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Assessment of the genetic relatedness of barley accessions (*Hordeum vulgare s.l.*) resistant to soil-borne mosaic-inducing viruses (BaMMV, BaYMV, BaYMV-2) using RAPDs

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Abstract Thirty-six *Hordeum vulgare* varieties and 12 *H. spontaneum* germplasms originating from different parts of the world and showing different reactions to the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) were analyzed for genetic similarity using RAPDs. On the basis of an analysis of 20 selected RAPD-primers corresponding to 544 bands genetic similarity according to Nei and Li (1979) was estimated to be between 0.685 and 0.964. Associations between the 48 genotypes were calculated using UPGMA-clustering and principal coordinate analysis. By applying these methods we were able to separate *H. spontaneum* accessions from *H. vulgare* varieties, and within these groups all the genotypes were clustered correctly according to their origin. Consequently, RAPD analysis can be considered a very useful and efficient tool for the fast estimation of genetic relationships in barley. The correlation between genetic similarity with respect to German varieties and adaptation of exotic barley varieties to German growing conditions is discussed.

Key words *Hordeum vulgare s.l.* · Barley yellow mosaic disease · Resistance · RAPDs · Genetic similarity

Introduction

Barley yellow mosaic disease has gained evident importance in most European winter barley growing areas due to its persistant spread and the high yield losses frequently observed in susceptible winter barley. The disease is caused by a complex of at least three viruses i.e., barley mild mosaic virus (BaMMV), barley yellow mosaic virus (BaYMV) and BaYMV-2 (Huth 1990; Huth and Adams 1990). All of these viruses – like other members of the bymovirus group (Usugi et al. 1989) – are transmitted by the soil-borne fungus *Polymyxa graminis* Led. (Toyama and Kusaba 1970). Therefore, chemical measures against the disease are neither effective nor acceptable for ecological and economical reasons. Consequently, high yield losses can only be prevented by growing resistant cultivars. The resistance of commercial German barley cultivars to BaMMV and BaYMV is presumed to be due to the gene *ym4*, which has been located on the long arm of chromosome 3 by trisomic and telotrisomic analysis (Kaiser and Friedt 1989, 1992) as well as by restriction fragment length polymorphism (RFLP) (Graner and Bauer 1993), isozyme (Le Gouis et al. 1995) and random amplified polymorphic DNA (RAPD) analysis (Ordon et al. 1995; Weyen et al. 1996). However, this gene is not effective against BaYMV-2, the resistance-breaking strain of BaYMV (cf. Bendiek et al. 1993), which was detected in Germany in 1987/1988 for the first time (Huth 1990). In extensive screening programs different types of resistance to the yellow mosaic-inducing viruses have been observed and different genes conferring resistance, at least to BaMMV, detected (Götz and Friedt 1993; Ordon and Friedt 1993; Ordon et al. 1993). Resistant germplasms are mainly derived from East Asia (Ordon et al. 1993) but have also been identified among accessions of *Hordeum spontaneum* Koch derived from Turkey (Erdogan et al. 1994) and Israel (Nevo and Ordon, unpublished data). Attempts to characterize these exotic germplasms by isozyme electrophoresis were not very efficient as out of 15 isozyme systems tested – corresponding to 26 loci – only 3 were polymorphic and 12 monomorphic (Le Gouis et al. 1995). Because of this low level of isozyme

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polymorphism within exotic germplasms and also in comparison to German commercial cultivars we used the RAPD technique to characterize these germplasms at the DNA level. This technique has already proven its usefulness for genetic fingerprinting in many species (e.g. Tinker et al. 1993; Virk et al. 1995; Abo-Elwafa et al. 1995; Maass and Klaas 1995; Sharma et al. 1995; Link et al. 1995; Grando et al. 1996; Loarce et al. 1996).

