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Using SSR markers for genetic diversity testing in barley genotypes with different sensitivity against Fusarium Head Blight*

Zastosowanie markerów SSR do testowania genetycznego zróżnicowania genotypów jęczmienia o różnej wrażliwości na fuzariozę kłosa

Fuzarioza kłosa (FHB) jest groźną chorobą zbóż szeroko rozpowszechnioną na całym świecie, wywoływaną przez grzyby z rodzaju *Fusarium* (*F. culmorum, F. graminearum* i in.). Porażenie jęczmienia tą chorobą może powodować nie tylko obniżkę plonu ale i akumulację w ziarnie niebezpiecznych wtórnych metabolitów grzyba — mykotoksyn (np. deoksyniwalenolu — DON). Nagromadzone mykotoksyny niekorzystnie wpływają na bezpieczeństwo żywności i obniżają jakość technologiczną ziarna. Celem badań było przetestowanie obecności markerów mikrosatelitarnych SSR (prostych powtórzonych sekwencji DNA) w siedmiu genotypach jęczmienia jarego (z określoną odpornością lub wrażliwością na FHB). Zbadano następujące formy: Chevron, Zao Zhou 3, PEC210, CI4196, 6NDRFG-1 (genotypy odporne) oraz PI383933 i Foster (genotypy wrażliwe). Wyniki zostaną wykorzystane do testowania 22 linii DH wytworzonych po skrzyżowaniu ww. genotypów w kombinacjach odporny × wrażliwy. Dotychczas przebadano genotypy rodzicielskie przy użyciu 35 markerów SSR z ogólnej liczby wybranych 60; 29 z tych markerów wykazało polimorfizm. Pozostałych sześć było monomorficznych i nie znajdą one zastosowania przy testowaniu linii DH. Częściowe wyniki analiz opracowano w formie binarnej macierzy i przekształcono w dendrogram charakteryzujący podobieństwo genetyczne analizowanych genotypów.

Słowa kluczowe: Fuzarioza kłosa, FHB, jęczmień, mikrosatelity, SSR

Fusarium Head Blight (FHB) or scab is a devastating disease of cereals widely spread all over the world and is caused by members of the genus *Fusarium* (*F. culmorum, F. graminearum*, and other ones). The FHB infection of barley may result in lower yields and in the accumulation of dangerous secondary metabolites of fungi — mycotoxins in the kernels (e.g. deoxynivalenol, DON). The accumulated mycotoxins have a negative effect on the safety and technological quality of the grain. The objective of the study was to test 7 parental genotypes of spring barley (with a recognised resistance

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or susceptibility to FHB) with SSR markers (Simple Sequence Repeats). We tested the following parental genotypes: Chevron, Zao Zhou 3, PEC210, CI4196, 6NDRFG-1 (resistant genotypes), and PI383933 and Foster (susceptible genotypes). The results of the analyses will be used to test 22 DH lines, which were derived after crossing the tested parental genotypes (resistant × susceptible). By now, a total of 35 SSR markers of parental genotypes were tested out of the total number of 60 selected microsatellite markers; 29 of the tested SSR markers showed a polymorphous character in the parental genotypes. The remaining 6 ones were monomorphous and will not be used to test the DH lines. Partial results of the analyses were elaborated into the form of a binary matrix and transformed into the form of a dendrogram, which characterised genetic affinity of the tested genotypes.

Key words: FHB, barley, microsatellites, SSR

INTRODUCTION

Fusarium Heat Blight (FHB), or scab, is a worldwide spread fungus disease of cereals caused by some species of the genus *Fusarium* (*F. graminearum, F. culmorum*, and other ones). During vegetation the FHB may considerably reduce yields. The pathogenic fungi disturb normal development of grain, not only reducing yields. The colour of the infected grain is irregular, the grain is wrinkled, its shape is deformed and the size differentiated. The *Fusarium* species, are also effective producers of dangerous mycotoxins — products of secondary metabolism. Such contaminants accumulated in the kernel may affect safety as well as quality of the end products in food production. Deoxynivalenol (DON), or vomitoxin, is a typical *Fusarium* mycotoxin; contamination with this toxin may cause undesirable excessive foaming of beer, so-called gushing (Capettini *et al.* 2003). Furthermore, consumption of products containing a high level of DON may seriously damage the human or animal digestive tract or immune system.

