated the susceptible cultivars uniformly showed the same genotype in this region, while inconsistent genotypes of most analyzed markers were observed in the resistant cultivars (Table 4). Of the 8 markers, only *LG1_v8_116.506Mbp* showed the consistent genotype in all the analyzed resistant cultivars. Therefore, *LG1_v8_116.506Mbp* was the widely available marker for MAS of race 2 resistant plants.

Discussion

Following the previous QTL mapping study for the resistance to fusarium wilt race 1 (Seki et al. 2020a), we succeeded in rapid genetic mapping of resistance to race 2 in lettuce, since F₂ population derived from a cross between ‘VI185’ and ‘ShinanoGreen’ with their genome-wide marker genotypes (Seki et al. 2020b) were already available for the QTL analysis. Consequently, a single major QTL, *qFOL 1.2*, derived from ‘VI185’ was efficiently identified in LG1. According to the genetic mapping using these ddRAD-seq and PCR-based markers, resistance to race 2 was probably determined by a single locus, and located between 116.468Mbp and 117.974Mbp in LG1 of the reference genome (Table 1, Table 4, Fig. 2). Previously, we developed a marker WF25-42 linked to the resistance locus to race 2 by RAPD and AFLP analysis (Aruga et al. 2012) and it also located at the position of 117.9Mbp in LG1 (data not shown), supporting that *qFOL 1.2* was the locus for the resistance to race 2.

In this study, we further developed the PCR-based markers for the *qFOL 1.2* (Table 3, Table 4, Fig. 2, Fig. S1). Genotyping in 42 cultivars using these PCR-based markers revealed that the resistant cultivars showed divergence in this genomic region (Table 3, Table 4), except for *LG1_v8_116.506Mbp*. Although disease by race 2 pathogen was known to be only occurred in Japan, ‘Grand Rapids’ derived from USA, ‘Oak leaf’ derived from Europe, and ‘Celtuce’ derived from China also showed the resistance to race 2 (Table 4). Accordingly, many lettuce cultivars in the worldwide natively possessed the resistance gene to race 2, and limited cultivars like ‘Salinas’ were susceptible to race 2. This was supported by the fact that

all of the susceptible cultivars had the same genotypes in this locus, implying that they were possibly derived from the same origin. The PCR-based marker, *LG1_v8_116.506Mbp*, can discriminate between resistant and susceptible to race 2 perfectly, suggesting that it is widely applicable to the selection of *qFOL1.2* in the breeding program and suggested to be closed to the causal gene for the resistance.

Notably, no disease resistance gene candidate was found in the annotation database for ‘Salinas’ genome even though this locus, between 116.468Mbp and 117.974Mbp in LG1, was located in a major resistance clusters (MRCs) (Table S1) (Christopoulou et al. 2015). Because ‘Salinas’ is a susceptible cultivar to race 2, it is possible that it may lack its disease resistance gene. Therefore, further studies, including genome resequencing analysis of the resistant cultivar, are necessary to determine the causal gene located in *qFOL1.2*.

Fusarium wilt frequently occurs under high-temperature environment (Tsuchiya et al. 2004), like summer season in Japan. Therefore, global warming advance was supposed to promote fusarium wilt occurrence in other countries. Three races of fusarium wilt (race 1,2, and 3) were already found in Japan (Fujinaga et al. 2001, 2003). Furthermore, its race 4 was recently identified in Europe (Gilardi et al. 2017, 2019; Claerbout et al. 2018). Considering difficulty of soil sterilization in the field, development of resistance cultivars is the most efficient strategy for avoiding disease. We already found significant QTLs in LG7 (*qFOL7.1*) and LG8 (*qFOL8.1*) that has major effect on race 1-resistance (Seki et al. 2020a). Marker-assisted selection of race 2-resistance in combination with QTLs for race 1-resistance provides the opportunity to develop cultivars with more durable resistance to fusarium wilt. The findings of this study will contribute to the pyramiding approach for disease resistance loci to multiple races of fusarium wilt.

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data analyzed during this study are included in this published article and its supplementary information files.

Code availability

Scripts for these analyses are deposited in https://github.com/KousukeSEKI/RAD-seq_scripts as (1) Parent_script.R, (2) RAD_mapping_script_BWAaln_script.txt, (3) Map_list_script.R, (4) Population_tagcount_script.R, (5) Genotype_script.R, (6) Convert_ABH_script.R, and (7) Post_data_correction_script.R.

Author contribution statement

KS, KK and HM planned the experiments. KS and MH performed the development of the mapping population. KS and MH performed bioassay for disease resistance. KS and YU performed the development of the ddRAD-seq library. KK and KT performed sequence analysis. KS and HM performed data analysis. KS wrote the manuscript. All authors read and approved the final manuscript.

