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Note

Harimaru: a new potato variety for a local specialty

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‘Harimaru’ is a new potato variety bred from a cross between ‘Saikai 35’ as a female parent and ‘Pike’ as a male parent. Marker selection was performed for 1,647 seedlings to combine resistance genes to late blight (*R1*), *Potato virus Y* (Ry_{chc}), *Potato virus X* (*Rx1*), and golden cyst nematode (*H1*). In total, 194 selected clones were evaluated in the field, among which the best clone was officially released as ‘Harimaru’. Its yield was slightly lower than the local standard variety, ‘May Queen’. However, it produces tasty potatoes, that do not become mushy with long boiling times despite its high starch content. ‘Harimaru’ may become a local specialty potato and its multiple resistance to potato viruses may allow cultivation using homemade seed tubers from the previous season’s crop.

Key Words: Harimaru, potato (*Solanum tuberosum* L.), homemade seed tuber, virus resistance, marker-assisted selection.

Introduction

The production and distribution of seed potato tubers is strictly regulated by the Plant Protection Act, and seed tubers must be certified by government officials in Japan, which often hampers the quick spread of new varieties and launching of seed potato-related businesses by private companies. A small lot of seed tubers of a new variety are hated at any stages from foundation seed to certified seed production and to commercial seed production and in seed tuber distribution. To avoid this well-established but strictly regulated seed tuber production system, a scientific approach might involve the use of true botanical seeds (Lindhout *et al.* 2011). An alternative is the use of a virus-resistant variety grown using homemade seed tubers from the previous season’s crop. Twelve viruses are known to infect and damage potatoes in Japan (Maoka *et al.* 2010). Among them, *Potato virus Y* (PVY) is the most important, causing particularly severe damage as a mixed infection with *Potato virus X* (PVX).

Disease resistance genes and DNA markers linked to these genes have been developed in potato (Ramakrishnan *et al.* 2015). Among them, resistance genes to PVX (*Rx1*), PVY (Ry_{chc}), and golden cyst nematode [*Globodera rostochiensis* (Woll.) Behrens] (*H1*) are available in Japan, and their DNA markers have been developed (Mori *et al.* 2010, 2011). The *Rx1* and Ry_{chc} genes show strain-nonspecific resistance. The *H1* gene shows strain-specific resistance to pathotypes Ro1 and Ro4. However, only pathotype Ro1 has been found in Japan. Late blight caused by *Phytophthora infestans* (Montague) de Bary is the most devastating disease in potato cultivation worldwide. One late blight resistance gene, *R1*, has been cloned, and the gene-specific marker is available (Ballvora *et al.* 2002). However, *R1*-mediated resistance has already been broken, and the chemical control has become routine practice in Japan.

In this study, we tried to combine *R1*, *Rx1*, Ry_{chc}, and *H1* genes into one clone using marker selection technology at the seedling stage. The *R1* gene, though not useful as mentioned above, was included to evaluate the efficiency of marker-assisted selection in a practical breeding program. Although the agronomically best clone lacked the *R1* gene, we successfully bred it as a locally adapted, tasty potato resistant to PVX, PVY, and golden cyst nematode. It was named ‘Harimaru’ and officially released in 2017.

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Materials and Methods

Parental clones

The pedigree of 'Harimaru' is shown in **Fig. 1**. 'Saikai 35' is a male and female fertile breeding clone carrying *Solanum phureja*-derived cytoplasm (P type, as defined by Hosaka and Sanetomo 2012), the PVY resistance gene (Ry_{chc}) derived from 'Konafubuki', and the golden cyst nematode resistance (*H1*) gene derived from Tunika, as well as resistance to bacterial wilt (Mori *et al.* 2012). 'Pike' is a US chip processing variety and possesses the *H1* gene derived from either 'Allegany' or 'Atlantic' and common scab resistance (Plaisted *et al.* 1998); it also contains the PVX resistance gene (*Rx1*) derived from 'Atlantic' (Ross 1986).