The most efficient protection against FHB are integrated cultivation measures, application of fungicides, or breeding and introduction of new resistant varieties (Steffenson, 2003). However, breeding of the new resistant improved genotypes is not simple. Resistance to FHB was found to be a complex property genetically determined by loci with a relatively low effect and variable level of the effect of environment (Kolb *et al.* 2001). A very important finding was the association between FHB resistance and the morphological character — number of rows of an ear. Two-rowed barley generally has a higher level of FHB resistance than six-row barley (Heta & Hiura 1963; Takeda & Heta 1989).

One possibility of identifying FHB resistant genotypes and thus supporting the deriving of new more resistant genotypes is to use molecular markers in a breeding programme (Zhu *et al.* 2001; Armstrong *et al.* 2001; Mueller and Wolfenbarger 1999). Micro-satellites, a type of DNA markers, appear suitable for this purpose.

MATERIAL AND METHODS

Seven genotypes of spring barley with recognised resistance or susceptibility to FHB were tested using SSR markers (Tab. 1) in order to select. Parent genotypes crossing and production of DH lines.

Table 1

Origin and characterisation of the initial parents
Pochodzenie i charakterystyka form rodzicielskich

Variety / Line Odmiana / Linia	Pedigree Pochodzenie	Country Kraj	FHB reaction Reakcja na FHB	Row no. Liczba rzędów kłosa
Chevron	CIho 1111 (PI38061) = Landrace from Luzerne	CHE	resistant odporna	6
PEC210	Embrapa 128	BRA		2
CI4196	PI 64275 (hang wang ta mai) = Landrace from Beijing	USA		2
ZAO ZHOU 3	Cultivar in East China, Zhejiang University, Hangzhou	CHIN	medium resistant	2
6NDRFG-1	PI 615583; North Dakota Agric. Experiment Station, USA	USA	średnio odporna	6
Foster	Robust/ 3/ Hanzen/Glenn/Karl	USA	susceptible	6
PI383933	Kanto Nijo2= Ko. 1018/Kyoto Nakate from Japan	USA	wrażliwa	6

Genomic DNA was isolated from seedlings (6 days old) using the Dneasy Plant Mini Kit isolation kit (Qiagen, GE). The DNA concentration was assessed using spectrophotometry.

We analysed 35 primer pairs. The primers were selected to cover the whole genome uniformly. The "forward" primers, intended for liquid electrophoresis, were marked with fluorescence marks (VIC, NED, 6-FAM, PET). The reaction mixture for PCR, total volume 25µl, contained 0.5 U of *Taq* polymerase (Promega, USA), 1x aliquot buffer, 0.1 mM of each dNTP, 0.3 M of each primer and 30 ng of template DNA. The reaction conditions of PCR – initial denaturation 2 min. at 93°C, then 30 cycles – denaturation 1 min. at 93°C, annealing 2 min. at 54°C, elongation 2 min. at 72°C.

Separation of PCR products:

— Horizontal electrophoresis – 30% non-denaturation PAA gel in TBE buffer followed by colouring with silver (0.2% $AgNO_3$) and SYBR Gold (Molecular Probes, USA), ELFO determined at 300V,
— Vertical electrophoresis: 3.5% MetaPhor agarose gel in TAE buffer (MetaPhor agarose (Cambrex, USA): agarose (Serva, USA) ratio was 2:1), followed by colouring with ethidium bromide, ELFO determined at 78V,
— Liquid electrophoresis – assessed on the ABI PRISM – AVANT Genetic Analyzer (Applied Biosystems, USA), fluorescent fragment analyses at the polymer POP4.

The resulting electrophoreograms were converted to binary matrices represented by the presence (1) or absence (0) of resulting alleles and then evaluated by means of statistical software FreeTree version 9.1 using the UPGMA dendrogram construction method and

similarity coefficient according to Jaccard. The software TreeView version 1.6 was used for the graphical expression of the matrix.

