FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2014, 312(31), 105–116

Katarzyna MOLIK, Edyta PAWŁOWSKA, Zuzanna KANTAREK, Paweł MILCZARSKI

QTL ANALYSIS OF CHLOROPHYLL CONTENT AND CHLOROPHYLL FLUORESCENCE PARAMETER IN MAPPING POPULATION OF RYE

ANALIZA QTL ZAWARTOŚCI CHLOROFILU I PARAMETRU FLUORESCENCJI CHLOROFILU W POPULACJI MAPUJĄCEJ ŻYTA

Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland

Streszczenie. Zdefiniowano QTL dla zawartości chlorofilu (Chc) i maksymalnej fotochemicznej aktywności PSII (F_v/F_m) żyta. Materiał do badań stanowiła populacja RIL mieszańca międzyliniowego DS2 × RXL10, złożona z 70 osobników pokolenia F_7 . Otrzymane wyniki poddano analizie statystycznej; stwierdzono istotne zróżnicowanie obu cech u form rodzicielskich i osobników w populacji mapującej. Nie zaobserwowano istotnej statystycznie korelacji pomiędzy Chc a F_v/F_m ; obliczone współczynniki odziedziczalności w szerokim zakresie (H_B) wynosiły odpowiednio 56 i 53%. Przy LOD $\ge 2,0$ wyznaczono 19 regionów QTL, w tym 9 dla zawartości chlorofilu i 10 dla całkowitej wydajności fotochemicznej PSII. Lokalizowały się one głównie w dystalnych lub centromerowych obszarach chromosomów: 1R, 3R, 5R, 6R, 7R, przy czym na chromosomach 1R, 5R i 6R znalazły się regiony wspólne dla QTL F_v/F_m1 i F_v/F_m5 (1R), Chc8 i F_v/F_m9 (5R) oraz F_v/F_m3 i F_v/F_m10 (6R). Otrzymane QTL dostarczają wstępnej wiedzy o dziedzicznym podłożu zawartości chlorofilu w życie i maksymalnej fotochemicznej aktywności PSII.

Key words: chlorophyll content, chlorophyll fluorescence, genetic mapping, rye, QTL. **Słowa kluczowe:** fluorescencja chlorofilu, mapowanie genetyczne, QTL, zawartość chlorofilu, żyto.

INTRODUCTION

The development of technology generating a large amount of molecular markers in a short time allowed for the acceleration of research aimed at identifying heritable basis of quantitative traits determined by multiple genes. The first stage of the study on the quantitative trait loci (QTL) mapping is the construction of a genetic map. On the map, one can localize regions, with a high probability of the occurrence of a gene responsible for the expression of a trait. Such studies are performed for the majority of crop species, including rye. In terms of rye, the studies concern mainly morphological, yielding, physiological and qualitative traits (Milczarski 2008; Börner et al. 2009; Masojć and Milczarski 2009; Miedaner et al. 2012; Myśków et al. 2014).

Corresponding author – Adres do korespondencji: MSc Katarzyna Molik, Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Juliusza Słowackiego 17, 71-434 Szczecin, Poland, e-mail: Katarzyna.Sobiech@zut.edu.pl

Most of these studies comprise the analyses of the most important agronomic trait - yield or its components. Photosynthetic efficiency constitutes the most important factor affecting plant growth, biomass accumulation and thus grain yield to a significant extent. One of the factors stimulating the intensity of photosynthetic process is synthesis of photosynthetic pigments, mainly chlorophylls. Their total content in leaves, measured in appropriate and under favorable conditions, can be used as a reliable marker of the efficiency of PSII photosystem (Vijayalakshmi et al. 2010). One of the parameters of PSII efficiency is a parameter of induced chlorophyll fluorescence – F_v/F_m , which is defined as the ratio of the maximum quantum efficiency of PSII photochemistry. It only provides the information on the maximum potential of photosynthetic apparatus, at which light energy absorbed by PSII is used for the total reduction of primary electron acceptor Q_A , and not for the real quantum productivity of PSII. The objective of this study was to identify QTLs, which control the chlorophyll content and the maximum PSII photochemical productivity on the genetic map of recombinant rye inbred lines (RIL) of DS2 × RXL10 hybrid cross.