Materials and methods

Thirty-six genotypes of *Hordeum vulgare* derived from Germany, East-Asia, Russia, Austria, Yugoslavia, Turkey, Bulgaria and the USA as well as 12 accessions of *H. spontaneum* derived from Turkey and Israel (kindly provided by Dr. M. Erdogan, Ege University Izmir, Turkey and Prof. E. Nevo, University of Haifa, Israel) showing different reactions to the viruses of the barley yellow mosaic virus complex (Table 1) were assayed for RAPD polymorphism. DNA isolation was carried out according to Bernatzky and

Table 1 Origin, number of rows (spike morphology) and reaction to BaMMV, BaYMV and BaYMV-2 of barley varieties analyzed for genetic similarity (cf. Götz and Friedt 1993; Ordon et al. 1993; Erdogan et al. 1994)

Variety/ accession	Origin	Number of rows	Reaction to		
			BaMMV	BaYMV	BaYMV-2
H. vulgare					
Brunhild	Germany	6	r	r	s
Colambo	Germany	2	r	r	s
Jana	Germany	6	r	r	s
Labea	Germany	6	r	r	s
Ogra	Germany	6	r	r	s
Sonate	Germany	2	r	r	s
Alraune	Germany	2	s	s	s
Corona	Germany	6	s	s	s
Gerbel	Germany	6	s	s	s
Igri	Germany	2	s	s	s
Magie	Germany	2	s	s	s
Trixi	Germany	2	s	s	s
Anson Barley	USA	6	r	s	s
Bulgarian 347	Bulgaria	6	r	s	s
Krasnodar 1920	Yugoslavia	6	r	s	s
Maksimirski 452	Yugoslavia	6	r	s	s
Russia 32	USSR	6	r	r	s
Russia 57	USSR	2	r	r	r
Turkey 235	Turkey	6	r	s	s
9043	Austria	6	r	r	–
9048	Austria	6	r	r	–
10247	Yugoslavia	6	r	r	s
Chikurin Ibaraki 1	Japan	6	r	r	r
Ea 52	Japan	6	s	r	r
Iwate Omugi 1	Japan	6	r	r	s
Kanto Nijo 19	Japan	2	r	r	r
Mihori Hadaka 3	Japan	6	r	r	r
Misato Golden	Japan	2	r	r	r
Mokusekko 3	China	6	r	r	r
Muju covered 2	Korea	6	r	r	r
Namji Milyang Native	Korea	6	r	r	r
Ou 1	Japan	6	r	r	r
Resistant Ym No. 1	Japan	2	r	r	r
Rokkaku 1	Japan	6	r	r	r
Taihoku A	Taiwan	6	r	r	r
Zairai Rokkaku	Japan	6	r	r	r
H. spontaneum					
09-01	Isreal	2	r	s	s
09-09	Israel	2	r	s	s
09-H27	Israel	2	r	s	s
09-35	Israel	2	r	s	s
09-39	Israel	2	r	s	s
09-43	Israel	2	r	s	s
25-30	Israel	2	r	s	s
Candarli	Turkey	2	r	s	s
Icemeler	Turkey	2	r	r	r
Kupalan	Turkey	2	r	s	s
Menemen	Turkey	2	r	s	s
Pinarbasi	Turkey	2	r	r	r

r = resistant, s = susceptible, – = not tested

Table 2 RAPD-primer selected for the analysis of genetic similarity

Primer	Sequence 5′ . . . 3′	Primer	Sequence 5′ . . . 3′
OPD-04	TCTGGTGAGG	OPK-17	CCCAGCTGTG
OPD-20	ACCCGGTCAC	OPL-01	GGCATGACCT
OPF-02	GAGGATCCCT	OPM-10	TCTGGCGCAC
OPF-05	CCGAATTCCC	OPM-13	GGTGGTCAAG
OPF-08	GGGATATCGG	OPN-01	CTCACGTTGG
OPJ-04	CCGAACACGG	OPN-02	ACCAGGGGCA
OPJ-10	AAGCCCGAGG	OPN-07	CAGCCCAGAG
OPJ-12	GTCCCGTGGT	OPN-16	AAGCGACCTG
OPK-08	GAACACTGGG	OPS-01	CTACTGCGCT
OPK-12	TGGCCCTCAC	OPS-08	TTCAGGGTGG

