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POLYMORPHISM OF MICROSATELLITE MARKERS ON CHROMOSOMES 3H AND 7H IN BARLEY GENOTYPES RESISTANT AND SUSCEPTIBLE TO *Rhynchosporium secalis*

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Abstract

NEVIMOVÁ, H., BEDNÁŘ, J., VYHNÁNEK, T.: *Polymorphism of microsatellite markers on chromosomes 3H and 7H in barley genotypes resistant and susceptible to Rhynchosporium secalis.* Acta univ. agric. et silvic. Mendel. Brun., 2009, LVII, No. 2, pp. 69–78

The objective of the present study was to explore the polymorphism of microsatellite markers localised on chromosomes 3H and 7H in 15 genotypes of barley (*Hordeum vulgare* L.), spring form (2n = 2x = 14 chromosomes, genome H^vH^v) from the collection of genetic resources of the Agricultural Research Institute Kroměříž, Ltd. showing various degrees of susceptibility to *Rhynchosporium secalis*. The selection of SSR markers was based on hitherto achieved knowledge according to which the greatest amount of resistance genes against *Rhynchosporium secalis* is localised on chromosomes 3H and 7H of barley. We selected 33 SSR markers for the analyses; 17 were localised on chromosome 3H of barley and 16 on chromosome 7H. Out of the total 33 SSR markers, 32 were polymorphous and one marker (*Bmac0282*) was monomorphic. In total we detected 172 alleles ranging between 101 and 235 bp; the average number of alleles per locus was 5.21. In terms of the polymorphism of the SSR markers localised on chromosomes 3H and 7H the highest polymorphism (60%) was detected in the *Bmag0006* and *Bmag0021* SSR markers; the lowest in the *Bmag0877* and *EBmac0713* markers, i.e. 20% and 13.3%, respectively. The average polymorphism based on analyses of 17 SSR markers on chromosome 3H was 37.6% and of 16 SSR markers on chromosome 7H was 31.3%. We also calculated the statistical indicators of the variability rate characteristics of the individual microsatellite markers: diversity index (DI) which ranged between 0.000 and 0.907 (on average 0.704); polymorphous information content (PIC) ranging between 0.000 and 0.906 (on average 0.679); and probability identity (PI) ranging between 0.006 and 1.000 (on average 0.137). On the basis of constructed dendrograms for SSR markers of both chromosomes together it was possible to divide the analysed set into cluster I of genotypes resistant and cluster II of genotypes susceptible and moderately susceptible to *Rhynchosporium secalis*, and was not possible in dendrograms of individual chromosomes.

polymorphism, microsatellite markers, resistance, *Rhynchosporium secalis*

At present, various molecular techniques are used to study DNA polymorphism, e.g. AFLP, RAPD, RFLP, SSR etc. Microsatellite markers (SSR – *Simple Sequence Repeats*) belong to DNA markers which are often used for genetic mapping and population studies thanks to their frequency, capacity to detect co-dominant alleles, the high level of polymorphism, broad dispersal in various genomes, simple execution of PCR reactions and easy repeatability among various laboratories (Liu et al., 1996). Last but not least they require a minimal amount of analysed DNA (Powell et al., 1996).

Leaf blotch (scald) is a fungal disease caused by the facultative pathogen *Rhynchosporium secalis* (Oudem.) J. J. Davis. It was described for the first time in the late 19th century (Häni et al., 1993) and is spread particularly in the northern parts of Central Europe. It invades a number of cereals, espe-

cially barley (spring and winter). In colder and more humid regions a commercially more important and regular incidence can be expected, although a more widespread occurrence was monitored also in higher altitudes inland. In the past decades it was reported that the disease appeared even in localities of lower altitude and drier climate (Minaříková and Míša, 1995). Infections usually appear at temperatures of 10–20 °C, at a low intensity of solar radiation and 95% air humidity (Beer, 1991). At present the importance of this disease is on the increase because the varieties of spring barley which are used particularly in the malt industry are more susceptible to leaf scald. Yield losses at a local level may reach 20% or even more.

Patil et al. (2003) reported that the genes determining resistance to *R. secalis* are localised primarily on chromosome 3H (*Rrs1*) and chromosome 7H (*Rrs2*) of barley. On the basis of analyses of dihaploid lines and backcrossing of barley loci determining resistance to *R. secalis* were detected on the long arm of chromosome 3H (*Rrs1* syn. *Rh*), on the short arm of chromosome 7H (*Rrs2*) and chromosome 4H (*Rrs3*). Donors of other genes of resistance *Rrs12*, *Rrs13* a *Rrs14* which are localised on the short arms of chromosomes 7H, 6H and 1H were described in wild barley (*Hordeum vulgare* ssp. *spontaneum*) (Gronnerod et al., 2002; Jensen et al., 2002; Genger et al., 2003; Patil et al., 2003). Genger et al. (2003) quoted loci on chromosomes 3HL and 7HS as majority loci.

The objective of the present study was to evaluate the variability of microsatellite markers localised on chromosomes 3H and 7H of barley in genetic resources which have a different susceptibility to *Rhynchosporium secalis*.

