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ASSOCIATION MAPPING OF EFFECTIVE LEAF RUST RESISTANCE GENES IN COLLECTION OF WINTER WHEAT

MAPOWANIE ASOCJACYJNE EFEKTYWNYCH GENÓW ODPORNOŚCI NA RDZĘ BRUNATNĄ W KOLEKCJI PSZENICY OZIMEJ

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Streszczenie. Rdza brunatna wywoływana przez *Puccinia triticina* przyczynia się do zmniejszenia plonu pszenicy na świecie. Opracowanie technologii sekwencjonowania następnej generacji (NGS) pozwala na skanowanie genotypów. Wybrane markery zasocjowane z cechami istotnymi rolniczo mogą być wykorzystane do selekcji genomowej. W prezentowanych badaniach 94 haplotypy zostały scharakteryzowane po względem 4599 markerów typu polimorfizm pojedynczy nukleotydu (SNP), reprezentujących dane DArTseq. Dane te były poddane analizie asocjacyjnej z rdzą brunatną w stadium rośliny dojrzałej przez trzy lata w trzech lokalizacjach. W celu identyfikacji asocjacji pomiędzy haplotypami a powierzchnią pod krzywą rozwoju choroby (AUDPC) i końcową oceną rdzy brunatnej (FLR) zastosowano mieszany model liniowy (MLM). Zidentyfikowano 13 markerów, które wyjaśniały 6,2–14,6% zmienności w AUDPC. Markery te reprezentowały pojedyncze loci rozmieszczone na chromosomach 1A, 2A, 2B, 3D, 4A, 6B i 7B. Markery zasocjowane z FLR wyjaśniały 8,5–21,0% zmienności. W przypadku FLR 20 markerów zasocjowanych ze zmienności fenotypową było rozmieszczonych na 10 chromosomach pszenicy. Chromosomy 2B, 3B i 5B były reprezentowane przez serie markerów. Scharakteryzowane genotypy mogą być krzyżowane w celu nagromadzenia markerów zasocjowanych ze zwiększeniem odporności na rdzę brunatną. Opcjonalnie, w celu precyzyjnej lokalizacji efektów stwierdzonych w badaniach asocjacyjnych, w odniesieniu do wcześniej zidentyfikowanych genów można testować dodatkowo populacje mapujące. Proponowane markery mogą być bezpośrednio wykorzystane w selekcji genomowej w celu zwiększenia odporności na rdzę brunatną w nowych odmianach pszenicy.

Key words: association mapping, DArTseq, leaf rust, *Triticum aestivum.*
Słowa kluczowe: mapowanie asocjacyjne, DArTseq, rdza brunatna, *Triticum aestivum.*

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is hexaploid plant with the genome size of 17 Gb (2n = 6x = 42) containing about 80% of repetitive sequences (Brenchley et al. 2012). Next to rice and maize, wheat is the third provider of calories in the world with the 729 million tons of total production. Area of cultivation in the world and in Poland accounts respectively 221 million

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and 2.3 million ha (FAOSTAT 2014). Leaf rust (Lr) disease caused by *Puccinia triticina* (Pt) reduces leaf assimilation area and contributes to decrease of yield by 15% (Strzembicka et al. 2013). Exploring of genetic variation is the most economical way to protect plants from this fungal disease. Pyramidization of adult plant resistance genes responsible for delay in pathogen development together with effective race specific genes may provide durable effects. Over 70 *Lr* genes were reported at Komugi – Wheat Genetic Resources Database (McIntosh et al. 2013).

Rapid progress in molecular biology entailed evolution of marker system and their application in selection process. Marker assisted selection (MAS) is often applied in selection of individual, selected qualitative traits (He et al. 2014). Development of next generation sequencing (NGS) technologies was connected with prompt and cost-efficient genotyping and sequencing of plant genomes (Deschamps et al. 2012). In result sets of genotypes can be scanned for selection of markers associated with changes of phenotype, and further application in genomic selection (GS). GS allows for complex characteristics of genotype and is applied for improvement of qualitative traits in large, diverse populations. In wheat, association mapping was applied for identification of markers related to agronomical important traits including disease resistance (Kertho et al. 2015; Ren et al. 2015).

Number of genotyping methods based on NGS was proposed including genotyping-by-sequencing (GBS), complexity reduction of polymorphic sequences (CRoPS), and restriction site-associated DNA sequencing (RAD-seq) (Poland et al. 2012; Saintenac et al. 2013; Sánchez--Sevilla et al. 2015). Prior to NGS based methods Diversity Arrays Technology (DArT) was applied for scanning of cereals genome (Wenzl et al. 2004; Bolibok-Brągoszewska et al. 2009; Zhang et al. 2011). This hybridization-based method used libraries developed using restriction enzyme targeting single copy, expressed regions of genomes (Gawronski et al. 2016). Next, sequencing of DArT libraries using NGS methods resulted in DArTseq technique (Li et al. 2015). In the present study we used haplotype scored with DArTseq method to identify regions related with adult plant resistance to leaf rust in common wheat.