RESULTS AND DISCUSSION

Seven parental spring barley genotypes were analysed by means of microsatellite markers. A total number of 35 microsatellite markers were tested, of which 29 were a polymorphic and provided 93 alleles. The remaining 6 SSR markers had a monomorphous character and were not used for testing of the prepared DH lines. Two SSR markers were found (Bmac139, Bmag131), which enable to differentiate susceptible and resistant genotypes. Results of the analyses were statistically evaluated and converted to dendrograms, which characterise genetic variability of the tested genotypes (Fig. 1). The dendrogram shows differentiation of the analysed genotypes distributed into 3 main groups. The first group are genotypes Chevron and PEC210, in literature recognised as resistant genotypes, the second group are genotypes CI4196, Zao Zhou 3 and 6NDRFG-1, validated in literature as medium resistant or resistant. The same group also includes the genotype Foster, which is declared in literature rather as a susceptible genotype. The last independent clade consists of the line PI383933, defined in literature as susceptible.

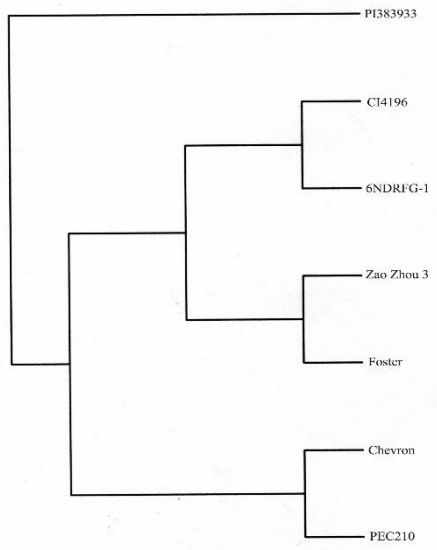

Fig. 1. Dendrogram characterising genetic affinity of the tested genotypes of spring barley
Rys. 1. Dendrogram charakteryzujący podobieństwo genetyczne badanych genotypów jęczmienia jarego

We compared various methods of detection of the SSR markers: the assessment of SSR markers on MetaPhor agarose gels coloured with ethidium bromide (Fig. 2), on PAA gels coloured with silver (Fig. 3 a) and SybrGold (Fig. 3 b) and assessment of the SSR markers on capillary electrophoresis (Fig. 4). All the abovementioned methods can be used for the

detection of polymorphism of microsatellite markers. Liquid electrophoresis provides the most accurate assessment of SSR markers.

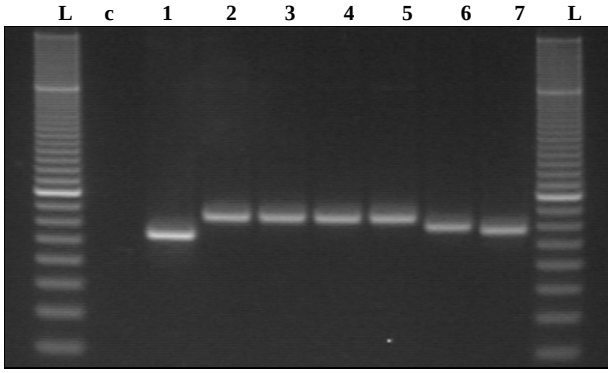

Fig. 2. MetaPhor agarose gel coloured with ethidium bromide — electrophoreogram of SSR products of the Bmag518 marker; (L) — 20 bp DNA ladder (Cambrex, USA), (c) —– negative control, (1) — Chevron, (2) — PEC210, (3) — Zao Zhou 3, (4) — 6NDRFG-1, (5) — CI4196, (6) — Foster, (7) — PI383933

Rys. 2. Żel agarozowy MetaPhor barwiony bromkiem etydyny — elektroforegram produktów SSR dla markera Bmag518; (L) – wzorzec DNA z interwałem 20bp (Cambrex, USA), (c) – kontrola negatywna, (opisy szczegółowe jak w tekście angielskim)