MATERIAL AND METHODS

Detection of polymorphism of microsatellite markers was conducted in 15 genotypes of barley (*Hordeum vulgare* L.), spring form (2n = 2x = 14 chromosomes, genome H^vH^v) from the collection of genetic resources of the Agricultural Research Institute Kroměříž, Ltd. of different susceptibility to *Rhynchosporium secalis* (Tab. I).

A total of 33 SSR markers obtained from the GrainGenes database (Ramsay et al., 2000) and from literature (Liu et al., 1996; Li et al., 2003; Varshney et al., 2007) were selected for the analyses. The total genomic DNA was isolated by DNeasy Plant Mini Kit (Qiagen, GE) from the first leaf (6–7 days old). The isolated DNA was quantified spectrophotometrically by measuring the absorbance of DNA at 260 nm. The total volume of the PCR reaction mixture was 25 µl and contained 18.4 µl deionised H_2O; 2.5 µl reaction buffer for polymerase Green GoTaq (Promega, USA); 1.0 µl of 100 mM dNTPs mixture (Promega, USA); 1.0 µl forward primer; 1.0 µl reverse primer; 0.1 µl of thermo-stable *Taq* DNA polymerase – 5 U (Promega, USA); and 1.0 µl template DNA. The PCR was conducted in the T3 Thermocycler combi (Biometra, GE) where the PCR program consisted of initial denaturation for 2 min at 93 °C, followed by 30 cycles of 1 min at 93 °C, 2 min at 54 °C, 2 min at 72 °C. The resulting products of the PCR reaction were separated electrophoretically on horizontal electrophoresis at 70 V on 1% agarose gels stained with ethidium bromide. Positive samples were separated on vertical electrophoresis at 300 V on 10% non-denaturation polyacrylamide gels in a TBE buffer stained with silver nitrate (0.2% $AgNO_3$) were selected. Polymorphic DNA products were scored as presence (1) or absence (0) of an allele and used to construct a binary matrix. The genetic similarity matrix of all genotypes was analysed using unweighted pair group method with arithmetic average (UPGMA) algorithm performed using FreeTree version 9.1 (Jaccard similarity coefficient); by means of TreeView version 1.6 dendrograms were drawn illustrating the genetic similarity of the tested genotypes. Further three statistical characteristics were calculated – the diversity index (DI), the probability identity (PI) and the polymorphous information content (PIC) according to Russell et al. (1997). The polymorphism value (%) was calculated as the ratio of alleles detected for a microsatellite marker and the number of analysed genotypes.

RESULTS AND DISCUSSION

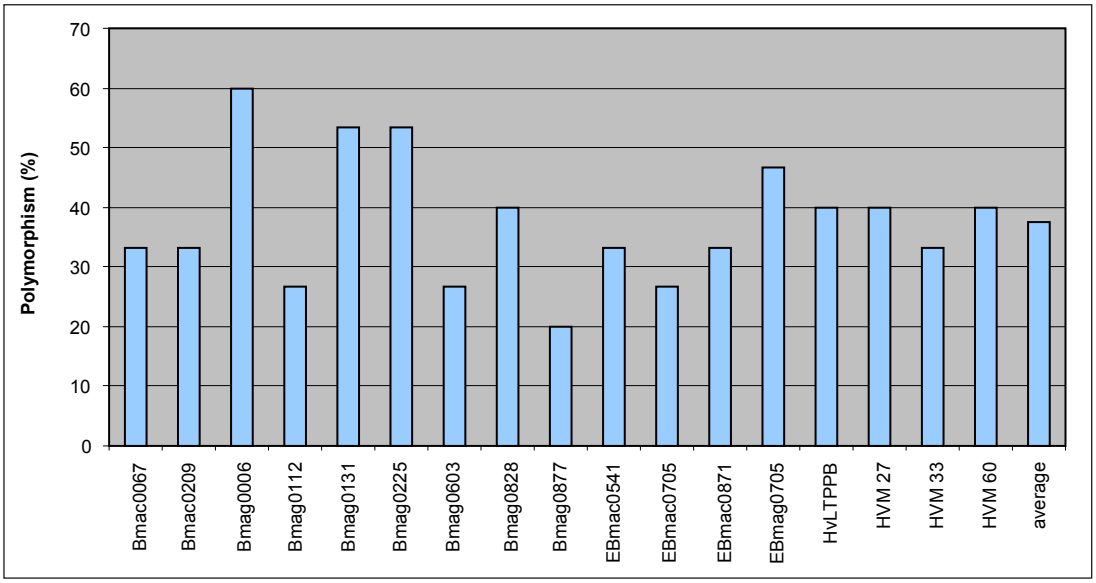
Out of the 33 SSR markers 32 were polymorphous and one was monomorphic (*Bmac0282* – 7H). 1–9 alleles per locus were detected. Altogether 172 alleles, i.e. on average 5.21 alleles per 1 locus were detected. The size of the amplified products (alleles) ranged between 101 bp and 235 bp. Hamza et al. (2004) used 17 microsatellite markers in 26 barley varieties and they discovered that the average number of alleles per locus was 3.6; a value lower than the results of the present study. To the contrary Struss and Plieske (1998) detected 130 alleles in 163 barley genotypes using 15 SSR markers; they found 5 to 14 alleles per locus, averaging 8.60 alleles per locus. Leišová et al. (2007) studied the genetic variability in 176 barley genotypes using 26 SSR markers and they detected as many as 328 alleles averaging 12.6 alleles per locus. It is apparent that the total number of alleles and the average number per locus is dependent on how extensive is the collection of genotypes to be analysed, including the diversity of their origin, and also on the number of the SSR markers.