MATERIAL AND METHODS

Plant material consisted of parental inbred lines: DS2 and RXL10 as well as 70 hybrids of F_7 (S₆) generation (RIL population), derived by SSD (Single Seed Descent) method by Milczarski (2010). From plants, in the earring stage, growing on experimental microplots in the Agricultural Academy in Szczecin (nowadays West Pomeranian University of Technology in Szczecin) between 2004–2005, three flag leaves from three different plants in two replications in each year were collected. On the cut leaves, the kinetics of fluorescence was measured with the use of PEA fluorometer (Hansatech, Kings Lynn, UK). The measurement was performed in the middle part of the leaf, after prior shading with a clip for a period of 20 minutes. The measurement of chlorophyll fluorescence was initiated from the illumination of previously shaded sample by the light of low intensity (3000 μ mol \cdot m⁻² \cdot s⁻¹ during 1s) in order to open the energy traps. The first registered parameter was the primary fluorescence (F_0). Then, the samples were illuminated by a saturating flash of light, which allowed for the registration of maximum fluorescence (F_M). This enabled determination of the maximum quantum efficiency of PSII from the equation: $F_V/F_M = (F_M - F_0)/F_M$. Among the obtained parameters, for further analyses maximum photochemical productivity of PSII (F_v/F_m) was selected. On the same leaves, the chlorophyll content (Chc) was determined using SPAD 502 chlorophyll meter (Minolta, Japan) according to the instruction. The obtained values in SPAD units are contractual and do not represent the absolute chlorophyll content, but are proportional to its amount.

The results of the measurements were subjected to statistical analysis with the use of Statistica 10.0 (StatSoft, Inc. 2010) and parameters: mean value of a trait (\bar{x}), minimum (min.) and maximum (max.) values, standard deviation (*SD*) and coefficient of variation (*CV*) were calculated. Distribution of the traits was evaluated by testing the significance of the deviation using the Kolmogorov-Smirnov test. The mean values of the traits tested at the turn of years in all subjects, were used to determine linear correlation coefficient. Data obtained from replicates in years was used for the evaluation of genotype-environment interactions.

Based on the analysis of variance, broad sense heritability (H_B) was calculated according to the formula proposed by Holland et al (2003). Significance of the differences between the parental lines was tested by Student's t test.

In this study, genetic maps of DS2 × RXL10 hybrid published by Milczarski et al. (2011), were used. These maps consisted mainly of DArT markers with the addition of a few markers of a different type: SCAR, SSR, RAPD. The number of markers on the maps was reduced by removing the majority of redundant ones. These maps were used to identify QTLs by using WinQTL Cartographer 2.5 program (Wang et al. 2011). QTL mapping was performed using composite interval mapping (CIM). It allows to establish the linkage between QTL and markers of the genetic map by identification of the chromosome region with the maximum probability of gene location determining a given trait. QTL position is identified in the region, for which the LOD (*logarithm of odds*) curve exceeds the critical level established in our study at LOD = 3.0. In addition, QTLs identified within LOD range of 2.0-3.0 were also considered, treating them as less certain *loci*. The parameters characterizing a particular QTL are: LOD critical value, position of the marker most strongly associated with a QTL (maximum of LOD curve), QTL interval, determination coefficient of a trait (R^2) and the additive effect of parental allele.

RESULTS

The basic assumption to identify QTLs using CIM method is the diversity of parental components and the trait distribution in the mapping population close to a normal distribution. The analysis of variance of investigated traits in the parental lines revealed that parents differ significantly in case of both F_v/F_m and Chc as well (Table 1).

Table 1. Mean values of two-year measurements of chlorophyll content (Chc) in SPAD units and maximal photochemical efficiency of PSII (F_v/F_m) (± standard deviation), for parental lines Tabela 1. Średnie zawartości chlorofilu (Chc), w jednostkach SPAD, i maksymalnej fotochemicznej wydajności PSII (F_v/F_m) (± odchylenie standardowe) dla linii rodzicielskich w okresie dwóch lat prowadzenia doświadczenia

Trait	Mean trait value for parental lines Wartość średnia cechy u rodziców (± SD)					
Cecha	DS2	RXL10	р			
Chc (SPAD)	43.067 ± 3.447	50.467 ± 3.489	0.023*			
F _v /F _m	0.827 ± 0.002	0.809 ± 0.009	0.009*			

p – probability of t Student test statistics – prawdopodobieństwo statystyki t testu Studenta.
* Significant differences of traite between neurot t to statystyki t testu Studenta.

