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Potato virus Y resistance gene, Ry_{chc}, mapped to potato chromosome 9

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Summary

Introduction

Potato virus Y (PVY) is one of the most important viruses affecting potato production systems. Three main strains have been distinguished: common strain (PVY^O), tobacco veinal necrosis strain (PVY^N) and stipple streak strain (PVY^C) (Stevenson, et al. 2001), among which PVY^N is problematic because it often causes a virtually symptomless infection on potato but causes a severe necrosis on nearby tobacco foliage. Conferring the genetic resistance to potato cultivars is always the most desirable solution.

Extreme resistance genes to PVY have been known from three different sources: Ry_{sto} from *Solanum stoloniferum* Schlechtd. et Bché. (Stelzner1950), Ry_{adg} from *S. tuberosum* L. subsp. *andigena* Hawkes (Munoz et al. 1975) and Ry_{chc} from *S. chacoense* Bitt.(Asama et al. 1982). Both Ry_{adg} and Ry_{sto} inherit monogenically with a dominant fashion and show strain non-specific resistance (Ross 1958, Munoz et al. 1975). Both genes have genetically localized at the approximately same position on the proximal end of potato chromosome 11 (Brigneti et al. 1997, Hämäläinen et al. 1997), which form a resistance gene cluster together with Na_{adg} (Potato virus A resistance gene), R_{Mc1} (nematode resistance gene) and *Sen1* (wart fungus resistance gene) (Gebhardt and

Valkonen 2001). Many cultivars carrying Ry_{sto} have been released mainly in Germany (Ross 1986). However, these cultivars show male sterility caused by association with the characteristic mitochondrial genome derived from *S. stoloniferum* (Lössl et al. 2000). Ry_{adg}, in turn, has been introduced into various breeding lines in the International Potato Center (CIP), Lima, Peru, but cultivar release has been still limited.

On the other hand, Ry_{chc} inherits in a dominant, monogenic fashion, as recently reported by a genetic analysis of PVY resistance in one of Japanese leading cultivars, 'Konafubuki' (Hosaka et al. 2001). Ry_{chc} is carried by a newly-released cultivar 'Sakurafubuki' (??), and could be useful due to no apparent association with inferior agronomic traits. By use of the RAPD marker 38-530 linked with Ry_{chc} (Hosaka et al. 2001) and a simple DNA extraction method (Hosaka 2004), a breeding program aiming at development of PVY resistance cultivars is in progress in Japan.

Since the linked marker 38-530 was mapped on chromosome 9 in a diploid segregating population from a selfed F1 hybrid of *S. chacoense* x *S. phureja*, Ry_{chc} was supposed to locate somewhere on the

same chromosome 9 (Hosaka et al. 2001). In this study, haploid plants were induced from ‘Konafubuki’, from which a resistant clone was selected and crossed with a susceptible clone to produce a diploid segregation population in order to directly map the Ry_{chc} gene.

Materials and methods

Seeds of the families 96H5 and 98H20 were supplied by K. Senda, Hokkaido Prefectural Kitami Agricultural Experiment Station, which were obtained by crossing ‘Konafubuki’ with pollen from a haploid inducer *S. phureja* Juz. et Buk., clones W902209-6 and W460, respectively. Seeds showing no embryo spot marker were sown and their somatic chromosome numbers were counted by K. Nakagawa, Kobe University.

A selected haploid plant 98H20-5 was crossed with diploid genotypes having Sli gene and no 38-530 marker band, which were all descended from a cross between 94H100-1 (a selected diploid clone from a *S. stenotomum-S. phureja* bulk population) and F_1-1 (an F_1 hybrid plant from *S. chacoense* 525-3 × *S. phureja* 1.22). The Sli gene

will be functioned to obtain selfed progeny (Hosaka and Hanneman 1988) and homozygotes of Ry_{chc} in future.

The procedures for DNA isolation and detection of RAPD and RFLP markers were described previously (Hosaka and Hanneman 1998). RFLP probes prefixed with “TG”, “CD” or “CT” were single-copy tomato probes obtained from S. D. Tanksley, Cornell University, NY, USA, and were localized on the tomato or potato genetic maps (Tanksley et al. 1992). The probes prefixed with “P” were single-copy DNA probes selected from a random genomic DNA library of *S. phureja* 1.22 (Hosaka and Spooner 1992), which have been localized on this potato genome (Hosaka and Hanneman 1998; Hosaka 1999).