MATERIAL AND METHODS

Plant material

Table 1. Common wheat cultivars used for association mapping
Tabela 1. Odmiany pszenicy zwyczajnej wykorzystane w badaniach asocjacyjnych

Cultivar (symbol) Odmiana (oznaczenie)	Year of registration Rok rejestracji	Country of origin Kraj pochodzenia	Breeder Hodowca
Estivus (PT.47)	2012	PL	Strube Research GmbH & Co. KG
Expert (PT.67)	2007	FR	Syngenta Seeds SAS
Forum (PT.36)	2012	PL	Nordsaat Saatzucht GmbH
Franz (PT.40)	2014	PL	Nordsaat Saatzucht GmbH
Hybery (PT.70)	2010	PL	Saaten-Union
KWS Dakota (PT.17)	2014	PL	KWS Lochow GmbH
KWS Loft (PT.20)	2014	PL	KWS Lochow GmbH
KWS Magic (PT.19)	2012	PL	KWS Lochow GmbH
KWS Tramp (PT.23)	2014	PL	KWS Lochow GmbH
Patras (PT.27)	2012	PL	Deutsche Saatveredelung AG
Platin (PT.46)	2012	PL	Strube Research GmbH & Co. KG
Praktik (PT.41)	2012	PL	RAGT 2n
Speedway (PT.37)	2012	PL	Nordsaat Saatzucht GmbH
Tobak (PT.4)	2011	PL	W. von Borries-Eckendorf GmbH & Co. KG

Breeders number (PT) is given in brackets – W nawiasie podano numer hodowlany (PT).

In total, 94 genotypes of common wheat were studied including 14 cultivars registered in Poland and France (Table 1) and 80 breeding lines (Małopolska Plant Breeders, Poland). Advanced breeding lines were annotated PT.1-3, PT.5-16, PT.18, PT.21-22, PT.24-26, PT.28-5, PT.38-39, PT.42-5, PT.48-65, PT.68-69, and PT.71-99. All genotypes were high yielding, valuable starting material for the new breeding cycle deposited by breeder (TD).

Leaf rust assessment

LR resistance was assessed in two locations at Lower Silesian region (Henryków: 50º39'07.3"N, 17º00'36.7"E, Kobierzyce: 50º58'02.7"N, 16º55'13.9"E), and single location in Subcarpathian region (Mikulice: 50º00'26.7"N, 22º26'25.2"E) for three years (2012–2014). The lines were sown in two 1 m long rows at the 20 x 7 cm spacing. Chemical protection was not applied during growth and leaf rust was scored under natural infection. Experiment was regularly inspected and scoring was carried out in 7–10 days intervals since epidemics occurred to allow clear differentiation among entries (the period from start of heading to early grain filling stage). Disease was assessed on a whole plot basis using a 0–9 scale (McNeal et al. 1971), where 0 is immune and 9 is very susceptible (Ziems et al. 2014). Disease was recorded 3-times during vegetative period and scores were used to calculate Area Under Disease Progress Curve (AUDPC) (Shaner and Finney 1977; Finckh et al. 1999).

Genotyping

Total genomic DNA was isolated from young leaves collected from 2-week old plants representing bulk of 12–15 individuals per genotype as previously described (Milligan 1992). DNA integrity was tested on agarose gels, while its quantity was measured spectrophotometrically. Genomic DNA was sent to Diversity Array Technology (Bruce, Australia) for profiling of approximately 50.000 SNP and DArT loci using wheat GBS 1.0 service.

Statistical analysis

In order to meet requirements of TASSEL 5 (http://www.maizegenetics.net, Bradbury et al. 2007) binary segregations of selected SNPs were reformatted and annotated. To obtain physical positions, sequences of DArTseq markers were queried against the wheat genome (Release 3.1 available from ftp://ftp.ensemblgenomes.org/pub/release-31/plants/fasta/triticum_ _aestivum/dna/) using BBmap tool (http://jgi.doe.gov/data-and-tools/bbtools/). Hits below threshold level of 13 (that corresponds to probability of below 95%) were discarded, and for each marker a single position with maximum probability was selected in Excel.

