US 20180112267A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2018/0112267 A1

KOISHIHARA et al. (43) Pub. Date: Apr. 26, 2018

(54) **MARKER ASSOCIATED WITH POWDERY MILDEW RESISTANCE IN PLANT OF GENUS FRAGARIA AND USE THEREOF**

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(21) Appl. No.: **15/558,663**

(22) PCT Filed: **Mar. 18, 2016**

(86) PCT No.: **PCT/JP2016/058711**

§ 371 (c)(1),
(2) Date: **Sep. 15, 2017**

(30) **Foreign Application Priority Data**

Mar. 18, 2015 (JP) 2015-054618
Mar. 4, 2016 (JP) 2016-042028

Publication Classification

(51) **Int. Cl.**
C12Q 1/6876 (2006.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/6876*** (2013.01); *C12Q 2600/13* (2013.01)

(57) **ABSTRACT**

This invention intends to develop many DNA markers for a plant of the genus *Fragaria* and detect powdery mildew resistance with high precision by using the many DNA markers. The marker associated with powdery mildew resistance in a plant of the genus *Fragaria* comprises a continuous nucleic acid region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 19 in the chromosome of the plant of the genus *Fragaria*.

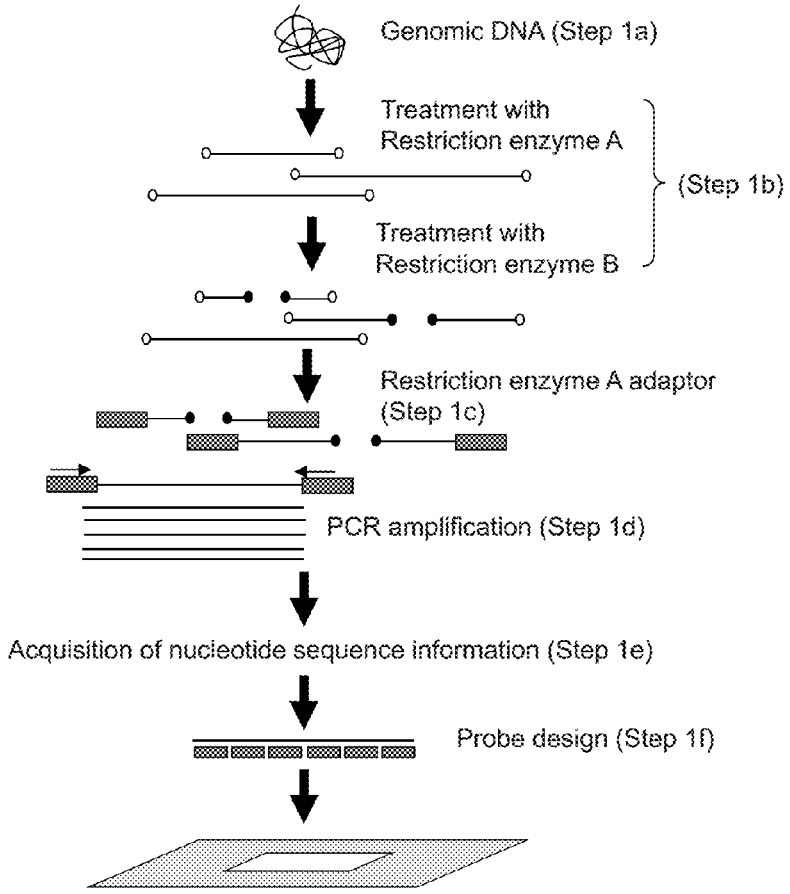


Fig. 1

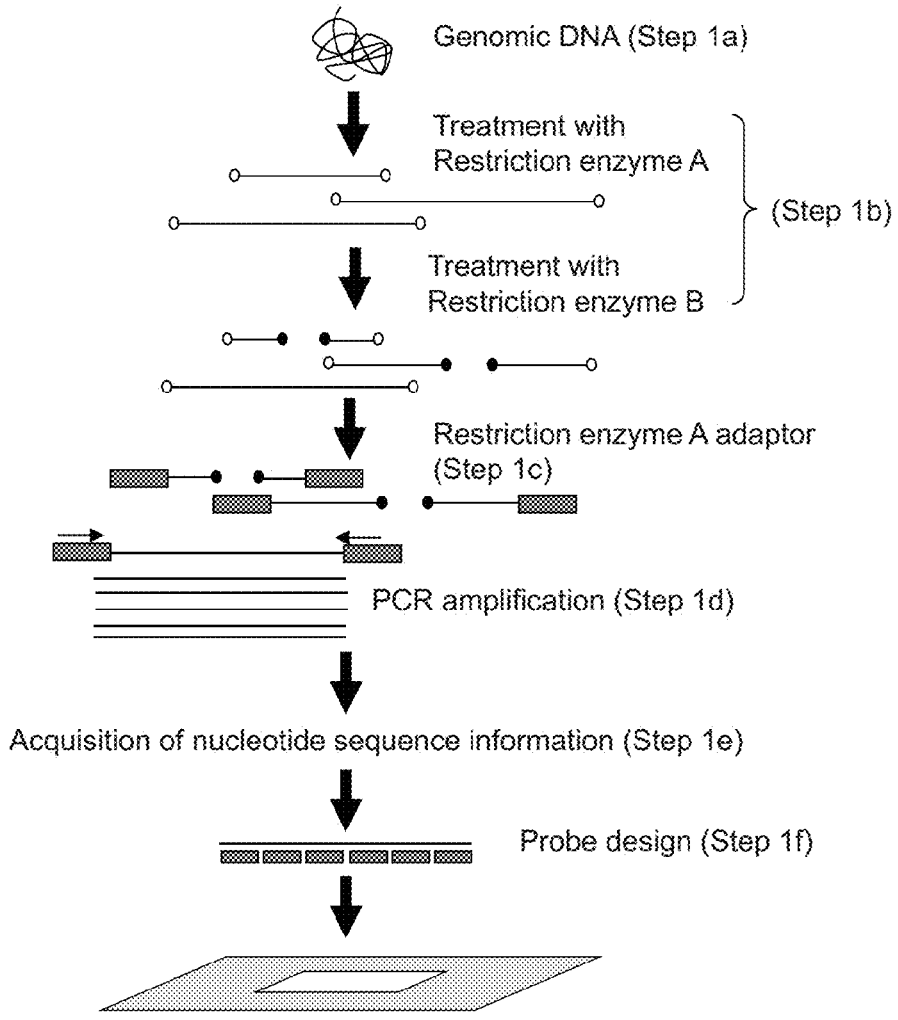

Genomic DNA (Step 1a)
Treatment with Restriction enzyme A
(Step 1b)
Treatment with Restriction enzyme B
Restriction enzyme A adaptor (Step 1c)
PCR amplification (Step 1d)
Acquisition of nucleotide sequence information (Step 1e)
Probe design (Step 1f)

Fig. 2

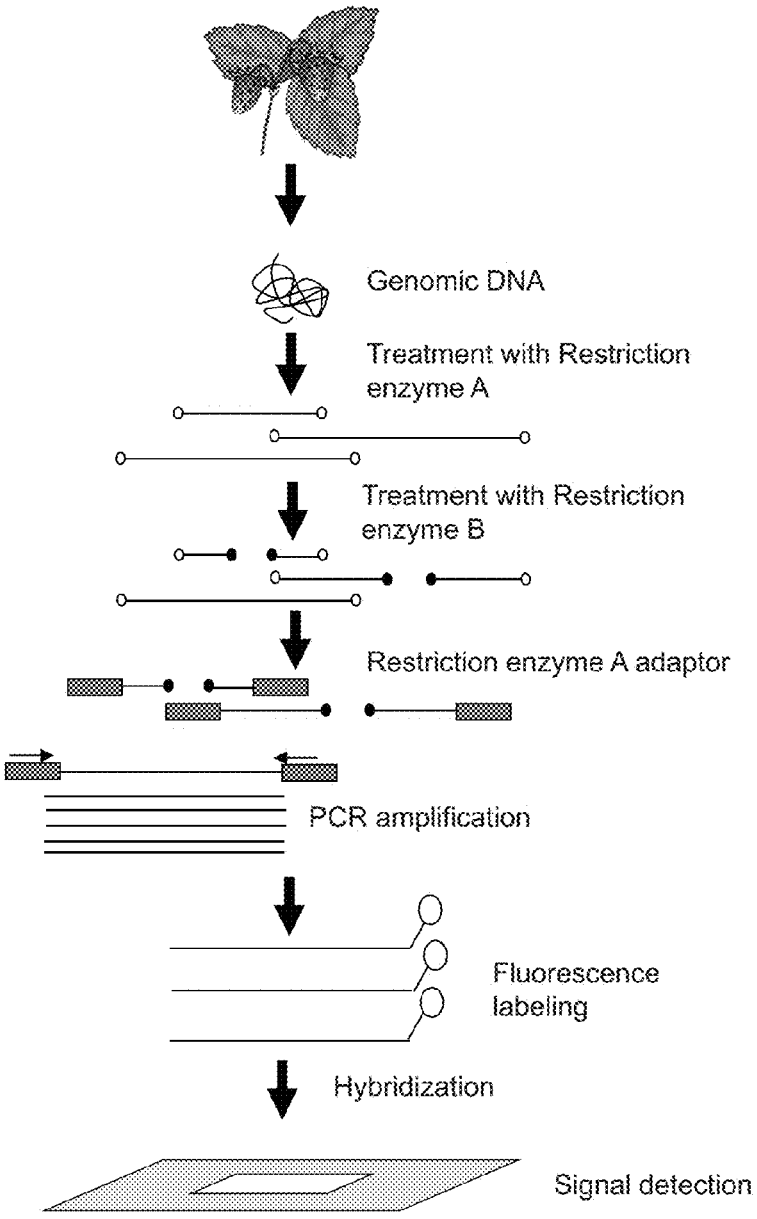

Genomic DNA
Treatment with Restriction enzyme A
Treatment with Restriction enzyme B
Restriction enzyme A adaptor
PCR amplification
Fluorescence labeling
Hybridization
Signal detection

Fig. 3

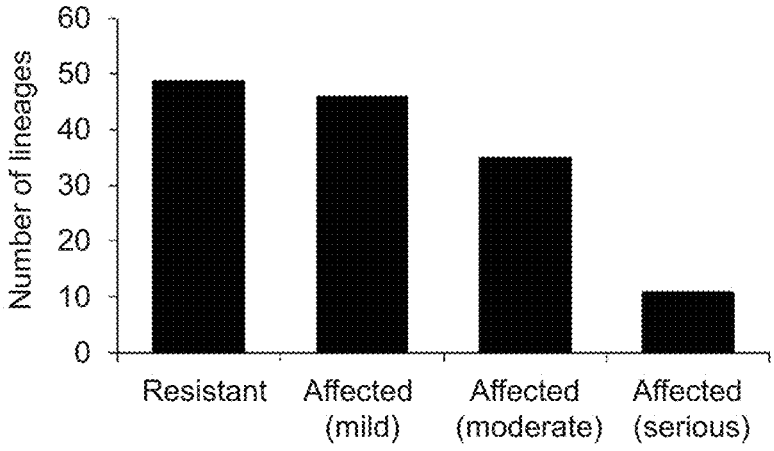
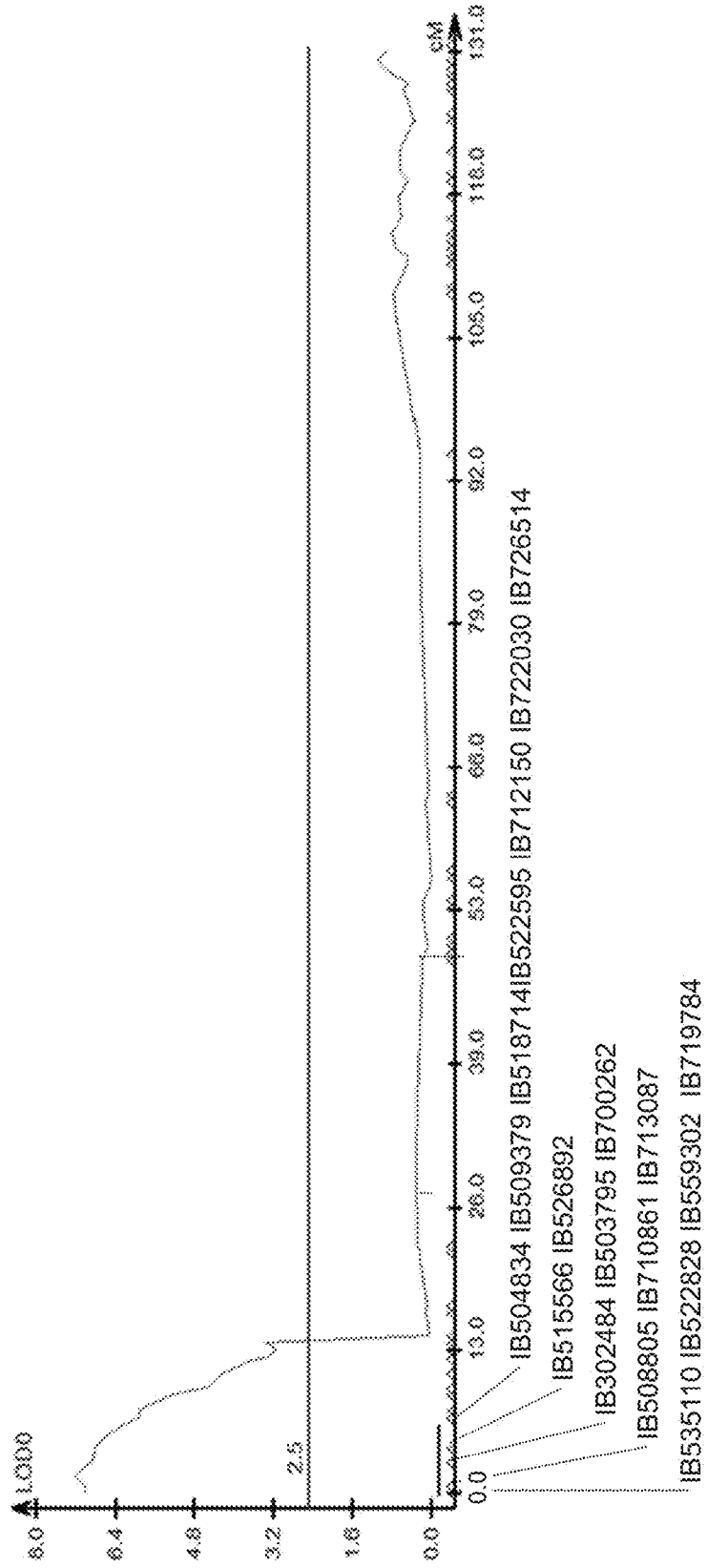

60
50
40
30
20
10
0
Number of lineages
Resistant
Affected (mild)
Affected (moderate)
Affected (serious)

Fig. 4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IB515566 IB526892
IB302484 IB503795 IB700262
IB508805 IB710861 IB713087
IB535110 IB522828 IB559302 IB719784

Fig. 5-1

Phenotypes concerning powdery mildew resistance/susceptibility of hybrid progenies (F1) in Populations A, B, and E

Miyazaki Natsu Haruka x 08 To-f		Miyazaki Natsu Haruka x Ohkimi		09s E-b 45c x Miyazaki Natsu Haruka	
Lineage	Powdery mildew resistance	Lineage	Powdery mildew resistance	Lineage	Powdery mildew resistance
A01	0	B01	0	E01	0
A02	1	B02	0	E02	1
A03	1	B03	0	E03	1
A04	0	B04	1	E04	1
A05	1	B05	0	E05	1
A06	0	B06	0	E06	0
A07	0	B07	1	E07	0
A08	1	B08	0	E08	0
A09	1	B09	0	E09	0
A10	1	B10	1	E10	0
A11	0	B11	1	E11	1
A12	0	B12	0	E12	1
A13	0	B13	1	E13	0
A14	0	B14	1	E14	0
A15	0	B15	1	E15	1
A16	0	B16	1	E16	1
A17	1	B17	0	E17	0
A18	1	B18	0	E18	0
A19	1	B19	0	E19	0
A20	0	B20	0	E20	0
A21	1	B21	0	E21	0
A22	0	B22	1	E22	0
A23	0	B23	0	E23	1
A24	1	B24	1	E24	1
A25	1	B25	1	E25	1
A26	0	B26	0	E26	1
A27	1	B27	0	E27	1
A29	1	B28	1	E28	1
A30	1	B29	0	E29	1
A31	1	B30	1	E30	0
A32	1	B31	1	E31	0
A33	1	B32	0	E32	0

•Fig. 5-2

Miyazaki Natsu Haruka x 08 To-f		Miyazaki Natsu Haruka x Ohkimi		09s E-b 45e x Miyazaki Natsu Haruka	
Lineage	Powdery mildew resistance	Lineage	Powdery mildew resistance	Lineage	Powdery mildew resistance
A34	1	B33	1	E33	0
A35	0	B34	1	E34	1
A36	1	B35	0	E35	1
A37	0	B36	1	E36	1
A38	0	B37	1	E37	1
A39	0	B38	0	E38	0
A40	0	B39	0	E39	1
A41	0	B40	0	E40	0
A42	1	B41	1	E41	0
A43	1	B42	0	E42	1
A44	0				
A45	0				
A46	1				
A47	0				
A48	0				
A49	0				
A50	1				
A51	0				

Powdery mildew resistance: 0: Not affected; 1: Affected

Fig.6-1

Linkage group	Marker name	Miyazaki Natsu Haruka	08 To-f	F1									
				1	2	3	4	5	6	7	8	9	10
08 To-f1 linkage group	IB535110	199	**26660**	**24842**	208	200	**23260**	250	**27722**	**25370**	238	205	**26497**
	IB522828	543	**7069**	**8093**	761	673	**8055**	936	**5854**	**7729**	763	737	**8666**
	IB559302	605	**9286**	**11105**	1110	837	**10461**	1215	**8031**	**9881**	973	873	**10931**
	IB719784	580	**7032**	**8543**	838	646	**8093**	953	**5791**	**7722**	815	740	**8827**
	IB508805	1099	**12677**	**12565**	**13385**	1167	**11477**	1097	**13138**	**12347**	1015	1487	**13826**
	IB710861	337	**18650**	**22355**	501	330	**24233**	498	**25865**	**24961**	425	454	**22266**
	IB713087	225	**48711**	**44036**	282	237	**44736**	245	**46674**	**47013**	239	311	**40090**
	IB302484	3989	**21780**	**20013**	**18057**	3939	**20318**	3303	**14997**	**18748**	3304	5139	**18413**
	IB503795	1955	**36530**	**31494**	**30441**	2359	**32142**	1724	**28720**	**30816**	2018	2636	**29436**
	IB700262	1277	**35877**	**31978**	**31396**	1109	**33664**	920	**29942**	**32891**	1253	1812	**31734**
	IB515566	257	**15574**	**17659**	**17125**	351	**17446**	295	**15311**	**16098**	328	533	330
	IB526892	4276	**54786**	**58495**	**55405**	4869	**54997**	4489	**59967**	**60237**	4866	4123	4128
	IB504834	473	**34291**	**23397**	**23418**	505	**23207**	437	**22750**	**24756**	659	450	291
	IB509379	2089	**48930**	**49368**	**51237**	1595	**45209**	1545	**50432**	**50588**	1505	1475	1877
	IB518714	4137	**76260**	**36523**	**22813**	4720	**26777**	4734	**28985**	**55310**	4864	5045	4141
	IB522595	674	**7849**	**6116**	**6673**	1381	**7009**	937	**5563**	**6715**	843	1352	1048
	IB712150	1695	**66456**	**34123**	**21797**	2179	**26399**	1838	**28761**	**53616**	2515	3248	2568
	IB722030	387	**25796**	**18780**	**17784**	362	**17611**	327	**17147**	**18460**	426	382	269
	IB726514	582	**37290**	**14863**	**8562**	878	**10983**	583	**11969**	**28035**	703	1294	855
Powdery mildew phenotype		Affected	**Resistant**	**Resistant**	Affected	Affected	**Resistant**	Affected	**Resistant**	**Resistant**	Affected	Affected	Affected