Cultivation practices

Seed tubers were planted in the field in early March 30-cm apart in 1-m-wide rows and fertilized with 18 kg 10 a^{-1} N, 18 kg 10 a^{-1} P, and 18 kg 10 a^{-1} K. Ordinary cultural practices were used until natural senescence in July.

Marker analysis

DNA was extracted from fresh leaves using the one-minute DNA extraction method (Hosaka 2004). The presence of four resistance genes (*R1*, *Rx1*, Ry_{chc}, and *H1*) were estimated using the following markers: *R1*-specific marker (76-2sf2: 5′-CACTCGTGACATATCCTCACTA-3′ and 76-2SR: 5′-CAACCCTGGCATGCCACG-3′, Ballvora *et al.* 2002), *Rx1*-linked marker (RxSP-S3: 5′-ATCTTGGTTTG AATACATGG-3′ and RxSP-A2: 5′-CACAATATTGGAAG GATTCA-3′, Ohbayashi and Komura 2004), Ry_{chc}-linked marker RAPD 38-530 (5′-TTCGAGCCAG-3′, Hosaka *et al.* 2001), and *H1*-linked marker (H1SP-S2: 5′-GAGCTCA GAGGTGAAAAATA-3′ and H1SP-A6: 5′-GGAACAAT GTTGAATGCAAG-3′, Ohbayashi *et al.* 2010). Diagnostic marker bands were amplified using polymerase chain reaction (PCR) in a volume of 10 µl consisting of 2 µl of template DNA, 5 µl of 2× Ampdirect® Plus (Shimadzu Co., Japan), 0.25 units of *Taq* DNA polymerase (NovaTaq™ Hot Start DNA Polymerase, Novagen, USA), and 1 µl each of 3 µM forward and reverse primers. The thermal conditions for PCR were similar to those described in the original literature. The detection procedure for the RAPD marker 38-530 is described in Hosaka (2004). The amplified product was separated on a 1.4% agarose gel in 1× TAE buffer (40 mM Tris-acetate and 1 mM EDTA).

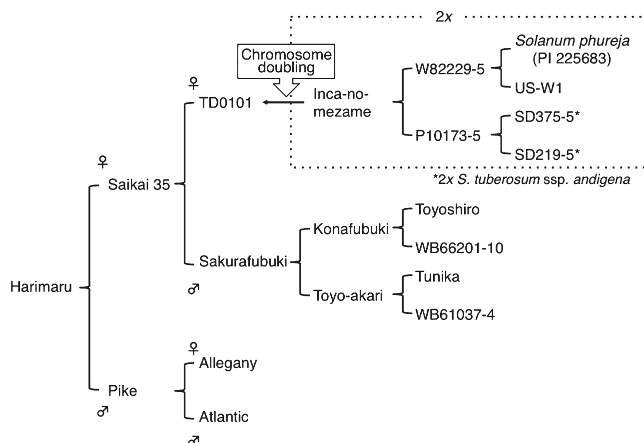

Fig. 1. Pedigree of 'Harimaru'.

Biological assay for disease resistance

Resistance to PVX, PVY, and golden cyst nematode (*G. rostochiensis* pathotype Ro1) was assayed by inoculation tests courtesy of the Hokkaido Agricultural Research Center, Sapporo.