PAST – PAlaeontological STatistics (Hammer et al. 2001) was used for cluster analysis based on Dice similarity coefficients. The population structure was estimated using silicoDArT markers and the software STRUCTURE version 2.3.3 (Pritchard et al. 2000). For population structure analysis, the 14804 best quality DArTseq markers showing low percentage of missing data (below 8.5%) were selected. Number of iterations was set at 100.000 with K value in the range of 1–10 (from K = 1 to K = 10) with three replications. Separate subpopulations were designated based on frequencies of alleles in each loci. Maximum values of ΔK(K) indicated

the real number of sub-populations in the population of 94 individuals. Analysis of variance for mixed ANOVA/ANCOVA model was carried out using Statistica v. 9.0 (Statsoft Inc. USA). Genotypes and environments were treated as random effects. Leaf rust evaluations were made on two replications for the genotype in each of the seven environments (Tsilo et al. 2014). Three varieties (Tonacja, Muszelka and Ozon) were sewed as control spreader lines every 20 plot. Broad-sense heritability (h^2) estimates were calculated from variance components according to Cherif et al. (2010).

DArTseq alleles with a frequency below 5 % were removed from datasets prior to association mapping. Mixed linear model (MLM) was applied with the five first principal components as covariates. Only markers revealing significant associations at $p \leq 0.01$ were taken into account.

RESULTS

Phenotypic evaluation

AUDPC showed high variation in all localizations and years for all the studied genotypes (Table 2). During winter 2011/2012 rapid temperature decrease below –10ºC together with missing ice sheet eliminated experiments set up in two locations. Minimum AUDPC values ranged from 20.2 to 61.9 and maximum values amounted 1336.7 in selected environments. Analysis of variance (Table 3) showed that variation of AUDPC was significantly affected by genotype ($F = 5.59$, $p < 0.0001$), and interaction of genotype with localization ($F = 1.62$, $p < 0.0003$). Interaction of localization with the year ($F = 39.16$, $p < 0.0001$) also strongly influenced observed variation in reaction to LR measured as AUDPC and estimate of broad-sense heritability (h^2) was 15.2 %. Due to low heritability of AUDPC we used also final score of leaf rust symptoms (FLR). The score was dependent on genotype ($F = 2.38$, $p < 0.037$) and showed better heritability of 26.3% (Table 3).

Table 2. Variation of AUDPC and FLR in three locations and years
Tabela 2. Zmienność AUDPC i FLR w trzech lokalizacjach i latach

Location Lokalizacja	Year Rok	AUDPC				FLR			
		mean średnia	min.	max.	SD	mean średnia	min.	max.	SD
Henryków	2013	267.0	20.2	997.2	273.1	2.9	0.0	6.0	1.8
	2014	461.3	61.9	954.0	260.7	4.8	2.0	7.0	1.3
Kobierzyce	2013	611.2	21.7	1336.7	473.4	5.1	0.0	8.0	3.1
	2014	452.0	23.3	1160.4	288.9	3.9	0.0	7.0	1.7
Mikulice	2012	193.8	55.8	1323.9	250.2	2.1	0.0	7.0	2.3
	2013	278.4	40.3	969.8	192.3	4.3	1.0	7.0	1.5
	2014	297.8	38.8	1004.4	218.6	3.7	0.0	7.0	2.0

AUDPC – Area Under Disease Progress Curve – powierzchnia pod krzywą rozwoju choroby, FLR – Final Leaf Rust score – końcowa ocena rdzy brunatnej, SD – standard deviation – odchylenie standardowe.

Table 3. Results of ANOVA analysis of AUDPC and FLR scores based on genotype, localization and year as a random factors
Tabela 2. Wyniki analizy wariancji AUDPC i FLR na podstawie genotypów, lokalizacji i lat jako czynników losowych

Source of variation Źródło zmienności	Effect – Efekt		Error – Błąd		F	p
	df	MS	df	MS		
AUDPC						
Genotype Genotyp (1)	93	325 363	68.79	58 255	5.59	0.0000
Localization Lokalizacja (2)	2	4 166 718	2.13	1 468 094	2.84	0.2509
Year Rok (3)	2	291 740	1.99	1 527 603	0.19	0.8397
1 × 2	186	63 021	210.93	38 790	1.62	0.0003
1 × 3	186	34 254	186.00	39 139	0.88	0.8179
2 × 3	2	1 532 488	186.00	39 139	39.16	0.0000
FLR						
Genotype Genotyp (1)	93	60.26	13.64	25.31	2.38	0.0371
Localization Lokalizacja (2)	2	273.13	1.92	132.00	2.07	0.3318
Year Rok (3)	2	139.37	2.01	156.05	0.89	0.5276
1 × 2	186	30.15	247.13	51.19	0.59	0.9999
1 × 3	186	51.81	186.00	51.09	1.01	0.4619
2 × 3	2	155.33	186.00	51.09		0.0502

AUDPC – Area Under Disease Progress Curve – powierzchnia pod krzywą rozwoju choroby, FLR – Final Leaf Rust score – końcowa ocena rdzy brunatnej, df – degrees of freedom – stopnie swobody, MS – the mean sum of squares – średnia suma kwadratów.