Fig.6-2

Linkage group	Marker name	F1									
		11	12	13	14	15	16	17	18	19	20
08 To-f1 linkage group	IB535110	25077	25651	23214	24771	24570	26310	199	202	206	25320
	IB522828	7511	9008	5811	6561	5708	9646	574	746	804	12014
	IB559302	10487	11213	8369	9342	8615	12731	604	984	700	10868
	IB719784	8415	8548	6420	7101	6195	10076	509	686	661	9052
	IB508805	12369	12694	10625	10527	10919	10906	809	858	954	15271
	IB710861	23235	17440	24325	25450	23458	22326	335	431	225	12972
	IB713087	43361	43950	42882	48315	42190	42796	241	327	237	41776
	IB302484	18184	19275	18891	18079	16989	17055	19108	2941	2587	17779
	IB503795	31458	31036	30296	32061	31032	29962	33654	1525	1627	33565
	IB700262	31160	30638	32516	34075	33197	33445	37431	1072	1018	29188
	IB515566	16697	17301	18188	17550	17381	17635	19539	356	333	18857
	IB526892	57577	62149	59909	59339	60279	56512	62304	3970	3818	61927
	IB504834	22788	25454	23436	24854	23554	22636	24783	393	631	19486
	IB509379	52095	49079	46615	47830	45914	47035	40048	994	1110	46390
	IB518714	31751	31453	32879	51190	28346	35178	41992	3754	3158	36901
	IB522595	5679	6081	6166	6235	5282	5827	6790	801	894	6942
	IB712150	31087	30754	31843	49824	27705	34194	38835	2046	2117	34815
	IB722030	16481	21131	15620	18257	16536	16704	20586	325	434	19645
	IB726514	13462	12527	13949	24357	11441	14669	17971	684	753	15189
Powdery mildew phenotype		Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Affected	Affected	Affected	Resistant

Fig.6-3

Linkage group	Marker name	F1								
		21	22	23	24	25	26	27	29	30
08 To-f1 linkage group	IB535110	258	27869	24926	205	203	28091	200	256	200
	IB522828	616	9816	9704	472	482	8270	488	441	526
	IB559302	701	11804	10933	522	654	10470	627	538	636
	IB719784	574	9543	8976	486	586	8317	547	458	508
	IB508805	1018	13869	14313	800	840	13879	775	924	845
	IB710861	260	24899	23080	244	315	22623	260	275	242
	IB713087	259	42546	42699	215	202	44500	206	257	249
	IB302484	3216	16893	18658	2124	3722	20797	4325	3695	4199
	IB503795	1486	28339	30392	1298	2248	24916	2470	2246	2183
	IB700262	853	30403	31029	1442	1239	36760	1322	1534	1287
	IB515566	280	17033	18881	275	306	18829	345	294	287
	IB526892	4020	64548	62282	5375	3396	57922	2848	3360	3130
	IB504834	337	23549	25545	710	469	26843	500	305	352
	IB509379	1060	50285	50467	991	1791	46704	949	1097	1439
	IB518714	3402	41598	33675	4624	5022	42470	3844	3789	4511
	IB522595	952	5319	6309	640	878	6287	715	553	1078
	IB712150	2001	41474	33667	2804	2870	40837	2644	2011	2979
	IB722030	301	17302	18266	395	362	21559	371	269	291
	IB726514	711	19915	15323	845	958	20002	920	668	958
Powdery mildew phenotype		Affected	Resistant	Resistant	Affected	Affected	Resistant	Affected	Affected	Affected

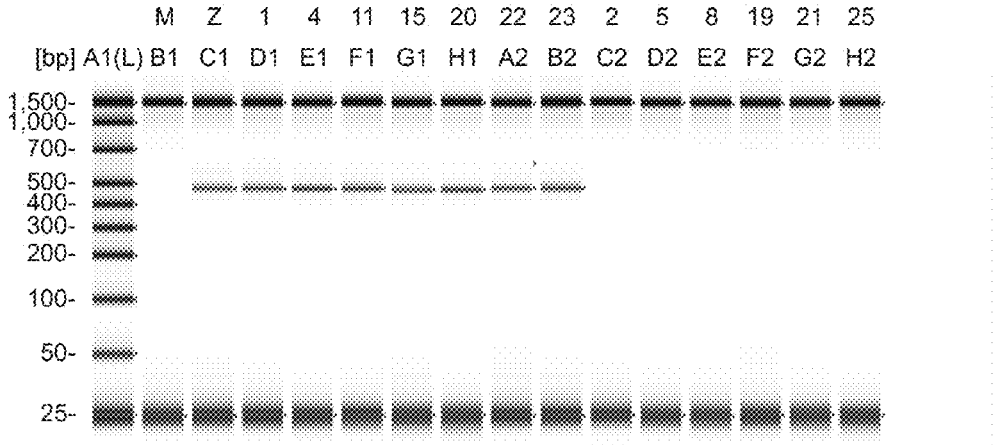
Fig.6-4

Linkage group	Marker name	F1									
		31	32	33	34	35	36	37	38	39	40
08 To-f1 linkage group	IB535110	201	208	200	264	**28161**	251	**23316**	**29131**	**25422**	**28024**
	IB522828	461	816	508	662	**8688**	646	**8598**	**7578**	**7993**	**8470**
	IB559302	519	1052	680	949	**10813**	798	**10245**	**10423**	**9680**	**10362**
	IB719784	445	747	601	670	**8382**	643	**7628**	**7489**	**7405**	**7709**
	IB508805	843	756	834	738	**13086**	901	**12437**	**13883**	**13275**	**14306**
	IB710861	243	467	258	325	**23893**	252	**26776**	**22530**	**21540**	**21825**
	IB713087	226	211	210	264	**43205**	250	**51545**	**44811**	**45307**	**45152**
	IB302484	4823	4860	4351	3245	**19581**	4430	**19562**	3947	**19035**	**21295**
	IB503795	2718	3224	2340	1891	**34874**	2669	**31619**	2102	**33998**	**32591**
	IB700262	1402	1579	1258	993	**37284**	1642	**32317**	1223	**35116**	**31979**
	IB515566	354	388	305	283	**20046**	309	**20773**	344	**18965**	**19749**
	IB526892	3785	3622	3812	3426	**58896**	3074	**63603**	3519	**56423**	**55261**
	IB504834	445	777	358	470	**25007**	485	**25708**	397	**25531**	**25394**
	IB509379	1388	1209	1310	1328	**46374**	1355	**43335**	3209	**48045**	**51945**
	IB518714	4001	5305	3998	3407	**36396**	3491	**47796**	4859	**52833**	**34317**
	IB522595	627	703	525	645	**6018**	672	**7242**	817	**5997**	**5779**
	IB712150	2545	3465	2440	1753	**34016**	2220	**43765**	2468	**49417**	**33640**
	IB722030	323	425	299	330	**18672**	335	**20196**	342	**20896**	**19726**
	IB726514	868	1109	803	628	**16481**	827	**23414**	744	**24442**	**15300**
Powdery mildew phenotype		Affected	Affected	Affected	Affected	**Resistant**	Affected	**Resistant**	**Resistant**	**Resistant**	**Resistant**

Fig.6-5

Linkage group	Marker name	F1											Concordance with phenotype
		41	42	43	44	45	46	47	48	49	50	51	
08 To-f1 linkage group	IB535110	**27473**	260	256	**22280**	**21605**	199	**22499**	**23365**	**25024**	267	**24260**	98.0%
	IB522828	**8355**	448	564	**7669**	**6992**	612	**7395**	**5838**	**8500**	601	**8517**	98.0%
	IB559302	**10996**	553	733	**9431**	**9411**	904	**9585**	**9385**	**10876**	736	**9845**	98.0%
	IB719784	**8400**	558	568	**7224**	**7246**	738	**7108**	**7049**	**9034**	627	**8140**	98.0%
	IB508805	**11650**	877	888	**11372**	**11936**	631	**10127**	**9604**	**11393**	695	**12442**	96.0%
	IB710861	**29585**	261	405	**24921**	**24913**	339	**27218**	**24476**	**23600**	268	**22528**	98.0%
	IB713087	**48317**	260	258	**51569**	**51391**	223	**50601**	**47555**	**45433**	268	**45072**	98.0%
	IB302484	**20603**	**18573**	3305	**19356**	**20069**	2633	**17642**	**18400**	**21514**	3776	**21901**	90.0%
	IB503795	**34614**	**28546**	1730	**33394**	**32937**	1508	**30402**	**32932**	**33507**	2118	**34331**	90.0%
	IB700262	**36117**	**32505**	991	**36260**	**33186**	884	**33115**	**33518**	**36227**	1146	**36118**	90.0%
	IB515566	**21969**	**20531**	328	**20861**	**21646**	266	**20230**	**18420**	**15982**	294	**18933**	92.0%
	IB526892	**60917**	**59343**	4217	**61549**	**59842**	4221	**59445**	**57065**	**63463**	4899	**55956**	92.0%
	IB504834	**26913**	**26972**	714	**24230**	**26505**	599	**25489**	**25313**	**26986**	826	**26481**	92.0%
	IB509379	**39270**	**44816**	3206	**43682**	**40891**	3916	**42680**	**48721**	**49624**	2303	**44050**	92.0%
	IB518714	**45207**	**26949**	4263	**39708**	**34384**	6057	**39447**	**25379**	**47109**	4546	**42783**	92.0%
	IB522595	**7429**	**6027**	727	**6893**	**7043**	600	**5745**	**5595**	**6709**	637	**5958**	92.0%
	IB712150	**42688**	**27239**	1777	**39259**	**33033**	2567	**38126**	**24578**	**45737**	1865	**41580**	92.0%
	IB722030	**19719**	**20103**	443	**18910**	**19356**	413	**18076**	**16538**	**20596**	442	**19927**	92.0%
	IB726514	**21197**	**10997**	636	**18220**	**14623**	820	**16505**	**10632**	**20683**	584	**20455**	92.0%
Powdery mildew phenotype		**Resistant**	Affected	Affected	**Resistant**	**Resistant**	Affected	**Resistant**	**Resistant**	**Resistant**	Affected	**Resistant**	-

•Fig. 7-1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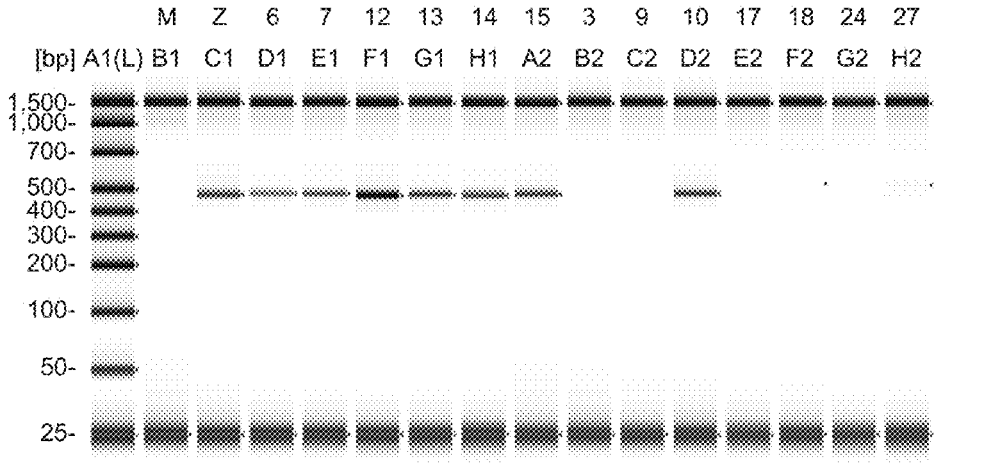
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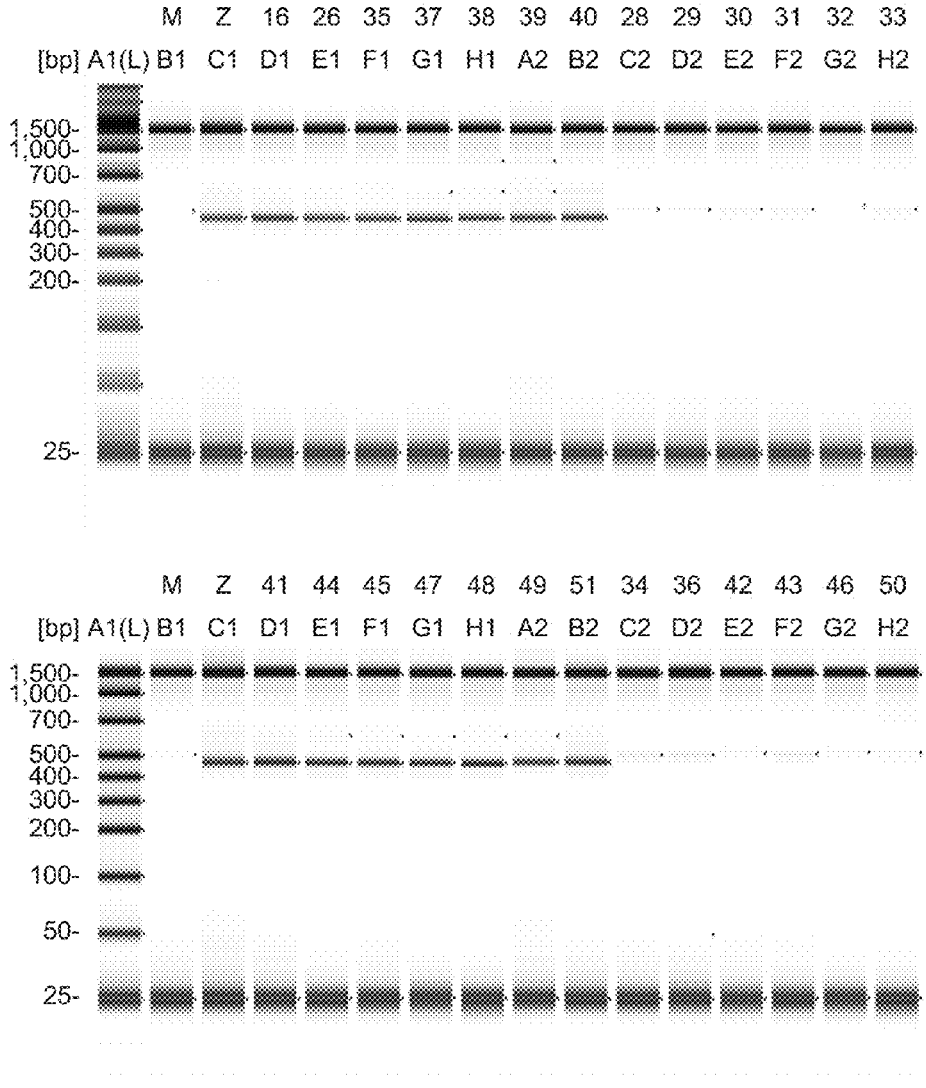
•Fig. 7-2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500-
400-
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200-
25-
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[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
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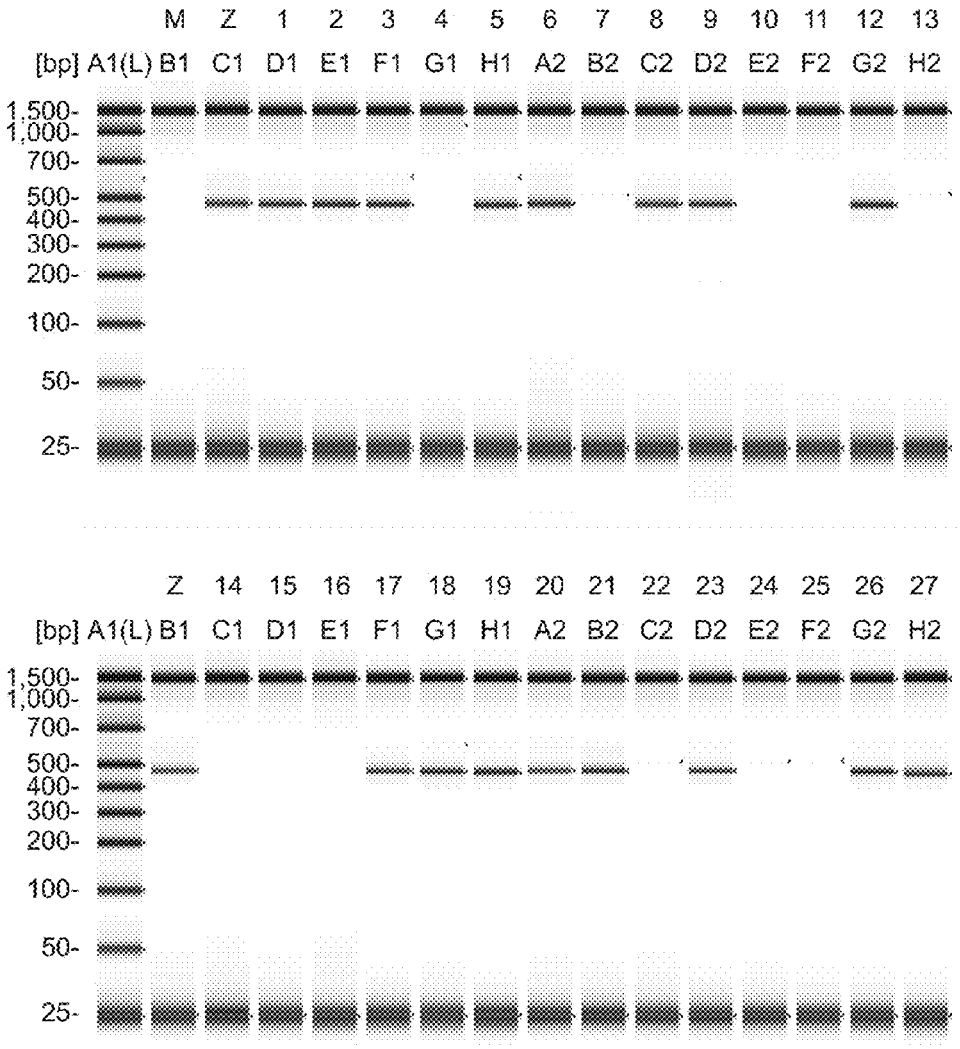
50-
25-