* Significant differences of traits between parental lines with $p \le 0.05$ – Istotne różnice cech pomiędzy liniami rodzicielskimi dla $p \le 0.05$.

In the case of F_v/F_m evaluated in both years, the coefficient of variation of the individuals in RIL population was very low and was estimated at a maximum of 3.3%. The reason for such low *CV* values was the low diversity of the trait (Table 2). Both traits had a normal distribution in a mapping population with the exception of F_v/F_m evaluated in 2005, where statistically significant deviation from the assumed trait distribution was reported by flattening and right shift of the curve (Table 2).

Table 2. Mean values of chlorophyll content (SPAD) and maximal photochemical efficiency of PSII for RIL population DS2 × RXL10 (\pm standard deviation), minimum (min.), maximum (max.), coefficient of variation (*CV*), Kolmogorov-Smirnov (K-S) test values and parameters of trait distribution – skewness and kurtosis

Tabela 2. Średnie zawartości chlorofilu (SPAD) i maksymalnej fotochemicznej wydajności PSII dla populacji RIL DS2 × RXL10 (± odchylenie standardowe), minimum (min.) maksimum (max.), współczynnik zmienności (*CV*), wartości testu Kołmogorowa-Smirnowa oraz parametry rozkładu cechy – skośność i kurtoza

Trait	Year	DS2 × RXL10 RIL population Populacja RIL DS2 × RXL10						
Cecha	Rok	mean średnia (± SD)	min.	max.	 CV [%]	K-S test test K-S	skewnesss skośność	kurtosis kurtoza
Chc (SPAD)	2004	45.045 ± 6.412	30.20	61.00	14.23	0.060	-0.033	0.063
	2005	41.679 ± 4.287	32.24	53.48	10.29	0.073	0.369	0.554
F _v /F _m	2004	0.821 ± 0.009	0.80	0.84	1.15	0.076	-0.417	0.496
	2005	0.808 ± 0.027	0.72	0.85	3.30	0.123*	-1.247	1.419

* Significant distortion of normal distribution with $p \le 0.05$ – Istotne odchylenie od rozkładu normalnego dla $p \le 0.05$.

The variance (mean squares) of investigated traits was significant for years, genotypes and interactions, except for Chc (Table 3). For this trait no significant interaction between years and genotypes was observed. Using the components of the analysis of variance, broad sense heritabilities were estimated (H_B). It was found, that the heritability of F_v/F_m , despite a little variation within the population, was high and was estimated at 0.53. A slightly higher rate of this parameter was obtained for Chc. The estimated level of phenotypic correlations between F_v/F_m and Chc was not statistically significant (Table 4). However, one reported the correlation between the same trait measured in subsequent years.

Table 3. Variance analysis of chlorophyll content (SPAD) and maximal photochemical efficiency of PSII for variables: year, genotype, interaction year × genotype and broad-sense heritability H_B Tabela 3. Analiza wariancji zawartości chlorofilu (SPAD) i maksymalnej fotochemicznej wydajności PSII dla roku, genotypu, interakcji rok × genotyp oraz współczynnik odziedziczalności H_B

		Trait					
Source of variation Źródło zmienności	Degrees of freedom Stoppie swobody	Chc		F _v /F _m			
	Ctopine owebody	mean squares średnie kwadraty	p	mean squares średnie kwadraty	p		
Year Rok	1	93.651*	0.038	0.008*	0.000		
Genotype Genotyp	69	4820.608*	0.000	0.050*	0.000		
Year × Genotype Rok × Genotyp	69	658.314	0.990	0.025*	0.000		
Error Błąd	140	2974.947	-	0.011	-		
H _B		0.56		0.53			

* Significance of variance with $p \le 0.05$ – Istotność wariancji na poziomie $p \le 0.05$.

Mean squares, number of degrees of freedom and probability of *F* statistics (*p*) were given – Podano średnie kwadraty, liczbę stopni swobody i prawdopodobieństwo statystyki F(p).