To duplicate clones of ‘Konafubuki’ haploid plants, the PVY-T (Japanease isolate of PVY^N) and PVY-O (Japanease isolate of PVY^O) strains were inoculated mechanically twice in two weeks interval and four weeks later leaves were sampled for ELISA test. To the plants of a segregation population, only PVY-T strain was innoculated one month after tuber planting, and three weeks after the inoculation, leaves were sampled for ELISA test. The plants that indicated negative reactions with the ELISA test were re-inoculated, and three weeks later ELISA test was performed again. The double-antibody sandwich method

(DAS-ELISA) was used for detection of PVY-T. Absorbance at 405nm was read in ELISA microplate reader (MTP-450, Corona). Absorbance values of healthy controls ranged from 0.00 to 0.01, and values above 0.1 were regarded as positive for PVY-T.

Linkage analysis was carried out by the program "MAPMAKER" (Lander et al. 1987) with Kosambi mapping function.

Results

Haploid plants from 'Konafubuki'

Out of 59 plants grown, 16 were diploid (2*n*=24), while 7 and 36 plants were of triploid and tetraploid levels, respectively. These 16 diploid plants were analyzed with a total of 75 RFLP probes for their haploidy as analysed by Samitsu and Hosaka (2002). The RFLP bands observed among diploid plants were all detected in 'Konafubuki'. As shown in Fig. 1, for example, various combinations of two bands out of four bands that 'Konafubuki' was holding were detected in diploid plants.

Hence, these diploid plants were confirmed to be euhaploids of 'Konafubuki'.

Twelve haploid plants could be evaluated for resistance to PVY (Table 1). Visual symptoms such as veinal necrosis on the innoculated leaves or mosaic on the upper leaves were observed on susceptible clones with PVY-O strain, while no symptom was observed with PVY-T strain except for 96H5-13, which showed veinal necrosis. ELISA tests showed the same results on the absorbance values with both PVY-O and PVY-T, and identified seven resistant and five susceptible plants. The ratio of 7 resistant: 5 susceptible plants fit the ratio of gametic sampling from a simplex genotype (x^2 = 0.33, P>0.10, for 1 :1 based on a random chromosome assortment model, or x^2 = 0.68, P>0.10, for 13:15 based on a random chromatid assortment model). The RAPD marker 38-530, linked with Ry_{chc} in 'Konafubuki', was present in nine plants, while it was absent in seven plants. The presence/absence of the marker band perfectly correlated with resistance phenotypes. One haploid plant (98H20-28) died early and most of the others grown showed very poor reproductivity. Only three plants flowered with reasonable pollen stainability (12.9-49.6%), while the others showed either no bud formation or bud dropping at a young stage of flower

formation. As a result, 98H20-5, that had Ry_{chc}, 38-530 marker band and reasonable fertility, was selected as a parent to generate a segregating population for mapping Ry_{chc}.

Production of a segregating population

The haploid plant 98H20-5 was crossed as female with 14 genotypes as male, while it was crossed as male with six genotypes as female (Table 2). Only three cross combinations set berries and produced seeds: 40 seeds from 97H32-6 x 98H20-5, 56 seeds from 98H20-5 × 94H89-43, and 235 seeds from 98H20-5 × 94H89-124, from which a segregating population (1H110 and 2H23 families) was generated.

Linkage analysis using the haploid population

Sixteen haploid plants were analyzed with a total of 75 single- or double-copy RFLP probes ranging from three to 29 probes per chromosome, from which 60 probes, with at least two probes per chromosome, generated 133 polymorphic bands among haploid plants. Linkage analysis for these 133 RFLP bands, a RAPD band 38-530 and

Ry$_{chc}$ resulted in 20 linkage groups at a LOD value of 3.0. The linkage group containing 38-530 and *Ry*$_{chc}$ also contained TG328 and TG429, both locating on chromosome 9 in potato genome.

Mapping population

One hundred nineteen hybrid plants (98H20-5 × 94H89-124) and their parents were grown for evaluation of PVY resistance with PVY-T strain and for RFLP mapping. The parental genotypes were reconfirmed; 98H20-5 (positive for 38-530 and *Ry*$_{chc}$) and 94H89-124 (negative for both). Virus tests were completed in 114 hybrid plants. The presence/absence of *Ry*$_{chc}$ was segregating as 46:68, showing slightly biased segregation (x^2 = 4.25, 0.05>P>0.01).