*Fig. 8-1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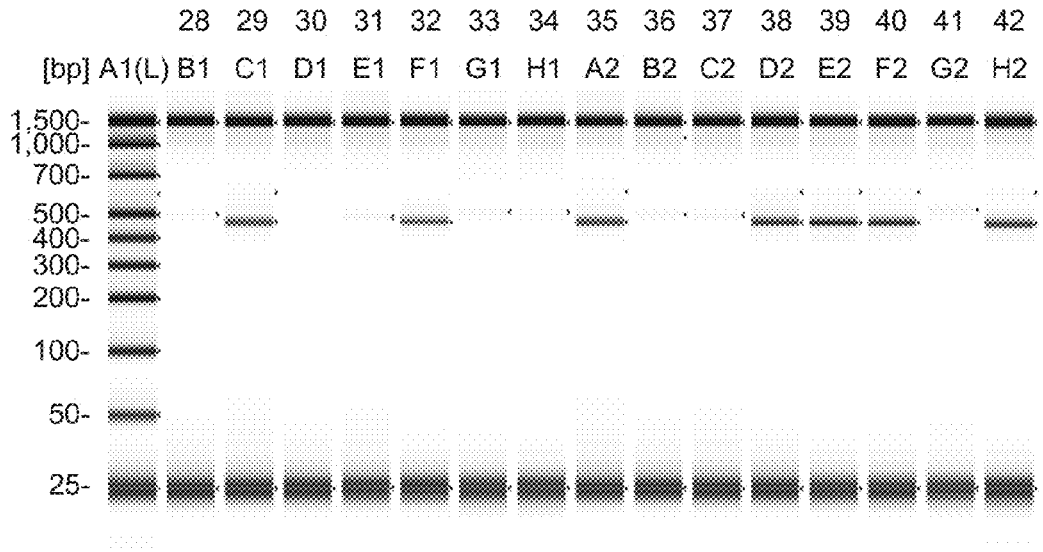
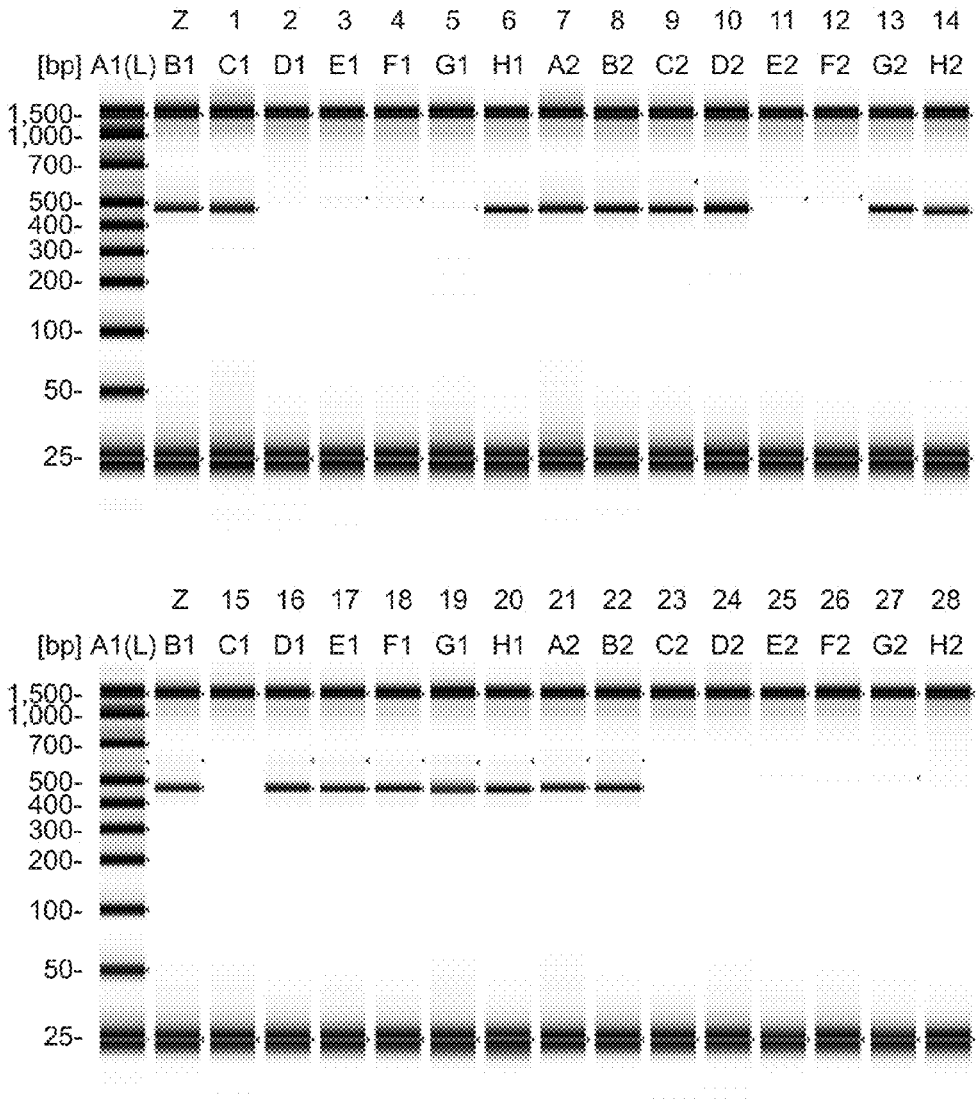
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
Z 14 15 16 17 18 19 20 21 22 23 24 25 26 27
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

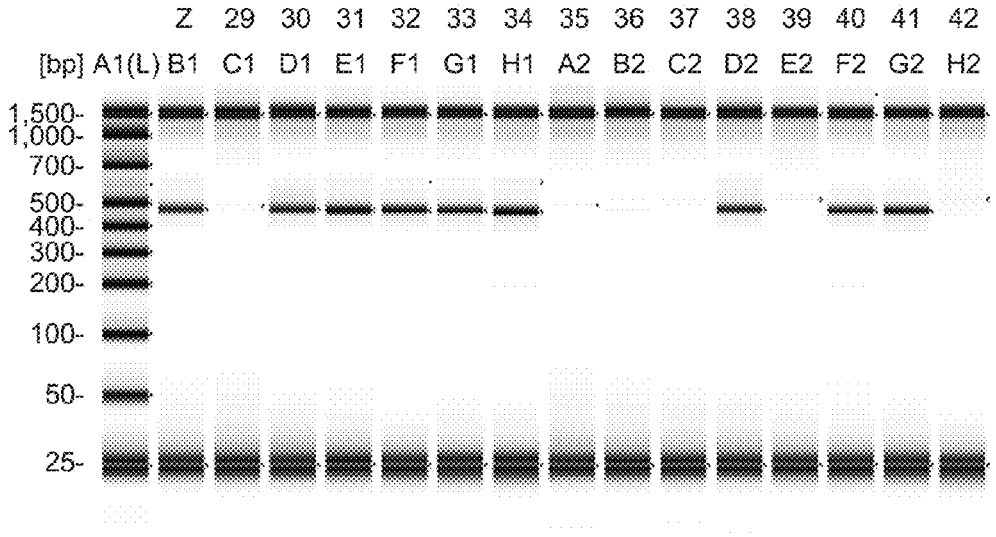
*Fig. 8-2


28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

•Fig. 9-1


Z 1 2 3 4 5 6 7 8 9 10 11 12 13 14
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
Z 15 16 17 18 19 20 21 22 23 24 25 26 27 28
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

•Fig. 9-2


Z 29 30 31 32 33 34 35 36 37 38 39 40 41 42
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

•Fig. 10-1

Miyazaki Natsu Haruka x 08 To-f			Miyazaki Natsu Haruka x Ohkimi			09s E-b 45e x Miyazaki Natsu Haruka		
Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker
A01	0	0	B01	0	0	E01	0	0
A02	1	1	B02	0	0	E02	1	1
A03	1	1	B03	0	0	E03	1	1
A04	0	0	B04	1	1	E04	1	1
A05	1	1	B05	0	0	E05	1	1
A06	0	0	B06	0	0	E06	0	0
A07	0	0	B07	1	1	E07	0	0
A08	1	1	B08	0	0	E08	0	0
A09	1	1	B09	0	0	E09	0	0
A10	1	**0**	B10	1	1	E10	0	0
A11	0	0	B11	1	1	E11	1	1
A12	0	0	B12	0	0	E12	1	1
A13	0	0	B13	1	1	E13	0	0
A14	0	0	B14	1	1	E14	0	0
A15	0	0	B15	1	1	E15	1	1
A16	0	0	B16	1	1	E16	1	**0**
A17	1	1	B17	0	0	E17	0	0
A18	1	1	B18	0	0	E18	0	0
A19	1	1	B19	0	0	E19	0	0
A20	0	0	B20	0	0	E20	0	0
A21	1	1	B21	0	0	E21	0	0
A22	0	0	B22	1	1	E22	0	0
A23	0	0	B23	0	0	E23	1	1
A24	1	1	B24	1	1	E24	1	1
A25	1	1	B25	1	1	E25	1	1
A26	0	0	B26	0	0	E26	1	1
A27	1	1	B27	0	0	E27	1	1
A29	1	1	B28	1	1	E28	1	1
A30	1	1	B29	0	0	E29	1	1
A31	1	1	B30	1	1	E30	0	0
A32	1	1	B31	1	1	E31	0	0
A33	1	1	B32	0	0	E32	0	0
A34	1	1	B33	1	1	E33	0	0

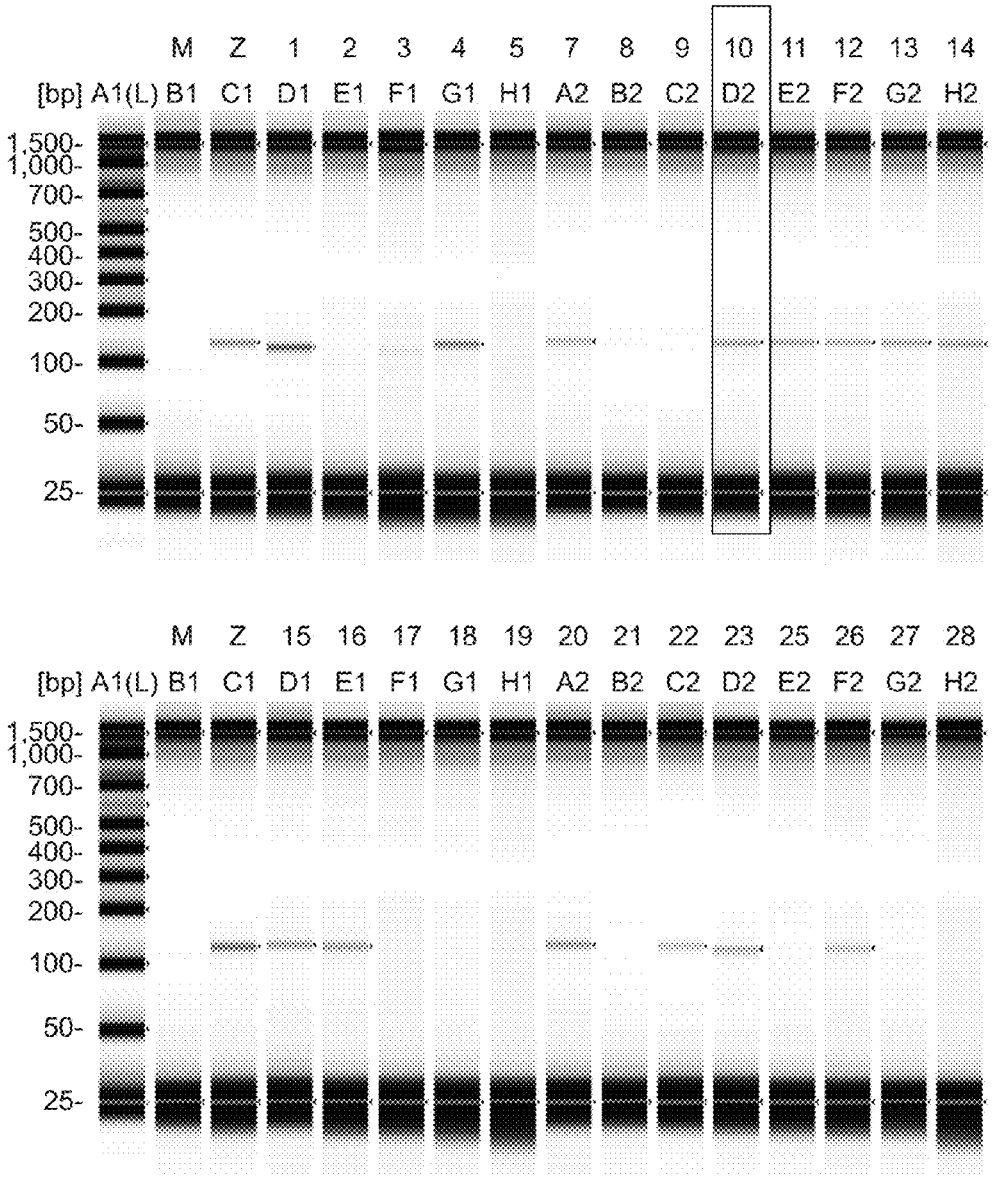
•Fig. 10-2

Miyazaki Natsu Haruka x 08 To-f			Miyazaki Natsu Haruka x Ohkimi			09s E-b 45e x Miyazaki Natsu Haruka		
Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker
A35	0	0	B34	1	1	E34	1	0
A36	1	1	B35	0	0	E35	1	1
A37	0	0	B36	1	1	E36	1	1
A38	0	0	B37	1	1	E37	1	1
A39	0	0	B38	0	0	E38	0	0
A40	0	0	B39	0	0	E39	1	1
A41	0	0	B40	0	0	E40	0	0
A42	1	1	B41	1	1	E41	0	0
A43	1	1	B42	0	0	E42	1	1
A44	0	0						
A45	0	0						
A46	1	1						
A47	0	0						
A48	0	0						
A49	0	0						
A50	1	1						
A51	0	0						

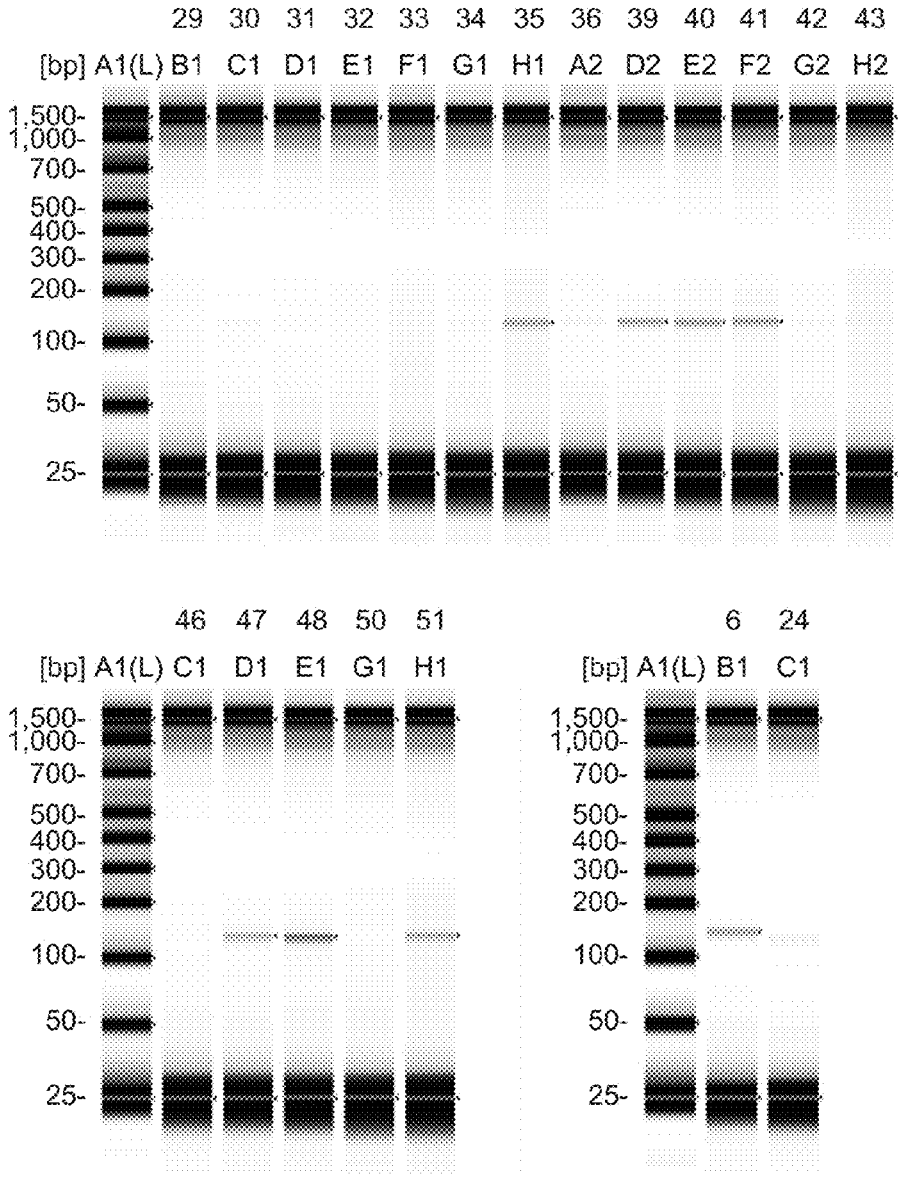
* Powdery mildew resistance: 0: Not affected; 1: Affected

* PCR base marker: 0: Band detected; 1: No band detected

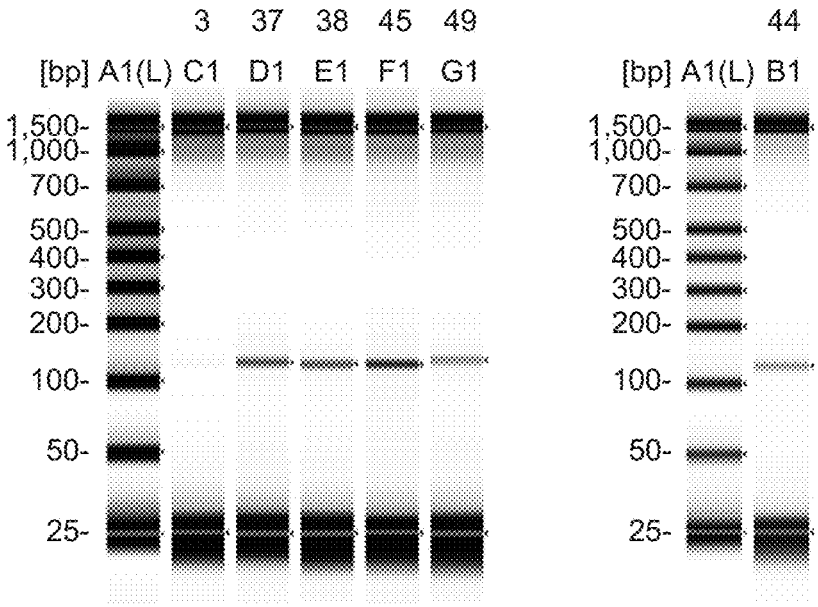
•Fig. 11-1


M Z 1 2 3 4 5 7 8 9 10 11 12 13 14
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
M Z 15 16 17 18 19 20 21 22 23 25 26 27 28
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