Table 4. Correlation matrix mean values between chlorophyll content and maximal photochemical efficiency of PSII for two-year measurements: 2004 (Chc2004, Fv/Fm2004) and 2005 (Chc2005, Fv/Fm2005)

Tabela 4. Macierz korelacji średnich zawartości chlorofilu i maksymalnej fotochemicznej aktywności PSII w populacji RIL, wraz z pomiarami z 2004 r. (Chc2004, Fv/Fm2004) i 2005 r. (Chc2005, Fv/Fm2005)

Cecha Trait	F _v /F _m 2004	Chc2004	F _v /F _m 2005	Chc2005	
F _v /F _m 2004	-				
Chc2004	-0.011	-			
F _v /F _m 2005	0.490	0.088	_		
Chc2005	0.169	0.649	0.138	-	

n – sample size – wielkość próby.

Statistically important correlations between phenotypic traits were marked grey, for n = 70 – Kolorem szarym zaznaczono statystycznie istotne korelacje pomiędzy cechami fenotypowymi, dla n = 70.

Averaged measurements of the traits were introduced to WinQTL Cartographer program to identify QTL regions determining F_v/F_m and Chc. A total of 19 QTLs were identified, including the most for F_v/F_m in 2005, and the least for Chc in 2005 and F_v/F_m in 2004, namely 9,3 and 3 respectively (Table 5). Identified QTLs were mapped on 1R, 3R, 5R, 6R and 7R chromosomes of rye (Fig. 1, Table 5). 14 of the identified QTLs showed the confidence of identification measured by the LOD test exceeding the critical value of 3.0. Among these *loci*, F_v/F_m9 was characterized by the highest level of LOD, exceeding 52. Distribution of QTLs on chromosomes was not uniform. They were mostly located in the distal regions of the chromosomes (1R, 5R, 6R and 7R) or near the centromere (3R and 6R). Three regions common for the QTLs were detected: one for different traits (*Chc8*, F_v/F_m9 on 5RL) and two for the same trait evaluated in different years (F_v/F_m1 , F_v/F_m5 on 1RS and F_v/F_m3 , F_v/F_m10 on 6RS). The remaining QTLs occurred individually.

DISCUSSION

The most important agronomic trait of crops, including rye, is yield. Among a number of traits affecting the yield of rye grains, a huge importance is attributed to the morphological features such as the size and the area of the flag leaf as well as the successive leaves, blade and physiological features such as chlorophyll content and photosynthetic activity (Xue et al. 2008). The measurement of the photosynthetic activity can be performed using fluorescence analysis of chlorophyll. It is a non-invasive and non-destructive method for plants, and at the same time it can be conducted in field research (Hur et al. 2009; Kalaji and Łoboda 2009). The results of these measurements inform about the progress of biophysical phenomena and biochemical processes occurring within photosystem II (PSII). Moreover, they are designed to assess the impact of external factors, including stress, on photosynthetic efficiency (Kalaji and Łoboda 2009). The intensity of photosynthetic process, including the efficiency of photosystem II is stimulated by the synthesis of photosynthetic pigments, especially chlorophyll (Vijayalakshmi et al. 2010). Increase in the concentration of chlorophyll in the plant can be an effective way to improve the production of biomass, and thus the yield (Wang et al. 2008).