References

- Abe A, Kosugi S, Yoshida K, et al (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. *Nat Biotechnol* 30:174–178. [https://doi.org/https://doi.org/10.1038/nbt.2095](https://doi.org/10.1038/nbt.2095)
- Aruga D, Tsuchiya N, Matsumura H, et al (2012) Analysis of RAPD and AFLP markers linked to resistance to *Fusarium oxysporum* f. sp. *lactucae* race 2 in lettuce (*Lactuca sativa* L.). *Euphytica* 187:1–9. <https://doi.org/10.1007/s10681-012-0665-5>
- Ashikari M, Matsuoka M (2006) Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends Plant Sci.* 11:344–350
- Baird NA, Etter PD, Atwood TS, et al (2008) Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. *PLoS One* 3:e3376. <https://doi.org/10.1371/journal.pone.0003376>

- Broman KW, Wu H, Saunak Sen ´, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinforma Appl NOTE* 19:889–890. <https://doi.org/10.1093/bioinformatics/btg112>
- Christopoulou M, Wo SRC, Kozik A, et al (2015) Genome-wide architecture of disease resistance genes in lettuce. *G3 Genes, Genomes, Genet* 5:2655–2669. <https://doi.org/10.1534/g3.115.020818>
- Claerbout J, Venneman S, Vandeveldel I, et al (2018) First report of *Fusarium oxysporum* f. Sp. *lactucae* race 4 on lettuce in Belgium. *Plant Dis.* 102:1037
- Fujinaga M, Ogiso H, Shinohara H, et al (2005) Phylogenetic relationships between the lettuce root rot pathogen *Fusarium oxysporum* f. sp. *lactucae* races 1, 2, and 3 based on the sequence of the intergenic spacer region of its ribosomal DNA. *J Gen Plant Pathol* 71:402–407. <https://doi.org/10.1007/s10327-005-0226-z>
- Fujinaga M, Ogiso H, Tsuchiya N, Saito H (2001) Physiological Specialization of *Fusarium oxysporum* f. sp. *lactucae*, a Causal Organism of Fusarium Root Rot of Crisp Head Lettuce in Japan. *J Gen Plant Pathol* 67:205–206. <https://doi.org/10.1007/pl00013012>
- Fujinaga M, Ogiso H, Tsuchiya N, et al (2003) Race 3, a new race of *Fusarium oxysporum* f. sp. *lactucae* determined by a differential system with commercial cultivars. *J Gen Plant Pathol* 69:23–28. <https://doi.org/10.1007/s10327-002-0009-8>
- Gilardi G, Franco Ortega S, van Rijswijk PCJ, et al (2017) A new race of *Fusarium oxysporum* f. sp. *lactucae* of lettuce. *Plant Pathol* 66:677–688. <https://doi.org/10.1111/ppa.12616>
- Gilardi G, Garibaldi A, Matic S, et al (2019) First Report of *Fusarium oxysporum* f. sp. *lactucae* Race 4 on Lettuce in Italy. *Plant Dis* 103:2680–2680. <https://doi.org/10.1094/pdis-05-19-0902-pdn>
- Kumar J, Choudhary AK, Solanki RK, Pratap A (2011) Towards marker-assisted selection in pulses: A review. *Plant Breed.* 130:297–313
- Matsumura H, Miyagi N, Taniai N, et al (2014) Mapping of the gynoecey in bitter melon (*Momordica charantia*) using RAD-seq analysis. *PLoS One* 9:. <https://doi.org/10.1371/journal.pone.0087138>
- Ogiso H, Fujinaga M, Saito H, et al (2002) Physiological Races and Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *lactucae* Isolated from Crisphead Lettuce in Japan. *J Gen Plant Pathol* 68:292–299. <https://doi.org/10.1007/pl00013094>
- Reyes-Chin-Wo S, Wang Z, Yang X, et al (2017) Genome assembly with in vitro proximity ligation data and whole-genome triplication in lettuce. *Nat Commun* 8:1–11. <https://doi.org/10.1038/ncomms14953>
- Seki K, Komatsu K, Hiraga M, et al (2020a) Identification of two QTLs for resistance to Fusarium wilt race 1 in lettuce (*Lactuca sativa* L.). *Euphytica* 216:1–11. <https://doi.org/10.1007/s10681-020-02713-8>

Seki K, Komatsu K, Tanaka K, et al (2020b) A CIN-like TCP transcription factor (*LsTCP4*) having retrotransposon insertion associates with a shift from Salinas type to Empire type in crisphead lettuce (*Lactuca sativa* L .). Hortic Res 7:1–14. <https://doi.org/10.1038/s41438-020-0241-4>