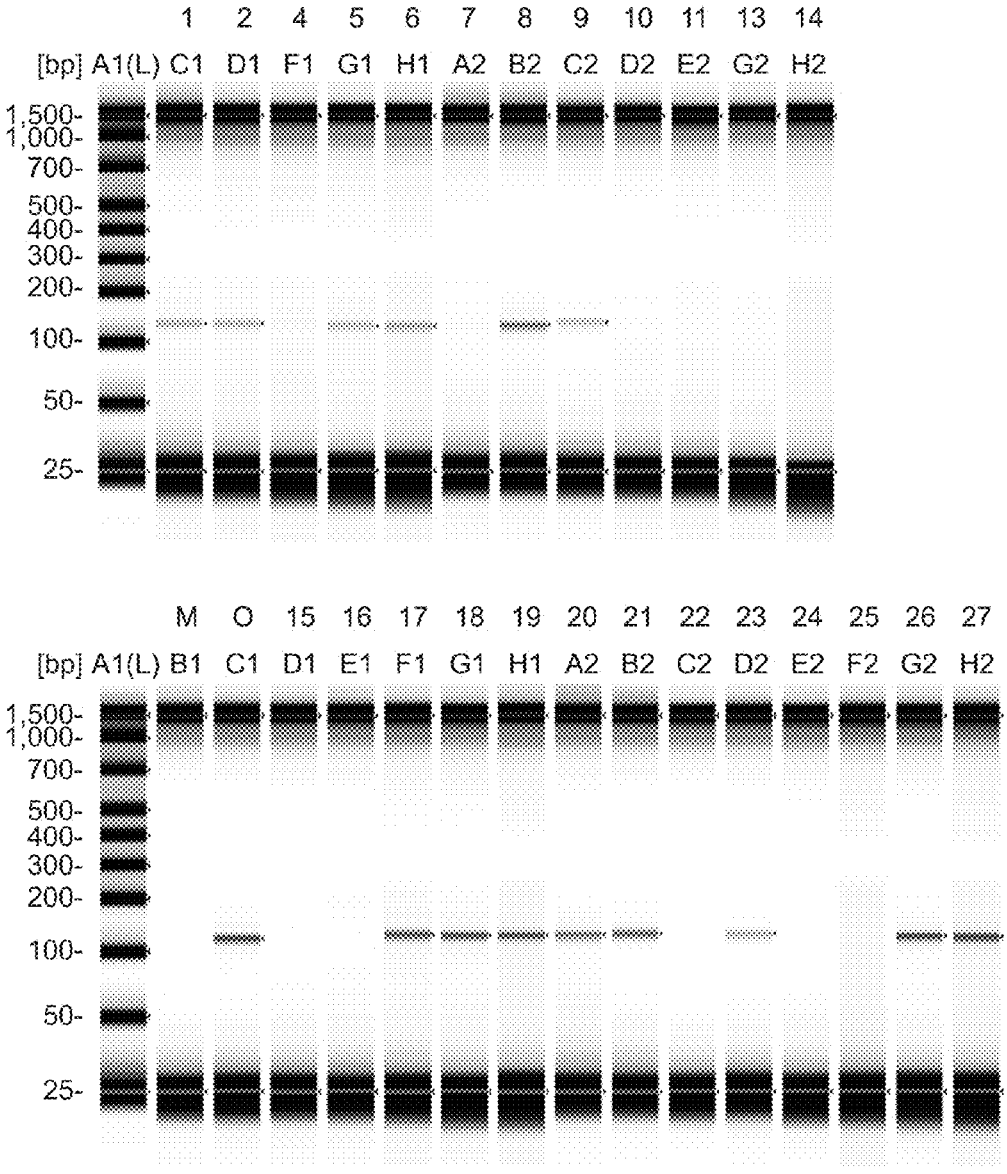
•Fig. 11-2


29 30 31 32 33 34 35 36 39 40 41 42 43
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
46 47 48 50 51
[bp] A1(L) C1 D1 E1 G1 H1
6 24
[bp] A1(L) B1 C1

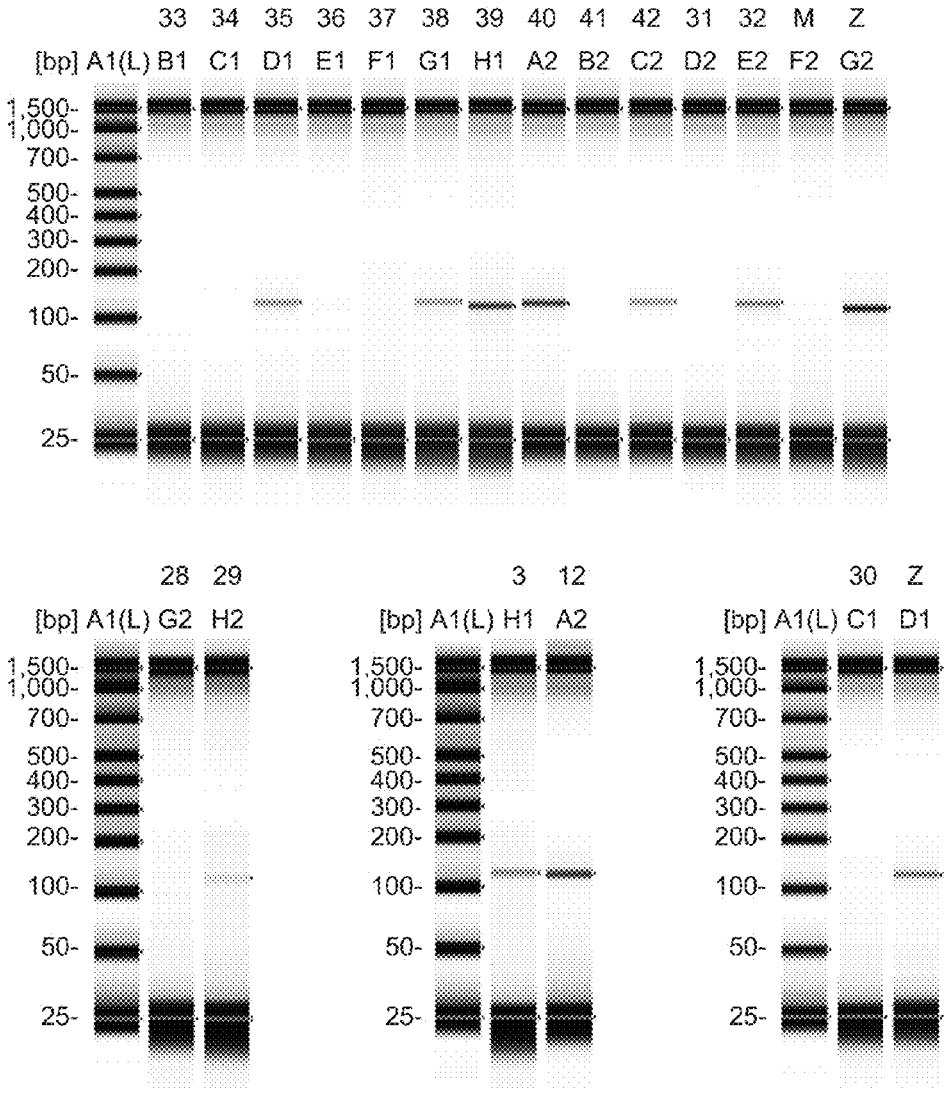
•Fig. 11-3


3
37
38
45
49
[bp]
A1(L)
C1
D1
E1
F1
G1
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
44
[bp]
A1(L)
B1
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

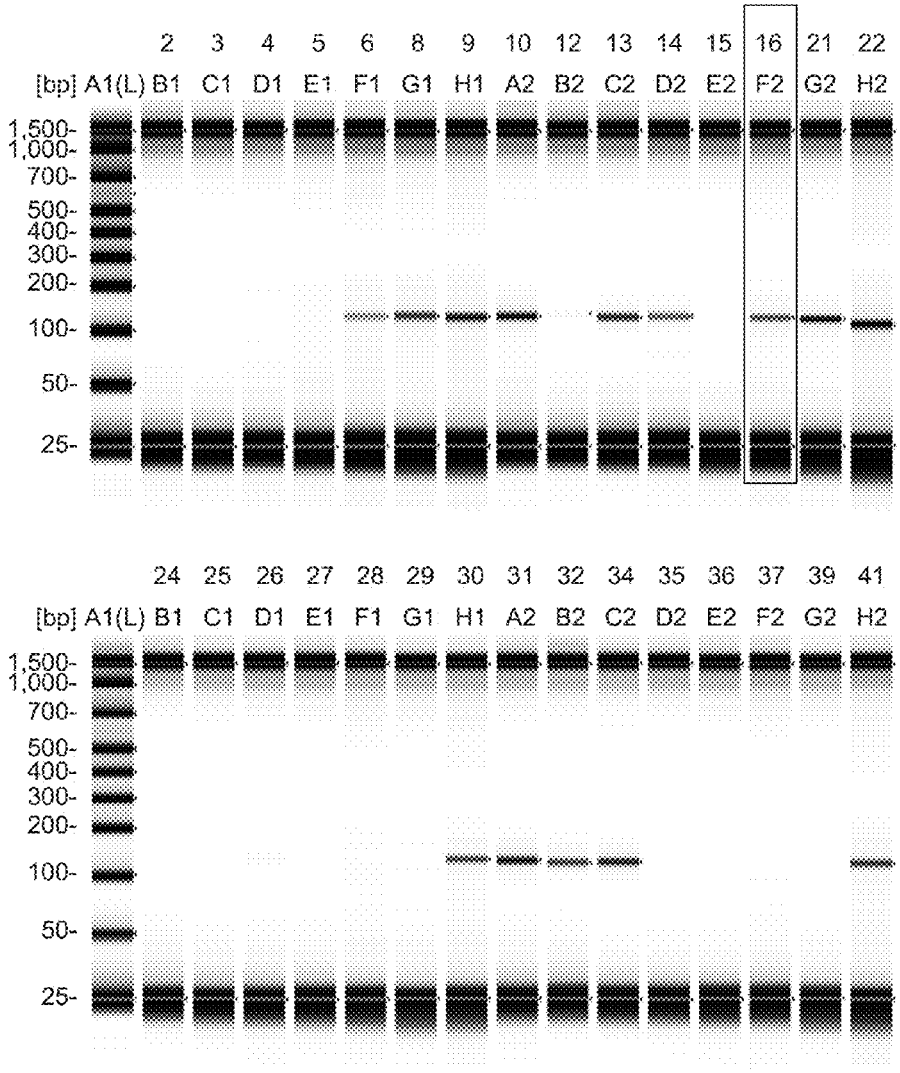
•Fig. 12-1


1 2 4 5 6 7 8 9 10 11 13 14
[bp] A1(L) C1 D1 F1 G1 H1 A2 B2 C2 D2 E2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
M O 15 16 17 18 19 20 21 22 23 24 25 26 27
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

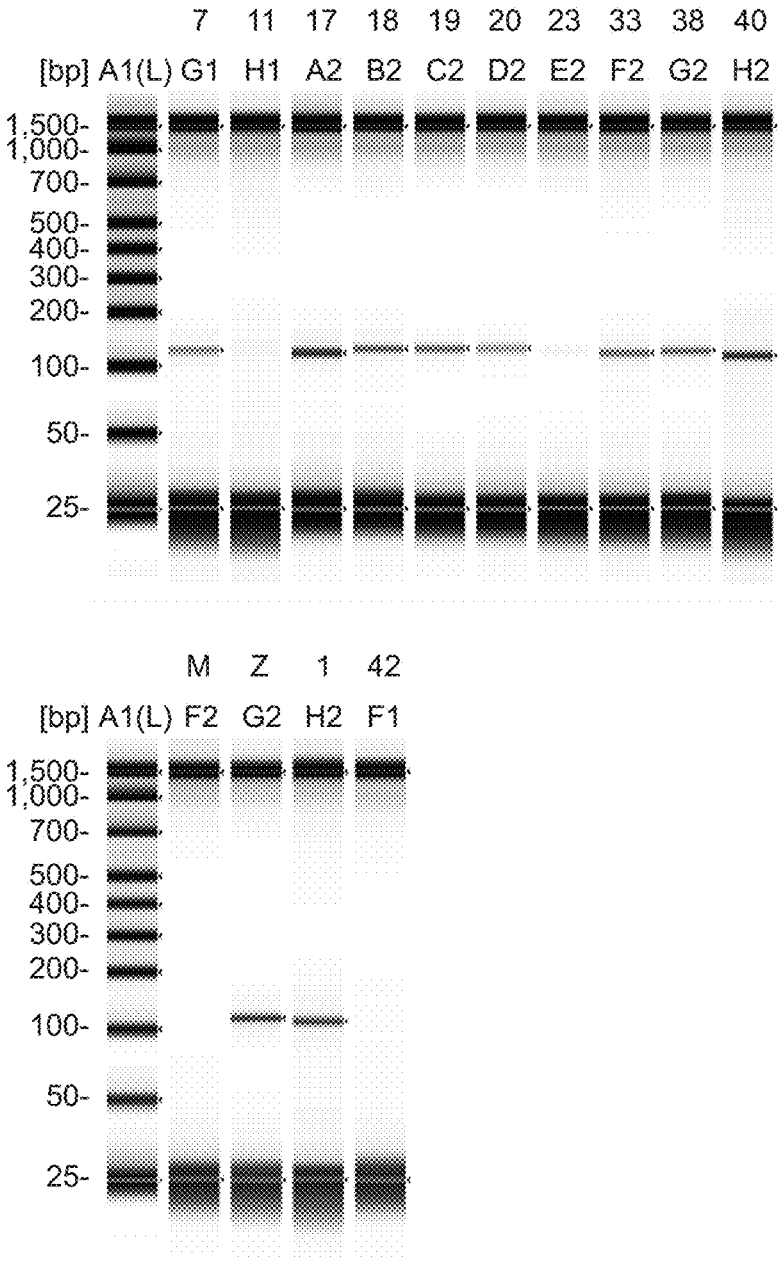
•Fig. 12-2


33 34 35 36 37 38 39 40 41 42 31 32 M Z
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
28 29
[bp] A1(L) G2 H2
3 12
[bp] A1(L) H1 A2
30 Z
[bp] A1(L) C1 D1

•Fig. 13-1


2 3 4 5 6 8 9 10 12 13 14 15 16 21 22
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
24 25 26 27 28 29 30 31 32 34 35 36 37 39 41
[bp] A1(L) B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

•Fig. 13-2


7 11 17 18 19 20 23 33 38 40
[bp] A1(L) G1 H1 A2 B2 C2 D2 E2 F2 G2 H2
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-
M Z 1 42
[bp] A1(L) F2 G2 H2 F1
1,500-
1,000-
700-
500-
400-
300-
200-
100-
50-
25-

•Fig 14-1

Miyazaki Natsu Haruka x 08 To-f			Miyazaki Natsu Haruka x Ohkimi			09s E-b 45e x Miyazaki Natsu Haruka		
Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker
A01	0	0	B01	0	0	E01	0	0
A02	1	1	B02	0	0	E02	1	1
A03	1	1	B03	0	0	E03	1	1
A04	0	0	B04	1	1	E04	1	1
A05	1	1	B05	0	0	E05	1	1
A06	0	0	B06	0	0	E06	0	0
A07	0	0	B07	1	1	E07	0	0
A08	1	1	B08	0	0	E08	0	0
A09	1	1	B09	0	0	E09	0	0
A10	1	**0**	B10	1	1	E10	0	0
A11	0	0	B11	1	1	E11	1	1
A12	0	0	B12	0	0	E12	1	1
A13	0	0	B13	1	1	E13	0	0
A14	0	0	B14	1	1	E14	0	0
A15	0	0	B15	1	1	E15	1	1
A16	0	0	B16	1	1	E16	1	**0**
A17	1	1	B17	0	0	E17	0	0
A18	1	1	B18	0	0	E18	0	0
A19	1	1	B19	0	0	E19	0	0
A20	0	0	B20	0	0	E20	0	0
A21	1	1	B21	0	0	E21	0	0
A22	0	0	B22	1	1	E22	0	0
A23	0	0	B23	0	0	E23	1	1
A24	1	1	B24	1	1	E24	1	1
A25	1	1	B25	1	1	E25	1	1
A26	0	0	B26	0	0	E26	1	1
A27	1	1	B27	0	0	E27	1	1
A29	1	1	B28	1	1	E28	1	1
A30	1	1	B29	0	0	E29	1	1
A31	1	1	B30	1	1	E30	0	0
A32	1	1	B31	1	1	E31	0	0
A33	1	1	B32	0	0	E32	0	0
A34	1	1	B33	1	1	E33	0	0

•Fig 14-2

Miyazaki Natsu Haruka x 08 To-f			Miyazaki Natsu Haruka x Ohkimi			09s E-b 45e x Miyazaki Natsu Haruka		
Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker	Lineage	Powdery mildew resistance	Results attained using PCR base marker
A35	0	0	B34	1	1	E34	1	0
A36	1	1	B35	0	0	E35	1	1
A37	0	0	B36	1	1	E36	1	1
A38	0	0	B37	1	1	E37	1	1
A39	0	0	B38	0	0	E38	0	0
A40	0	0	B39	0	0	E39	1	1
A41	0	0	B40	0	0	E40	0	0
A42	1	1	B41	1	1	E41	0	0
A43	1	1	B42	0	0	E42	1	1
A44	0	0						
A45	0	0						
A46	1	1						
A47	0	0						
A48	0	0						
A49	0	0						
A50	1	1						
A51	0	0						

* Powdery mildew resistance: 0: Not affected; 1: Affected

* PCR base marker: 0: Band detected; 1: No band detected

MARKER ASSOCIATED WITH POWDERY MILDEW RESISTANCE IN PLANT OF GENUS FRAGARIA AND USE THEREOF

TECHNICAL FIELD

[0001] The present invention relates to a marker associated with powdery mildew resistance that enables selection of a plant line of the genus *Fragaria* exhibiting resistance against powdery mildew and use thereof.

BACKGROUND ART

[0002] With the development of DNA markers (also referred to as genetic markers or gene markers), both useful and undesirable traits can be rapidly and efficiently identified when improvement in plant varieties is intended. The development of DNA markers has advanced for a wide variety of practical plants as well as for model plants such as *Arabidopsis thaliana* and *Oryza saliva*. Thus, such markers significantly contribute to improvement in plant varieties.

[0003] Plant epidemic prevention 52: 14-17, Uchida, Inoue, 1998 reports that there are at least 2 pathogenic races of powdery mildew fungi of strawberries in Japan. Also, Plant epidemic prevention 52: 14-17, Uchida, Inoue, 1998 implies that, on the basis of the results of investigation concerning sensitivity and resistance to powdery mildew fungi, powdery mildew resistance of strawberries is controlled by at least one oligogene. However, Plant epidemic prevention 52: 14-17. Uchida, Inoue, 1998 does not disclose or suggest DNA markers associated with powdery mildew resistance of strawberries.

[0004] Bulletin of the Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, No. 51: 7-12, Yamamoto et al., 2003 discloses that a linkage map was prepared with the use of hybrid lines of strawberry varieties "Toyonoka" and "Houkou-wase" and DNA markers detecting powdery mildew resistance were selected. Bulletin of the Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, No. 51: 7-12, Yamamoto et al., 2003 discloses that 29 linkage groups of "Toyonoka"-specific markers (a total of 109 markers, full-length: 1451.7 cM) and 21 linkage groups of "Houkou-wase"-specific markers (a total of 88 markers, full-length: 1205.7 cM) were obtained and that QTL analysis was conducted on the basis of the results of investigation concerning the onset of powdery mildew. According to Bulletin of the Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, No. 51: 7-12, Yamamoto et al., 2003, however, the LOD value attained by prospective linkage groups is about 1.22.

[0005] Summary of achievements, Miyagi Prefectural Agriculture and Horticulture Research Center, Chiba, Itabashi, 2008 discloses that resistance to strawberry powdery mildew can be attained via aggregation of a plurality of resistant genes and that linkage maps of 30 linkage groups (137 DNA markers, full length: 1,360 cM) were prepared with the use of *F. virginiana* (the original species) having resistance to strawberry powdery mildew-afflicted variety "Sachinoka." According to Summary of achievements, Miyagi Prefectural Agriculture and Horticulture Research Center, Chiba, Itabashi, 2008, QTLs are designated at 3 positions as a result of the QTL analysis using the results of examination and linkage maps of strawberry powdery mildew.

SUMMARY OF THE INVENTION

Objects to be Attained by the Invention

[0006] To date, the DNA marker technologies concerning powdery mildew resistance of strawberries as described above could not be regarded as sufficient in terms of the logarithm of odds (LOD) and the contribution ratio, and such markers could not be evaluated as excellent markers.

[0007] Under the above circumstances, it is an object of the present invention to develop many DNA markers in plants of the genus *Fragaria*, which are polyploids with complex genomic structures, and to provide markers associated with powdery mildew resistance that enable evaluation of powdery mildew resistance with high accuracy with the use of such many DNA markers and to provide a method of using such markers.