Population structure and association mapping

DArTseq analysis revealed SNP polymorphism in 6558 loci. Physical in-silico mapping was used to localize markers on genome of wheat. We successfully determined localization for 4599 SNP markers. Additionally, 14804 high quality dominant silicoDArT markers were used to infer population structure. Two subpopulations could be discerned based on ΔK(K) values (Fig. 1). Cluster analysis based on Dice similarities showed that three pairs of lines were closely (>0.95) related (PT2-PT3, PT33-PT34, and PT39-PT79) and lines containing higher percentage of missing data were eliminated from the dataset.

MLM was applied to identify associations between 4599 SNP markers and leaf rust symptoms determined with AUDPC and FLR parameters. In case of AUDPC we found 13 markers that explained from 6.2 to 14.6% of variation in AUDPC (Table 4). The associated markers generally presented unique loci distributed on chromosomes 1A, 2A, 2B, 3D, 4A, 6B and 7B. However, when using 40 Mbp window, the regions on 1A, 2B, and 4A can be pooled. At chromosome 2B predicted distance between markers 1081114.6 and 2268857.48 was 3.3 Mbp only. Distribution of p-values over chromosomes is shown on Manhattan plot (Fig. 2).

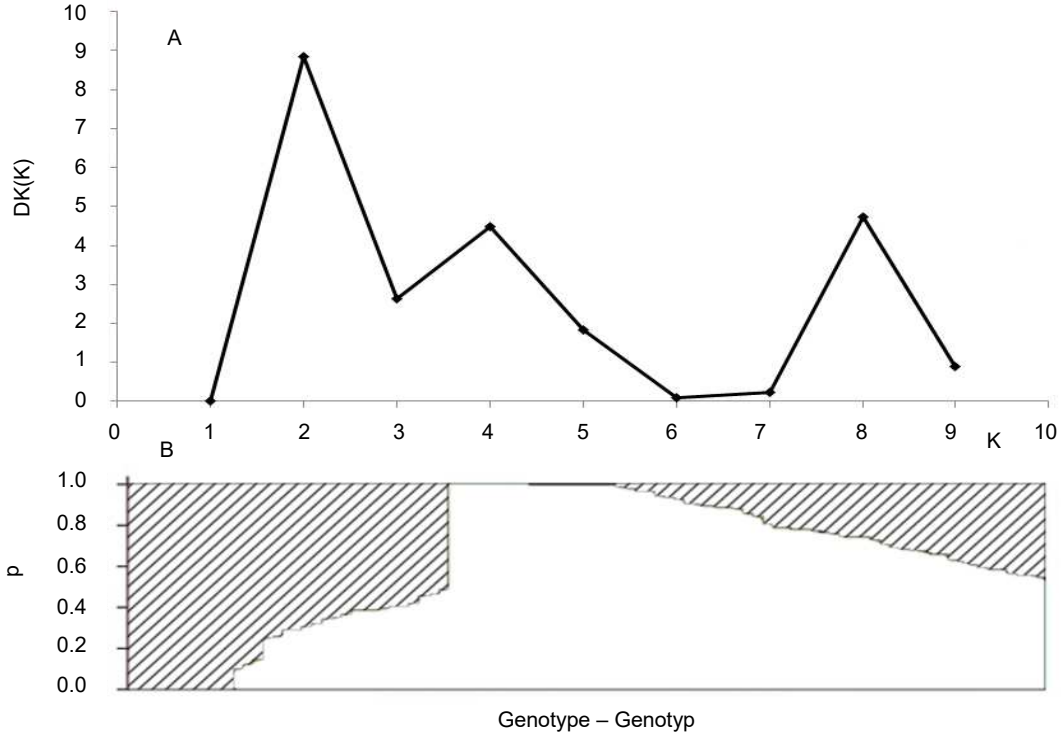

Fig. 1. Analysis of population structure: A – the highest value of ΔK(K) indicates the presence of two subpopulations (K) in the set of 94 genotypes, B – distribution of genotypes in two subpopulations (marked with dashed lines and plain white). Values of p correspond to probability that genotype represents the first or the second subpopulation
Ryc.1. Analiza struktury populacji: A – największa wartość ΔK(K) wskazuje na obecność dwóch subpopulacji (K) w zestawie 94 genotypów, B – rozmieszczenie genotypów w dwóch subpopulacjach (oznaczonych ukośnymi liniami i białym polem). Wartości p odpowiadają prawdopodobieństwu, że genotyp reprezentuje pierwszą lub drugą subpopulację

