Production of a Novel Virus-Resistant Barley Line Introgression to the *rym1* Locus with High Malting Quality Using DNA Marker Assisted Selection

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ABSTRACT

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The *rym1* is completely resistant to the *Barley yellow mosaic virus* (BaYMV)-I, -II, the *Barley mild mosaic virus* (BaMMV)-Ka1 and -Na1, and is acceptably resistant to BaYMV-III. However, in breeding programs for BaYMV resistance, *rym1* was not commonly introgressed into the established cultivars. This present study introduces a novel resistant gene, *rym1,* to a malting barley variety, 'Mokkei 01530', which was bred from a cross between a donor for *rym1,* 'Y4' line and a high malting quality variety, 'Haruna Nijo'. This paper describes the malting quality and the agronomic performance of the line. The results indicate that 'Mokkei 01530', carrying *rym1,* is completely resistant to BaYMV-I and has an acceptable level of resistance to BaYMV-III. In comparison with its high quality parent, 'Haruna Nijo', the malt produced by 'Mokkei 01530' had similar levels of all malting quality characteristics. Furthermore, its agronomic performance was similar to 'Haruna Nijo'.

Key words: Barley yellow mosaic disease, malting quality, novel resistant gene, *rym1*.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important crop both for brewing and for animal feed. It is grown extensively in Europe, in parts of East Asia, North America, Australia and New Zealand. The production of East Asian and European winter barley is seriously damaged by BaYMV and BaMMV[5,7]. The viruses are transmitted by a soil-borne fungus, *Polymyxa graminis*[21], and barley seedlings show extreme symptoms such as yellow streaking and brown necrotic patches. In Japan, BaYMV occurrence is widespread and the virus causes serious damage to two-rowed malting barley[9]. Based on pathogenicity tests on various barley cultivars, the BaYMVs identified in Japan are generally classified into three strains, BaYMV-I, -II and -III[8], while in Europe, they are classified into two strains, BaYMV-1 and -2[6]. Two strains of BaMMV, -Ka1 and -Na1, have been isolated in Japan[14].

The most common approach for the prevention of infection with BaYMV and BaMMV is the introgression of the resistance genes identified in barley germplasm accessions into modern barley cultivars. In Japan, using 'Mokusekko 3' as a cross parent, many BaYMV-resistant malting barley cultivars have been developed. Though 'Mokusekko 3' harbours at least two BaYMV resistance genes, *rym1* and *rym5,* only *rym5* was used for barley breeding against BaYMV. Another gene resistant to all strains of BaYMV is *rym3*. The *rym3* gene was found in Haganemugi[10] and Ea 52[22] and displays resistance to all strains of BaYMV in Japan. However, *rym3* is not effective against BaMMV. The locations of the *rym1* locus and *rym5* locus in restriction fragment length polymorphism (RFLP) linkage maps have recently been reported[12]. In addition, Okada et al.[15] have reported that *rym1* is completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1, and is acceptably resistant to BaYMV-III. However, in the breeding programs for BaYMV resistance, *rym1* was not commonly introgressed into the established cultivars. Assuming a close linkage between *rym1* and an unfavourable quantitative trait locus (QTL) for malting barley, *rym1* could be easily dropped by eliminating the QTL. On the other hand, the elimination of *rym1* might be due to the linkage drag between *rym1* and QTLs with the unfavourable agronomic characteristics derived from 'Mokusekko 3'. From our previous report the *rym1* locus was found to be located on chromosome 4H and it is likely that the *rym1* locus and the blue aleurone (*Bl*) locus are linked with a recombinant value of about 4.25% to 12.59%[15]. In Japan, *Bl* tends to be regarded as an unfavourable character and it has been eliminated in malting barley-breeding programs. A consequence was that, in past breeding programs for BaYMV resistance, *rym1* was not introgressed into the established cultivars. The present study breaks the tight linkage between *rym1* and *Bl,* introduces only *rym1* to a modern malting barley cultivar, using DNA markers, and investigates agronomic performance including virus resistance and malting quality.