Means for Attaining the Objects

[0008] The present inventors have conducted concentrated studies in order to attain the above objects. As a result, they discovered markers linked to powdery mildew resistance by preparing many markers in plants of the genus *Fragaria* and conducting linkage analysis between phenotypic expression and markers in hybrid progeny lines. This has led to the completion of the present invention.

[0009] The present invention includes the following.

[0010] (1) A marker associated with powdery mildew resistance in plants of the genus *Fragaria* comprising a continuous nucleic acid region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 19 in the chromosome of the plant of the genus *Fragaria*.

[0011] (2) The marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to (1), wherein the nucleic acid region comprises any nucleotide sequence selected from the group consisting of nucleotide sequences as shown in SEQ ID NOs: 1 to 19 or a part of the nucleotide sequence.

[0012] (3) The marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to (1), wherein the nucleic acid region is located in a region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 7 in the chromosome of the plant of the genus *Fragaria*.

[0013] (4) A method for producing a plant line of the genus *Fragaria* with improved powdery mildew resistance comprising:

[0014] a step of extracting a chromosome of a progeny plant whose at least one parent is a plant of the genus *Fragaria* and/or a chromosome of the parent plant of the genus *Fragaria*; and

[0015] a step of determining the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria* according to any one of (1) to (3) in the chromosome obtained above.

[0016] (5) The method for producing a plant line of the genus *Fragaria* according to (4), wherein the step of determination comprises conducting a nucleic acid amplification reaction using a primer that specifically amplifies the marker associated with powdery mildew resistance in the plant of

the genus *Fragaria* to determine the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria*.

[0017] (6) The method for producing a plant line of the genus *Fragaria* according to (4), wherein the step of determination involves the use of a DNA chip comprising a probe corresponding to the marker associated with powdery mildew resistance in the plant of the genus *Fragaria*.

[0018] (7) The method for producing a plant line of the genus *Fragaria* according to (4), wherein the progeny plant is a seed or seedling and the chromosome is extracted from the seed or seedling.

[0019] This description includes part or all of the content as disclosed in the descriptions and/or drawings of Japanese Patent Application Nos. 2015-054618 and 2016-042028, which are priority documents of the present application.

Effects of the Invention

[0020] The present invention provides novel markers associated with powdery mildew resistance in plants of the genus *Fragaria* that are linked to powdery mildew resistance among various traits of plants of the genus *Fragaria*. With the use of the markers associated with powdery mildew resistance in plants of the genus *Fragaria* according to the present invention, powdery mildew resistance in hybrid lines of the plants of the genus *Fragaria* can be tested. Thus, plant lines of the genus *Fragaria* with improved powdery mildew resistance can be identified in a very cost-effective manner.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. **1** schematically shows a process for producing a DNA microarray used for obtaining markers in chromosomes of plants of the genus *Fragaria*.

[0022] FIG. **2** schematically shows a step of signal detection using a DNA microarray.

[0023] FIG. **3** shows a characteristic diagram showing the results of inspection concerning the onset and extent of strawberry powdery mildew of hybrid progeny lines of the "Miyazaki Natsu Haruka" and "08 To-f."

[0024] FIG. **4** shows a characteristic diagram showing the results of QTL analysis concerning powdery mildew resistance (the 1st linkage group of "08 To-f").

[0025] FIG. **5-1** shows a characteristic diagram showing the results of inspection concerning the onset and extent of powdery mildew in hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A), hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B), and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0026] FIG. **5-2** shows a characteristic diagram showing the results of inspection concerning the onset and extent of powdery mildew in hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A), hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B), and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0027] FIG. **6-1** shows a characteristic diagram showing the results of comparison of the array signal values of the markers associated with powdery mildew resistance of strawberries and the phenotypes of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0028] FIG. **6-2** shows a characteristic diagram showing the results of comparison of the array signal values of the markers associated with powdery mildew resistance of strawberries and the phenotypes of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0029] FIG. **6-3** shows a characteristic diagram showing the results of comparison of the array signal values of the markers associated with powdery mildew resistance of strawberries and the phenotypes of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0030] FIG. **6-4** shows a characteristic diagram showing the results of comparison of the array signal values of the markers associated with powdery mildew resistance of strawberries and the phenotypes of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0031] FIG. **6-5** shows a characteristic diagram showing the results of comparison of the array signal values of the markers associated with powdery mildew resistance of strawberries and the phenotypes of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0032] FIG. **7-1** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker 1B535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0033] FIG. **7-2** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0034] FIG. **8-1** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B).

[0035] FIG. **8-2** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B).

[0036] FIG. **9-1** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0037] FIG. **9-2** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0038] FIG. **10-1** shows a characteristic diagram summarizing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A), hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B), and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0039] FIG. **10-2** shows a characteristic diagram summarizing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB535110 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A), hybrid progeny lines of "Miyazaki Natsu

Haruka" and "Ohkimi" (Population B), and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0040] FIG. **11-1** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0041] FIG. **11-2** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0042] FIG. **11-3** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A).

[0043] FIG. **12-1** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B).

[0044] FIG. **12-2** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B).

[0045] FIG. **13-1** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0046] FIG. **13-2** shows electrophoresis images showing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0047] FIG. **14-1** shows a characteristic diagram summarizing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A), hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B), and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

[0048] FIG. **14-2** shows a characteristic diagram summarizing the results of PCR carried out with the use of a primer that specifically amplifies the marker IB522828 of hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" (Population A), hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (Population B), and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (Population E).

EMBODIMENTS FOR CARRYING OUT THE INVENTION

[0049] Hereafter, the markers associated with powdery mildew resistance in plants of the genus *Fragaria* of the present invention, the method for using the same, in particular, a method for producing plant lines of the genus *Fragaria* using the markers associated with powdery mildew resistance in plants of the genus *Fragaria* are described.

[Markers Associated with Powdery Mildew Resistance in Plants of the Genus *Fragaria*]

[0050] The marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to the present invention is a particular region in the chromosome of a plant of the genus *Fragaria* that makes it possible to identify traits of powdery mildew resistance of a plant of the genus *Fragaria*. By determining the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria* in the progeny lines obtained from existing plants of the genus *Fragaria*, specifically, whether or not a line of interest has powdery mildew resistance can be determined. In the present invention, the term "strawberry powdery mildew" refers to a disease resulting from infection with *Sphaerotheca aphanis* (*Podosphaera aphanis*), leading to development of lesions, as described in Ann. Phytopathol. Soc. Jpn., 64: 121-124, 1998.

[0051] In the present invention, strawberry powdery mildews are preferably caused by infection with fungi that are pathogenic for 7 varieties other than "Toyonoka" and "Harunoka" among 9 strawberry varieties; i.e., "Toyonoka," "Nyohou," "Reikou," "Himiko," "Houkou-Wase," "Dana." Kougyoku," "Harunoka." and "Fukuba" (Race 0, Japanese Journal of Phytopathology Vol. 63, No. 3, p. 226).

[0052] The term "a marker associated with powdery mildew resistance in plants of the genus *Fragaria*" refers to a marker linked to traits of a high degree of powdery mildew resistance. When the marker associated with powdery mildew resistance in plants of the genus *Fragaria* is present in a given plant of the genus *Fragaria*, for example, such plant can be determined to have a high degree of powdery mildew resistance. In particular, the marker associated with powdery mildew resistance in plants of the genus *Fragaria* may be considered to be a region linked to a causal gene (or causal genes) of traits such as powdery mildew resistance in plants of the genus *Fragaria.*

[0053] The term "plants of the genus *Fragaria*" used herein refers to all plants belonging to the rosaceous genus *Fragaria* (*Fragaria* L.). Specific examples of plants of the genus *Fragaria* include hybrids of general strawberry cultivars, *Fragaria ananassa* (i.e., *Fragaria*×*ananassa*). Examples of plants of the genus *Fragaria* include plants of *F. virginiana* that are progenitor species of strawberry cultivars and plants of wild species, such as *F. chiloensis. F. vesca, F. iinumae. F. nipponica. F nilgerrensis. F. nubicola. F bucharica. F daltoniana. F. ortentalis, F. cortmbosa, F. moschata*, and *F. iturupensis*. Further. "plants of the genus *Fragaria*" encompass known varieties and lines of strawberry cultivars (*F.*×*ananassa*). Known varieties and lines of strawberry cultivars are not particularly limited, and any varieties and lines that can be used inside or outside Japan are within the scope thereof. For example, strawberry varieties grown in Japan are not particularly limited. Examples thereof include Toyonoka, Sanchigo. June berry, Nyohou, Pisutoro, Rindamore. Tochiotome, Aisutoro, Tochinomine, Akihime, Benihoppe, Tochihime, Sachinoka, Keikiwase, Sagahonoka. Aiberry, Karen berry, Red pearl, Satsumaotome, Fukuoka S6 (Amaou), Nohime, Hinomine, and Houkou-wase.

[0054] The presence or absence of the marker associated with powdery mildew resistance in plants of the genus *Fragaria* can be determined in the above plants of the genus *Fragaria* and progeny lines of the above plants of the genus *Fragaria*. In a progeny line, either the mother plant or father

plant may be a plant of the genus *Fragaria* described above. A progeny line may result from sibling crossing or may be a hybrid line. Alternatively, a progeny line may result from so-called back crossing.

[0055] It is particularly preferable that the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria* be determined in strawberry cultivars (*F.×ananassa*). In addition, it is preferable that the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria* be determined in improved lines resulting from various varieties and lines of the strawberry cultivars described above. In such a case, powdery mildew resistance of strawberries can be evaluated in produced new varieties. Accordingly, it is preferable that a new variety be derived from a line having powdery mildew resistance in strawberries as either the mother plant or father plant.

[0056] The marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to the present invention has been newly identified by QTL (Quantitative Trait Loci) analysis using a genetic linkage map containing 8,218 markers acquired from the strawberry variety "Miyazaki Natsu Haruka" and 8,039 markers acquired from the strawberry line "08 To-f" and data concerning powdery mildew resistance of strawberries. QTL analysis is carried out with the use of the genetic analysis software of QTL Cartographer (Wang S., C. J. Basten and Z.-B. Zeng, 2010, Windows QTL Cartographer 2.5., Department of Statistics, North Carolina State University, Raleigh, N.C.) in accordance with the composite interval mapping (CIM) method.

[0057] Specifically, a region exhibiting a LOD score equivalent to or higher than a given threshold (e.g., 2.5) was found in the gene linkage maps by the QTL analysis. A size of a region that is lower than the peak by 1 LOD is approximately 6.8 cM (centimorgan), and this region is included in the 1st linkage group of the strawberry line "08 To-f." The unit "morgan (M)" relatively indicates a distance between genes on the chromosome, and such distance is represented in terms of a percentage of the crossing-over value. In the chromosome of a plant of the genus *Fragaria*, "1 cM" is equivalent to approximately 400 kb. This region has a peak whose LOD score is approximately 7.3. This implies the presence of a causal gene (or causal genes) that improve(s) powdery mildew resistance in plants of the genus *Fragaria* at such peak or in the vicinity thereof.

[0058] The 6.8-cM region comprises the 19 types of markers shown in Table 1 in the order shown in Table 1. The marker names indicated in Table 1 were acquired exclusively for the present invention.

TABLE 1

Seq ID No	Marker name	Nucleotide sequence information
1	IB535110	GGTGGAATTCATATACCATTTATTTAACAGAAGA GGCTTGTAAGTTATCGATCAATCGATACAAGGTA TAGTGTTGTGATTTTTTCAAGCTAAGATCATCTA ATATCATTCTTTTTTGCAGTTATGCTGGTATGTA AGCCTCTGGGTCTGATCAAATGAGAGTGTATCTA GAACTTTCAACTTGATACTTTGACCATATCGTTT GAGTTTGCCTCATGAAATTTGATTGCAATCTACT ACTGTTTATCTTGCACTCTTTGATGATAGATAAC GCAGCCATGCGTTGAGCACAGACCGAACTACACA TATATGAATCGGAGCCATGGATGCAGCCTTAGTT

TABLE 1-continued

Seq ID No	Marker name	Nucleotide sequence information
		TCAGGTACTTTGATTATCAATAGTTTCAGCCGCA GTAACAAACAACTATGGCCCTTTCGCATTTTATG AATGTCTCATCTGTTCCTGTCTATACTTGAAATA ATATTATTACATACCAAATACTACTTCGTTGTCC GACGTAAGTATATTAATCTATTTGAACAGCTATG GAGTTCCAATTTTAAATGCATGAAGTAGGAGAAA ATTTAGAAACCATGAATTAAGATATTAGAATTCC TACATCATCACCACCCAGAGCCAAGAGAGTTTGG TGGTGTTTCAATTTCAGCCCAAGTTTTCTCTATT CGTCGTCTCCTTCTCCCTCTCCTCCATTATTTCC ATTACATGACAGTTGAAACGCTTTCTCCCGATCG TGTACAATTCATTTTCGATTGAGCATCTTGAGCA GAACTCTGATCACTATTAATTCACTTTCTGATGG CGTTGAGCAGCCAAACTGGGT
2	IB522828	TCCAAGACACTTGACGATATCAGACGCAAAGGGT CGTCATATAATCCACTACTGCTGCTTTGACGCCT ACTGCAATAGCATATTCCTATGAATCACCCACCG TGGCAGGCTGGCAGTGTTTTGGCTGTGAATGATG AAGATGATGATGAAATTTGGGTTATGCTCAAGTG GTGCAAACTTTTGAAAGCAACGTGAGCTTTAACG AAGCCCAACCCAAATTAAGTCCTACATTTGAGAG AGACTCTGAGATGAGTGAGATCAGTGCATCATTC TTTGAATCATTCAACAATATCCACTTTCAAAACA AATTTTTCTCTCTTTTGGGTAAACAAACAAGTTT TGAATAGGTTTCCTTCTTCTGTAACAAGGACTTG CTACAGAAATGGACCGATAACAACCTGCTGTTCC AGAGGACTCCCCATTCTTCTGTGTAAGGCTTCTG GAGCTCGATGATATCAAAGAAGGGAGGAAGGTAC CTTTGCTTATGTCTCTTTCTTTAATCTTCTCAAA GCTTGTAACTTTGAAAGCTGAAACATGCATTTGC TTCAGTACTGATCTTGTTTTT
3	IB559302	CTGGAAGTTCCTGTACATAGGTATATAGTTAGAC TTAGTCACAATGCATAATGGTGGGTTCAAATTAG AGGCAAAACAAGCCATAAACAGATAAAGATACAG CTAAAAACCAAGGCCAAGGGAATAGAAACACAGT AAACATGAAAATTTGAATTGTCCTTCACGGTACA GGGTACAGATTTCAAACTTTTTAGCTGCAAAAAG TTCATAAATCAAGCAGAACCTTTTTCTTTATTGT CCTGCAAGACTTATCTATAAAGGCTTATAATTTC AAGTGTTTGGAAAAAAAAAATGTAAAATAAAAAC AGAACAACAACTGGAATTAACAGAATCATAGAAC TGAAGCAAAGCTCTTTAGTTTCTACTTTCTAGTG AACATGTAAAGATCTCAACTTTCAACTCTCAAGA TTATCAAGCTGTGAAATTAAGTAAACACATGTTC CTAAAAAAAGTGGAAAATGTAAAGGTTTTATCTT TCACGCTAATCAAACAAGATCAGAACTTCTCCAC ACAAAAAAAAACAAGATCAGTA
4	IB719784	TTTTTCTTTAGGAGTACGCAAGTCTGCATACCAT GCGACGATCATCTCAAAAAGATAGTAAGTGACCA TGTAAAAATCATTTACCCTCTCAAAATCCCGCCG CCCCCCCACGCCACGATTTCCATTATGTATTCTA TATTTACATATCTCTACAATAGACAAACACTTTC CTCTTTCTTTAGACATGTTACTGAGACCTCACCT ACAAATTTTTCTGACCATCTTAACGCAAAATTTA CAGATCCGGTGATCCGGTAATCCATTTAACCCGA TAAAACATATAAGTGTCGTACATTCCATTTAGAA TCTCTCAATAATAATGCTACATGAGTGTCACTAA TGCTAT
5	IB508805	TTCCATATATATACATTAGAATCCTCACTTGCTG ATATTATATGTTTCCCATCTGAAGTGAAAGTGGC AGATCGCAGGTTTGCTGCATTCTTTAATCCTAAA CAAGAGACGAGAGATGAGGTTTAGCAAAAGGAAA TGTCATATATCACATCTAAAATTCACAAACATGT GGCATAAAATTATGCCAAAGGAGTGTAAAATTTG TTTGCAGACAAAGGAATCTCATGAAAAGAGCTTA CGAAATGCACATACCCTTGTATTTTCCAACCACA TTCAAACCATGAAGAATTCTGACTTGTGAATCGG CGCAAGTGACCATTACTTTGTCAGGATCATGAGG GAAATACTGCATTAATAACATAATTTAGAAAAGA AAAAAGAATGGATCCCTAACGAATAGGTAACAAA

TABLE 1-continued

Seq ID No	Marker name	Nucleotide sequence information
		CACAAGAAACCAAAAGAAGAATACAAAAGTATTA GCTACCTCAAAGCCTGTTATCTTT
6	IB710861	AAGAATGAAGAATGTAAAGAGACACTGTCCAGCT TTGAAAAATCTGATCTTGGTCTTAATCAGCGTGG TAATCAAGGCCTTCATGGAATGGTTTGAGCAAGT CGATCAGCTAAAGTATTGTGCGTAAAAATTTTGT GTAGTGTCAAACCGGTGATGTTACTACTGTCAAA CTGGTGATGATACTACTGAGATTGTCAATGATTC AGACGCAGATCATGTTTTCTATTGATCCATTTCT TGTTTAACTCTTTATCCAGAGATGACCTTTCGAT CTTCTCATATTTTTCGTAAAAAGAATAAGGTTGC AAATGCTTTAGCTAACCACGGTACGTCATTAACA TAGCTAGTTTGGTAAGATTCACATATTCCTTTTA TTTTGTTATATTGTAGTAGTGACCTTATGAGTCT TTCCCAATTTCGGTTTCTTAGTTTTGTTTCGTTG TTATTTTGTTACGAGAGATTTTGGTCTAATCCTC CTCTCTTGATGTTTCTCTTTTTTCTTTTGTAATG CATAAGAGTGTTCAGAGGTTATTCCTCTCTCACT CATCTTTCAGCCAAAAAAAAAAATTTGCATTAAT TTATTGAAAGTTTTGCTTCATGTGTGT
7	IB713087	AGATATATTCGTCGTCAGAGCCACCACTTCTGCT TGTTGCTGCCTTAACCATGGAGCCTTCTTGTTCA TTCATAGCCTCGTGAACAGAAATGCTGCTATTGG ATTGTTTCATTTACTAATCAGCTCTTCTTTGTCG TGCTCAAACAGTGCACGGGCCCCACATTTCTTCA CCTTCATGTAGCTGCATAAAGGGCGTTTCATGCA TCTGTTGTACCAAGATTCCATCTTTCTCTTCTTT TTGATTTGATTCAGTTGATGTTATTAGAAATACT TGGAGAATTTAATCAATGGGTCTCAGAGTCTATG GATGGTATTTGGTAACAAACGGGTCTGATTGATA TGGTTATCCTTGTTCAAACATTTGGAACCTTAGA ATGTTTCCAACTGATATTGAGTTCAATACTTGCA GGAATTCTAATCTGTGATTTAGTATAAAACTATG AATAAACCAATGGTTTACAGGGAATATACAGCAG GGCAATGGTTT
8	IB302484	CTGTAAAAATCAAAGGCAAGCACTTGATGAAAAA GAAGGTTGGTGATTTTGGATTAGATGGGCATCCA TCGTATATAGGCTCTAATATCTTTTGTGGTTGAT TAAACAAATGAGGATCTCTGTAATAAGTGGAGAT TCTTATCATTTCCCACATCTGAGAAACTCTGAAA TAAACAAAAAGAAAGAGAAAAAGGCTTTCACGAC AATATGGGTGAAGCATGGGGTCCTAACTCCTAAG TTGTAATACCTGTGTTTGTTAAACTACTATACAT AGCAACTCTTGGTGTTGCTCGGTCTAAGG
9	IB503795	CTGTAAAAATCAAAAGCAAGCACTTGATGAAAAA GAAGGTTGGTGATTTTGGACGAGATGGACATCCA TCATATATAGGGTCTAATATCTTTTGTGGTTGAT TAAACATATGAGGATCTCTGTAATAAGTGGAGAT TCTTATCATCTCCCACATCTGAGAAACTCAGAAA CAAACAAAAAGAAAGAGAAAAAGGCTTTCACGAC AATATGGGTGAAGCATGGGTCCTAAGTTCGTAAT CTCTGTGTTTGTTAAACAACTATAATCTATATAT AGTAACTCTTGGTGTTGCTCGGTCTAAGGTTGTA CCAATCAGTGTCTTAGATAGACAAAGTCGGTGGA AGGTGGCAGTAACATATCACAAAGTCTGTTGTGA GGGTTGCAACAATATAACGCAACTGTAAACTGTC ACATCAGTTTACAAACTCTACTTACATAAATTTT ATTTAGTGTTCAACGTTCAAACATTACATTCTAT CATATTTCGGTGCATGACATACTTCGCGTTTTGG AC
10	IB700262	TGTGGCAAATTACAGACCAAAAGATCTATCTGTC TATCAATGCCGACCTATTCTCATATGGTTTTGGC TTCTATGTGGTGAAGGTTCAACGTTGTTGTTGTT AAGGAAGGTCATCTTGGACTTTTATTTTGTTTCC AAGTTCTATTTATTAATTTCATATGAAAATGATA TATACCTACAGAAGCTAACATTACCCGTGAAATA TTGAACACCCTTTTGATGTCTATACTTCAATAAT GTCTGTCAGATGATTAAGGCAAACTATCTTTTAT GGCATCTAAATTGGTTAATTCGATTCGTTTTGAT