Table 4. Markers associated with Area Under Disease Progress Curve (AUDPC)
Tabela 4. Markery zasocjowane z powierzchnią pod krzywą rozwoju choroby (AUDPC)

Marker Marker	Localization Lokalizacja (Mbp)	Df	p	R^2 [%]	Add	Dom	SNP (effect) – (efekt)	
1167826.16	1A(12.8)	89	0.005	10.0	65.2	743.8	A(–678.6)	C (–809.0)
1038442.51	1A(51.0)	89	0.009	6.2	–	–	G(–282.2)	
1218489.22	2A(253.5)	88	0.009	9.2	148.5	13.7	C(134.8)	T (–162.2)
1081114.6	2B(311.8)	83	0.006	10.5	–210.2	38.4	C(–248.6)	T (171.7)
2268857.48	2B(315.1)	64	0.009	8.3	–	–	A(265.7)	
992276.39	2B(344.3)	79	0.005	10.7	–107.2	613.7	C(–720.9)	T (–506.4)
2262703.20	3B(95.9)	89	0.006	9.5	100.8	–239.4	C(340.2)	T (138.6)
1396283.13	3D(4.3)	87	0.005	7.7	–	–	C(–293.2)	
1190058.59	4A(169.5)	63	0.008	9.6	–	–	C(327.0)	
1109386.42	4A(193.7)	69	0.006	8.2	–	–	C(–365.9)	
1042298.66	4A(215.8)	67	0.004	14.6	–247.6	55.2	A(–302.8)	C (192.4)
1125870.58	6B(165.8)	85	0.001	10.2	–	–	A(305.6)	
1165713.27	7B(34.1)	70	0.004	12.3	–8.4	–335.6	A(327.2)	T (343.9)

Df – degrees of freedom – stopnie swobody, p – significance levels for the F-tests – poziomy istotności dla testów F, R^2 – regression coefficient for generalized least squares model – współczynnik dla modelu regresji liniowej najmniejszych kwadratów, Add – additive effect – efekt addytywny, Dom – dominance effect – efekt dominacji.
Position of SNP in marker sequence was attached to marker name. Predicted physical localization on chromosome is given. Changes of AUDPC values are predicted for the selected nucleotides (SNP) – Pozycję SNP w sekwencji markera dołączono do nazwy markera. Podano przewidywaną lokalizację fizyczną na chromosomach. Dla wybranych nukleotydów (SNP) przewidziano zmiany wartości AUDPC.

