# Genetic Difference in Barley Yellow Mosaic Virus Resistance between Mokusekko 3 and Misato Golden

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# Genetic Difference in Barley Yellow Mosaic Virus Resistance between Mokusekko 3 and Misato Golden

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Mokusekko 3, a Chinese landrace, is an important genetic resource for resistance to Barley Yellow Mosaic Virus (BaYMV). Using this genotype as a cross parent, the resistant varieties including Misato Golden were developed. Misato Golden carries one partial dominant resistance gene (*Ym*) which is tightly linked to the esterase-isozyme-geneblock $Est_1$-$Est_2$-$Est_4$ on chromosome 3, as found previously in hybrid populations (KONISHI *et al.* 1989). Whereas, in Mokusekko 3, another gene(s) in addition to *Ym* is responsible for resistance to BaYMV, which is related to the marker gene *K* for hooded lemma on chromosome 4.
KEY WORDS: *Hordeum vulgare*, Barley Yellow Mosaic Virus (BaYMV), resistance, esterase, linkage

## Introduction

Barley Yellow Mosaic Virus (BaYMV) causes one of the most important diseases in Japanese malting-barley and European winter-barley. Resistant varieties are the only possibility for preventing considerable yield losses caused by this soil-borne virus. Therefore, extensive screening programmes have been conducted to find suitable genetic sources of resistance against BaYMV (e.g. TAKAHASHI *et al.* 1973, FRIEDT *et al.* 1985, KAWADA and TSURU 1987).

One of the most important resistant varieties, Mokusekko 3, carries a partial dominant resistance gene *Ym* and an additional gene with slight resistance effect (TAKAHASHI *et al.* 1973). Using a few cross combinations of BaYMV resistant bred lines derived from Mokusekko 3, KONISHI *et al.* (1989) found a close linkage relationship between *Ym* and the esterase-isozyme-geneblock $Est_1$-$Est_2$-$Est_4$ at the terminal of the long arm of chromosome 3.

Because of unsuitable agronomic and malting features of Mokusekko 3, Japanese barley breeders have introduced its BaYMV resistance into two-rowed malting barley (by repeated back and multiple crosses) in an effort to produce new resistant varieties (e.g. Misato Golden, Nishino Gold, Mikamo Golden and Kinuyutaka). However, it is recently shown that these varieties are susceptible to a new Japanese BaYMV type III, although the original parent Mokusekko 3 is still resistant (KASHIWAZAKI *et al.* 1989, IIDA 1990).

The objective of this study was to clarify the difference in genetic constitution of resistance to BaYMV between Mokusekko 3 and Misato Golden, a resistant variety developed at Tochigi Agricultural Experiment Station (SEKO 1987, KOBAYASHI *et al.* 1987).

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## Materials and Methods

Resistant varieties Mokusekko 3 and Misato Golden were crossed with Colsess I, a susceptible linkage stock carrying the marker gene *K* (hooded lemma) on chromosome 4.

The $F_2$ individuals were propagated in a non-infected field at the Research Institute for Bioresources of Okayama University in Kurashiki, and the $F_3$ progenies were grown at Tochigi and Kyushu Agricultural Experiment Stations. Fields at these stations have been infected by BaYMV type I which is widely distributed in Japan. Segregation for reaction to BaYMV was examined at Tochigi Agricultural Experiment Station only, since the infection at Kyushu Agricultural Experiment Station was insufficient due to less rainfall after sowing and milder winter than usual. Esterase-isozyme-patterns were evaluated in individual $F_2$ plants before transplanting to the field. A detailed description of esterase-isozyme-test has already been given by KONISHI *et al.* (1989).

## Results

Segregation for reaction to BaYMV in the $F_3$ progenies of Misato Golden×Colsess I fits the expected segregation ratio of 1:2:1 for RR (homozygous for resistance) : RS (heterozygous for resistance/susceptibility) : SS (homozygous for susceptibility) ($\chi^2=3.40$), indicating that one resistance gene (*Ym*) acts in Misato Golden (Table 1). In the case of Mokusekko 3×Colsess I, the segregation deviates from the expected 1:2:1 ratio ($\chi^2=28.07$), due to more resistant and less susceptible progenies than expected (Table 2). This result suggests that Mokusekko 3 contains the other gene(s) besides *Ym* which is responsible for resistance to BaYMV.