Tanksley (1986) using equal quantities of leaf tissue from 10 plants of each variety grown in the greenhouse. Polymerase chain reaction (PCR) mixtures (25 µl) contained 25 ng genomic DNA, 0.4 m*M* dNTPs, 6 m*M* $MgCl_2$, 0.3 µ*M* of a random 10 mer primer (Operon Technologies) and 0.2 U *Taq* DNA-polymerase (Red Goldstar, Eurogentec) with the corresponding reaction buffer. The mixture was overlaid with mineral oil, and amplification was carried out in a DNA thermocycler 480 (Perkin Elmer) using the following temperature profile: an inital denaturation step (94°C/4 min) was followed by 45 cycles of 94°C/1 min, 36°C/1 min and 72°C/2 min. The polymerization step was extended for 3 s/cycle. Fragments were separated on a 2% agarose gel (NuSieve agarose, FMC), stained in ethidium bromide and visualized on an UV screen (254 nm). In order to identify primers giving polymorphic distinct and reliable bands we tested the primer kits OP-D, OP-F, OP-J, OP-K, OP-L, OP-M, OP-N and OP-S on the varieties 'Resistant Ym No. 1' and 'Sonate' for polymorphism and distinctness of bands. Out of these primers those listed in Table 2 were selected for the analysis of genetic similarity. RAPD patterns were scored using the software package RFLPscan 2.0, and genetic similarity was estimated according to Nei and Li (1979). On the basis of these data UPGMA-clustering was carried out using the software package NTSYS-pc 1.7 (Rohlf 1992), and an analysis of principal coordinates was conducted using the statistical software package SPSS/PC + 5.0.

Results

RAPD patterns generated by the selected primers were polymorphic and distinct. Based on the analysis of 20 RAPD primers (Table 2) corresponding to 544 different fragments ranging from 2691 to 280 bp we estimated the genetic similarity (Nei and Li 1979) to be between 0.685 ('09-09' *versus* 'Corona') and 0.964 ('09-39' *versus* '09-43').

Associations between the 48 genotypes calculated on these data by UPGMA-clustering are presented in Fig. 1. The cophenetic correlation as a measure of goodness-of-fit for the cluster analysis was estimated to be $r = 0.962$, revealing a very good fit (Rohlf 1992). In general, seven groups of genotypes can be distinguished. The first one consists of BaMMV resistant *H. spontaneum* accessions originating from Israel ('09-H27' to '09-01'). In this case it is interesting to note that var '25-30' – although from Israel – does not belong directly to this cluster. This may be due to the fact that varieties from '09-H27' to '09-01' come from the same region (Mt. Meron), while '25-30' was collected in a different region, designated Atli (E. Nevo, personal communication). This cluster is followed by the Turkish *H. spontaneum* accessions 'Pinarbasi' to 'Candarli'. In contrast to the Israeli genotypes which revealed genetic similarity estimates between 0.881 and 0.964, with the exception of '25-30', genetic similarity is lower within the Turkish *H. spontaneum* accessions (0.808–0.887). Next to this cluster are the resistant *H. vulgare* varieties derived from East Asia ('Iwate Omugi 1' to 'Chikurin Ibaraki 1') followed by 3 more or less isolated genotypes, i.e. 'Anson Barley' derived from the USA as well as 'Russia 32' and 'Russia 57' from Russia. The fourth cluster comprises three Japanese malting barley cultivars ('Resistant Ym No. 1' to 'Kanto Nijo 19'), and the last 3 groups contain European barley varieties. The latter comprise resistant varieties from the Eastern part of Europe ('Turkey 235' to '10247') and resistant and susceptible German cultivars, which can be subdivided into six-rowed ('Ogra' to 'Brunhild') and two-rowed cultivars ('Magie' to 'Alraune'), respectively.

Similar results were obtained using principal coordinate analysis (Fig. 2). PC1 in accounting for 55.6% of the total variation clearly separates the Israelian *H. spontaneum* accessions from the Turkish *H. spontaneum* germplasms and the *H. vulgare* varieties, respectively, while PC2, accounting for 16.2% of the variation, separates the resistant East-Asian *H. vulgare* varieties from the other genotypes. As in the cluster analysis the Japanese malting barley varieties are more closely grouped to the European barley cultivars. Again Israelian *H. spontaneum* accession '25-30' is separated from the rest of the Israelian germplasms and more closely grouped to the Turkish accessions.