TABLE 1-continued

Seq ID No	Marker name	Nucleotide sequence information
		TTTGTTTTCTCTACTAATTCTGACAATCGAAAAA CCGAACGTGTTAGTCTAGAAATGACGTATTATAA AACACAGGTGTTCCATTTCTAATTTTTCTGCATA ACACCTGCTTTCAGTTGTGATTAGAAAAACATCT TTAAGTTGACATTT
11	IB515566	TAACTTCAGGGAGCTAAAGATCATGGGTCGTTTC GACGTCAGATTCGCTTCAACATTAGTTGGTACTT ATCTTCCTAATCTCAAGGTCATGAGCCTGCGGTG TTCGCAGCTGGTTAGGGAAGCTTTGATCACTGTA TTGGACGGGTTACCACAGCTAGAAGTCCTCAATA TAGCACATTGTGTGCTTCTGATTGAACCCCCGCG CCGTAATCAGCCTCTCCAAATTGTTGAGGAGGTT GATGAAGTTATTCTTGAGAAGGCTGCTCGGTTAG AGAGATTCATAACGTGCACGCAAATAGACCGGTG CATCCTGTGCCAAAGGGCCAGAAACGACGGGGGG ATTATGAAATGGTATAAATATGAAGAAGGGCTCT GGAAACAAGATGAGGTGAACACTCTTGCTCTTTG ATTCTATTCGAGTGTGTTATGCTTGTAA
12	IB526892	CTTCCTATCTGTGACAACAATCCTAACCTTCAAT GAATAGGAGAAGTAGACTATCTCTACCAAATATA CATATATACAGGACTATATGTTTCAAATTATATG TATCCAGATTGGAAAAGTTTGCCATCAGATTATT TGCGGTGTAGCATTGTTTGTAAATCATGGAATTG CGTAGCAAACGATAATCGAATCCAACAAGCTAAG ATGATGTCAAATTCTCATCACCCTCCTATGCTCT TGATTCCTGCAAAAGAAGAAGATACATGGAACTT GTACAACATTATGGAAAAAAAAAGGTTCTTGATAT GCAAGTCACAGTGCCACCTAACTATAAACGGTTT TCTGGATCCTCAAAGGGATGGTTGATAGCTTTGG ATGAGAATTTTGTAGTAACACTGATAAATCCTTT CTCTAGAGTTAAGGGAAGGAGAGAGAAAGAAAAT TCAATCATTCGGCTTCCTCCTTTGAATCATCAAC AATCGACAATAAGATTACGAGGTGAAGAGTATC
13	IB504834	TGTAGCGGAGGGATTGTTTTGTCATTTCAAAACT GAGGGACTTTTTTTTTTATTGAAATTAAACTGAG GGCCTTGCAAGCCGTAGGCGTTGGTACTGGACGG TGCCGTTTTCTTTGATCGAAGTTTTTATGGCAAG GGGTTTAATTGTCCTTTCAAAAATGTTAGAAGTG AAATTTGGGTCAGATGGATGAAGGTTTTCTTGTG TCCATATATACGAGTGTATTATGTTTCGTCGATG TATCGATGATTTATATTAAATTTCAGATTTTAAT TTTGAGACATGAAAAACATTTATAATTTAAGTGA TTTTGTGTTTCTAGCCTTATAGT
14	IB509379	AGTGCTATGGAATATGTCTTCGGTTCAACCTTTG TGTGCAAGACTATTAATGCTGCAAAGGAGGTGAG AGGTTGATTATCGTGCTGTAGGCTGATTATATAG TATTGTCCTTTTAAACACTTGTAATCTAAGCAGG AAAGCGGCATGACCCAATCTGGTTCTCTATGAAT GTTTCCTAGGTTGCTTTTAACAGGGAAGTTCGTA CCCTAGTGTCACTCTTGAAGGTGATATCTTCCAG CCCAGTGGTCTTTTGACTGGTGGAAGCCGCAAGT AAGCCACTGTTCTTTTTCCTCCAGTTTAGATTTC ATGCTTTACCCCCTTCCTCTTGAGTATATCTGTT GTTAGCTCTCTCTGACTAATTTTCCATACTTGTG TTGTCCTTATCATTTATCAATTCAAAGTACATAT ACTTCTAGCCAGTTTTCCTTCTAAAGCAAAAATT TCCTGTCACAGGGGTGGGGGAGATCTGTTAAG
15	IB518714	TAGGTGATATTTGACGTGCAAGTGTCCAAAATAA TCTCATAAGGCCTAACTCCCCCATCGTCACAATT TGACCATCAAACTATCTCCAGCGCTACCTGTTGT CGGCACCCTCTACCGACGTTATTTCACAACCATT TTAATTAACGTTCGATTTGTTTCAGTGAAAAACA AACAGTTGGTAGTAAAAGATCATGGTAAAAAGCA GACTGCGTGGTGGGGTGGATGTACACAACGCGGA GTAGAACGCTTAAAGTTTTTCACACCACTAATAA TATATTATACATATTATATAATACAAAACCTGTA ATTATAAATATACATAATATATTCTTAAGAAAAC TTTGCGAGGTAAAAGTGGTGGCGGCAAGGCACTT

TABLE 1-continued

Seq ID No	Marker name	Nucleotide sequence information
		TGAGTGATTAGAATTGGGAGGTTTTGGTGGTGGA TGACACTGAATATAGTGCCGGATGCTTGCCGGGT
16	IB522595	AAATTGTTTCCATATGATACGGTTCAACATGACA CTTACATAGTTACATTAGCATAGAAGTCAACATT GCCTCTCTTTCTCACAACTGATCAAACTCTACCT GATCAGGCAGGCCAATCAAGAGAGGATTTGACTG CATTTCAGCAAAATAAGCACATATGCAACACCCT ATGCACATATACAAGAAGTGGCACATTGCCTTCA CATTTGCCTAAAAGTACATAAAACTAACAGAAGC ATCCATGAAAGCTCCATGGCAACCACTTCTCAAC TCCATTGCCTAGTTAAACAATGTAGATCATAATT AAAACAGATATTTGAGGAGCAGGAAA
17	IB712150	CAAACCGGGTTTAGACTTGCTACGATCAAGTTGT TCTTCAATCTGCTCTGCCATTCTCCCTACATCAT AGACACCCGGAAAGTGTGAGGGCTAATGTGATTG CCAACAATATAATTGATGCTTTGAATAGAGGGGT GAACTTGGATGACAAGGAGAGTAAGGATAGTGGT GTTTCGCATTTGACTGATTTGAATTGGGAGGTTT TGGTGGTGGATGACACTGAATATAGTGCCGGATG CTTGCCGGGTGGGAAGATTGTGGTCTGCTCAGGG CTGCTCAAGCATTATTTTAGTGATGCGGAGATAG CTATGGTAATTGCTCATGAGGTACGATGACTAGT TGTGTAGTGTTTCTGTTCAAAGTGCTAAAACAAT GTGGGCTGCTAACTTCTCCTCTGTCTTGTGATTG CAAGCTAGGTTGGGCATACTGTGGCTCGACACCA AGCTGAGTTAGTCACAAAGTTCCTGTGGC
18	IB722030	TGTAGCGGAGGGATTGTTTTGTCATTTCAAAACT GAGGGACTTTTTTTTTATTGAAATTAAACTGAGG GCCTTGCAAGCCGTAGGCGTTGGTACTGGACGGT GCCGTTTTCTTTGATCGAAGTTTTTATGGCAAGG GGTTTAATTGTCCTTTCAAAAATGTTAGAAGTGA AATTTGGGTCAGATGGATGAAGGTTTTCTTCTGT CCATATATACGAGTGTATTATGTTTCGTCGATGT ATCGATGATTTATATTAAATTTCAGATTTTAATT TTGAGACATGAAAAACATTTATAATTTAAGTGAT TTTGTGTTTCTAGCGTTATAGTGCGTATGAATGA GACACAACGTACAAAAAAGTTGAGATAAGAAAAT GACCCATAAATTATTTTGGTTTTAATTTATGTAA GCGATATTTTTAGGTTGGTTGATTATGAATTTAT GTACATTAAAATTCAAAATATTTTTTTGGCACAT TAGATTGTAAACTTGAATCAATAGTACTTGACGT CGTTAGCATGATTGAATTGTCAAATGTTGTATAT TTTGAAAGGTAAAAAGGTACCTCTCTTCACTTCA TCTTTTTTGTCTCTAAACCACACCAAGACTTTGC GCAAAGCCCTCCATCTTTACATCAAATGGTGATA TTCTAAGTCGCATACCAAAACCCCGATCTCCAAG ACTCGACTCCCAAATCTGGAGATGGAGGTGACAA CACGACTAGAATCACAGCTTTGGTACTATCATGA CAATAAGTTGAACAACTTTGGTCGTCTGGGTATG CT
19	IB726514	GAAAACCCCATCATCTTTAATCCTTTGCTGAGGG GAAGCACAAGGGCTCAACAGCTATAACATTGAGC AACTACTATAGTTAGTCCTGTGATTGGAAGTGCC AAGGGTCTTCAAAATAACCGGGGCAATCTATGGC CATGGTTCTATGTATATACATAATCCTCTATCCT AGTTATGCTACCAAATATGTTCTGAGACATAATC GTTCTTCTGTTGCTCGGAACAATGCAGAAAACTT AAAATAGTAAAAGTGTTGTTATAGAATCTCCTCA AAATTTTAGACCATTTTAGGGAAATTCTATCAGT GTTTCAATCGTTAGACACTTCAAGTCCTAGTATA CTAATCCAAAAGCCTCACTACAAAAATACATGAA GACATTTACATGCGACCATACTAGCCTTCCTCTA TCAGAACGAACCAACACTAAGAAGAGCATCATAG GATACATAATCCTCTATCCGTAAACAAATGACAA TCAGAAGAAACA

[0059] Specifically, the marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to the present invention is a continuous nucleic acid region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 19 in the chromosome of the plant of the genus *Fragaria*. The peak in the 6.8-cM region is located in a region sandwiched between the marker comprising the nucleotide sequence as shown in SEQ ID NO: 1 (1B535110) and the marker comprising the nucleotide sequence as shown in SEQ ID NO: 7 (1B713087).

[0060] A continuous nucleic acid region in the 6.8-cM region shown in Table 1 can be used as the marker associated with powdery mildew resistance in plants of the genus *Fragaria*. The term "nucleic acid region" used herein refers to a region comprising a nucleotide sequence having 95% or less, preferably 90% or less, more preferably 80% or less, and most preferably 70% or less identity to the other region in the chromosome of the plant of the genus *Fragaria*. As long as the degree of identity between the nucleic acid region as the marker associated with powdery mildew resistance in plants of the genus *Fragaria* and the other region is within the range described above, such nucleic acid region can be specifically detected in accordance with a conventional technique. The degree of identity can be determined using, for example, BLAST with the default parameters.

[0061] A nucleic acid region serving as the marker associated with powdery mildew resistance in plants of the genus *Fragaria* can comprise at least 8, preferably 15 or more, more preferably 20 or more, and most preferably 30 nucleotides. As long as the number of nucleotides constituting the nucleic acid region as the marker associated with powdery mildew resistance in plants of the genus *Fragaria* is within such range, such nucleic acid region can be specifically detected in accordance with a conventional technique.

[0062] In particular, the marker associated with powdery mildew resistance in plants of the genus *Fragaria* is preferably selected from a region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 7 among the 19 types of markers included in the 6.8-cM region because the peak is located in the region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 7.

[0063] The marker associated with powdery mildew resistance in plants of the genus *Fragaria* can be a nucleic acid region including a single type of marker selected from among the 19 types of markers shown in Table 1. For example, use of a nucleic acid region including a marker comprising the nucleotide sequence as shown in SEQ ID NO: 1 (IB535110), which is located in a position nearest to the peak, as the marker associated with powdery mildew resistance in plants of the genus *Fragaria* is preferable. In such a case, the nucleotide sequence of the nucleic acid region including the marker can be identified by a method of flank sequence analysis, such as inverse PCR using primers designed based on the nucleotide sequence of the marker.

[0064] Alternatively, a plurality of regions may be selected from a nucleic acid region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 19 in the chromosome of the plant of the genus *Fragaria* as the marker associated with powdery mildew resistance in the plant of the genus *Fragaria*.

[0065] In addition, any of the above 19 types of markers can be directly used as markers associated with powdery

mildew resistance in plants of the genus *Fragaria*. Specifically, one or more regions selected from the 19 regions comprising the nucleotide sequences as shown in SEQ ID NOs: 1 to 19 can be used as markers associated with powdery mildew resistance in plants of the genus *Fragaria*. For example, use of a marker comprising the nucleotide sequence as shown in SEQ ID NO: 1 (IB535110), which is located in a position nearest to the peak, as a marker associated with powdery mildew resistance in plants of the genus *Fragaria* is preferable. Alternatively, a region sandwiched between the marker comprising the nucleotide sequence as shown in SEQ ID NO: 2 (IB522828) and the marker comprising the nucleotide sequence as shown in SEQ ID NO: 3 (IB559302) can be used as a marker associated with powdery mildew resistance in plants of the genus *Fragaria*, for example.

[Identification of Marker in Plants of the Genus *Fragaria*]

[0066] In the present invention, as described above, the markers associated with powdery mildew resistance in plants of the genus *Fragaria* were identified from among the 8,218 markers acquired from the strawberry variety "Miyazaki Natsu Haruka" and the 8,039 markers acquired from the strawberry line "08 To-f." Such 8,218 markers and 8,039 markers are described below. These markers can be identified with the use of a DNA microarray in accordance with the methods disclosed in JP 2011-120558 A or WO 2011/074510.

[0067] Specifically, probes used for the DNA microarray are designed in the manner shown in FIG. **1**. That is, genomic DNA is first extracted from "Miyazaki Natsu Haruka" or "08 To-f" (Step 1a). Subsequently, the extracted genomic DNA is digested with one or more restriction enzymes (Step 1b). In an embodiment shown in FIG. **1**, two types of restriction enzymes, Restriction enzyme A and Restriction enzyme B, are used in that order to digest genomic DNA. Restriction enzymes are not particularly limited, and examples of restriction enzymes that can be used include PstI, EcoRI, HindIII, BstNI, HpaII, and HaeIII. Restriction enzymes can be adequately selected by taking, for example, the frequency of recognition sequence appearance into consideration, so as to yield a genomic DNA fragment with 20 to 10,000 nucleotides upon complete digestion of genomic DNA. When a plurality of restriction enzymes are used, it is preferable that the genomic DNA fragment comprise 200 to 6,000 nucleotides after all the restriction enzymes are used. When a plurality of restriction enzymes are used, in addition, the order in which restriction enzymes are subjected to treatment is not particularly limited. Under common treatment conditions (e.g., a solution composition or temperature), a plurality of restriction enzymes may be used in the same reaction system. While Restriction enzyme A and Restriction enzyme B are successively used in that order so as to digest genomic DNA in an embodiment shown in FIG. **1**, specifically. Restriction enzyme A and Restriction enzyme B may be simultaneously used in the same reaction system to digest genomic DNA. Alternatively, Restriction enzyme B and Restriction enzyme A may be successively used in that order, so as to digest genomic DNA. In addition, 3 or more restriction enzymes may be used.

[0068] Subsequently, adaptors are bound to the genomic DNA fragment treated with restriction enzymes (Step 1c). The adaptors used herein are not particularly limited, provided that such adaptors can be bound to the both ends of the genomic DNA fragment obtained through the treatment with restriction enzymes. An example of an adaptor that can be used is an adaptor comprising a single strand that is complementary to a protruding end (a sticky end) formed at both ends of the genomic DNA fragment obtained through the treatment with restriction enzymes and having a primer-binding sequence to which a primer used at the time of amplification can hybridize (details are described below). Alternatively, an adaptor comprising a single strand complementary to the protruding end (a sticky end) and having a restriction enzyme recognition site to be incorporated into a vector at the time of cloning can be used.

[0069] When genomic DNA is digested with a plurality of restriction enzymes, a plurality of adaptors corresponding to relevant restriction enzymes can be used. Specifically, a plurality of adaptors each comprising a single strand complementary to any of a plurality of types of protruding ends resulting from digestion of genomic DNA with a plurality of types of restriction enzymes can be used. In such a case, a plurality of adaptors corresponding to a plurality of restriction enzymes may have common primer-binding sequences enabling hybridization of common primers. Alternatively, such adaptors may have different primer-binding sequences, so that different primers can hybridize thereto.

[0070] When genomic DNA is digested with a plurality of restriction enzymes, in addition, an adaptor corresponding to a restriction enzyme selected from among the plurality of restriction enzymes used or adaptors corresponding to a subset of restriction enzymes selected from among the plurality of restriction enzymes used can be prepared.

[0071] Subsequently, a genomic DNA fragment comprising adaptors bound to both ends thereof is amplified (Step Id). When adaptors comprising primer-binding sequences are used, primers that can hybridize to such primer-binding sequences may be used, so that the genomic DNA fragment can be amplified. Alternatively, a genomic DNA fragment comprising adaptors added thereto may be cloned into a vector using the adaptor sequences, and primers that can hybridize to particular regions in such vector may be used, so as to amplify the genomic DNA fragment. An example of an amplification reaction of the genomic DNA fragment with the use of primers is PCR.