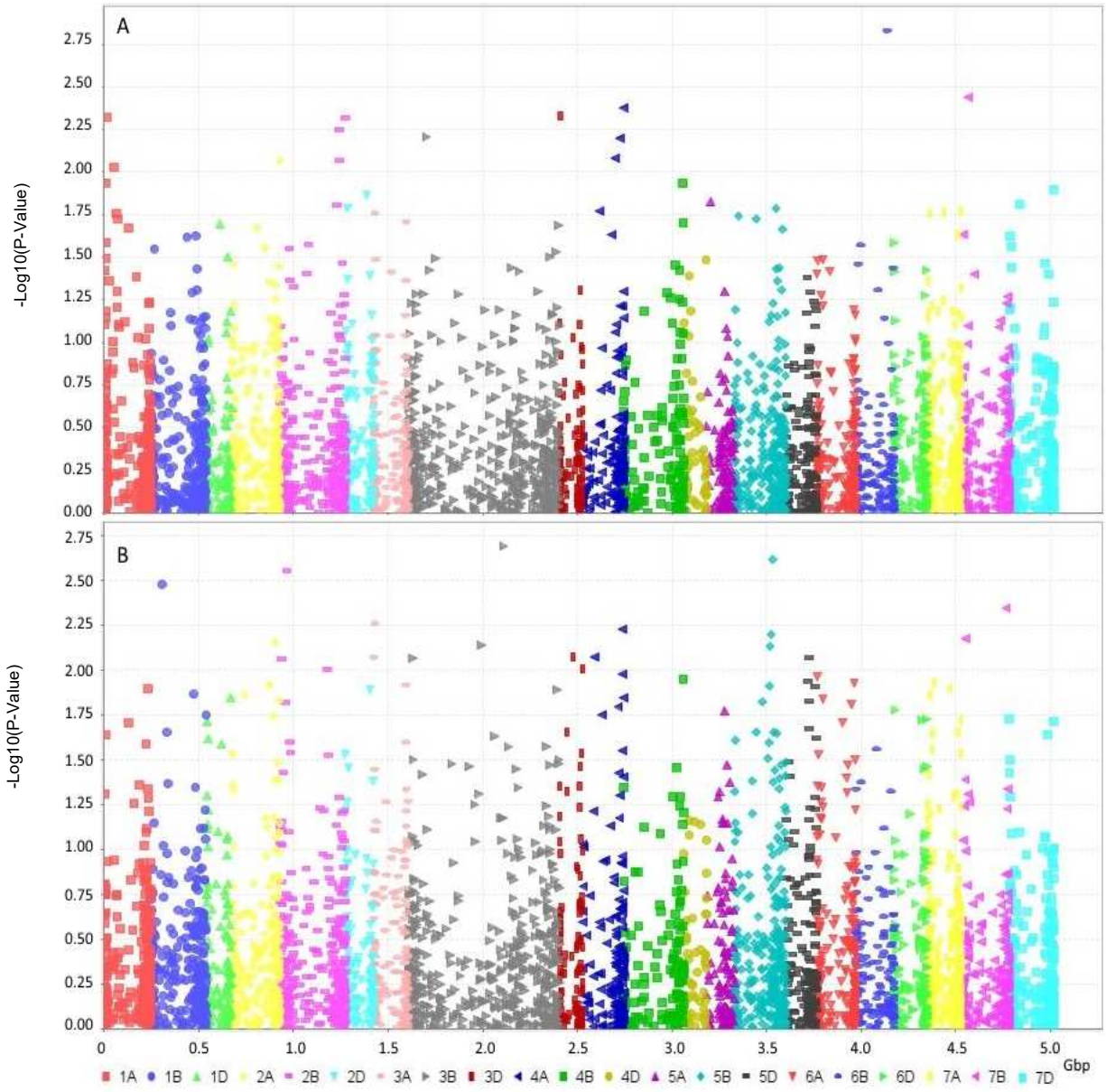

Fig. 2. Manhattan plot showing distribution of p-values for AUDPC (A) and FLR (B) over chromosomes. Threshold of significance is 2.00 that is equal to p = 0.01. Distances between markers expressed in Gbp (10^9 base pairs)

Ryc. 2. Wykres punktowy typu Manhattan pokazujący rozkład wartości p dla AUDPC (A) i FLP (B) na chromosomach. Wartość krytyczna istotności wynosi 2.00, co odpowiada p = 0.01. Odległości pomiędzy markerami wyrażono w Gbp (10^9 par zasad)

For FLR, 20 markers associated with phenotypic variation were distributed over 10 wheat chromosomes. Each of 2B, 3B and 5B chromosomes was represented by three markers. In case of 5B these markers were located in region 187.8-201.8 Mbp. Significant effects corresponding to selected nucleotides were predicted. The markers associated with FLR explained from 8.5 to 21.0 % of variation (Table 5).

Table 5. Markers associated with Final Leaf Rust score (FLR)
Tabela 5. Markery zasocjowane z końcową oceną rdzy brunatnej (FLR)

Marker Marker	Localization Lokalizacja (Mbp)	Df	p	R^2 (%)	Add	Dom	SNP (effect) – (efekt)	
2268264.7	1B(58.5)	79	0.003	16.8	–1.2	–0.5	A(–0.718)	G (1.765)
980420.47	2A(229.7)	89	0.007	12.3	–0.7	–2.6	C(1.899)	T (3.304)
998362.7	2B(8.6)	66	0.009	10.9	–	–	C(–1.534)	
1003352.25	2B(36.8)	91	0.003	14.4	–0.5	–2.6	C(2.113)	T (3.161)
995362.32	2B(249.7)	89	0.010	11.5	0.9	0.5	C(0.395)	T (–1.414)
1128097.64	3A(1.2)	78	0.009	13.4	–0.7	–2.5	A(1.718)	G (3.19)
1233670.35	3A(6.6)	68	0.006	21.0	1.0	–1.5	A(2.501)	G (0.584)
985909.31	3B(24.9)	88	0.009	8.5	–	–	C(2.118)	
1090318.34	3B(387.6)	91	0.007	11.9	1.0	2.5	C(-1.518)	G (–3.439)
3021892.8	3B(502.6)	91	0.002	15.2	1.8	–3.4	A(5.154)	T (1.62)
2261562.59	3D(72.6)	86	0.008	12.0	0.6	–2.8	C(3.428)	T (2.214)
2322447.32	3D(121.7)	76	0.010	12.8	–0.9	–1.2	A(0.335)	G (2.164)
996058.26	4A(59.9)	75	0.009	14.3	–0.9	3.4	A(-4.248)	T (–2.537)
1211884.7	4A(208.8)	72	0.006	15.4	–1.1	1.4	C(-2.482)	T (-0.227)
980383.11	5B(187.8)	90	0.007	12.0	1.0		C(-2.658)	T (-4.633)
993999.36	5B(194.6)	91	0.006	12.3	–0.9	1.6	C(-2.467)	T (-0.643)
1030395.38	5B(201.8)	91	0.002	11.1	–	–	C(1.858)	
1212116.45	5D(121.7)	67	0.009	11.4	–	–	C(2.033)	
2260678.59	7B(18.0)	89	0.007	13.0	–0.9	–0.2	C(-0.645)	G (1.069)
1028859.50	7B(233.3)	83	0.005	14.3	–0.9	–1.3	C(0.356)	G (2.184)