On chromosome 3H the polymorphism of 17 microsatellite markers and on chromosome 7H of 16 SSR markers were explored. Graph 1 shows the polymorphism detected in SSR markers localised on chromosome 3H. The SSR marker *Bmag0006* showed the highest degree of polymorphism (60%) and marker *Bmag0877* the lowest (20%). The average degree of polymorphism determined on the basis of analyses of 17 SSR markers on chromosome 3H was 37.6%.

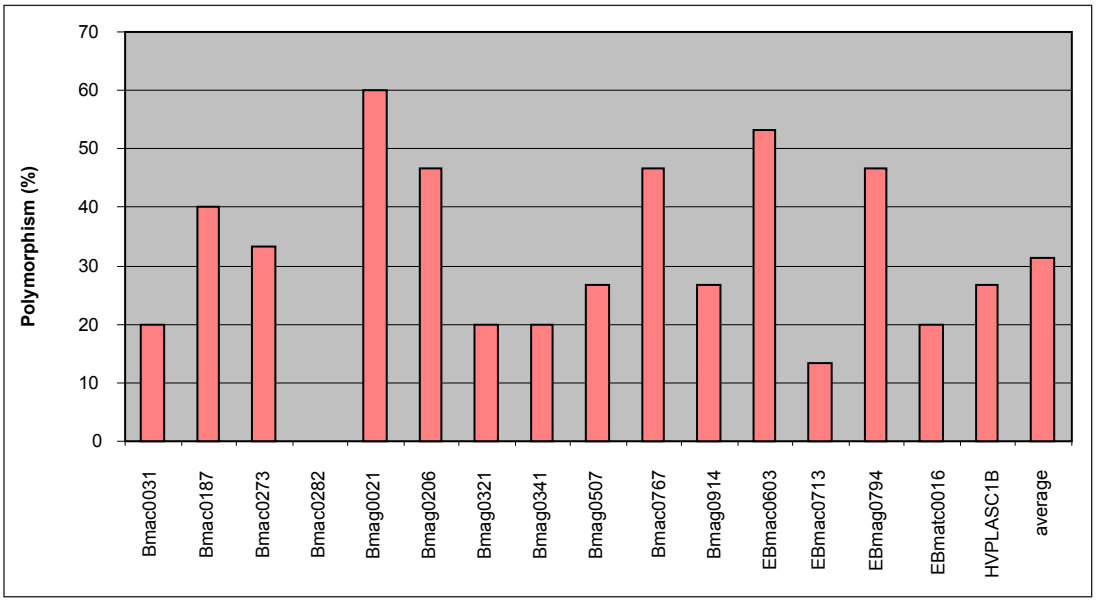
I: *Characteristics of the barley genotypes*

Genotype	taxon	country of origin *	Pedigree	resistance genes	susceptibility to Rhynchosporium secalis
Abyssinian	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nutans* SCHUEBL	ETH	S- CI 668	*Rh*	susceptible
Atlas	*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL	USA	S- Coast	*Rh2*	resistant
Atlas 46	*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL.	USA	Hanna/Atlas	*Rh, Rh2, Rh3*	resistant
Atlas 57	*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL.	USA	Lion/Atlas	*Rh2*	resistant
Bay	*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL.	USA	Minnesota 450/Spartan	*Rh3*	resistant
Cambrinus	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nutans* SCHUEBL.	NED	Balder/Streg Franken III	not described	susceptible
Clipper	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nutans* SCHUEBL.	AUS	Prior/Proctor	not described	moderately susceptible
Jet	*Hordeum vulgare* L. subsp. *vulgare* var. *nigrum* (WILLD.) LINK	USA	introduced from Ethiopia	*rh5, rh6, rh7*	resistant
Kitchin	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN.	USA	Moravian/ Deficiens	*Rh9*	resistant
Kompakt	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nutans* SCHUEBL.	SVK	Galan/KM-A10	*Rh3, Rh12*	moderately susceptible
Korál	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nutans* SCHUEBL.	CSK	Hana/4/ CarlsbergII/ F.Union// Alsa/3/ Celech. hanacky/J25	*Rh2*	moderately susceptible
La Mesita	*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL.	MEX	S-field California Mariout	*Rh4, Rh10*	resistant
Nigrinudum Abyssinum	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nigrinudum* VAV.	ETH	regional or primitive cultivar	*rh8*	resistant
Psaknon	*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum VIL.*	AUS	breeding cultivar	*Rh3*	resistant
Rapid	*Hordeum vulgare* L. subsp. *distichon* (L.) KOERN. var. *nutans* SCHUEBL.	CSK	Voldagsen/ Kneifel// Diamant/3/ Denar	*Rh2,Rh4*	moderately susceptible

* AUS – Australia, CSK – Czechoslovakia, ETH – Ethiopia, MEX – Mexico, NED – Netherlands, SVK – Slovakia, USA – Unites States of America



1: *Values of polymorphism detected in SSR markers localised on chromosome 3H of barley*



2: *Values of polymorphism detected in SSR markers localised on chromosome 7H of barley*

Graph 2 shows the values of polymorphism detected in the individual SSR markers on chromosome 7H. The highest degree of polymorphism (i.e. 60%) was detected in marker *Bmag0021*; on the contrary the lowest in marker *EBmac0713*, i.e. 13.3%. Zero polymorphism was determined only in marker *Bmac0282* and the average polymorphism based on analyses of 16 SSR markers on chromosome 7H was 31.3%.

