SHORT COMMUNICATION

Use of STS Marker Linked to *ym4* Gene for the Genotyping of Winter Barleys

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Abstract: The codominant STS marker MWG838, linked to BaYMV/BaMMV resistance gene *ym4*, was introduced into a winter barley breeding program. Lines of F_2 and F_3 progenies were created from crosses of the gene *ym4* donor genotype Romanze and five different gene acceptors. Homozygous recessive, homozygous dominant, and heterozygous individuals were clearly identified and selected within F_2 individuals by the used DNA marker linked to *ym4* gene. F_3 lines created by self-pollination from selected *ym4ym*4 F_2 genotypes were reverified and included into further testing and breeding. The *ym4* gene-linked presence of DNA marker was confirmed in progenies from all parental combinations.

Keywords: winter barley; BaYMV; BaMMV; *ym4* gene; marker assisted selection; STS marker

Barley mild mosaic virus (BaMMV) and *Barley yellow mosaic virus* (BaYMV) are considered as very important winter barley pathogens in Europe transmitted by the soil-borne fungus *Polymyxa graminis*. The most effective way to avoid yield losses is resistance breeding and growing of resistant cultivars. Resistance of the newest European winter barleys is based on single recessive gene *ym4*. This gene does not confer resistance to strain BaYMV-2, therefore other sources of resistance were searched for and identified (LE GOUIS *et al*. 2000; KONISHI *et al*. 1997). The application of linked molecular markers can considerably speed up the incorporation of resistance genes and their combination in plant breeding. Several types of molecular markers for resistance genes against BaYMV/BaMMV are available at present. The RFLP and advanced PCR-based markers were identified for resistance genes *ym5*, *ym9*, *ym11* (ORDON *et al*. 1999), *ym3* (SAEKI *et al*. 1999), *ym8* (BAUER *et al*. 1997), *ym1* (KONISHI *et al*. 1997), and *ym7* (GRANER *et al*. 1995). Molecular markers usable for the *ym4* gene detection are based on two mapping approaches. GRANER and BAUER (1993) performed RFLP mapping and identified two RFLP markers – MWG838 and MWG10, flanking this gene at a distance of 1.2 ± 1.2 cM. A distance between *ym4* gene and MWG838 marker was later adjusted to 3.3 cM (GRANER & BAUER 1993). PCR-based RAPD marker OPZ04 cosegregated with RFLP MWG10 marker and was located at 1.6 cM, OPL14 was located at 3.2 cM, and others at longer distances from the *ym4* gene were identified later (ORDON *et al*. 1995; WEYEN *et al*. 1996).

The first massive occurrence of these viruses in winter barley fields was detected in Slovakia in the last vegetation season (2001/2002). The aim of this study was to verify applicability of molecular marker for the genotyping of breeding lines.

The donor of the *ym4* gene was winter barley resistant cultivar Romanze. Cultivars Torrent, Copia, Luxor, Kamil, and line KM-1448 were used as acceptors of this gene. Total DNA was extracted from small segments of young leaves according to KLIMYUK *et al*. (1993). Used primers were based on the MWG838 marker sequence (BAUER & GRANER 1995). PCR, digestion of PCR products with *Rsa*I, and agarose electrophoresis were performed according to TUVESSON *et al*. (1998).

Presence or absence of STS marker MWG838, linked to the *ym4* gene, was tested in donor genotype Romanze and all acceptor genotypes before crossing. Its presence in Romanze and absence in all acceptor genotypes was confirmed. Progenies were created in all five combinations of parents. DNA fragments amplified from resistant and sensitive parents differ in *Rsa*I restriction site position. After having been digested with *Rsa*I both dominant and recessive homozygous genotypes as well as heterozygous genotypes, i.e. marker-based resistant and

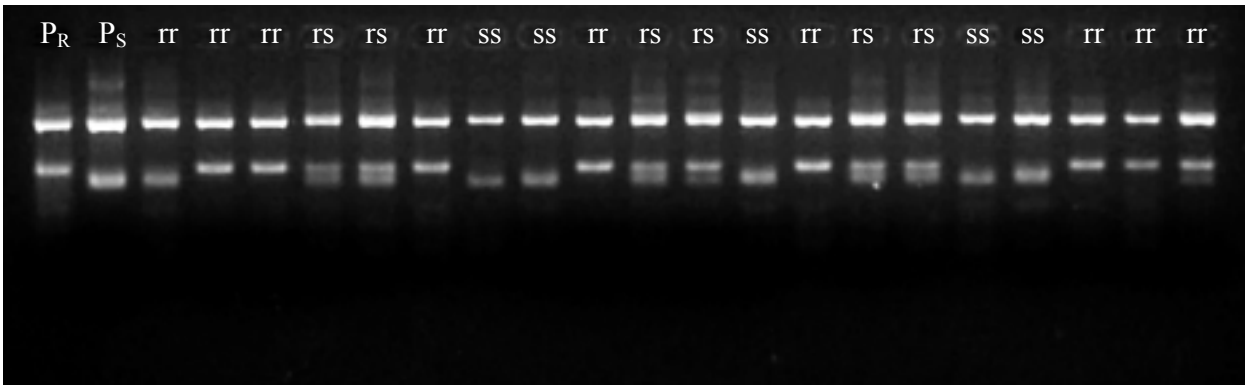

Fig. 1. Segregation of F_2 progeny from Luxor × Romanze cross in loci *ym4* analysed by STS pair primers MWG838 (P_R – resistant parent, P_S – sensitive parent, rr – homozygote *ym4ym4*, rs – heterozygote *Ym4ym4*, ss – homozygote *Ym4Ym4*)