Df – degrees of freedom – stopnie swobody , p – significance levels for the F-tests – poziomy istotności dla testów F, R^2 – regression coefficient for generalized least squares model – współczynnik dla modelu regresji liniowej najmniejszych kwadratów, Add – additive effect – efekt addytywny, Dom – dominance effect – efekt dominacji. Position of SNP in marker sequence was attached to marker name. Predicted physical localization on chromosome is given. Changes of FLR values are predicted for the selected nucleotides – Pozycję SNP w sekwencji markera dołączono do nazwy markera. Podano przewidywaną lokalizację fizyczną na chromosomach. Dla wybranych nukleotydów przewidziano zmiany wartości FLR.

DISCUSSION

Heritability estimates for leaf rust severity calculated in our study were low both for AUDPC and FLR scores (15.2% and 26.3%, respectively). In another studies higher estimates of heritability (0.33–0.92) for adult plant leaf rust resistance were recorded (Bjarko and Line 1988; Buerstmayr et al. 2014; Tsilo et al. 2014). These discrepancies can be explained by high variation of *Puccinia triticina* population naturally occurring in the three regions of southern Poland resulting from natural infection. Our results suggest, that variable weather conditions in stages from plant heading to early maturity influence more dynamics of disease development that final leaf rust score.

Advanced marker technologies including diversity array technology (DArT) markers were applied to find associations with resistance to foliar diseases, and grain yield in wheat (Crossa et al. 2007; Bansal et al. 2013; Zhao et al. 2013; Gowda et al. 2014; Juliana et al. 2015; Kertho et al. 2015; Aoun et al. 2016; Li et al. 2016). SNP polymorphisms can be directly associated

with resistance to leaf rust as previously found in case of *Lr1* and *Lr34* gene (Tyrka et al. 2004; Cao et al. 2010). However, the accuracy of genomic selection depends on the relatedness between the members of the set in which marker effects are estimated based on evaluation data and the types for which performance is predicted (Gowda et al. 2014). Moreover, association mapping can produce spurious marker-trait associations if not corrected for population structure and relatedness among individuals (Kertho et al. 2015). In the present study, high-yielding materials selected by breeders were used for association mapping under natural infection to identify markers useful for effective leaf rust resistance genes that can be applied in on-going breeding process. DArT markers can be easily adapted as tool for selection after allele mining through re-sequencing of specific fragments. However, the development of gene specific primers in wheat may be hampered by the size of genome (17 Gbp), the high repeat content of about 80%, and by the high fragment homology resulting from the presence of the three genomes (A, B and D) and gene family members (Babben et al. 2015).

Association mapping (AM) is an important tool to discover markers applicable in wide genetic background. Most of the markers identified using AM approach validate regions or quantitative trait loci (QTL) already reported, but some new chromosome regions for disease resistance can also be identified in the wheat genome (Crossa et al. 2007; Maccaferri et al. 2010). In numerous association studies stringent threshold limiting significance of association was set to 0.001 (Lex et al. 2014; Kherto et al. 2015; Aoun et al. 2016). However, in case of TASSEL 5.0 false discover rate of 0.01 is accepted (Li et al. 2016) and high R^2 values (6.2–21.0) found in our studies were attributed to the selected markers of leaf rust resistance. Another factor that possibly contributed to low p-values is the low number of genotypes used for the association mapping.

Association studies can lead to discovery of new regions influencing leaf rust resistance. Mixed linear models identified 65 significant markers associated with leaf rust in set of 567 winter wheat landrace accessions. Eleven effects associated with resistance to Lr identified on chromosomes 3A, 5A, and 6D, were not previously reported in *T. aestivum* (Kertho et al. 2015). Similarly, six regions on 7AL, 1D, 2BL, 5BL, and 7AS were possibly novel loci for leaf rust resistance (Li et al. 2016).