In the set of analysed barley genotypes on average 34.5% of the 33 tested microsatellite markers were polymorphous. The highest degree of polymorphism (97%) was determined in the following genotypes: Abyssinian, Atlas, Atlas 46, Atlas 57, Bay, Cambrinus, Clipper, Kompakt, La Mesita, Nigrinudum Abyssinum and Rapid. The lowest degree of polymorphism (87.9%) was detected in the genotype Kitchin.

For each microsatellite marker statistical indicators were calculated for characteristics of the rate of variability within the analysed collection (Tab. II). The diversity index (DI) of the tested microsatellite markers ranged between 0.000 in the SSR marker *Bmac0282* and 0.907 in the markers *Bmac0067* and *EBmag0705*; the average DI value was 0.704. The polymorphous information content (PIC) was found to range between 0.000 (*Bmac0282*) and 0.906 (*Bmac0067* and *EBmag0705*) and the average PIC value was 0.679. The average value of the probability identity (PI) was 0.137 and ranged between 0.006 (*Bmac0067* and

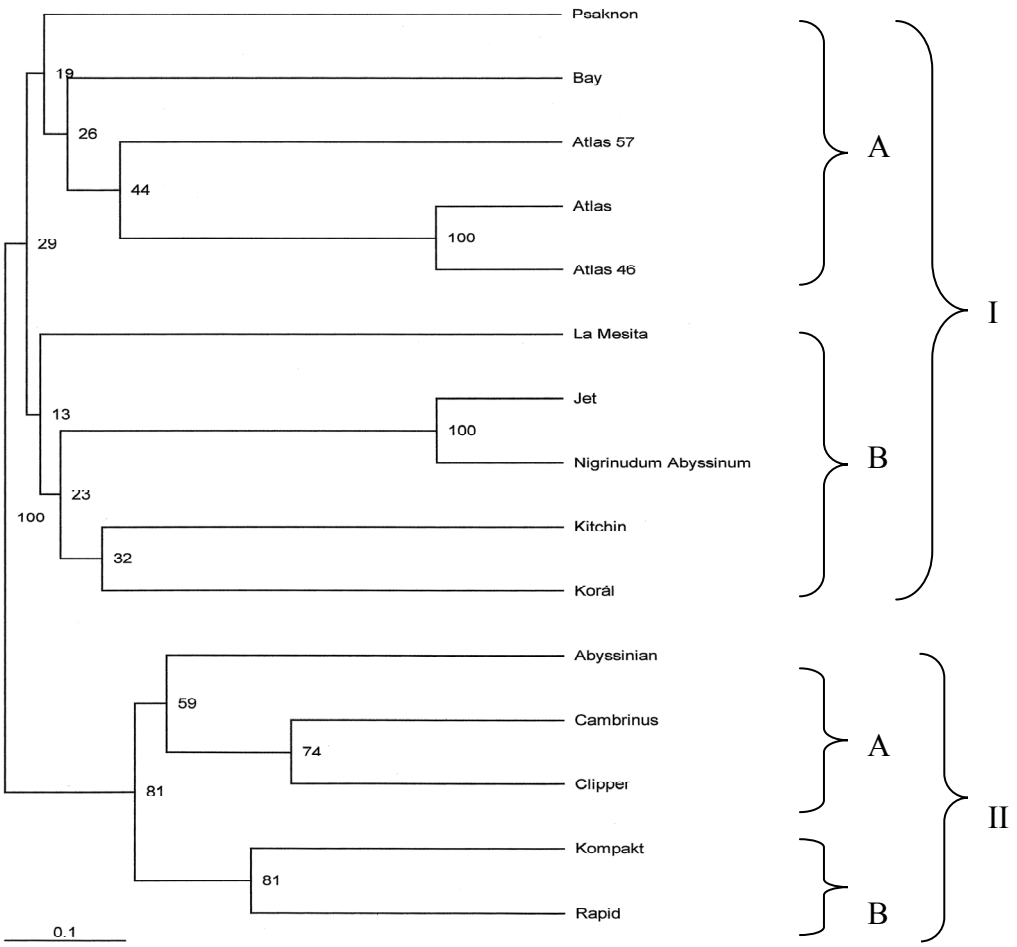
EBmag0705) and 1.000 (*Bmac0282*). Russell et al. (1997) discovered average values lower than those calculated in the present study (PI 0.290; PIC 0.501 and DI 0.842); the reason may be that fewer microsatellite markers were used (11). Kolodinska Brantestam et al. (2007) and Leišová et al. (2007) obtained higher PIC and DI values when using a comparable number of SSR markers. The differences were probably due to the fact that the analysed collection of genotypes was more extensive and ranged from 180 to 200 barley genotypes.