Results

Breeding process

All breeding processes were performed at the Food Resources Education and Research Center, Kobe University. In November 2007, 'Saikai 35' was pollinated with the pollen of 'Pike'. From 77 pollinations, 33 berries were obtained. On February 10, and September 14, 2008, 1,170 and 1,232 seeds (families 8H14 and 8H18, respectively) were pretreated by soaking in 2,000 ppm gibberellic acid (GA_3) solution for 48 hr and sown; from these, 905 and 758 seedlings were raised in 10.5-cm black vinyl pots. Using molecular markers, 194 seedlings were selected as positive for all four resistance genes. In 2009, 2 hills from each of the 194 clones were grown in the field, from which 15 clones were selected based on tuber quality (shape, uniformity and taste). In 2010, 10 hills from each of the 15 clones were grown, from which three clones were selected and named 10H15, 10H16, and 10H17. In 2011, 60 hills per clone were grown, from which 10H16 and 10H17 were selected. In 2012, 324 hills per clone were grown, from which 10H16 was selected as the best. It was named 'Harimaru', and official registration paperwork for this clone was filed. 'Harimaru' was officially registered on February 24, 2017 and announced as a new variety on March 7, 2017. 'Harimaru' is a name coined by the combination of 'Harima' (a region where this variety was bred) and 'maru' (='round', indicating the tuber shape).

Seedling selection using molecular markers

DNA was extracted from 905 seedlings of the family 8H14, of which three DNA samples were accidentally lost during the process. This population was first screened by an *R1*-specific marker. Then, possible *R1* gene holders were analyzed by an *Rx1*-linked marker. Two hundred thirty-four seedlings were positive for both markers. From the family 8H18, 742 seedlings were surveyed first by an *Rx1*-linked marker, and then by an *R1*-specific marker. Two hundred thirteen seedlings were positive for both markers. A total of 447 seedlings likely possessing both *R1* and *Rx1* markers were surveyed for the presence of the RAPD marker 38-580

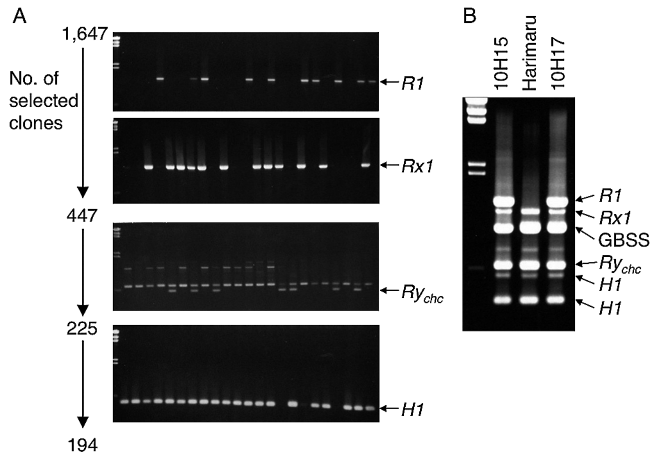

Fig. 2. Marker selection. Selection process (A) and reconfirmation of genotypes by the multiplex PCR method of Mori *et al.* (2011) (B).

Table 1. Segregation of DNA markers in progeny of 'Saikai 35' × 'Pike'

Gene-specific or linked marker	Observed (+:–)	Postulated genotype for 'Saikai 35' × 'Pike'	Expected ratio (+:–)[a]	P-value by χ^2 test[a]
R1 and *Rx1*	447:1197	Nulliplex × simplex	1:3	0.0403
			169:615	<0.0001
Ry_{chc}	225:222	Simplex × nulliplex	1:1	0.8872
			13:15	0.0977
H1	194:31	Simplex × simplex	3:1	0.0001
			559:225	<0.0001
		Simplex × duplex	11:1	0.0031
			347:45	0.2795

[a] Upper column for random chromosome assortment model and lower column for random chromatid assortment model.

for the Ry_{chc} gene, resulting in 225 positive seedlings. Then the *H1*-linked marker was used. Consequently, 194 seedlings were selected as potentially containing the four resistance genes (**Fig. 2A**).

The segregation ratios of these DNA markers were analyzed (**Table 1**). The parental clone 'Saikai 35' has marker bands for Ry_{chc} and *H1*, while 'Pike' has bands for *R1*, *Rx1* and *H1*. The hybrid seedlings possessing both *R1* and *Rx1* markers were significantly over-represented, likely because PCR conditions were not optimized for amplification of the *R1*-specific marker. The *H1*-linked marker also significantly deviated toward over-representation from the segregation ratios in the progeny of simplex × simplex as expected either by a random chromosome assortment model or by a random chromatid assortment model. If we assume 'Pike' is duplex for this marker, a better fitting to the expected ratio can be obtained.