Using both AUDPC and FLR we found 4 common regions with markers associated to leaf rust. These markers were derived from 2AL (229.7–253.5 Mbp), 2BL (249.7–311.8 Mbp), 4AL (208.8–215.8 Mbp), and 7BS (18.0–34.1 Mbp). Regions located at chromosome 2A showed highly consistent associations with leaf rust in durum wheat (Maccaferri et al. 2010) and common wheat (Kertho et al. 2015). *Lr11* gene is located at 2A (McIntosh et al. 2013) and was suggested to be responsible for this effect (Kertho et al. 2015). Mapping information for *Lr11* is not available to allow for comparison with markers found in chromosomes where these resistance genes are located. Another gene located on short arm of chromosomes 2A is *Lr17*. In this region (9.7 cM – *QLr.cim-2AL* – 12.7 cM), 78 DArTseq markers were identified in common wheat (Li et al. 2015). On consensus map, marker 980420 associated with FLR was found outside of this region, and may possibly be related with unknown Lr resistance gene.

Genes *Lr50* and *Lr58* derived respectively from *T. timopheevii* subsp. *armeniacum*, and *Ae. triuncialis* were identified on 2BL chromosome arm (Brown-Guedira et al. 2003; Kuraparthy et al. 2007). Effective gene located on 4AL is *Lr28* introduced from *Ae. speltoides* (Cherukuri

et al. 2005). In association studies, LG_Wsnp12474 associated with LR on chromosome 4A was positioned adjacent to the seedling resistance genes *Lr28* (Gowda et al. 2014). In durum wheat *Lr72* gene was mapped on 7BS chromosome arm (Herrera-Foessel et al. 2014). Association of DArT markers with leaf rust resistance confirm significance of markers from chromosomes 2BL, 7BS (Bansal et al. 2013).

Important adult plant resistance to leaf rust is provided by *Lr46* gene located on long arm of chromosome 1B. We found single DArTseq marker (2268264) physically mapped to 1B chromosome at position of 58 Mbp (total length of 1B chromosome accounts for 295 Mbp). BLAST search in EnsemblePlants database confirmed localization of this sequence on chromosome 1B. *Lr46* gene was located distally on 4BL, and flanked by DArTseq markers 2289154 and 999754 (Li et al. 2015). Localization of marker 2268264 in region of *Lr46* cannot be confirmed and need additional validation. Development of consensus wheat genetic map integrating microsatellite, DArT and DArTseq markers can be useful for this purpose (Tyrka at al. 2015). Similarly, five DArTseq markers associated with AUDPC and FLR were physically located on chromosome 4A (Table 4,5) where *Lr28* and *Lr30* genes were previously located. Only for 1042298 marker, genetic position was available and agreed with physical mapping, however in the lack of integrated maps no structural comparisons are feasible.

CONCLUSIONS

The characterized genotypes can be crossed to accumulate of markers associated with improved resistance. Alternatively, mapping populations can be developed to map more precisely the resistance loci identified in association studies. The markers proposed can be directly used in genomic selection to improve leaf rust resistance in modern wheat cultivars.

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Abstract. Leaf rust disease caused by *Puccinia triticina* contributes to decrease of wheat yield worldwide. Development of Next Generation Sequencing (NGS) technologies allows for scanning of genotypes. Next, selected markers associated with agronomically important traits can be used for genomic selection. In the present study 94 haplotypes scored for 4599 Single Nucleotide Polymorphism (SNP) markers extracted from DArTseq data were associated with reaction to leaf rust at adult stage estimated for three years in three locations. Mixed Linear Model (MLM) was applied to identify associations between haplotypes Area Under Disease Progress Curve (AUDPC) and Final Leaf Rust score (FLR). We found 13 markers that explained from 6.2 to 14.6% variation in AUDPC. These markers generally presented unique loci distributed over chromosomes 1A, 2A, 2B, 3D, 4A, 6B and 7B. The markers associated with FLR explained from 8.5 to 21.0% of variation. For FLR, 20 markers associated with phenotypic variation were distributed over 10 wheat chromosomes. Chromosomes 2B, 3B and 5B were represented by multiple markers. The characterized genotypes can be crossed to accumulate markers associated with improved leaf rust resistance. Alternatively, mapping populations can be developed to map more precisely the resistance loci identified in association studies. The markers proposed can be directly used in genomic selection to improve leaf rust resistance in modern wheat cultivars.

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