sensitive individuals in F_2 progenies (Fig. 1), were clearly differentiated. Total number of analysed individuals of different F_2 progenies ranged from 49 to 150. Calculated values of χ^2 and probability ($P > 0.05$) of the observed F_2 genotype segregation ratio from crosses Copia × Romanze, Kamil × Romanze, Torrent × Romanze, and Luxor × Romanze indicate that differences between the expected (1:2:1) and observed segregation ratio are not statistically significant. F_2 individuals with marker-based genotype *ym4ym4* were self-pollinated and all obtained F_3 plants were reanalysed with the same marker. Among 20 F_3 plants of Torrent × Romanze combination, one marker-based dominant homozygous genotype *Ym4Ym4* was also found out. It should be explained by incorrect evaluation and selection at the level of individuals or perhaps by outcross in the field of F_2 generation. No homozygous dominant or heterozygous genotypes were discovered in 27 and 20 reanalysed F_3 individuals of Copia × Romanze and Kamil × Romanze progenies, respectively. Analyses of F_3 plants proved successful transfer and introduction of the marker linked with *ym4*. A set of winter barley lines selected by the molecular marker will be included in the breeding program and the resistance of these lines will be tested in field conditions to compare the marker reliability. Gene introduction, selection, gene pyramiding, and backcrossing procedures for different resistance genes to BaYMV/BaMMV can efficiently be enhanced by the use of molecular markers. Marker assisted introduction should be essential also in the creation of barley genotypes resistant to the virus complex BaYMV/BaMMV.

References

BAUER E., GRANER A. (1995): Basic and applied aspects of the genetic analysis of the *ym4* virus resistance locus in barley. Agronomie, **15**: 469–473.

BAUER E., WEYEN J., SCHIERMAN A., GRANER A., ORDON F. (1997): Molecular mapping of novel resistance genes against barley mild mosaic virus (BaMMV). Theor. Appl. Genet., **95**: 1263–1269.

GRANER A., BAUER E. (1993): RFLP mapping of the *ym4* virus resistance gene in barley. Theor. Appl. Genet., **86**: 689–693.

GRANER A., BAUER E., KELLERMANN A., PROESELER G., WENZEL G., ORDON F. (1995): RFLP analysis of resistance to the barley yellow mosaic virus complex. Agronomie, **15**: 475–479.

KLIMYUK V.I., CARROLL B.J., THOMAS C.M., JONES J.D.G. (1993): Alkali treatment for rapid preparation of plant material for reliable PCR analysis. Plant J., **3**: 493–494.

KONISHI T., BAN T., IIDA Y., YOSHIMI R. (1997): Genetic analysis of disease resistance to all strains of BaYMV in a Chinese barley landrace, Mokusekko 3. Theor. Appl. Genet., **94**: 871–877.

LE GOUIS J., HARIRI D., BAHRMAN N., JESTIN L. (2000): Resistance of old French barley cultivars to barley mosaic viruses. J. Plant Dis. Protect., **107**: 433–438.

ORDON F., BAUER E., FRIEDT W., GRANER A. (1995): Marker-based selection for the *ym4* BaMMV-resistance gene in barley using RAPDs. Agronomie, **15**: 481–485.

ORDON F., SCHIERMANN A., PELLIO B., DAUCK V., BAUER E., STRENG S., FRIEDT W., GRANER A. (1999): Application of molecular markers in breeding for resistance to the barley yellow mosaic virus complex. J. Plant Dis. Protect., **106**: 256–264.

SAEKI K., MIYAZAKI C., HIROTA N., SAITO A., ITO K. (1999): RFLP mapping of BaYMV resistance gene *rym3* in barley (*Hordeum vulgare*). Theor. Appl. Genet., **99**: 727–732.

TUVESSON S., V POST L., ÖHLUND R., HAGBERG P., GRANER A., SVITASHEV S., SCHEHR M., ELOVSSON R. (1998): Molecular breeding for the BaMMV/BaYMV resistance gene *ym4* in winter barley. Plant Breeding, **117**: 19–22.

WEYEN J., BAUER E., GRANER A., FRIEDT W., ORDON F. (1996): RAPD-mapping of the distal portion of chromosome 3 of barley, including the BAMMV/BaYMV resistance gene *ym4*. Plant Breeding, **115**: 285–287.

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Abstrakt

ŠUDYOVÁ V., ZÁVODNÁ M., HUDCOVICOVÁ M., KRAIC J. (2002): **Použitie STS markera viazaného s génom *ym4* v určovaní genotypu jačmeňa ozimného**. Czech J. Genet. Plant Breed., **38**: 129–131.

Kodominantný STS marker MWG838, geneticky viazaný s génom rezistencie *ym4* proti komplexu vírusov BaYMV/BaMMV, bol introdukovaný do šľachtiteľského programu jačmeňa ozimného. Vytvorené boli línie generácie F_2 a F_3 z krížení medzi donorom génu *ym4* genotypom Romanze a 5 rôznymi akceptormi tohto génu. V generácii F_2 boli potvrdené homozygotne recesívne, homozygotne dominantné a heterozygotné jedince pomocou použitého DNA markera viazaného s génom *ym4*. F_3 línie, vytvorené samoopelením vybratých genotypov *ym4ym4* F_2, boli znova preverené pomocou DNA markera a následne začlenené do šľachtenia jačmeňa ozimného. Prítomnosť DNA markera viazaného s génom *ym4* bola potvrdená v potomstvách všetkých kombinácií kríženia rodičov.

Kľúčové slová: jačmeň ozimný; BaYMV; BaMMV; *ym4*; selekcia pomocou markerov; STS marker

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