II: *The sizes of SSR alleles in 15 genotypes of the spring form of barley and their statistical analysis* *

SSR marker	localisation	Number of alleles	Assumed size of alleles	Detected size of alleles (bp)	DI	PI	PIC
Bmac0031	7H	3	175	168–182	0.602	0.110	0.587
Bmac0067	3H	5	171	165–190	0.907	0.006	0.906
Bmac0187	7H	6	177	163–235	0.880	0.009	0.879
Bmac0209	3H	5	176	168–199	0.880	0.009	0.879
Bmac0273	7H	5	186	178–187	0.818	0.034	0.809
Bmac0282	7H	1	195	192	0.000	1.000	0.000
Bmag0006	3H	9	174	158–190	0.867	0.014	0.862
Bmag0021	7H	9	143	135–143	0.862	0.014	0.857
Bmag0112	3H	4	196	157–186	0.684	0.095	0.662
Bmag0131	3H	8	149	138–158	0.862	0.011	0.858
Bmag0206	7H	7	239	239–270	0.827	0.029	0.816
Bmag0225	3H	8	162	137–160	0.816	0.031	0.806
Bmag0321	7H	3	218	198–223	0.587	0.240	0.513
Bmag0341	7H	3	215	208–218	0.541	0.291	0.456
Bmag0507	7H	4	147	118–150	0.627	0.127	0.608
Bmag0603	3H	4	120	112–142	0.693	0.119	0.653
Bmag0767	7H	7	151	134–198	0.782	0.059	0.762
Bmag0828	3H	6	124	120–175	0.738	0.088	0.709
Bmag0877	3H	3	153	140–150	0.560	0.289	0.463
Bmag0914	7H	4	170	156–170	0.640	0.152	0.606
EBmac0541	3H	5	106	106–110	0.649	0.134	0.623
EBmac0603	7H	8	149	135–180	0.836	0.025	0.827
EBmac0705	3H	4	150	148–178	0.658	0.069	0.649
EBmac0713	7H	2	168	159–162	0.500	0.375	0.375
EBmac0871	3H	5	180	175–200	0.756	0.320	0.749
EBmag0705	3H	7	155	122–210	0.907	0.006	0.906
EBmag0794	7H	7	197	149–210	0.782	0.025	0.779
EBmatc0016	7H	3	143	136–149	0.561	0.294	0.458
HvLTPPB	3H	6	221	200–222	0.800	0.034	0.790
HVM27	3H	6	192	180–189	0.720	0.070	0.709
HVM33	3H	5	157	143–164	0.649	0.149	0.616
HVM60	3H	6	115	104–118	0.738	0.082	0.712
HVPLASC1B	7H	4	110	101–111	0.516	0.208	0.511
Average		5.21	166.18		0.704	0.137	0.679

* DI – diversity index, PI – probability identity, PIC – polymorphous information content

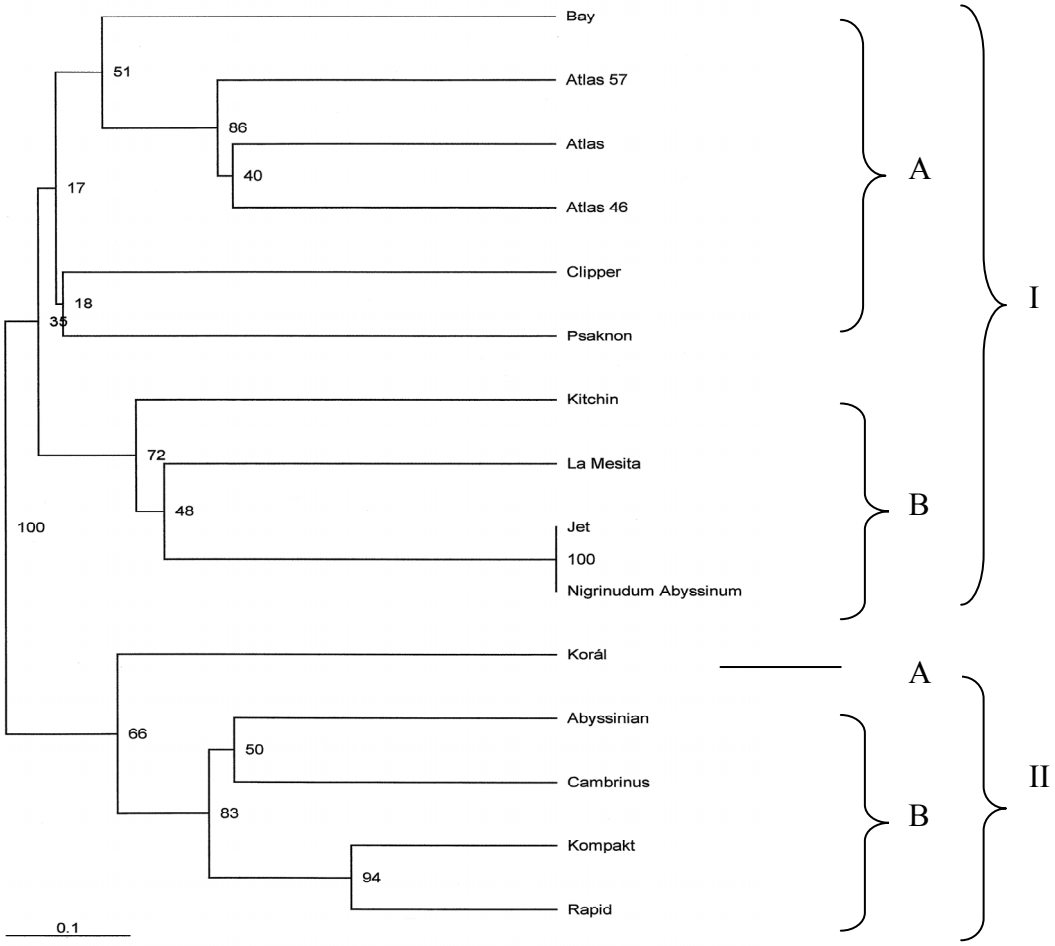
The results were processed and graphically presented in three dendrograms (Figs. 1–5) on the basis of variability of the microsatellites of the individual chromosomes; the main clusters are marked with Roman numerals I and II, subclusters with letters A and B and they are further divided into subgroups 1 and 2. The value of bootstrapping expresses the reliability of conclusions (Flegr, 2005); in our case the highest possible value equals 100.