[0072] When genomic DNA is digested with a plurality of restriction enzymes and a plurality of adaptors corresponding to relevant restriction enzymes are ligated to the genomic DNA fragments, adaptors would be ligated to all genomic DNA fragments resulting from the treatment with the plurality of restriction enzymes. In such a case, primer-binding sequences contained in the adaptors may be used to perform a nucleic acid amplification reaction. Thus, all resulting genomic DNA fragments can be amplified.

[0073] When genomic DNA is digested with a plurality of restriction enzymes and an adaptor corresponding to a restriction enzyme selected from among the plurality of restriction enzymes used or adaptors corresponding to a subset of restriction enzymes selected from among the plurality of restriction enzymes used are ligated to the genomic DNA fragments, alternatively, the genomic DNA fragments comprising the recognition sequences for the selected restriction enzymes at both ends thereof can be selectively amplified among the resulting genomic DNA fragments.

[0074] Subsequently, nucleotide sequences of the amplified genomic DNA fragments are determined (Step 1e), one or more regions of a nucleotide length shorter than that of the genomic DNA fragment and corresponding to at least a part of the genomic DNA fragment are identified, and the one or more identified regions are designed as probes in strawberry cultivars (Step 1f). A method for determining nucleotide sequences of genomic DNA fragments is not particularly limited. For example, a conventional technique involving the use of a DNA sequencer in accordance with the Sanger's method can be employed. A region to be designed herein is of, for example, a 20-to 100-nucleotide length, preferably a 30- to 90-nucleotide length, and more preferably a 50- to 75-nucleotide length, as described above.

[0075] As described above, many probes are designed using genomic DNAs extracted from strawberry cultivars, and oligonucleotides comprising target nucleotide sequences are synthesized on a support based on the nucleotide sequences of the designed probes. Thus, a DNA microarray can be produced. With the use of the DNA microarray produced as described above, the 8,218 markers and the 8,039 markers including the 19 types of markers associated with powdery mildew resistance in plants of the genus *Fragaria* as shown in SEQ ID NOs: 1 to 19 can be identified.

[0076] More specifically, the present inventors obtained the signal data with the use of the DNA microarray concerning 8,215 markers obtained from the strawberry variety "Miyazaki Natsu Haruka," the strawberry line "08 To-f," and hybrid progeny lines thereof (147 lines). They then obtained the genotype data from the obtained signal data, and, on the basis of the obtained genotype data, they obtained the positional information for markers in the chromosomes in accordance with a genetic distance calculation formula (Kosambi) using genetic map production software (AntMap, Iwata, H., Ninomiya, S., 2006, AntMap: Constructing genetic linkage maps using an ant colony optimization algorithm. Breed Sci., 56: 371-378). On the basis of the positional information for the obtained markers, in addition, a genetic map datasheet was prepared using the Mapmaker/EXP ver. 3.0 (A Whitehead Institute for Biomedical Research Technical Report, Third Edition, January, 1993). As a result, the 8,218 markers and the 8,039 markers including the 19 types of markers associated with powdery mildew resistance in plants of the genus *Fragaria* as shown in SEQ ID NOs: 1 to 19 are identified.

[Use of Markers Associated with Powdery Mildew Resistance in Plants of the Genus *Fragaria*]

[0077] With the use of the markers associated with powdery mildew resistance in plants of the genus *Fragaria*, whether or not plants of the genus *Fragaria* whose powdery mildew resistance remains unknown (e.g., progeny lines) have powdery mildew resistance can be determined. The use of markers associated with powdery mildew resistance in plants of the genus *Fragaria* includes an embodiment of the use of a method that specifically amplifies a nucleic acid fragment comprising the markers and an embodiment of the use of a DNA microarray comprising probes corresponding to the markers.

[0078] The method that specifically amplifies a nucleic acid fragment comprising markers associated with powdery mildew resistance in plants of the genus *Fragaria* is a method of so-called nucleic acid amplification. Examples of methods of nucleic acid amplification include a method involving the use of a primer designed so as to specifically amplify a target nucleic acid fragment and a method of specifically amplifying a target nucleic acid fragment without the use of a primer.

[0079] A primer that specifically amplifies a target nucleic acid fragment is an oligonucleotide that can amplify a nucleic acid fragment comprising a marker associated with powdery mildew resistance in plants of the genus *Fragaria* as defined above by a method of nucleic acid amplification. Methods of nucleic acid amplification involving the use of primers are not particularly limited, and any method may be employed, provided that a nucleic acid fragment is amplified. A representative example is a polymerase chain reaction (PCR). Examples of other methods include, but are not limited to, conventional techniques, such as rolling circle amplification (RCA), cycling probe technology (CPT), isothermal and chimeric-primer-initiated amplification of nucleic acids (ICAN), loop-mediated isothermal amplification of DNA (LAMP), strand displacement amplification (SDA), nucleic-acid-sequence-based amplification (NASBA), and transcription-mediated amplification (TMA).

[0080] When PCR is selected from among such nucleic acid amplification reactions, for example, a pair of primers are designed so as to sandwich markers associated with powdery mildew resistance in plants of the genus *Fragaria* in the chromosome of the plant of the genus *Fragaria*. When the LAMP method is employed, 4 types of primers are designed so as to sandwich the markers associated with powdery mildew resistance in plants of the genus *Fragaria* in the chromosome of plants of the genus *Fragaria*.

[0081] A method of nucleic acid amplification to be performed without the use of a primer is not particularly limited, and an example thereof is a method of ligase chain reaction (LCR). When the method of LCR is employed, a plurality of oligonucleotides that hybridize to nucleic acid fragments containing the markers associated with powdery mildew resistance in plants of the genus *Fragaria* are designed.

[0082] When the markers associated with powdery mildew resistance in plants of the genus *Fragaria* are present in the target plants of the genus *Fragaria*, as described above, nucleic acid fragments containing the markers can be obtained as amplification products according to methods of nucleic acid amplification. When a nucleic acid fragment of interest is amplified via a method of nucleic acid amplification using, as a template, the chromosome extracted from the target plant of the genus *Fragaria*, in other words, the target plant of the genus *Fragaria* can be determined to have powdery mildew resistance.

[0083] Methods for detecting an amplified nucleic acid fragment are not particularly limited. Examples thereof include a method in which a solution resulting after the amplification reaction is subjected to agarose electrophoresis, and a fluorescent intercalator, such as ethidium bromide or SYBR green, is allowed to bind thereto, so as to observe specific fluorescence, a method in which a fluorescent intercalator is added to a solution used for nucleic acid amplification, so as to detect fluorescence after the amplification reaction, and a method in which nucleic acid amplification is carried out with the use of a fluorescence-labeled primer, so as to detect fluorescence after the amplification reaction.

[0084] When the markers associated with powdery mildew resistance in plants of the genus *Fragaria* are detected

via a method of nucleic acid amplification, an amplified fragment containing such markers can contain, for example, 30 to 10.000, preferably 50 to 5,000, and more preferably 70 to 2,000 nucleotides, although the number of nucleotides would vary depending on the principle of the method of nucleic acid amplification.

[0085] When evaluating the powdery mildew resistance of plants of the genus *Fragaria*, a plurality of markers associated with powdery mildew resistance in plants of the genus *Fragaria* may be detected. Specifically, a plurality of regions selected from nucleic acid regions sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 19 in the chromosome of plants of the genus *Fragaria* may be designated as the markers associated with powdery mildew resistance in plants of the genus *Fragaria*, and the plurality of markers associated with powdery mildew resistance in plants of the genus *Fragaria* may be detected. For example, a plurality of regions selected from among 19 regions consisting of nucleotide sequences as shown in SEQ ID NOs: 1 to 19 may be designated as the markers associated with powdery mildew resistance in plants of the genus *Fragaria*, and the plurality of regions may be detected.

[0086] For example, the region comprising the nucleotide sequence as shown in SEQ ID NO: 1 (IB535110) and the region comprising the nucleotide sequence as shown in SEQ ID NO: 2 (IB522828) may be designated as the markers associated with powdery mildew resistance in plants of the genus *Fragaria*, and these regions may be subjected to nucleic acid amplification, so as to determine the presence or absence of the markers associated with powdery mildew resistance in plants of the genus *Fragaria*. Alternatively, a region sandwiched between the region comprising the nucleotide sequence as shown in SEQ ID NO: 2 (IB522828) and the region comprising the nucleotide sequence as shown in SEQ ID NO: 3 (IB559302) may be designated as the marker associated with powdery mildew resistance in plants of the genus *Fragaria*, and the region may be subjected to nucleic acid amplification, so as to determine the presence or absence of the marker associated with powdery mildew resistance in plants of the genus *Fragaria*.

[0087] According to an embodiment in which a DNA microarray comprising probes corresponding to the markers associated with powdery mildew resistance in plants of the genus *Fragaria* is used, the probes are oligonucleotides that can hybridize specifically to the markers associated with powdery mildew resistance in plants of the genus *Fragaria* as defined above under stringent conditions. Such an oligonucleotide can be designed as, for example, a partial region comprising 10, 15, 20, 25, 30, 35, 40, 45, 50, or more continuous nucleotides in the nucleotide sequence of the marker associated with powdery mildew resistance in plants of the genus *Fragaria* as defined above or a complementary strand thereof or the entire region of the nucleotide sequence. The DNA microarray comprising probes may be, for example, a microarray comprising a planar substrate of glass or silicone as a carrier, a bead array comprising microbeads as carriers, or a three-dimensional microarray comprising probes immobilized on the inner wall of a hollow fiber.

[0088] With the use of the DNA microarray thus produced, whether or not a plant of the genus *Fragaria* whose phenotypic characteristics with regard to powdery mildew resistance remain unknown (e.g., a progeny line) exhibits a phenotype indicating excellent powdery mildew resistance can be determined. Alternatively, the marker associated with powdery mildew resistance in plants of the genus *Fragaria* may be detected in accordance with a conventional technique, and whether or not the target plants of the genus *Fragaria* have excellent powdery mildew resistance may be determined by a method other than the method involving the use of a DNA microarray. An example of a method other than the method involving the use of a DNA microarray that can be employed is so-called FISH (fluorescence in situ hybridization) involving the use of the probes described above.

[0089] A method involving the use of a DNA microarray is described in greater detail. As shown in FIG. **2**, genomic DNA is first extracted from a target plant of the genus *Fragaria*. A target plant of the genus *Fragaria* is a plant of the genus *Fragaria* with unknown phenotypic characteristics in terms of powdery mildew resistance (e.g., a progeny line) and/or a parent plant of the genus *Fragaria* used when producing a progeny line, which is to be evaluated as to excellent powdery mildew resistance.

[0090] Subsequently, the extracted genomic DNA is digested with the restriction enzyme used when preparing the DNA microarray described in the [Identification of markers in plants of the genus *Fragaria*] section above, so as to prepare a plurality of genomic DNA fragments. The resulting genomic DNA fragments are then ligated to adaptors used when preparing the DNA microarray. The genomic DNA fragments comprising adaptors added to the both ends are then amplified using the primers used when preparing the DNA microarray. Thus, the genomic DNA fragments derived from the target plant of the genus *Fragaria* corresponding to the genomic DNA fragment amplified in Step Id when preparing a DNA microarray can be amplified.

[0091] In this step, among the genomic DNA fragments comprising adaptors added thereto, specific genomic DNA fragments may be selectively amplified. When a plurality of adaptors corresponding to the plurality of restriction enzymes are used, for example, genomic DNA fragments comprising specific adaptors added thereto can be selectively amplified. When genomic DNA is digested with a plurality of restriction enzymes, adaptors are selectively added to the genomic DNA fragments having protruding ends corresponding to specific restriction enzymes among the resulting genomic DNA fragments. Thus, genomic DNA fragments comprising the adaptors added thereto can be selectively amplified. By selectively amplifying specific genomic DNA fragments, as described above, these fragments can be concentrated.

[0092] Subsequently, the amplified genomic DNA fragments are labeled. Any conventional material may be used as a label. Examples of labels that can be used include fluorescent molecules, pigment molecules, and radioactive molecules. This step can be omitted with the use of a labeled nucleotide in the step of genomic DNA fragment amplification. That is, a genomic DNA fragment is amplified with the use of a labeled nucleotide in the above step, so that the amplified DNA fragment is labeled.

[0093] Subsequently, a labeled genomic DNA fragment is brought into contact with a DNA microarray under given conditions, so as to allow a probe immobilized on a DNA microarray to hybridize to the labeled genomic DNA fragment. It is preferable that hybridization be carried out under highly stringent conditions. Under highly stringent condi-

tions, whether or not the marker associated with powdery mildew resistance in plants of the genus *Fragaria* is present in the target plant of the genus *Fragaria* can be determined with higher accuracy. Stringent conditions can be adjusted based on reaction temperature and salt concentration. Specifically, higher stringency can be realized by increasing temperature or decreasing salt concentration. When a probe comprising 50 to 75 nucleotides is used, for example, hybridization can be carried out at 40° C. to 44° C. in 0.2% SDS and 6×SSC, so that higher stringency can be realized.

[0094] Hybridization between a probe and a labeled genomic DNA fragment can be detected based on a label. After the hybridization reaction between the labeled genomic DNA fragment and the probes, specifically, unreacted genomic DNA fragments or the like are washed, and a label bound to the genomic DNA fragment that had specifically hybridized to the probes are then observed. In the case that the label is a fluorescent material, for example, the fluorescent wavelength thereof is detected. When a label is a pigment molecule, the pigment wavelength thereof is detected. More specifically, apparatuses such as fluorescence detectors or image analyzers used for conventional DNA microarray analysis can be used.

[0095] By the method involving nucleic acid amplification or the method involving the use of a DNA microarray, as described above, whether or not the target plant of the genus *Fragaria* has the marker associated with powdery mildew resistance in plants of the genus *Fragaria* can be determined. As described above, a marker associated with powdery mildew resistance in plants of the genus *Fragaria* is linked to traits of excellent powdery mildew resistance. If a marker associated with powdery mildew resistance in plants of the genus *Fragaria* is present, accordingly, the target plant can be determined to be of a line or variety excellent in powdery mildew resistance.

[0096] According to the method described above, in particular, it is not necessary to have the target plant of the genus *Fragaria* grow to the extent that the target plant can actually be subjected to the test as to powdery mildew resistance. For example, seeds of progeny lines or young seedlings germinated from such seeds can be used. With the use of the markers associated with powdery mildew resistance in plants of the genus *Fragaria*, accordingly, cost of the field for growing the target plant of the genus *Fragaria* and cost for growing the plant can be reduced to a significant extent. Also, the use of markers associated with powdery mildew resistance in plants of the genus *Fragaria* eliminates the need to actually infect plants with microorganisms causing powdery mildew (i.e., *Sphaerotheca aphanis*). Thus, expenditures required for equipment such as a large-scale greenhouse for an exclusive purpose, a field for an exclusive purpose, or a facility isolated from the outside can be reduced.

[0097] When producing new varieties of the plants of the genus *Fragaria*, it is particularly preferable that several tens of thousands of types of hybrid species be first produced via crossing and evaluation take place prior to or instead of seedling selection with the use of the markers associated with powdery mildew resistance in plants of the genus *Fragaria*. Thus, the number of plants to be grown in the actual field can be reduced to a significant extent, and the labor and expenditures required for the production of new varieties of plants of the genus *Fragaria* can be reduced to a significant extent.

[0098] When producing new varieties of plants of the genus *Fragaria*, alternatively, the presence or absence of the markers associated with powdery mildew resistance in plants of the genus *Fragaria* in the parent varieties to be used for crossing is first evaluated, and parent varieties with excellent powdery mildew resistance can be selected. By producing progeny lines with the preferential use of parent varieties with excellent powdery mildew resistance, progeny lines with excellent powdery mildew resistance can develop at high frequency. Thus, the number of plants necessary to cultivate in order to produce superior lines can be reduced to a significant extent, and the labor and expenditures required for the production of new plant varieties of the genus *Fragaria* can be reduced to a significant extent.

EXAMPLES

[0099] Hereafter, the present invention is described in greater detail with reference to the examples, although the technical scope of the present invention is not limited to these examples.

1. Preparation of DNA Microarray Probe

(1) Materials

[0100] The strawberry varieties: "Miyazaki Natsu Haruka" and "08 To-f," were used.

(2) Treatment with Restriction Enzyme

[0101] Genomic DNA was extracted from these strawberry varieties using the Dneasy Plant Mini Kit (Qiagen). The extracted genomic DNA (150 ng) was treated with the PstI restriction enzyme (5 units, NEB) at 37° C. for 1 hour.

(3) Ligation of Adaptors

[0102] The PstI sequence adaptors (5'-CACGATGGATC-CAGTGCA-3' (SEQ ID NO: 20) and 5'-CTGGATC-CATCGTGCA-3' (SEQ ID NO: 21)) and T4 DNA ligase (200 units, NEB) were added to the genomic DNA fragment (150 ng) treated in (2) above, and the resultant was subjected to ligation at 16° C. for 1 hour, 55° C. for 20 minutes, and then 37° C. for 30 minutes. Subsequently, the BstNI restriction enzyme (6 units, NEB) was added to the treated sample, and the sample was then treated at 60° C. for 1 hour.

(4) Amplification by PCR

[0103] The PstI sequence adaptor recognition primer (5'-GATGGATCCAGTGCAG-3' (SEQ ID NO: 22)) and Taq polymerase (1.25 units, PrimeSTAR, Takara Bio Inc.) were added to the sample treated with the BstNI restriction enzyme (15 ng) obtained in (3) above, and the DNA fragment was amplified by PCR (30 cycles of 98° C. for 10 seconds, 55° C. for 15 seconds, and 72° C. for 1 minute, and treatment at 72° C. for 3 minutes, followed by storage at 4° C.).

(5) Acquisition of Genome Sequence

[0104] The nucleotide sequence information of the genomic DNA fragment amplified by PCR in (4) above was determined using Hiseq 2000 (Miseq, Illumina).

(6) Design of Probes and Preparation of DNA Microarray

[0105] On the basis of the genome sequence information acquired in (5) above, 50 to 60 bp probes were designed. On

the basis of the nucleotide sequence information of the designed probes, a DNA microarray comprising these probes was produced.

2. Acquisition of Signal Data

(1) Materials

[0106] The strawberry varieties: "Miyazaki Natsu Haruka" and "08 To-f," and 147 hybrid progeny lines thereof were used.

(2) Treatment with Restriction Enzyme

[0107] Genomic DNA was extracted from these strawberry varieties and the hybrid progeny lines using the Dneasy Plant Mini Kit (Qiagen). The extracted genomic DNA (150 ng) was treated with the PstI restriction enzyme (6 units, NEB) at 37° C. for 1 hour.

(3) Ligation of Adaptors

[0108] The PstI sequence adaptors (5'-CACGATGGATC-CAGTGCA-3' (SEQ ID NO: 20) and 5'-CTGGATC-CATCGTGCA-3' (SEQ ID NO: 21)) and T4 DNA ligase (200 units, NEB) were added to the genomic DNA fragment (150 ng) treated in (2) above, and the resultant was subjected to ligation at 16° C. for 1 hour, 55° C. for 20 minutes, and then 37° C. for 30 minutes. Subsequently, the BstNI restriction enzyme (6 units, NEB) was added to the treated sample, and the sample was then treated at 60° C. for 1 hour.

(4) Amplification by PCR

[0109] The PstI sequence adaptor recognition primer (5'-GATGGATCCAGTGCAG-3' (SEQ ID NO: 22)) and Taq polymerase (1.25 units, PrimeSTAR, Takara Bio Inc.) were added to the sample treated with the BstNI restriction enzyme (15 ng) obtained in (3) above, and the genomic DNA fragment was amplified by PCR (30 cycles of 98° C. for 10 seconds, 55° C. for 15 seconds, and 72° C. for 1 minute, and treatment at 72° C. for 3 minutes, followed by storage at 4° C.).