Shimazu J, Yamauchi N, Shirakawa T (2009) Heredity of resistance to race 2 of *Fusarium oxysporum* f. sp. *lactucae* in a lettuce (*Lactuca sativa*) cultivar “Celtuce.” Breed Res 11:155–158

Tsuchiya N, Fujinaga M, Ogiso H, Usui T (2004) Resistance tests and genetic resources for breeding fusarium root rot resistant lettuce. J Japan Soc Hort Sci 73:105–113

Voorrips RE (2002) MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. J Hered 93:77–78. <https://doi.org/10.1093/jhered/93.1.77>

Tables

Table 1. Segregation of the resistance to Fusarium wilt race 2 in F₂ population derived from ‘VI185’ and ‘ShinanoGreen’

Population	Total number of plants	Number of plants showing the disease severities			Segregation ratio	χ^2 (1 : 2 : 1)
		0–10	10–90	90–100		
VI185	10	10	0	0	-	-
ShinanoGreen	10	0	0	10	-	-
F ₂	96	17	49	30	1 : 2.9 : 1.8	0.17

Table 2. Identified QTL for disease resistance to Fusarium wilt race 2 by composite interval mapping and genetic effects.

Trait name	Linkage Group	Marker	Genetic distance (cM)	Physical position (cM)	Threshold LOD	LOD	Additive Effect	Dominant Effect	R ² (%)
<i>qFOL1.2</i>	LG1	LG1_v8_113.602Mbp to LG1_v8_130.852Mbp	9.55	114.49 - 124.04	4.55	71.15	-48.18	-6.64	96.47

Table 3. Primers for the PCR-based markers in *qFOL1.2*

Primer name	Primer sequence (5'–3')	Marker type [*]
LG1_v8_116.394Mbp_F LG1_v8_116.394Mbp_R	CTCAACGGTTGGGCTTGAC AGTGCTCACCATGTCGATCT	SSR
LG1_v8_116.452Mbp_ShinanoGreen_F LG1_v8_116.452Mbp_ShinanoGreen_R LG1_v8_116.452Mbp_VII85_F LG1_v8_116.452Mbp_VII85_R	CATGTCAATGGAGGGTTTAGG GATAAGCTTAGTACCCTTAATAAG GTCGAATTACTTCCCGAAC TGCAG CTCTTCAACCCACCAATCC	ARMS
LG1_v8_116.468Mbp_F LG1_v8_116.468Mbp_R	TGAACGGTTCTATGGTTTTG AAGGTTGAGGAAAAATAAGCACA	INDEL
LG1_v8_116.506Mbp_F LG1_v8_116.506Mbp_R	TCCCAACAGAATCCTCAACG TCTTGATGGCATTITGGTCA	SSR
LG1_v8_117.090Mbp_F LG1_v8_117.090Mbp_R	AACGATTTTGTATGGGTGATTCAA TTGCCACCATCACTACCAAA	SSR
LG1_v8_117.175Mbp_F LG1_v8_117.175Mbp_R	TGGTTTTCAGCCACATAAA CACGTGTGCCTCTACGACGA	INDEL
LG1_v8_117.451Mbp_F LG1_v8_117.451Mbp_R	CGTCGTTTAGGGAGTTGGAA AAAGGCGGTACACATTTTGG	INDEL
LG1_v8_117.549Mbp_F LG1_v8_117.549Mbp_R	TTTATTTTCGATCCGCCACT TCATCGGTCTCAAAGAATTGC	INDEL
LG1_v8_117.892Mbp_F LG1_v8_117.892Mbp_R	GCACATGCATTTTGTAAAC TGCCTTTACCAGAGTGTTTCG	INDEL
LG1_v8_117.974Mbp_ShinanoGreen_F LG1_v8_117.974Mbp_VII85_F LG1_v8_117.974Mbp_R	GAGAGTGCATGGGTTTCGAT CCATATCAATGCACACAGGTT CCACCGACTGCAATACCAAC	ARMS

* SSR: Simple Sequence Repeat, INDEL: INsertion and DEletion, ARMS: Amplified Refractory Mutation System.

Table 4. Resistance to Fusarium wilt race 2 and the genotype of PCR-based markers at *qFOL 1.2* in 42 lettuce cultivars.