MATERIALS AND METHODS

Parent and breeding. A BaYMV-resistant F_4 progeny, derived from 'Ko A' × 'Mokusekko 3', carrying only *rym1,* was screened by DNA markers linked to *rym1* (MWG2134, MWG2159) and linked to *rym5* (ABC172) as illustrated in Fig. 1. Its progeny, 'Y4', carrying only *rym1* isolated from 'Mokusekko 3', proved homozygous for resistance. A cross was made between 'Y4' as the female parent and 'Haruna Nijo' as the male parent at

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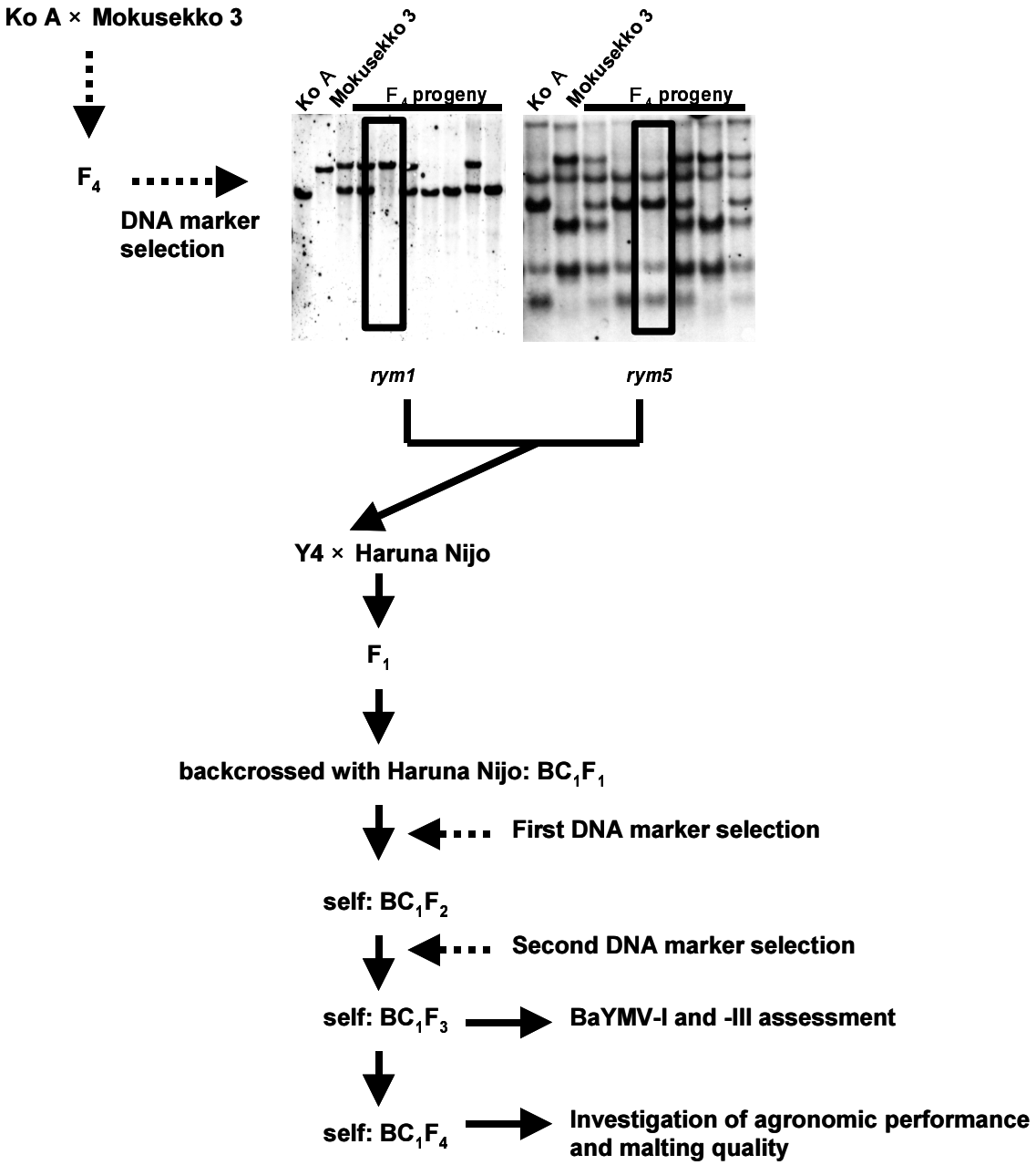

Fig. 1. The breeding strategies for BaYMV resistance introgression to 'Haruna Nijo' using the *rym1* locus.