Discussion

Contrary to the results obtained from isozyme electrophoresis (Le Gouis et al. 1995) RAPD analysis revealed a large genetic diversity, both among varieties resistant to the soil-borne mosaic-inducing viruses and in comparison to German cultivars. Like RFLP analysis (Graner et al. 1994; Melchinger et al. 1994; O'Donoughue et al. 1994) the use of RAPD-PCR is a very suitable and efficient tool for the genetic characterization of barley. Using only 20 RAPD primers we were able to differentiate *H. spontaneum* accessions from *H. vulgare* germplasms, and all the varieties were correctly grouped according to their origin (Figs. 1, 2). In this respect it is interesting to note that the Japanese malting barley varieties 'Resistant Ym No. 1', 'Kanto Nijo 19' and 'Misato Golden' are more closely grouped to European barley cultivars than the other East Asian varieties. This may be due to the fact that these Japanese varieties obtained their malting quality from European barley cultivars, e.g. 'Union' (Muramatsu 1976;

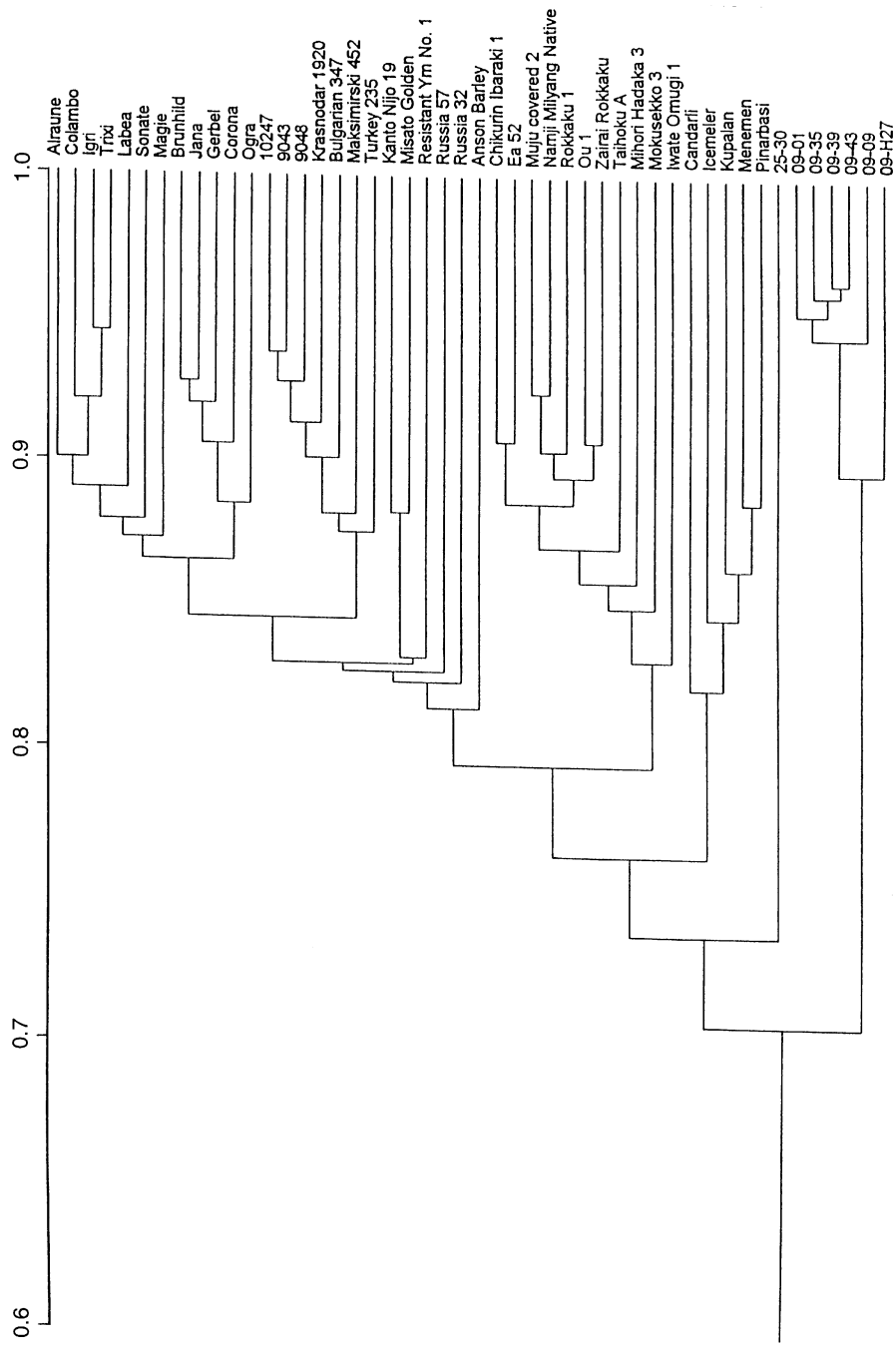
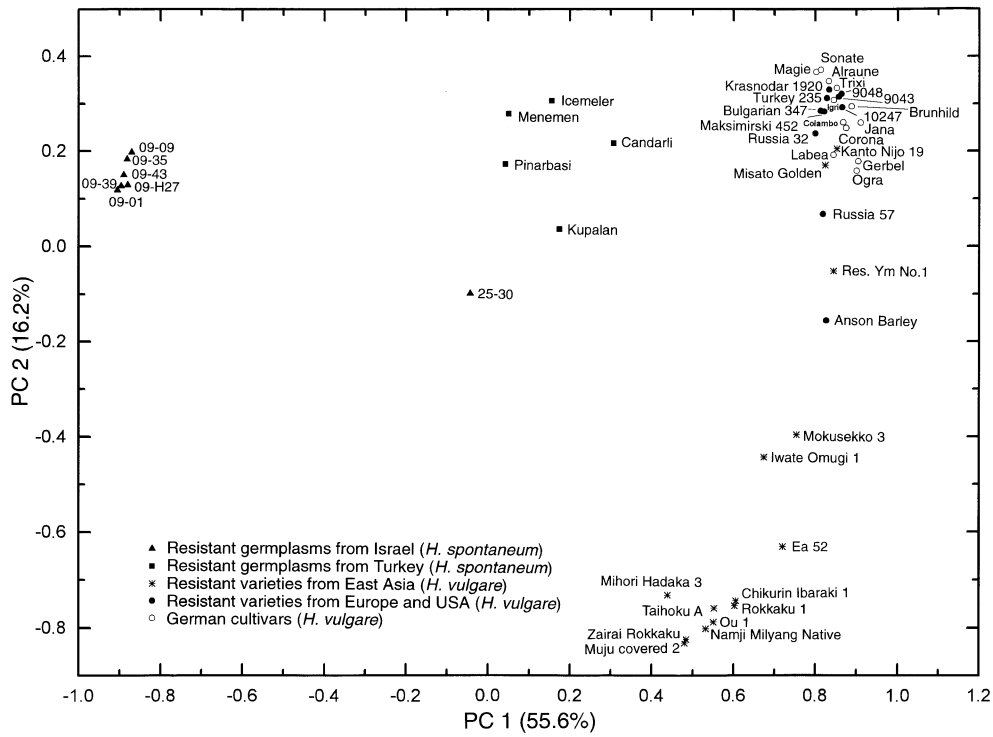


Fig. 1 Association among barley varieties (*H. vulgare* and *H. spontaneum*) showing different reactions to soil-borne mosaic-inducing viruses (BaMMV, BaYMV, BaYMV-2) revealed by cluster analysis performed on genetic similarity estimates (Nei and Li 1979) calculated from PCR data of 20 RAPD primers corresponding to 544 bands

Kobayashi et al. 1987). Furthermore, vars 'Chikurin Ibaraki 1' and 'Ea 52' are grouped together within the cluster of the East Asian varieties. This clearly reflects the genetic relationship between these genotypes since 'Ea 52' is a gamma-ray induced mutant of 'Chikurin Ibaraki 1' (Ukai and Yamashita 1980). However, genetic changes may not be limited to BaYMV resistance and earliness (Ukai 1984; Götz and Friedt 1993) because the genetic similarity between these varieties was estimated at only 0.908. This result suggests that the induced mutation in 'Ea 52' has led to reasonable changes in the genome compared to 'Chikurin Ibaraki 1'. In this respect, Chalhoub et al. (1995) reported on differences between these varieties with respect to their reaction to two PAV-like isolates of barley yellow dwarf virus (BYDV).