(5) Labeling

[0110] The DNA fragment amplified in (4) above was purified through a column (Qiagen), and a labeled sample was then prepared using a NimbleGen One-Color DNA Labeling kit (Roche Diagnostics K.K.) in accordance with the NimbleGen Arrays User's Guide.

(6) Hybridization and Signal Detection

[0111] Hybridization was carried out by the array CGH (aCGH) method involving the use of the Agilent in-situ oligo DNA microarray kit using the labeled sample obtained in (5) above and the DNA microarray prepared in 1. above. Signals from the samples were detected. 3. Identification of QTL associated with powdery mildew resistance of strawberries and selection of selection markers

(1) Preparation of Gene Map Data Sheet

[0112] From the signal data of the 147 hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f," the genotype data of "Miyazaki Natsu Haruka"-type 8,218 markers and "08 To-f"-type 8,039 markers were obtained. On the basis of the genotype data, the gene mapping data of the markers were obtained in accordance with the genetic distance calculation formula (Kosambi) using the genetic map production software (AntMap, Iwata. H., Ninomiya, S., 2006, AntMap: Constructing genetic linkage maps using an ant colony optimization algorithm. Breed Sci. 56: 371-378).

(2) Acquisition of Phenotype Data of Strawberry Powdery Mildew

[0113] Seeds of the 147 hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" were grown to seedlings in a greenhouse, the resulting seedlings were transplanted in an outdoor field in spring on the following year, and the onset and extent of strawberry powdery mildew was inspected in summer (FIG. **3**). Affected plants were evaluated in terms of the severity at 3 different stages: mild, moderate, and severe.

[0114] In this example, the plants were naturally infected with powdery mildew fungi indigenous in the soil of Morioka, Iwate, Japan.

(3) Analysis of Quantitative Trait Loci (QTL)

[0115] On the basis of the genetic map data obtained in (1) above and the results of strawberry powdery mildew test obtained in (2) above (i.e., the onset and extent of powdery mildew). QTL analysis was carried out by the composite interval mapping (CIM) method with the use of the genetic analysis software (QTL Cartographer, Wang S., C. J. Basten, and Z.-B. Zeng, 2010, Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, N.C.). The LOD threshold was designated to be 2.5. As a result, the presence of the gene associated with powdery mildew resistance of strawberries (LOD value: 7.3) was detected in a region between the 1B535110 marker and the 1B726514 marker in the 1st linkage group of "08 To-f" (Table 2, FIG. **4**).

(4) Selection of Selection Marker

[0116] Markers in the vicinity of the region of the strawberry powdery mildew resistant gene in a region from 0 cM to 6.83 cM of the 1st linkage group were selected as selection markers (FIG. **4**, Table 1).

TABLE 2

QTL concerning strawberry powdery mildew resistance

Variety	Linkage group	Position (cM)	Range (cM)	Flanking markers	LOD value	Effect*	Contribution rate (%)
08 To-f	1	0.0	6.8	IB535110-IB726514	7.3	−0.8	15.7

*Extent of powdery mildews (0: none; 1: mild; 2: moderate; 3: severe)

[0117] In Table 2, the column of the effects indicates an influence of the QTL on the onset and extent of powdery mildews (0: none; 1: mild; 2: moderate; 3: severe). If the

numeral value indicating the effects is a negative value, specifically, an extent of powdery mildew is lowered, and such QTL is linked to a trait that improves the powdery mildew resistance.

[0118] As shown in FIG. **4**, a marker located in the vicinity of such peak is inherited in linkage with a causal gene (or causal genes) capable of improving the powdery mildew resistance. This indicates that such marker may be used as the marker associated with powdery mildew resistance in plants of the genus *Fragaria*. Specifically, the 19 types of markers shown in FIG. **4** were found to be usable as the markers associated with powdery mildew resistance in plants of the genus *Fragaria*.

4. Selection of Unknown Line

(1) Acquisition of Phenotype Data of Strawberry Powdery Mildew

[0119] Separately from the lines described in "3. (2) Acquisition of phenotype data of strawberry powdery mildew" above, seeds of the hybrid progeny lines of "Miyazaki Natsu Haruka" and "08 To-f" were grown to seedlings in a greenhouse (50 lines, hereafter referred to as "Population A"), the resulting seedlings were transplanted in an outdoor field in autumn, and the onset and extent of strawberry powdery mildew was inspected in summer on the following year. In addition, hybrid progeny lines of "Miyazaki Natsu Haruka" and "Ohkimi" (42 lines, hereafter referred to as "Population B") and hybrid progeny lines of "Miyazaki Natsu Haruka" and "09s E-b 45e" (42 lines, hereafter referred to as "Population E") were grown to seedling, transplanted, and then inspected in terms of the onset and extent of powdery mildew in the same manner (FIGS. **5-1** and **5-2**).

(2) Extraction of Genomic DNA

[0120] Separately, genomic DNAs were extracted from the strawberry varieties: "Miyazaki Natsu Haruka" and "08 To-f," and Population A, respectively, using the Dneasy Plant Mini Kit (Qiagen).

(3) Treatment with Restriction Enzyme and Ligation of Adaptor

[0121] The extracted genomic DNA (150 ng) was treated with the PstI restriction enzyme (5 units, NEB) at 37° C. for 1 hour, the PstI sequence adaptors (5'-CACGATGGATC-CAGTGCA-3' (SEQ ID NO: 20) and 5'-CTGGATC-CATCGTGCA-3' (SEQ ID NO: 21)) and T4 DNA ligase (200 units, NEB) were added to the sample treated with PstI, and the resultant was subjected to the reaction at 16° C. for 1 hour, 55° C. for 20 minutes, and then 37° C. for 30 minutes. The BstNI restriction enzyme (6 units, NEB) was added to the treated sample, and the sample was then treated at 60° C. for 1 hour.

(4) Amplification of DNA Fragment

[0122] The PstI sequence adaptor recognition primer (5'-GATGGATCCAGTGCAG-3' (SEQ ID NO: 22)) and Taq polymerase (1.25 units, PrimeSTAR, Takara Bio Inc.) were added to the sample treated with the BstNI restriction enzyme (15 ng) obtained in (3) above, and the DNA fragment was amplified by PCR (30 cycles of 98° C. for 10 seconds, 55° C. for 15 seconds, and 72° C. for 1 minute, and treatment at 72° C. for 3 minutes, followed by storage at 4° C.).

(5) Labeling

[0123] The DNA fragment amplified in (4) above was purified through a column (Qiagen), and a labeled sample was then prepared using a NimbleGen One-Color DNA Labeling kit (Roche Diagnostics K.K.) in accordance with the NimbleGen Arrays User's Guide.

(6) Hybridization and Signal Detection

[0124] Hybridization was carried out by the array CGH (aCGH) method involving the use of the Agilent in-situ oligo DNA microarray kit using the fluorescence-labeled sample obtained in (6) above and the array prepared in 1. above. Signals from the samples were detected.

(7) Test of Selection Marker

[0125] In Population A, the markers in the vicinity of the region of the strawberry powdery mildew resistant gene were selected (Table 1), the array signal values regarding the selection markers and the phenotypes of Population A were compared, and the degrees of consistency were found to be 90.0% to 98.0% (FIGS. **6-1** to **6-5**). In FIGS. **6-1** to **6-5**, high array signal values were underlined. The results indicate that the use of the markers shown in Table 1 enables selection of lines that are excellent and lines that are poor in terms of powdery mildew resistance.

5. Selection and Test Using PCR Base Marker 1

(1) Extraction of Genomic DNA

[0126] Genomic DNAs were extracted from the strawberry varieties: "Miyazaki Natsu Haruka," "08 To-f," "Ohkimi," and "09s E-b 45e," Population A (51 lines), Population B (42 lines), and Population E (42 lines), using the Dneasy Plant Mini Kit (Qiagen).

(2) Preparation of Primer

[0127] With the use of PCR primer analytic software (Primer 3), primers that recognize the sequences of IB535110 were prepared on the basis of the sequence information thereof (SEQ ID NO: 1) (35110_v1F: ACA-CATATATGAATCGGAGCCA (SEQ ID NO: 23); 35110_v1R: GCTCAAGATGCTCAATCGAA (SEQ ID NO: 24)).

(3) Amplification by PCR and Test of Selection Marker

[0128] The above pair of the primers (35110_v1F and 35110_v1R) and Taq polymerase (1.25 units, Tks Gflex DNA Polymerase, Takara Bio Inc.) were added to the genomic DNAs (15 ng each) of the hybrid progeny lines: Population A. Population B, and Population E, and the genomic DNAs were amplified by PCR (30 cycles of 94° C. for 1 minute, 98° C. for 10 seconds, 60° C. for 15 seconds, and 68° C. for 30 seconds, followed by storage at 4° C.). The PCR-amplified DNA fragment was confirmed using the TapeStation D1000 (Agilent). The results attained for Population A, Population B, and Population E are shown in FIGS. **7-1** and **7-2**, FIGS. **8-1** and **8-2**, and FIGS. **9-1** and **9-2**, respectively. In FIGS. **7-1** to **9-2**, lane M represents "Miyazaki Natsu Haruka" and lane Z represents "08 To-f."

These results are summarized in FIGS. **10-1** and **10-2**. In FIGS. **10-1** and **10-2**, underlines are provided when phenotypes are not consistent with the results attained with the use of PCR markers. As shown in FIGS. **7-1** to **10-2**, the degree of consistency between band patterns and phenotypes is very high (i.e., 98.5%) and the method of nucleic acid amplification involving the use of primers that specifically amplify IB535110 enables selection of lines that are excellent and lines that are poor in terms of powdery mildew resistance.

6. Selection and Test Using PCR Base Marker 2

(1) Extraction of Genomic DNA

[0129] Genomic DNAs were extracted from the strawberry varieties: "Miyazaki Natsu Haruka," "08 To-f." "Ohkimi," and "09s E-b 45e," Population A (51 lines), Population B (42 lines), and Population E (42 lines), using the Dneasy Plant Mini Kit (Qiagen).

(2) Preparation of Primer

[0130] With the use of PCR primer analytic software (Primer 3), primers that recognize the sequences of IB533828 were prepared on the basis of the sequence information thereof (SEQ ID NO: 2) (22828_v6F: CTTTGACGCCTACTGCATIA (SEQ ID NO: 25) and 22828_v6R: GGTUGGGCTTCGTTAAATCT (SEQ ID NO: 26)).

(3) Amplification by PCR and Test of Selection Marker

[0131] The above pair of the primers (22828_v6F and 22828_v6R) and Taq polymerase (1.25 units, Tks Gflex DNA Polymerase, Takara Bio Inc.) were added to the genomic DNAs (15 ng each) of the hybrid progeny lines: Population A, Population B, and Population E, and the genomic DNAs were amplified by PCR (30 cycles of 94° C. for 1 minute, 98° C. for 10 seconds, 60° C. for 15 seconds, and 68° C. for 30 seconds, followed by storage at 4° C.). The PCR-amplified DNA fragment was confirmed using the TapeStation D1000 (Agilent). The results attained for Population A, Population B. and Population E are shown in FIGS. **11-1** to **11-3**, FIGS. **12-1** and **12-2**, and FIGS. **13-1** and **13-2**, respectively. In FIGS. **11-1** to **13-2**, lane M represents "Miyazaki Natsu Haruka," lane Z represents "08 To-f," and lane O represents "Ohkimi." These results are summarized in FIGS. **14-1** and **14-2**. In FIGS. **14-1** and **14-2**, underlines are provided when phenotypes are not consistent with the results attained with the use of PCR markers. As shown in FIGS. **11-1** to **14-2**, the degree of consistency between band patterns and phenotypes is very high (i.e., 98.5%) and the method of nucleic acid amplification involving the use of primers that specifically amplify 1B522828 enables selection of lines that are excellent and lines that are poor in terms of powdery mildew resistance.

[0132] All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

SEQUENCE LISTING

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gttatgctgg tatgtaagcc tctgggtctg atcaaatgag agtgtatcta gaactttcaa 180

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gaaaatttag aaaccatgaa ttaagatatt agaattccta catcatcacc acccagagcc 600

aagagagttt ggtggtgttt caatttcagc ccaagttttc tctattcgtc gtctccttct 660

ccctctcctc cattatttcc attacatgac agttgaaacg ctttctcccg atcgtgtaca 720

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ttggctgtga atgatgaaga tgatgatgaa atttgggtta tgctcaagtg gtgcaaactt 180

ttgaaagcaa cgtgagcttt aacgaagccc aacccaaatt aagtcctaca tttgagagag 240

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aaaacaaatt tttctctctt ttgggtaaac aaacaagttt tgaataggtt tccttcttct 360

gtaacaagga cttgctacag aaatggaccg ataacaacct gctgttccag aggactcccc 420

attcttctgt gtaaggcttc tggagctcga tgatatcaaa gaagggagga aggtaccttt 480

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agacatgtta ctgagacctc acctacaaat ttttctgacc atcttaacgc aaaatttaca 240

gatccggtga tccggtaatc catttaaccc gataaaacat ataagtgtcg tacattccat 300

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aaatgcacat acccttgtat tttccaacca cattcaaacc atgaagaatt ctgacttgtg 300

aatcggcgca agtgaccatt actttgtcag gatcatgagg gaaatactgc attaataaca 360

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aaagaagaat acaaaagtat tagctacctc aaagcctgtt atcttt 466

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tactactgag attgtcaatg attcagacgc agatcatgtt ttctattgat ccatttcttg 240

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acatattcct tttattttgt tatattgtag tagtgacctt atgagtcttt cccaatttcg 420

gtttcttagt tttgtttcgt tgttattttg ttacgagaga ttttggtcta atcctcctct 480

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cagctcttct ttgtcgtgct caaacagtgc acgggcccca catttcttca ccttcatgta 180

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tttggaacct tagaatgttt ccaactgata ttgagttcaa tacttgcagg aattctaatc 420

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tgtaataagt ggagattctt atcatttccc acatctgaga aactctgaaa taaacaaaaa 180

gaaagagaaa aaggctttca cgacaatatg ggtgaagcat ggggtcctaa ctcctaagtt 240

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tgtaataagt ggagattctt atcatctccc acatctgaga aactcagaaa caaacaaaaa 180

gaaagagaaa aaggctttca cgacaatatg ggtgaagcat gggtcctaag ttcgtaatct 240

ctgtgtttgt taaacaacta taatctatat atagtaactc ttggtgttgc tcggtctaag 300

gttgtaccaa tcagtgtctt agatagacaa agtcggtgga aggtggcagt aacatatcac 360

aaagtctgtt gtgagggttg caacaatata acgcaactgt aaactgtcac atcagtttac 420

aaactctact tacataaatt ttatttagtg ttcaacgttc aaacattaca ttctatcata 480

tttcggtgca tgacatactt cgcgttttgg ac 512

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gaagctaaca ttacccgtga aatattgaac acccttttga tgtctatact tcaataatgt 240

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tttgattttg ttttctctac taattctgac aatcgaaaaa ccgaacgtgt tagtctagaa 360

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agctttgatc actgtattgg acgggttacc acagctagaa gtcctcaata tagcacattg 180

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ccggtgcatc ctgtgccaaa gggccagaaa cgacgggggg attatgaaat ggtataaata 360

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tgatatgcaa gtcacagtgc cacctaacta taaacggttt tctggatcct caaagggatg 360

gttgatagct ttggatgaga attttgtagt aacactgata aatcctttct ctagagttaa 420

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gacaataaga ttacgaggtg aagagtatc 509

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<212> TYPE: DNA
<213> ORGANISM: Fragaria x ananassa

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aagtttttat ggcaaggggt ttaattgtcc tttcaaaaat gttagaagtg aaatttgggt 180

cagatggatg aaggttttct tctgtccata tatacgagtg tattatgttt cgtcgatgta 240

tcgatgattt atattaaatt tcagatttta attttgagac atgaaaaaca tttataattt 300

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ttatttcaca accattttaa ttaacgttcg atttgtttca gtgaaaaaca aacagttggt 180

agtaaaagat catggtaaaa agcagactgc gtggtggggt ggatgtacac aacgcggagt 240

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cctgtaatta taaatataca taatatattc ttaagaaaac tttgcgaggt aaaagtggtg 360

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agtgctaaaa caatgtgggc tgctaacttc tcctctgtct tgtgattgca agctaggttg 420

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<213> ORGANISM: Fragaria x ananassa

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agtttttatg gcaaggggtt taattgtcct ttcaaaaatg ttagaagtga aatttgggtc 180

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cgatgattta tattaaattt cagattttaa ttttgagaca tgaaaaacat ttataattta 300

agtgattttg tgtttctagc cttatagtgc gtatgaatga gacacaacgt acaaaaaagt 360

tgagataaga aaatgaccca taaattattt tggttttaat ttatgtaagc gatattttta 420

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ttgtaaactt gaatcaatag tacttgacgt cgttagcatg attgaattgt caaatgttgt 540

atattttgaa aggtaaaaag gtacctctct tcacttcatc ttttttgtct ctaaaccaca 600

ccaagacttt gcgcaaagcc ctccatcttt acatcaaatg gtgatattct aagtcgcata 660

ccaaaacccc gatctccaag actcgactcc caaatctgga gatggaggtg acaacacgac 720

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cattgagcaa ctactatagt tagtcctgtg attggaagtg ccaagggtct tcaaaataac 120

cggggcaatc tatggccatg gttctatgta tatacataat cctctatcct agttatgcta 180

ccaaatatgt tctgagacat aatcgttctt ctgttgctcg gaacaatgca gaaaacttaa 240

aatagtaaaa gtgttgttat agaatctcct caaaatttta gaccatttta gggaaattct 300

atcagtgttt caatcgttag acacttcaag tcctagtata ctaatccaaa agcctcacta 360

caaaaatata tgaagacatt tacatgcgac catactagcc ttcctctatc agaacgaacc 420

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<223> OTHER INFORMATION: Synthetic DNA

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<400> SEQUENCE: 23

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<400> SEQUENCE: 25

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1. A marker associated with powdery mildew resistance in plants of the genus *Fragaria* comprising a continuous nucleic acid region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 19 in the chromosome of the plant of the genus *Fragaria*.

2. The marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to claim **1**, wherein the nucleic acid region comprises any nucleotide sequence selected from the group consisting of nucleotide sequences as shown in SEQ ID NOs: 1 to 19 or a part of the nucleotide sequence.

3. The marker associated with powdery mildew resistance in plants of the genus *Fragaria* according to claim **1**, wherein the nucleic acid region is located in a region sandwiched between the nucleotide sequence as shown in SEQ ID NO: 1 and the nucleotide sequence as shown in SEQ ID NO: 7 in the chromosome of the plant of the genus *Fragaria*.

4. A method for producing a plant line of the genus *Fragaria* with improved powdery mildew resistance comprising:

a step of extracting a chromosome of a progeny plant whose at least one parent is a plant of the genus *Fragaria* and/or a chromosome of the parent plant of the genus *Fragaria*; and

a step of determining the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria* according to claim **1** in the chromosome obtained above.

5. The method for producing a plant line of the genus *Fragaria* according to claim **4**, wherein the step of determination comprises conducting a nucleic acid amplification reaction using a primer that specifically amplifies the marker associated with powdery mildew resistance in the plant of the genus *Fragaria* to determine the presence or absence of the marker associated with powdery mildew resistance in the plant of the genus *Fragaria*.

6. The method for producing a plant line of the genus *Fragaria* according to claim **4**, wherein the step of determination involves the use of a DNA chip comprising a probe corresponding to the marker associated with powdery mildew resistance in the plant of the genus *Fragaria*.

7. The method for producing a plant line of the genus *Fragaria* according to claim **4**, wherein the progeny plant is a seed or seedling and the chromosome is extracted from the seed or seedling.

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