Trait Cecha	Year Rok	QTL symbol Symbol QTL	Chromosome Chromosom	QTL peak position in cM (QTL interval) Pozycja piku QTL w cM (przedział QTL)	Nearest marker Najbliższy marker (cM)	LOD	Additive effect Efekt addytywny	R ² [%]
		Chc1	1R	200.30 (192.5–203.3)	XrPt401180 (3.0)	4.69	2.45	27.03
		Chc2	5R	125.60 (125.3–126.6)	XrPt508209 (0.0)	4.02	-1.73	13.70
		Chc3*	6R	66.1	XrPt400119 (0.0)	2.09	-1.18	5.84
	2004	Chc4*	6R	83.9 (82.9–84.0)	XrPt505336 (0.1)	2.18	1.33	8.13
Chc		Chc5*	6R	228.7 (228.7–229.7)	XrPt505447 (0.0)	2.77	1.39	8.91
U		Chc6	7R	259.1 (257.5–258.1)	XrPt389927 (0.0)	6.23	-2.27	23.81
		Chc7	3R	94.8 (92.5–94.8)	XrPt66766 (1.0)	3.46	7.64	21.28
	2005	Chc8	5R	171.3 (170.3–173.3)	XrPt399270 (9.9)	17.78	19.16	46.15
		Chc9	6R	215.3 (214.7–215.3)	XrPt400168 (0.0)	4.75	-7.29	19.78
		F√/F _m 1	1R	25.3 (25.2–25.3)	XrPt506276 (0.0)	4.12	0.12	15.65
	2004	F√/F _m 2	3R	121.5	XrPt410986 (0.0)	3.32	-0.11	12.93
F√F _m		<i>F</i> √ <i>F</i> _m 3	6R	7.9 (7.9–8.0)	XrPt410992 (0.1)	6.41	-0.17	27.39
		F√/F _m 4	1R	14.6 (8.6–14.7)	XrPt400359 (0.1)	2.24	-0.11	10.40
		F√/F _m 5	1R	25.3 (25.2–25.3)	XrPt506276 (0.0)	5.05	0.13	17.77
		<i>F</i> _v / <i>F</i> _m 6	3R	62.4 (62.4–64.4)	XrPt399942 (0.0)	3.43	-0.09	8.98
	2005	<i>F</i> √ <i>F</i> _m 7	3R	85.0 (85.0–85.2)	XrPt346908 (0.2)	3.11	0.12	15.60
		<i>F</i> _v / <i>F</i> _m 8*	5R	22.9 (22.8–24.9)	XrPt411232 (0.0)	2.56	-0.09	8.48
		<i>F</i> _v / <i>F</i> _m 9	5R	172.3 (164.3–175.3)	XrPt399270 (8.9)	52.82	0.40	44.41
		F _v /F _m 10	6R	8.0 (7.9–8.0)	XrPt410992 (0.0)	4.84	-0.13	17.49

Table 5. Characteristic of QTLs controlling chlorophyll content and maximal photochemical efficiency of PSII for RIL mapping population DS2 × RXL10 Tabela 5. Charakterystyka QTL kontrolujących zawartość chlorofilu oraz całkowitą fotochemiczną aktywność PSII dla populacji mapującej RIL DS2 × RXL10

* QTLs with LOD score = 2.00–3.00 – QTL o niższej wiarygodności, z wartością LOD = 2,00–3,00.



Fig. 1. Localization of QTLs for chlorophyll content (Chc) and maximal photochemical efficiency of PSII (F_v/F_m) for RIL population, for two-year measurements (2004, 2005). Nearest markers for QTLs were marked

Ryc. 1. Lokalizacja QTL zawartości chlorofilu (Chc) i całkowitej aktywności fotochemicznej PSII (F_v/F_m) w populacji RIL dla pomiarów wykonanych w latach 2004 i 2005. Zaznaczone markery zlokalizowano najbliżej obszaru QTL

Quantitative traits are determined both by environmental and genetic factors. Understanding of the genetic basis of a particular quantitative trait is possible by using genetic maps and tools for the identification of QTL. The high-density map used in this study consists of 1391 markers of DS2 x RXL10 population (Milczarski et al. 2011). However, for the analysis of QTL, a smaller number of individuals was available at the time of measurement. It should be emphasized, that such number of mapping population is insufficient for the precise localization of QTLs, and the obtained results should be treated as preliminary recognition of hereditary basis of investigated traits. In this paper an attempt was made to determine the probable number of QTLs determining maximum photochemical productivity of PSII (F_v/F_m) and chlorophyll content (Chc). Similarly as in this study, a number of authors (Li et al. 2006; Zhang et al. 2010; Kumar et al. 2012; Czyczyło-Mysza et al. 2013) have not confirmed statistically significant correlation between the content of chlorophyll and F_v/F_m in plants remaining in physiological condition. This situation changes dramatically when the plants are subjected to e.g. prolonged drought-stress conditions (Zhang et al. 2010; Kumar et al. 2012). It suggests a high sensitivity of the analyzed traits to external factors. The F_v/F_m parameter selected for mapping is one of many possible to obtain from chlorophyll fluorescence analysis. In the literature, this parameter as well as its components have been widely used in the studies of QTL mapping of chlorophyll fluorescence under both, optimal plant growth (Yang et al. 2007; Guo et al. 2008; Liang et al. 2010; Yin et al. 2010; Zhang et al. 2010; Czyczyło-Mysza et al. 2013), as well as drought-stress conditions (Yang et al. 2007; Kumar et al. 2010), or cold-stress conditions (Fracheboud et al. 2002; Jompuk et al. 2005; Guo et al. 2008).