Esterase-isozyme-pattern in the $F_2$ individuals of both cross combinations (Misato Golden×Colsess I; Mokusekko 3×Colsess I) segregates in the expected 1:2:1 ratio for AA

Table 1. Linkage relationship between BaYMV resistance and esterase isozymes in $F_3$ progenies of Misato Golden (RR, AA)×Colsess I (SS, BB)

| | | Reaction to BaYMV* | | | |
|---|---|---|---|---|---|
| | | RR | RS | SS | Total |
| Esterase** | AA | 48 | 4 | 0 | 52 |
| | AB | 1 | 105 | 5 | 111 |
| | BB | 0 | 1 | 63 | 64 |
| | Total | 49 | 110 | 68 | 227 |

* RR = homozygous for resistance
RS = heterozygous for resistance/susceptibility
SS = homozygous for susceptibility
** AA = homozygote *Ca-null-Nz/Ca-null-Nz*
AB = heterozygote *Ca-null-Nz/Pr-Fr-Su*
BB = homozygote *Pr-Fr-Su/Pr-Fr-Su*
$\chi^2$(BaYMV) = 3.40; $\chi^2$(Est) = 1.38; $\chi^2_L$ = 406.16
Recombination value (%) = 2.45 ± 0.73

Table 2. Linkage relationship between BaYMV resistance and esterase isozymes in $F_3$ progenies of Mokusekko 3 (RR, AA) × Colsess I (SS, BB)

| | | Reaction to BaYMV* | | | |
|---|---|---|---|---|---|
| | | RR | RS | SS | Total |
| Esterase** | AA | 71 | 1 | 0 | 72 |
| | AB | 18 | 112 | 3 | 133 |
| | BB | 11 | 23 | 35 | 69 |
| | Total | 100 | 136 | 38 | 274 |

* and ** See the footnote in Table 1.
$\chi^2$(BaYMV) = 28.07; $\chi^2$(Est) = 0.30; $\chi^2_L$ = 278.91

(homozygous for the female parental pattern) : AB (heterozygous for the both parental patterns) : BB (homozygous for the male parental pattern) (Tables 1 and 2) with $\chi^2$ values of 1.38 and 0.30, respectively.

A close relationship exists between reaction to BaYMV and esterase-isozyme-pattern in the $F_3$ progenies of Misato Golden × Colsess I (Table 1). Almost all the homozygous resistant progenies (RR) are homozygous for the esterase-isozyme-pattern (AA) which is controlled by the same allelic combination $Est_1^{Ca}$-$Est_2^{null}$-$Est_4^{Nz}$ (following designated as *Ca-null-Nz*) as that of the resistant parent, Misato Golden. Homozygous susceptible progenies (SS) are homozygous for $Est_1^{Pr}$-$Est_2^{Fr}$-$Est_4^{Su}$ (following designated as *Pr-Fr-Su*) which is same as the susceptible parent, Colsess I, and heterozygous progenies for BaYMV reaction (RS) are heterozygous for the esterase-isozyme-pattern (*Ca-null-Nz/Pr-Fr-Su*), with a few exceptions only. From these results, a recombination value between the loci for reaction to BaYMV and esterase-isozyme-pattern is estimated to be 2.45 ± 0.73%.

In the $F_3$ progenies of Mokusekko 3 × Colsess I, different results were obtained (Table 2). Almost all the homozygous resistant progenies (RR) show the same esterase-isozyme-pattern *Ca-null-Nz* as the resistant parent, Mokusekko 3, and most of the homozygous susceptible progenies (SS) are homozygous for the *Pr-Fr-Su* pattern. However, among these resistant progenies (RR), there are also *Pr-Fr-Su* homozygotes and *Ca-null-Nz/Pr-Fr-Su* heterozygotes. These results indicate that one resistance gene of Mokusekko 3 (*Ym*) is linked to the esterase-isozyme-geneblock as in Misato Golden, but the other resistance gene(s) seems to be independent of this geneblock for esterase isozymes.

The relationship between reaction to BaYMV and hooded lemma was also studied. In the $F_3$ progenies of the cross Misato Golden × Colsess I (Table 3), the chi-square test for linkage shows that the BaYMV resistance gene *Ym* and the marker gene *K* are inherited independently ($\chi^2_L$ = 9.22; P = 0.10–0.05), whereas in the case of Mokusekko 3 × Colsess I (Table 4) a weak relationship between BaYMV resistance and hooded lemma is observed.