Cultivars name	No. of plants tested	The disease index				Disease severity ¹	Disease phenotype	Genotype of PCR-based markers ²							
		0	1	2	3			LG1_y8_116.468Mbp	LG1_y8_116.506Mbp	LG1_y8_117.090Mbp	LG1_y8_117.175Mbp	LG1_y8_117.451Mbp	LG1_y8_117.549Mbp	LG1_y8_117.892Mbp	LG1_y8_117.974Mbp
VII85	10	10	0	0	0	0	Resistance	A	A	A	A	A	A	A	A
Banchu Red Fire	9	9	0	0	0	0	Resistance	B	A	C	A	A	A	B	N.D.
Escort	9	9	0	0	0	0	Resistance	N.D.	A	B	A	N.D.	N.D.	N.D.	N.D.
Alex BB	10	10	0	0	0	0	Resistance	B	A	C	B	N.D.	N.D.	B	N.D.
HaruP	10	10	0	0	0	0	Resistance	N.D.	A	B	C	B	N.D.	N.D.	N.D.
Salad Bowl	9	9	0	0	0	0	Resistance	A	A	A	A	A	A	A	A
ShinanoLead	10	10	0	0	0	0	Resistance	A	A	A	A	A	A	A	A
Taugh V	10	10	0	0	0	0	Resistance	A	A	A	A	A	A	A	A
Valu sine	8	8	0	0	0	0	Resistance	B	A	C	B	N.D.	N.D.	B	N.D.
White Paris	10	10	0	0	0	0	Resistance	B	A	C	B	N.D.	N.D.	B	N.D.
Oak Leaf	10	9	1	0	0	3.3	Resistance	B	A	C	B	N.D.	N.D.	B	N.D.
Grand Rapids	10	9	0	1	0	6.7	Resistance	A	A	A	A	A	A	A	A
Celuce	9	7	2	0	0	7.4	Resistance	B	A	A	B	N.D.	N.D.	B	N.D.
Blizzard	10	9	0	0	1	10	Resistance	A	A	A	A	A	A	A	A
Flamingo	10	7	3	0	0	10	Resistance	B	A	C	A	A	A	B	N.D.
Lollo Rossa	10	7	3	0	0	10	Resistance	A	A	A	A	A	A	A	A
Lushina67	10	8	1	1	0	10	Resistance	A	A	A	A	A	A	A	A
ShinanoPower	10	9	0	0	1	10	Resistance	A	A	A	A	A	A	A	A
Lushina66	8	7	0	0	1	12.5	Resistance	A	A	A	A	A	A	A	A
Alex S1	10	8	0	1	1	14.7	Resistance	B	A	C	B	N.D.	N.D.	B	N.D.
Vally	10	5	5	0	0	14.7	Resistance	A	A	A	A	A	A	A	A
EarlyImpulse	10	3	6	1	0	24.7	Resistance	B	A	C	B	N.D.	N.D.	B	N.D.
Randy	10	3	6	0	1	30	Resistance	A	A	A	A	A	A	A	A
Waldmann's Green	9	6	0	0	3	33.3	Resistance	A	A	A	A	A	A	A	A
Wide View No.8	10	6	0	2	2	33.3	Resistance	A	A	A	A	A	A	A	A
ShinanoGreen	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Mayer	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
NaganoVenus	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Olympia	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Patriot	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Raptor	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Salinas	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
ShinanoFresh	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
ShinanoHope	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
ShinanoStar	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
ShinanoSummer	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Summer Land	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
SummerAce	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Sun Vally	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
V Lettuce	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
Version	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B
VP2000	10	0	0	0	10	100	Susceptible	B	B	B	B	B	B	B	B

¹ Since the all cultivars was homogeneous, cultivars with disease severity less than 33.3 were evaluated as resistant, more than 33.4 were as susceptible.

² "A" indicates the phenotype of VII85, "B" indicates the phenotype of ShinanoGreen, "C" indicates the phenotype different from "A" and "B", "N.D." indicates No Band.

Figures

Figure 1

Correlation between the genotypes of LG1_v8_116.506Mbp and phenotypes (disease severity to Fusarium wilt). Twenty individuals of each of F2:3 plants derived from a cross 'VI185 (resistant)' and 'ShinanoGreen (susceptible)' were applied to genotyping and phenotype scoring. In genotype, "A" or "B" allele was derived from 'VI185' or 'ShinanoGreen', respectively.

Figure 2

Fine genetic map of qFOL1.2 on LG1. Genetic distances (cM) were shown between the markers. “(RAD)” and “(PCRbased)” in the marker name indicated RAD-seq markers and PCR-based markers, respectively. “FW2-LG1 (Fusarium Wilt resistance to race 2 on LG1)” indicated the position of the causal gene for resistance to Fusarium wilt race 2.

Supplementary Files

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