In the majority of the above-mentioned studies, authors used chlorophyll fluorescence parameters to investigate the genetic basis of photosynthesis, and to compare the effect of stress on different genotypes. Despite the small variation of F_v/F_m parameter and CV in the range of 1.1–3.3%, in the course of this study 10 QTLs were identified, localized on 1R, 3R, 5R and 6R chromosomes. Four of them co-localized in two regions, F_v/F_m1 and F_v/F_m5 on the short arm of 1R chromosome, and F_v/F_m3 and F_v/F_m10 on 6RS. After elimination of QTLs which repeated their position in the same region, the number of QTLs for the F_v/F_m parameter was 8, while F_v/F_m4 and F_v/F_m8 loci were mapped below the accepted threshold of LOD equal to 3.0. The number of identified QTLs for F_v/F_m , in the studies conducted by other authors, investigating especially wheat, was different and was estimated between three (Liang et al. 2010; Zhang et al. 2010) to nine (Czyczyło-Mysza et al. 2013).

In terms of F_v/F_m9 QTL very high values of both, LOD test and R² were obtained. The presence of a gene essential for the PSII functioning in the interval mapping could be the reason for such high values of the parameters describing QTL. Unfortunately, the closest marker is located at a distance of approximately 9 cM, which prevents from verification of such thesis. Chlorophyll content expressed in SPAD units was conditioned by the presence of 9 QTLs, localized on 1R, 3R, 5R, 6R and 7R chromosomes. No co-localization of QTLs detected in different growing seasons was observed, although some *loci* were located on the same chromosome arms (6RS and 6RL) in close proximity. A more detailed analysis of QTL additive effects of Chc from 6R chromosome showed that the indication of the activity of DS2 allele line for *loci* located near each other are different, which indicates dissimilarity of detected QTLs.

Milczarski and Masojć (2002) analyzing QTLs for Chc in the mapping population of the same hybrid but obtained by sibling mating (F₅ sib), identified 4 loci on 1RS, 3RS (C), 4RL and 5RL chromosomes. Comparing our results with the results of their study, it was found that the approximate common locations refer to QTLs identified on 3R and 5R chromosomes. The highest values of the parameters describing the QTLs were reported for Chc8 localized in the distal segment of 5R chromosome. Although there was no correlation between Chc and F_v/F_m , F_v/F_m 9 was also mapped in this region. For both QTLs, the values of parameters describing QTLs seem to be overestimated and should be treated with caution. Similarly as for $F_{v}/F_{m}9$, the distance of the most associated marker with the QTL is estimated at more than 9 cM and no data on the function of this marker is available. Nevertheless, it is an extremely important QTL region. In this area, a gene encoding recessive dwarfism in RXL10 line was localized. From studies conducted by Milczarski (2010), it can be concluded, that this gene exhibits pleiotropy towards a number of traits. In this area QTLs for: spike length, number of spikelets per spike, number of grains per spike, weight of grains per ear, weight of 1000 seeds and grain yield of plant were also identified. The analysis of the additive effect of allele from DS2 line for both QTLs indicates, that the donor of favorable alleles which increases the probability of both traits is DS2 line, although RXL10 line is characterized by a higher content of chlorophyll in leaves. The presented distribution of QTLs on rye chromosomes ensures complex and polygenic control of two important traits for the functioning of the photosynthetic apparatus: chlorophyll content (Chc) and maximum photochemical productivity of PSII (F_v/F_m).

CONCLUSIONS

The study allowed for the preliminary identification of 9 and 10 QTLs (including 2 QTLs co-localizing on 1R and 6R) for the chlorophyll content and the maximum photochemical productivity of PSII, respectively. Determination of the location of the QTLs determining F_v/F_m has been the first such analyses for rye. Detected QTLs broaden the knowledge on rye genome and provide new data useful for comparative analyses.