With respect to the German cultivars the use of 20 selected RAPD primers led to a differentiation between two-rowed and six-rowed cultivars (Fig. 1). Furthermore, it is interesting to note that the highest genetic similarity within the German varieties was found between the cvs 'Trixi' and 'Igri' (0.945), which in comparison to the other German cultivars are quite closely related due to their pedigree (Fischbeck 1992).



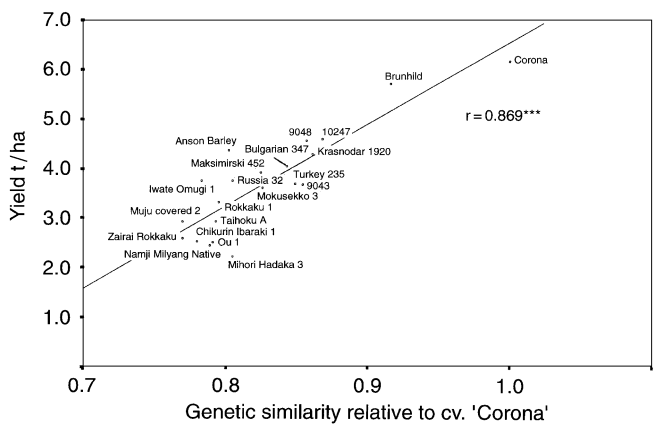


Fig. 3 Relationship between genetic similarity and grain yield of exotic barley germplasms resistant to soil-borne mosaic-inducing viruses in comparison to the adapted cultivar 'Corona' (data for grain yield taken from Ordon and Friedt 1994, and unpublished data)

In conclusion, the use of 20 selected RAPD primers produced estimates of the genetic relationship that are in a very close accordance with the known data about the origin and pedigree of the varieties tested.

In addition to the estimation of genetic relationships among varieties resistant to soil-borne viruses, the results presented here have an impact on pratical plant breeding. From our results it may be concluded that varieties more closely related to German cultivars, e.g. Japanese malting barley cultivars and varieties derived from the Eastern part of Europe (Figs. 1, 2), seem to be better suited to the rapid introgression of different resistance genes, i.e. combining these genes with those for superior agronomic performance. Conversely, more distantly related varieties may be better suited to be used in recurrent selection programs for an exploitation of their resistance. Concerning the six-rowed resistant material this assumption is partly proven by the results presented in Fig. 3. Based on yield data from two-year trials of exotic varieties (Ordon and Friedt 1994; Ordon and Friedt unpublished data) and the genetic similarity data obtained in this study a highly significant correlation between these parameters is obvious (Fig. 3). Besides grain yield exotic varieties showing a closer genetic similarity to German cultivars, like '10247', 'Krasnodar 1920' and 'Bulgarian 347', are in general better adapted to German growing conditions (cf. Ordon and Friedt 1994). However, the more distantly related East Asian varieties like 'Ou1', 'Muju covered 2' or 'Taihoku A' are the only sources which combine resistance to BaYMV-2 with BaMMV resistance genes that are different from *ym4* (Götz and Friedt 1993; Ordon and Friedt 1993). Unfortunately, the Japanese two-rowed malting barley varieties ('Resistant Ym No. 1', 'Misato Golden' and 'Kanto Nijo 19'), although being more closely related to European barley cultivars (Figs. 1, 2) and resistant to all of the yellow mosaic-inducing viruses (Table 1), carry BaMMV resistance genes allelic to *ym4* (Götz and Friedt 1993). Therefore, the more distantly related East-Asian varieties are best suited to transfer resistance to BaYMV-2 into adapted cultivars by simultaneously broadening the genetic

base of BaMMV resistance. Due to agronomic shortcomings the incorporation of these resistance genes into adapted varieties requires extensive and time-consuming breeding programs which may be enhanced today by the use of haploidy technique (Foroughi-Wehr and Wenzel 1990) and by an application of efficient marker-based selection procedures (Graner et al. 1995, 1996).

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