A later assay using more accurate markers for Ry_{chc} and *H1* genes (Takeuchi *et al.* 2008) and a deliberately designed multiplex PCR condition (Mori *et al.* 2011) indicated 'Harimaru' does not have the *R1* gene (**Fig. 2B**).

Growth habit and morphological characteristics

Plant, leaf, flower, sprout, and tuber characteristics of

Fig. 3. Plant, leaf, flower, sprout and tuber characteristics of 'Harimaru'.

'Harimaru' are shown in **Fig. 3**. The vine has a spreading shape. The plant height (47.6–68.4 cm) is comparable to that of 'May Queen' (52.5–64.5 cm). No anthocyanin coloration was observed in the leaf and stem. The stem is slender, and there are more stems (6.7) than in 'May Queen' (3.5–4.8). The flower is pale violet and the number of flowers is intermediate. 'Harimaru' matures approximately 140 days after planting on March 5, longer than 'Kita-akari' (127 days after planting). Tubers are short ovate with medium-netted yellow skin and shallow pink eyes. The flesh is bright yellow. The tuber dormancy of 'Harimaru' is slightly shorter (80 days) than 'Kita-akari' (96 days).

Tuber yield and quality

Marketable yield (>30 g) of 'Harimaru' was 2,713 kg 10 a^{-1} (4-year mean in 2014–2017) (**Table 2**), slightly lower than that of 'May Queen', a standard variety in that region (4-year mean of 3,236 kg 10 a^{-1} in 2006–2009). The mean tuber weight of 'Harimaru' (3-year mean = 67 g) was much lower than that of 'May Queen' (4-year mean = 88 g). As shown in **Fig. 4**, tubers smaller than L size (the highest price) predominated the yield. Tubers of 'Harimaru' constantly showed high specific gravity (=high starch content) (**Table 2**). Nevertheless, they do not fall apart with long boiling times. 'Harimaru' tubers have a nutty flavor originally derived from the Andean diploid potato (*S. phureja*). Although a formal sensory evaluation test for the taste was not carried out, we did several taste tests at our institution,

Table 2. Yield of 'Harimaru'

Year	Seed tuber planted (g)	Total yield (kg 10 a^{-1})	Marketable yield (kg 10 a^{-1})[a]	Mean tuber weight (g)	Specific gravity
2012	Whole (30–60 g)	–	3,150[b]	50.6	1.097
2013	Whole (30–60 g)	–	3,719[b]	56.7	1.084
2014	Whole (30–50 g)	3,198	2,642[c] (82.6%)	–	1.117
2015	1/2 or 1/3-cut (30–50 g)	3,143	2,797[c] (89.0%)	67.2	1.101
2016	Whole (30–50 g)	2,592	2,257[c] (87.1%)	65.2	1.097
2017	Whole (30–50 g)	3,743	3,157[c] (84.3%)	67.7	1.093

[a] Marketable yield comprises tubers greater than 20 g[b] or 30 g[c] and free of external defects. The percentage of marketable yield over total yield is shown in parenthesis.

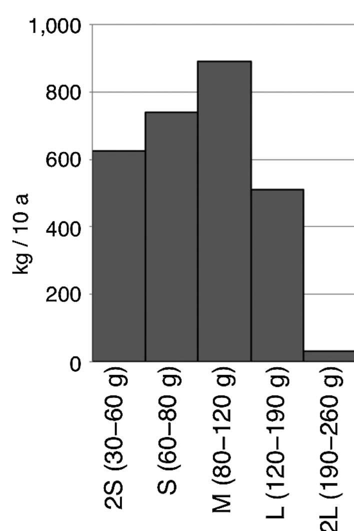

Fig. 4. Size distribution for total yield (2,797 kg 10 a^{-1}) in 2015.

restaurants, and grocery stores and always received a favorable review.