3: *Dendrogram of analysed genotypes drawn on the basis of analyses of SSR markers localised on chromosome 3H in barley*

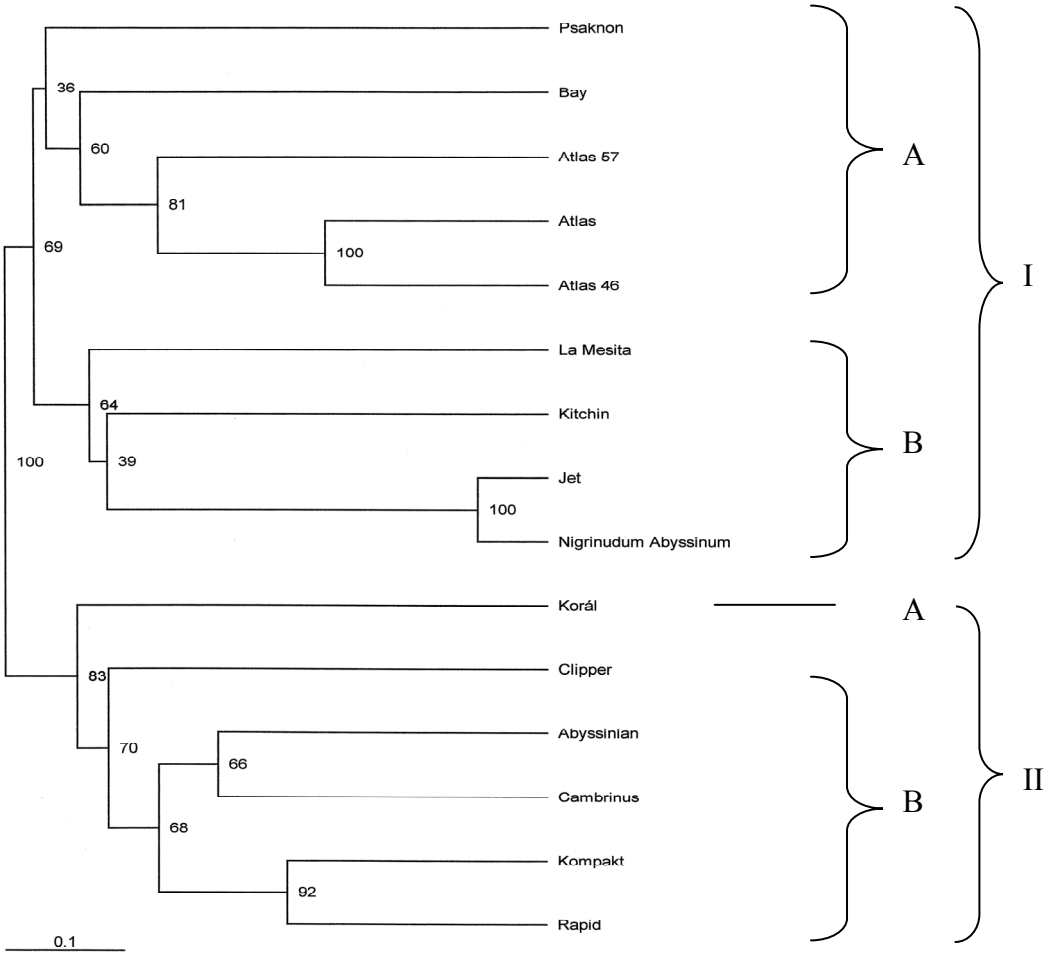
Based on analyses of markers on chromosome 3H (Fig. 3) a dendrogram dividing the genotypes into two basic clusters: genotypes resistant and susceptible to *Rhynchosporium secalis* was drawn. Each cluster is made up of two subclusters. Sub-cluster A of cluster I contains the resistant genotypes Atlas, Atlas 46, Atlas 57, Bay and Psaknon. Subcluster B of cluster I contains resistant genotypes Jet, Kitchin, Korál, La Mesita, Nigrinudum Abyssinum and moderately susceptible genotype Korál. The genotypes Jet and Nigrinudum Abyssinum (bootstrapping 100) showed the highest genetic similarity. The second cluster (II) is made up of the susceptible genotypes Abyssinian and Cambrinus and the moderately susceptible genotypes Clipper, Kompakt and Rapid.