Table 3. Interrelationship between BaYMV resistance and hooded lemma in $F_3$ progenies of Misato Golden (RR, kk) × Colsess I (SS, KK)

| | | Reaction to BaYMV* | | | |
|---|---|---|---|---|---|
| | | RR | RS | SS | Total |
| Lemma** | KK | 14 | 35 | 14 | 63 |
| | Kk | 22 | 41 | 41 | 104 |
| | kk | 13 | 34 | 13 | 60 |
| | Total | 68 | 110 | 49 | 227 |

* See the footnote in Table 1.
**KK = homozygous for hooded lemma
Kk = heterozygous for hooded/awned lemma
kk = homozygous for awned lemma
$\chi^2$(BaYMV) = 3.40; $\chi^2$(lemma) = 1.67; $\chi^2_L$ = 9.22

Table 4. Interrelationship between BaYMV resistance and hooded lemma in $F_3$ progenies of Mokusekko 3 (RR, kk) × Colsess I (SS, KK)

| | | Reaction to BaYMV* | | | |
|---|---|---|---|---|---|
| | | RR | RS | SS | Total |
| Lemma** | KK | 13 | 36 | 11 | 60 |
| | Kk | 53 | 74 | 23 | 150 |
| | kk | 34 | 26 | 4 | 64 |
| | Total | 100 | 136 | 38 | 274 |

* See the footnote in Table 1.
** See the footnote in Table 3.
$\chi^2$(BaYMV) = 28.07; $\chi^2$(lemma) = 2.58; $\chi^2_L$ = 15.69

## Discussion

TAKAHASHI *et al.* (1973) demonstrated that Mokusekko 3 carries a partial dominant gene (*Ym*) and a gene with slight effect on resistance to BaYMV. Using the $F_2$ population of a single cross between Mokusekko 3 and a susceptible linkage stock Colsess IV, they found a linkage relationship between *Ym* and the marker gene *K* for hooded lemma on chromosome 4 (recombination value of 29.37%).

The $F_1$ plants of crosses between German resistant varieties (e.g. Franka) and Mokusekko 3 are resistant, and no susceptible individuals segregate in the $F_2$ generation (FRIEDT and FOROUGHI-WEHR 1986, FRIEDT *et al.* 1989). From these results, it was concluded that the respective resistance genes are either allelic or very tightly linked.

However, KAISER *et al.* (1989) showed that the "German" resistance gene is inherited independently of the marker gene *K* on chromosome 4. Using trisomic analysis, KAISER and FRIEDT (1989) localized the gene for resistance to BaYMV of German resistant varieties on chromosome 3.

Corresponding results were presented by KONISHI and MATSUURA (1987). Almost all the Japanese BaYMV resistant strains bred from Mokusekko 3 carry the same esterase-isozyme-pattern as Mokusekko 3, which is controlled by the allelic combination *Ca-null-Nz* at three closely linked loci ($Est_1$-$Est_2$-$Est_4$) at the terminal of the long arm of chromosome 3. Linkage analysis of BaYMV resistant bred lines derived from Mokusekko 3 revealed that *Ym* is very tightly linked (recombination values ranged from 1.26 to 5.01%) to this esterase-isozyme-geneblock (KONISHI *et al.* 1989).

Furthermore, the present results indicate a very close linkage relationship (recombination value $2.45 \pm 0.73\%$) between the BaYMV resistance gene *Ym* of Misato Golden and the esterase-isozyme-geneblock $Est_1$-$Est_2$-$Est_4$ on chromosome 3. However, the gene *Ym* is inherited independently of the gene *K* for hooded lemma on chromosome 4.

On the other hand, BaYMV resistance in Mokusekko 3 is conditioned by an additional gene(s) besides the partial dominant gene *Ym*, resulting in more resistant and less susceptible $F_3$ progenies than expected. A weak relationship between BaYMV resistance and hooded lemma suggests the existence of the second resistance gene which is related to the marker gene *K* (hooded lemma) on chromosome 4.