Mapping Ry$_{chc}$

In our unpublished data, the RAPD marker 38-530 was mapped on chromosome 9 in the F2 population derived by selfing from the clone F1-1. 'Konafubuki' haploids supported the possible localization of both *Ry*$_{chc}$ and 38-530 on chromosome 9 as described above. Thus,

RFLP probes mapped on chromosome 9 were mainly used in this study. RFLP bands unique to 98H20-5 were analyzed for linkage with Ry_{chc}. Only one recombinant in 114 plants (or 0.9% recombination frequency) was found between Ry_{chc} and molecular markers 38-530, CT210, TG328, CT71 and CT220, among which no recombinant was found in 119 plants. Since these RFLP loci were localized on the most terminal region of chromosome 9 in potato or tomato genome (Tanksley et al. 1992), Ry_{chc} mapped 0.9 cM distal to these markers and expanded the total map length of chromosome 9 (Fig. 2).

Discussion

The extreme PVY resistance gene Ry_{chc} was mapped on the most terminal region of potato chromosome 9.

Table 1. Characterization of haploid plants from ‘Konafubuki’

Plant	2n	Resistance	38-530 marker	Pollen stainability
96H5-3	24	R	+	(buds dropped)
96H5-5	24	R	+	(buds dropped)
96H5-13	24	S	-	12.9%
98H20-2	24	R	+	(buds dropped)
98H20-5	24	R	+	27.0%
98H20-6	24	S	-	(no buds)
98H20-16	24	R	+	(no buds)
98H20-18	24	S	-	49.6%
98H20-20	24	R	+	(no buds)
98H20-28	24	ND	+	ND
98H20-37	24	S	-	(buds dropped)

98H20-44	24	R	+	(buds dropped)
98H20-48	24	ND	+	(no buds)
98H20-67	24	S	-	(buds dropped)
98H20-68	24	ND	-	(buds dropped)
98H20-83	24	ND	-	(buds dropped)

ND no data; R resistant, S susceptible; + present, - absent

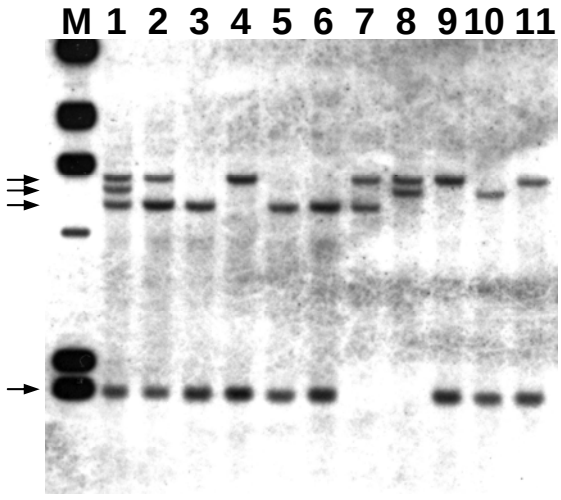
Table 2. Pollinations with 98H20-5, a haploid clone of ‘Konafubuki’.