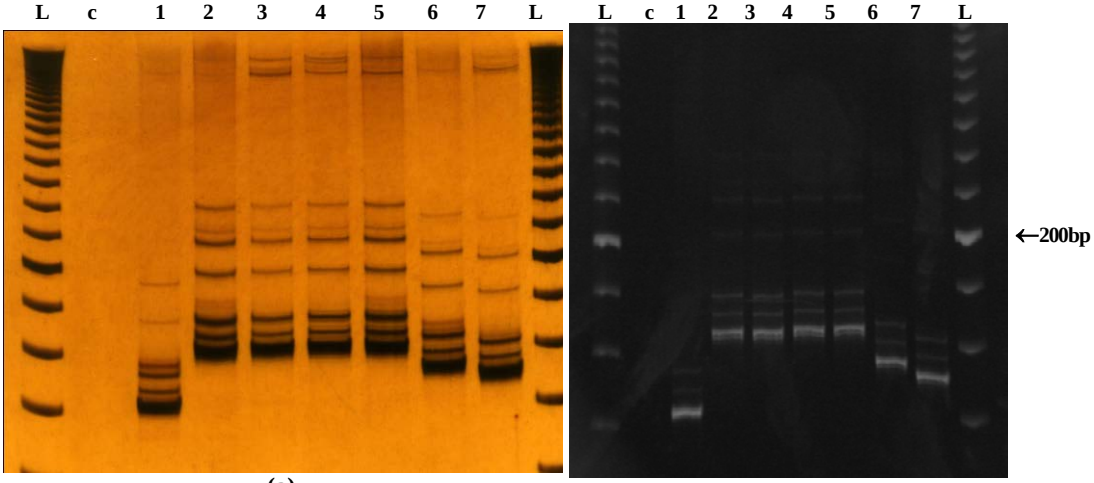

Fig. 3. (a) PAA gel coloured with silver, (b) PAA gel coloured with SybrGold — electrophoreograms of SSR products of the Bmag518 marker; (L) — 20bp DNA ladder (Cambrex, USA), (c) — negative control, (1) — Chevron, (2) — PEC210, (3) — Zao Zhou 3, (4) — 6NDRFG-1, (5) — CI4196, (6) — Foster, (7) — PI383933

Rys. 3. (a) Żel poliakrylamidowy barwiony srebrem, (b) żel poliakrylamidowy barwiony złotem (SYBRGold) – elektroforegramy produktów SSR dla markera Bmag518 (opisy szczegółowe jak w tekście angielskim)

The assessment with vertical electrophoresis on PAA gels is a less accurate method, but sufficiently sensitive. As a convenient method of DNA visualisation on gels we recommend staining with silver rather than colouring with SybrGold, it appears to be more sensitive and also makes the preservation of gels possible. The tested methods showed that the least sensitive method of detection of SSR markers was analysis on horizontal electrophoresis on MetaPhor agarose gels. However, this method is undemanding and can be considered as an alternative in all cases where more accurate vertical electrophoresis or liquid electrophoresis is not available.

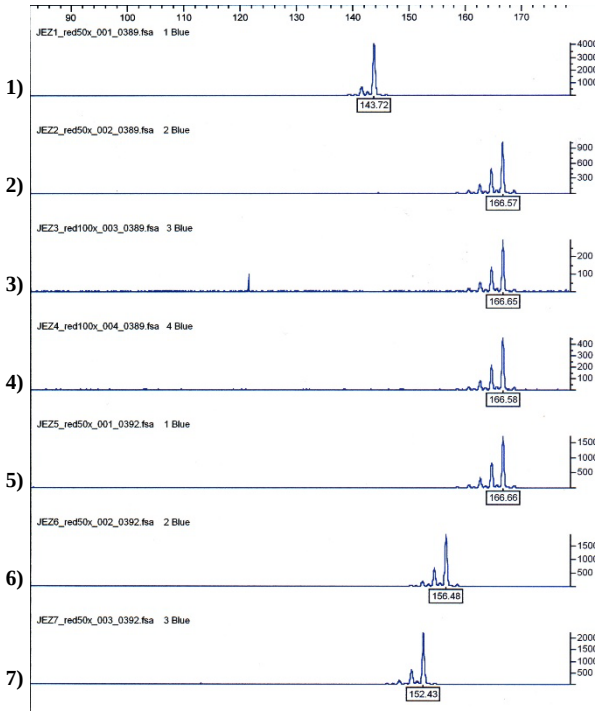

Fig. 4. Result of analysis with capillary electrophoresis — SSR products of the Bmag518 marker;— Chevron, (2) — PEC210, (3) — Zao Zhou 3, (4) — 6NDRFG-1, (5) — CI4196, (6) — Foster, (7) — PI383933

Rys.4. Wynik analizy elektroforezą kapilarną — produkty SSR dla markera Bmag518 (opisy szczegółowe jak w tekście angielskim)

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