the Plant Bioengineering Research Laboratories (PBRL), Sapporo Breweries Ltd., Gunma, Japan in 1999. 'Haruna Nijo', bred by Sapporo Breweries Ltd., is a Japanese malting variety with high malting and brewing quality. However, it is susceptible to BaYMV and BaMMV. F_1 plants were grown in a summer nursery and then backcrossed with 'Haruna Nijo'. Further, the selection was made from 321 BC_1F_1 plants using DNA markers. After marker selection of BC_1F_1, hetero types were selected and selfed and a second marker selection of progeny homozygous for *rym1* made from BC_1F_2. These selections were carried out from the F_1 to BC_1F_4 generations at PBRL up to 2001. Cultivation for malting quality analysis and investigation of agronomic characters were carried out at Kizaki, Gunma, Japan in the 2001–2002 season.

DNA extraction and marker assisted selection (MAS). An RFLP linkage map and significant QTLs of *rym1* and *rym5* were reported by Miyazaki et al.[12]. DNA was extracted from the mature leaves of the plants and their parents as described in the standard protocol of the Cethyl Trimethyl Ammonium Bromide (CTAB) method[13]. Then, 3 μg of each DNA sample were individually digested with *Bam*HI or *Dra*I. The DNA digests were electrophoresed on 0.8% agarose gel and transferred to nylon membranes by the capillary method[16,17]. The prehybridization, hybridization, detection and probe labeling procedures were performed according to the *Gene Images*™ system (Amersham Pharmacia Biotech) manual.

Resistance test of BaYMV-I and -III. In the assessment of resistance to BaYMV-I barley samples were grown

Table I. Reaction of cultivars and lines to BaYMV-I and -III.

Cultivars and lines	Resistant gene	Reaction to BaYMV-I R/S*	Reaction to BaYMV-III		
			Score of mosaic	Score of yellowing	R/S*
Amagi Nijo	—	S	5.5	4.0	S
Haruna Nijo	—	S	5.5	4.0	S
Misato Golden	*rym5*	R	5.5	3.5	S
Mikamo Golden	*rym5*	R	5.5	4.0	S
Mokusekko 3	*rym1, rym5*	R	0.0	0.0	R
Mokkei 01512	—	S	5.0	3.0	S
Mokkei 01523	—	S	5.5	3.0	S
Mokkei 01530	*rym1*	R	4.5	2.0	R

*R, resistant; S, susceptible.

in a field infected with only BaYMV-I and the reaction was investigated in the 2001–2002 season. Parents of the cross, susceptible checks and differential genotypes were planted at the same time. The disease reaction was evaluated individually based on the mosaic symptoms on the leaves. If at least one leaf with mosaic symptoms was detected, the plant was scored as susceptible.

In the 2001–2002 season, 40 seedlings per BC_1F_4 line were grown in a field infected with only BaYMV-III and investigated for their reaction to BaYMV-III. The assessment of resistance to BaYMV-III was based on the mosaic symptoms and yellowing on the leaves. The scoring of symptoms and assessment used the observed values of 0.0 to 6.0.

Micromalting and malting quality analysis. Two hundred and fifty grams of grain (>2.5 mm screen) were micromalted in an Automatic Micromalting System (Phoenix Biosystems, Torrens Park, Australia). Prior to germination, the steeping moisture was set to approximately 43.0–43.5% by adjusting the steep time of each sample (steeping utilized a 5-hour air rest after every 7 hours steep in water, 15°C). Germination lasted 6 days at 15°C, and the kilning scheme was 10 hours at 45°C, 8 hours at 55°C, 3.5 hours at 65°C, 3.5 hours at 75°C and 4 hours at 83.5°C. Malt samples were evaluated by the EBC analytical methods (Analytica-EBC 4.5.)[1], including hot water extract, total and soluble nitrogen on malt, Kolbach index, diastatic power, apparent attenuation limit, Hartong index (VZ45), wort colour, wort beta-glucan and friability.