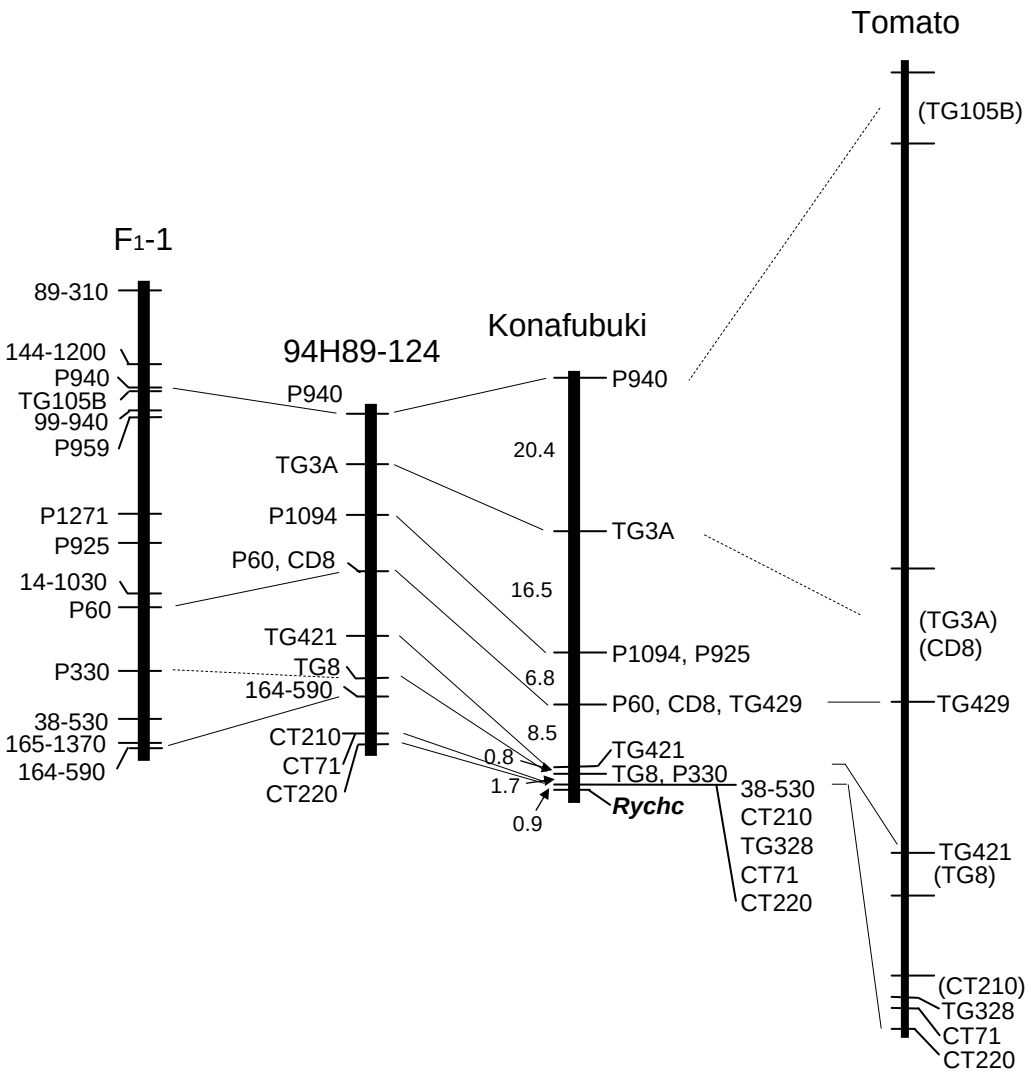
Female	Male	Total no. of pollinations	Total no. of berries	Total no. of seeds
14 genotypes	98H20-5	202	1	40
98H20-5	6 genotypes	30	8	291

Legend of Figures

Fig. 1. A Southern-blot of *Hin*dIII-digested total DNAs probed with P1120 probe. M, *Hin*dIII-digested lambda DNA; 1, Konafubuki; 2, Kita-akari; 3, 96H5-3; 4, 96H5-13; 5, 98H20-2; 6, 98H20-5; 7, 98H20-16; 8, 98H20-18; 9, 98H20-20; 10, 98H20-37; 11, 98H20-44. Arrows indicated four bands corresponding to four alleles of 'Konafubuki'.

Fig. 2. Chromosome 9 linkage maps of F_1-1, 94H89-124 and Konafubuki in comparison with tomato chromosome 9 (Tanksley et al. 1992). Ry_{chc} was mapped at the most distal end of Konafubuki chromosome 9.


M 1 2 3 4 5 6 7 8 9 10 11


Tomato
(TG105B)
F1-1
89-310
144-1200
P940
TG105B
99-940
P959
P1271
P925
14-1030
P60
P330
38-530
165-1370
164-590
94H89-124
P940
TG3A
P1094
P60, CD8
TG421
TG8
164-590
CT210
CT71
CT220
Konafubuki
P940
20.4
TG3A
16.5
P1094, P925
6.8
P60, CD8, TG429
8.5
0.8
TG421
1.7
TG8, P330
0.9
Rychc
38-530
CT210
TG328
CT71
CT220
(TG3A)
(CD8)
TG429
TG421
(TG8)
(CT210)
TG328
CT71
CT220