Disease and pest resistance

Inoculated leaves and the upper leaves were sampled 10 days and four weeks after inoculation, respectively, and were examined by enzyme-linked immunosorbent assay (ELISA) and reverse-transcription polymerase chain reaction (RT-PCR) methods, as described by Maoka *et al.* (2010). PVX was not detected by either method. After inoculation of PVY strain O, hypersensitive reaction was observed on the surface of the inoculated leaves. PVY was not detected by ELISA. However, RT-PCR did produce a faint band, indicating a slight presence of PVY in the inoculated and the upper leaves. This is a normal response of Ry_{chc}-mediated resistance (Maoka *et al.* unpublished). 'Harimaru' is highly resistant to golden cyst nematode. It is, however, susceptible to late blight (*P. infestans*). For other diseases such as common scab and bacterial wilt, 'Harimaru' has not been tested.

Discussion

Recently, a new variety 'Nagasaki Kogane' was bred by applying marker-assisted selection at the second field-grown stage and it has *H1* and Ry_{chc} genes (Sakamoto *et al.* 2017). We successfully bred a tasty potato, 'Harimaru', with multiple disease resistances. This is the first incidence of marker-assisted selection applied at a seedling stage and the first variety possessing *H1*, *Rx1* and Ry_{chc} genes in Japan. 'Harimaru' produced slightly lower tuber yield than the local standard variety. However, its taste and cooking quality are appreciated by people in the region; thus, its growing area is gradually increasing.

When we started, the marker selection technology was still at a premature stage. We performed PCR only for positive clones for the next marker, resulting in more than 2,500 PCR for 1,647 seedlings, which is less efficient compared to the currently used multiplex PCR technique (Mori *et al.* 2011). For amplification of the *R1* gene-specific marker, we used an annealing temperature of 55°C as described in the literature (Ballvora *et al.* 2002). This condition might induce unspecific amplification, resulting in over-representation of *R1* and *Rx1* positive seedlings. The multiplex PCR technique (Mori *et al.* 2011) modified the annealing temperature to 68°C for the *R1*-specific marker. The *H1*-linked marker that we used was perfectly matched with the presence of *H1* gene in Japanese potato varieties (K. Hosaka, unpublished data). However, the present data indicated the possibility of duplex status for the marker in the US variety 'Pike'. According to the biological assay of golden cyst nematode resistance in the progenies of 'Pike', 'Pike' is undoubtedly simplex for the *H1* gene (T. Igarashi, Calbee Potato Inc., personal communication). Thus, of the four homologous chromosomes of potato chromosome 5 in 'Pike', one has the marker and *H1* gene, while the second has only the marker. The multiplex PCR technique (Mori *et al.* 2011) uses two more tightly linked markers sandwiching the *H1* gene (Takeuchi *et al.* 2008).

For normal potato breeding, it takes at least ten years from crossing to applying for variety registration. However, the breeding process of 'Harimaru' was completed in six years because adaptability in several different locations was not tested. Wide adaptability was not required for local farmers who need specialty potatoes in their regions.

'Harimaru' has extreme resistance genes *Rx1* and Ry_{chc}, which makes it possible to use a part of the product stored for next season's seed tubers. Such a cultivation system using homemade seed tubers is an old-fashioned or traditional farming system in the Andes. The other viruses rarely occur or, even if infected, cause mild or no symptoms, resulting in no significant yield losses. *Potato spindle tuber viroid* (PSTV) is another feared pathogen, but its infection of potato has never been reported in Japan. Further incorporation

of resistance genes to these viruses and a viroid may allow cultivation using homemade seed tubers, which may become a new, sustainable potato cultivation system.

Acknowledgments

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