The resulting dendrogram of polymorphism of microsatellites of chromosome 7H (Fig. 4) also consists of two main clusters; however only cluster I is divided into two larger subclusters. The second cluster (II) consists of the genotype Korál (A) and the larger subcluster B which contains the genotypes Abyssinian, Cambrinus, Kompakt and Rapid. A high genetic similarity (94) was determined in the moderately susceptible genotypes Kompakt and Rapid and the resistant genotypes Jet and Nigrinudum Abyssinum (100). In contrast the lowest genetic similarity (18) was determined in the moderately susceptible genotype Clipper and the resistant genotype Psaknon.



4: *Dendrogram of analysed genotypes drawn on the basis of analyses of SSR markers localised on chromosome 7H in barley*

If a combination of data from both (3H and 7H) analysed chromosomes was used for drawing the dendogram (Fig. 5), the analysed collection of genotypes was divided into two clusters; both the first (I) and the second (II) clusters were then divided into two subclusters. Low genetic variability was confirmed in the genotypes Atlas, Atlas 46 and Atlas 57 which arises from the pedigrees of these genotypes which are given in Tab. I. The value in genotypes Atlas and Atlas 46 was as high as 100. This value (100) was also detected in genotypes Jet and Nigrinudum Abyssinum which have black kernels (variety *nigrum* and *nigrinudum*, respectively); and they are regional or primitive cultivars from Ethiopia. By means of this dendrogram the analysed genotypes of barley, spring form, were divided into resistant and susceptible to barley scald; the resistant genotypes are in cluster I and the susceptible and moderately susceptible ones in cluster II.



5: *Dendrogram of analysed genotypes drawn on the basis of analyses of SSR markers localised on chromosomes 3H and 7H in barley*

SUMMARY

The objective of the study was to explore the polymorphism of microsatellite markers on chromosomes 3H and 7H of barley in barley genotypes resistant and susceptible to *Rhynchosporium secalis*. The collection of 15 analysed genotypes of barley (*Hordeum vulgare* L.), spring form, originated from the collection of genetic resources of the Agricultural Research Institute Kroměříž, Ltd., i.e. Abyssinian, Atlas, Atlas 46, Atlas 57, Bay, Cambrinus, Clipper, Jet, Kitchin, Kompakt, Korál, La Mesita, Nigrinudum Abyssinum, Psaknon and Rapid. For the analyses 33 SSR markers were selected, 17 of which were localised on barley chromosome 3H and 16 SSR markers were localised on barley chromosome 7H. The microsatellite markers were selected from the database GrainGenes and from literature where the sequence of primers, motives and localisation are given. The total genomic DNA was isolated by means of the isolation kit DNeasy Plant Mini Kit from 6–7-day-old plants. The concentration of isolated DNA was detected spectrophotometrically. On this basis samples suitable for separation on vertical electrophoresis at 300 V on 10% non-denaturation polyacrylamide gels in a TBE buffer stained with silver nitrate (0.2% $AgNO_3$) were selected. The obtained electrophoreograms were converted to a binary matrix. The processed results were statistically evaluated with the programme FreeTree version 9.1 using cluster analysis UPGMA and Jaccard similarity coefficient. Using the programme TreeView version 1.6 three dendrograms illustrating the genetic similarity of the tested genotypes were drawn. Out of the total 33 SSR markers 32 showed a polymorphous and one marker (*Bmac0282* – 7H) a monomorphic character. Altogether 172 alleles were detected and the average number of alleles per 1 locus was 5.21 (1–9 alleles). The size of the amplified products (alleles) ranged from 101 bp to 235 bp. The highest degree of polymorphism of the 17 SSR markers localised on chromosome 3H was detected in marker *Bmag0006*, i.e. 60%, and the lowest (20%) degree of polymorphism in the SSR marker *Bmag0877*. The average degree of polymorphism based on analyses of 17 SSR markers on chromosome 3H was 37.6%. The highest degree of polymorphism of the 16 SSR markers localised on chromo-

some 7H was detected in marker *Bmag0021*, i.e. 60%, and the lowest in marker *EBmac0713*, i.e. 13.3%. The average degree of polymorphism resulting from analyses of 16 SSR markers on chromosome 7H was 31.3%. Three statistical characteristics were then quantified – the diversity index (DI), probability identity (PI) and polymorphous information content (PIC). Of the tested microsatellite markers the DI ranged between 0.000 in the SSR marker *Bmac0282* and 0.907 in markers *Bmac0067* and *EBmag0705*. The average DI value was 0.704. PI ranged between 0.006 in markers *Bmac0067* and *EBmag0705* and 1.000 in marker *Bmac0282*; the average value was 0.137. The PIC value ranged between 0.000 in marker *Bmac0282* and 0.906 in markers *Bmac0067* and *EBmag0705*. The average PIC value was 0.679. Based on the variability of the microsatellites of the individual chromosomes the results were statistically processed into two dendrograms. The moderately susceptible genotype Clipper in the dendrogram of chromosome 3H (Fig. 3) resp. Korál in the dendrogram of 7H (Fig. 4) was clustered with resistant genotypes. On the basis of results processed for both chromosomes together it was possible to divide the analysed set into cluster I of genotypes resistant and cluster II of genotypes susceptible and moderately susceptible to *Rhynchosporium secalis* (Fig. 5).