RESULTS

The infection of barley seedlings by BaYMV-I or -III grown in the field was so effective that the resistant plants were easily distinguishable from the susceptible ones. The seedlings of the susceptible controls 'Amagi Nijo' and 'Haruna Nijo' were entirely infected by BaYMV-I. However, the cultivar/line carrying the resistance gene was not infected by BaYMV-I (Table I). The susceptible control 'Amagi Nijo', 'Haruna Nijo' and our breeding lines with an absence of the *rym1* gene, 'Mokkei 01512' and 'Mokkei 01523', displayed severe mosaic symptoms, (symptom score of 5.0 to 5.5), and severe yellowing, (yellowing leaves scores of 3.0 to 4.0), in the BaYMV-III infested field. Further, the *rym5* carrying cultivars, 'Misato Golden' and 'Mikamo Golden', also displayed mosaic symptoms, (symptom score of 5.5), and yellowing, (yellowing leaves score of 3.5 and 4.0). On the other hand, our resistant breeding line 'Mokkei 01530', that carries *rym1,* was susceptible, showed numerous mosaics, (symptom scores of 4.5), similar to the susceptible cultivars. However, the yellowing of leaves score of 2.0 indicated mild yellowing (Table I).

The important agronomic traits data of 'Mokkei 01530' are summarized in Table II comparing it with the Japanese malting variety, 'Haruna Nijo'. 'Mokkei 01530' shows similar agronomic potential to 'Haruna Nijo' in all traits.

As shown in Table III all the malting quality character values of 'Mokkei 01530' were in the desirable range and compared favourably with its high quality parent, 'Haruna Nijo',

DISCUSSION

This study introduces only *rym1* to the modern malting barley cultivar using DNA markers and investigates virus resistance and malting quality. The above results indicate that 'Mokkei 01530' was completely resistant to BaYMV-I and had an acceptable level of resistance to BaYMV-III and field resistance. The agronomic performance and

Table II. Important agronomic characteristics of Mokkei 01530 in comparison with the control variety Haruna Nijo.

Character	Mokkei 01530	Haruna Nijo
Heading date	2002/4/6	2002/4/7
Culm length (cm)	91.7 ± 3.0	84.6 ± 6.1
Panicle length (cm)	5.3 ± 0.07	5.1 ± 0.3
Panicle number/m^2	812.5 ± 73.2	839.7 ± 56.1
Number of grains per panicle	22.8 ± 0.09	22.1 ± 2.1
Grain yield (kg/a)	47.3 ± 2.0	50.2 ± 5.6
Yield of plump (kg/a)	38.1 ± 2.1	40.1 ± 3.7
Plumpness (>2.5 mm) (%)	80.6 ± 0.9	80.1 ± 1.6
1000 kernel weight (g)	33.0 ± 0.9	31.9 ± 0.5
Plump kernel weight (g)	36.1 ± 0.09	35.3 ± 1.2

Table III. Malting quality of Mokkei 01530 in comparison with the control variety Haruna Nijo malt as determined in a micromalting test.

Character	Mokkei 01530	Haruna Nijo
Hot water extract (%, db)	83.7	84.2
Total nitrogen on malt (%, db)	1.57	1.67
Soluble nitrogen on malt (%, db)	0.66	0.68
Kolbach index	42.2	40.7
Diastatic power (°WK)	218	227
Apparent attenuation limit (%)	84.5	84.3
Hartong index (VZ45)	37.6	37.2
Wort β-glucan (mg/l)	66	79
Friability (%)	88.6	86.4

malting quality of the introgression to the *rym1* line, 'Mokkei 01530', were comparable to the standard and acceptable level of normal malting barley variety (Tables II, III). The *rym1* gene has not been commonly introgressed into the established cultivars in previously breeding programs. However, in this program, application of DNA marker-assisted selection (MAS) to malting barley lead to successful breeding of novel virus resistance into a high malting quality barley line in only 3 years. MAS has been advocated as a highly efficient breeding method because it offers rapid and precise selection of the target gene[3,11,23]. Application of MAS has been described practically by many breeders[4,16,18,19] and others. Thus, DNA marker technology and MAS can potentially increase the efficiency of traditional breeding programs by speeding up the time for release of a new variety, lowering plant population requirements and eliminating costly field evaluation.

However, the RFLP method for marker-assisted selection is inadequate for breeding; the detection steps are laborious and impractical for the high throughput analysis required by plant breeders. For this purpose, the PCR-based markers detectable by electrophoresis have become more applicable. Of these markers, microsatellite markers are valuable because they are co-dominant and can detect high levels of allelic diversity[20]. In rice more than 300 microsatellite markers covering around the whole genome have been reported[2,19]. Marker-assisted selection usually requires the screening of hundreds of individuals derived from crosses of interest, with DNA extraction being one of the time-consuming steps. It is necessary to develop simpler and easier methods for breeders.

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