The close linkage between *Ym* and esterase-isozyme allelic combination or genotype *Ca-null-Nz* at the $Est_1$-$Est_2$-$Est_4$ complex loci in the cross Misato Golden × Colsess I is of practical importance for barley breeding. Due to this tight linkage relationship, breeders can screen indirectly for *Ym* genotypes by choosing genotypes with esterase-isozyme-pattern *Ca-null-Nz* after starch gel electrophoresis at the seedling stage. This screening-system is also effective if Mokusekko 3 is used as a cross parent. Although in the $F_3$ progenies of Mokusekko 3 × Colsess I no recombination value between loci for BaYMV resistance and esterase-isozyme-pattern can be estimated due to the presence of an additional resistance gene(s), almost all the $F_3$ progenies carrying the same esterase-isozyme-pattern as Mokusekko 3, are homozygous for BaYMV resistance.

Recently new BaYMV types occurred in some limited areas of Japan (KASHIWAZAKI *et al.* 1989) and Europe (HUTH 1989). A Japanese new BaYMV type III induces susceptibility in Misato Golden (OGAWA *et al.* 1987) and other varieties carrying *Ym* (e.g. Nishino Gold, Mikamo Golden and Kinuyutaka), whereas Mokusekko 3 is still resistant (KASHIWAZAKI *et al.* 1989, IIDA 1990). In Europe another new BaYMV type attacks barley varieties carrying the "German" gene for BaYMV resistance (BEATON 1989).

Fortunately, resistance genes against the new BaYMV types are available in Japan (e.g. Mokusekko 3, Ishukushirazu, Haganemugi; KASHIWAZAKI *et al.* 1989) as well as Europe (BEATON 1989). Because of extension of the new BaYMV types, barley breeders are requested to introduce these resistance genes in the former resistant varieties to combine "old" and "new" resistance genes in one variety.

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# 木石港3とミサトゴールデンの大麦縞萎縮病抵抗性に関する遺伝的差異

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木石港3は大麦縞萎縮病に対して抵抗性遺伝子(*Ym*)をもつことが知られている重要な遺伝資源である. 本報では, 木石港3とそれに由来する抵抗性品種ミサトゴールデンとの大麦縞萎縮病抵抗性に関する遺伝的差異を明かにするとともに, 両者のもつ抵抗性遺伝子の連鎖分析を試みた.

これら2品種に罹病性の Colsess I を交配し, $F_2$個体についてエステラーゼ同位酵素遺伝子型を調べるとともに, $F_3$系統における大麦縞萎縮病の発病程度を調査した. ミサトゴールデンとの組合せの$F_3$系統で抵抗性ホモ(RR):抵抗性/罹病性ヘテロ(RS):罹病性ホモ(SS)の分離比が1:2:1によく適合したことから, ミサトゴールデンは抵抗性遺伝子 *Ym* をもつと考えられる. さらに, 抵抗性とエステラーゼ同位酵素遺伝子型との間には高い連鎖関係が認められ, 両遺伝子座間の組換価は2.45%と推定された. 一方, 木石港3の場合には系統の分離が1:2:1の分離比から著しく歪み, RR 系統数が期待値より多く, 逆に SS 系統が少なくなったことから, 木石港3は *Ym* とさらに別の抵抗性遺伝子をもつと考えられる. この組合せでも, 抵抗性とエステラーゼ同位酵素遺伝子型との関連性が認められ, 木石港3と同じ同位酵素遺伝子型を示す系統のほとんどすべてが抵抗性ホモ(RR)であった. しかし, 罹病性親と同じ同位酵素遺伝子型の系統にも RR があり, さらに, 抵抗性と第4染色体の標識遺伝子である三叉芒遺伝子(*K*)との間にも関連性がみられた. これらのことから, 木石港3は第3染色体長腕末端部に座乗するエステラーゼ同位酵素遺伝子と密接に連鎖する抵抗性遺伝子 *Ym* のほかに, 第4染色体上の *K* 遺伝子と連鎖する別の抵抗性遺伝子をもつものと推測される.

以上の結果から, 木石港3及びそれに由来する抵抗性品種や系統を用いて大麦縞萎縮病抵抗性遺伝子 *Ym* を導入する場合, 雑種個体の幼苗の第1葉からの粗抽出液を用い, でんぷんゲル電気泳動法でエステラーゼ同位酵素遺伝子型を調べ, 木石港3と同じ同位酵素遺伝子型を示す個体をとれば, 容易に抵抗性個体を選抜することができると結論した. さらに, 縞萎縮病の新しい型への抵抗性を付与するために, 木石港3でみられた *Ym* とそれ以外の遺伝子も取り込むことが必要であり, その選抜法についても早期に確立することが望まれる.