SOUHRN

Polymorfismus mikrosatelitních markerů na 3H a 7H chromozomu u genotypů ječmene rezistentních a náchylných k *Rhynchosporium secalis*

Cílem práce bylo studium polymorfismu mikrosatelitních markerů na 3H a 7H chromozomu ječmene u genotypů ječmene rezistentních a náchylných k *Rhynchosporium secalis*. Soubor patnácti analyzovaných genotypů ječmene setého (*Hordeum vulgare* L.) jarní formy pochází z kolekce genetických zdrojů Zemědělského výzkumného ústavu Kroměříž, s.r.o.: Abyssinian, Atlas, Atlas 46, Atlas 57, Bay, Cambrinus, Clipper, Jet, Kitchin, Kompakt, Korál, La Mesita, Nigrinudum Abyssinum, Psaknon a Rapid. K analýzám bylo vybráno celkem 33 SSR markerů, z nichž 17 bylo lokalizováno na 3H chromozomu ječmene a 16 SSR markerů bylo lokalizováno na 7H chromozomu ječmene. Mikrosatelitní markery byly vybrány z databáze GrainGenes a z literatury, kde je uvedena sekvence primerů, motiv a lokalizace. Celková genomická DNA byla izolována pomocí izolačního kitu DNeasy Plant Mini Kit od firmy Qiagen (GE) z listů rostlin ve stáří 6–7 dní. Koncentrace izolované DNA byla zjišťována spektrofotometricky. Výsledné produkty PCR reakce byly elektroforeticky separovány na horizontální elektroforéze při 70 V na 1% agarózových gelech barvených ethidium bromidem, tzv. kontrolní elektroforéza pro ověření úspěšnosti PCR reakce. Na jejím základě byly vybrány vzorky vhodné pro separaci na vertikální elektroforéze při 300 V na 10% nedenaturačních polyakrylamidových gelech v TBE pufru barvených dusičnanem stříbrným (0,2 % $AgNO_3$). Získané elektroforeogramy byly převedeny do podoby binární matice. Takto zpracované výsledky byly statisticky vyhodnoceny počítačovým programem FreeTree verze 9.1 s použitím konstrukční metody shlukové analýzy UPGMA a podobnostního koeficientu Jaccard. Poté byly pomocí programu TreeView verze 1.6 sestaveny tři dendrogramy znázorňující genetickou podobnost testovaných genotypů. Z celkového počtu 33 SSR markerů vykazovalo 32 polymorfní a jeden marker (*Bmac0282* – 7H) monomorfní charakter. Celkem bylo detekováno 172 alel a průměrný počet alel na 1 lokus byl 5,21 (1–9 alel). Velikost amplifikovaných produktů (alel) byla od 101 bp do 235 bp. Nejvyšší stupeň polymorfismu 17 SSR markerů lokalizovaných na 3H chromozomu byl zjištěn u markeru *Bmag0006*, a to 60%. Nejnižší 20% polymorfismus byl u SSR markeru *Bmag0877*. Průměrný polymorfismus zjištěný z analýz 17 SSR markerů na 3H chromozomu byl 37,6 %. Nejvyšší polymorfismus ze 16 SSR markerů lokalizovaných na 7H chromozomu byl detekován u markeru *Bmag0021* ve výši 60 %, nejnižší naopak u markeru *EBmac0713* 13,3 %. Průměrný polymorfismus zjištěný z analýz 16 SSR markerů na 7H chromozomu byl 31,3%. Dále byly vypočteny tři statistické charakteristiky – index diverzity (DI), pravděpodobnost identity (PI) a polymorfní informační obsah (PIC). Index diverzity testovaných mikrosatelitních markerů se pohyboval v rozmezí od 0,000 u SSR markeru *Bmac0282* do 0,907 u markerů *Bmac0067* a *EBmag0705*. Průměrná hodnota DI byla 0,704. Pravděpodobnost identity se pohybovala od 0,006 u markerů *Bmac0067* a *EBmag0705* do 1,000 u markeru *Bmac0282* s průměrnou hodnotou 0,137. Polymorfní informační obsah byl zjištěn v rozsahu od 0,000 u markeru *Bmac0282* do 0,906 u markerů *Bmac0067* a *EBmag0705*. Průměrná hodnota PIC byla 0,679. Na základě variability mikrosatelitů lokalizovaných na jednotlivých chromozomech byly získané výsledky statisticky zpracovány do podoby dvou dendrogramů. V dendrogramu sestaveném na základě chromozomu 3H (Obr. 3) tvořil středně náchylný genotyp Clipper, resp. středně náchylný genotyp Korál v dendrogramu vytvořeném na základě chromozomu 7H (Obr. 4), klaster s rezistentními genotypy. Na základě statistického vyhodnocení výsledků získaných z obou chromozomů je možné analyzované genotypy ječmene rozdělit do klasteru I s rezistentními genotypy a klasteru II s genotypy náchylnými a středně náchylnými k *Rhynchosporium secalis* (Obr. 5).

polymorfismus, mikrosatelitní markery, rezistence, *Rhynchosporium secalis*

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