REFERENCES

- Börner A., Korzun V., Voylokov A.V., Weber W.E. 1999. Detection of quantitative trait *loci* on chromosome 5R of rye (*Secale cereale* L.). Theor. Appl. Genet. 98, 1087–1090.
- Czyczyło-Mysza I.M., Tyrka M., Marcińska I., Skrzypek E., Karbarz M., Dziurka M., Hura T., Dziurka K., Quarrie S.A. 2013. Quantitative trait *loci* for leaf chlorophyll fluorescence parameters, chlorophyll and carotenoid contents in relation to biomass and yield in bread wheat and their chromosome deletion bin assignments. Mol. Breed. 32, 189–210.
- **Fracheboud Y., Ribaut J.M., Vargas M., Messmer R., Stamp P.** 2002. Identification of quantitative trait *loci* for cold tolerance of photosynthesis in maize (*Zea mays* L.). J. Exp. Bot. 53(376), 1967–1977.
- **Guo P., Baum M., Varshney R.K., Graner A., Grando S., Ceccarelli S.** 2008. QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought. Euphytica 163, 203–214.

- **Holland J.B., Nyquist W.E., Cervantes-Martinez C.T.** 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breed. Rev. 22, 9–112.
- Hura T., Hura K., Grzesiak M.T. 2009. The usefulness of chlorophyll fluorescence parameters in harvest prediction in 10 genotypes of winter triticale under optimal growth conditions. Plant Biosyst. 143, 496–503.
- Jompuk C., Frachenboud Y., Stamp P., Leipner J. 2005. Mapping of quantitative trait *loci* associated with chilling tolerance in maize (*Zea mays* L.) seedlings grown under field conditions. J. Exp. Bot. 56(414), 1153–1163.
- **Kalaji M.H., Łoboda T.** 2009. Fluorescencja chlorofilu w badaniach stanu fizjologicznego roślin. Warszawa, Wydaw. SGGW. [in Polish]
- Liang Y., Zhang K., Zhao L., Liu B., Meng Q., Tian J., Zhao S. 2010. Identification of chromosome regions conferring dry matter accumulation and photosynthesis in wheat (*Triticum aestivum* L.). Euphytica 171, 145–156.
- **Masojć P., Milczarski P.** 2009. Relationship between QTLs for preharvest sprouting and alpha--amylase activity in rye grain. Mol. Breed. 23, 75–84.
- Miedaner T., Hübner M., Korzun V., Schmiedchen B., Bauer E., Haseneyer G., Wilde P., Reif J.C. 2012. Genetic architecture of complex agronomic traits examined in two testcross populations of rye (*Secale cereale L.*). BMC Genomics 13, 706.
- **Milczarski P., Masojć P.** 2002. The mapping of QTLs for chlorophyll content and responsiveness to gibberellic GA₃ and abscisic ABA acids in rye. Cell. Mol. Biol. Lett. 7, 449–455.
- Milczarski P. 2008. Identyfikacja QTL wybranych cech morfologicznych związanych z wyleganiem żyta (Secale cereale) [Identification of QTLs of morphological traits related to lodging of rye (Secale cereale L.)]. Biul. Inst. Hod. Rośl. 250, 211–216.
- Milczarski P. 2010. Identyfikacja i mapowanie porównawcze QTL warunkujących wybrane cechy ilościowe w populacjach mapujących dwóch mieszańców międzyliniowych żyta (Secale cereale L.) [Identification and comparative mapping of QTLs controlling selected quantitative traits in two mapping populations of rye (Secale cereale L.)]. Szczecin, ZUT, 86. [in Polish]
- Milczarski P., Bolibok-Brągoszewska H., Myśków B., Stojałowski S., Heller-Uszyńska K., Góralska M., Brągoszewski P., Uszyński G., Kilian A., Rakoczy-Trojanowska M. 2011. A high density consensus map of rye (*Secale cereale* L.) based on DArT markers. PLoS ONE 6(12), www.10.1371/journal.pone.0028495.
- Myśków B., Hanek M., Banek-Tabor A., Maciorowski R., Stojałowski S. 2014. The application of high-density genetic maps of rye for the detection of QTLs controlling morphological traits. J. Appl. Genet. 55, 15–26.
- StatSoft, Inc. 2010. STATISTICA (data analysis software system), version 10.0. www.statsoft.com.
- Vijayalakshmi K., Fritz A.K., Paulsen G.M., Bai G., Pandravada S., Gill B.S. 2010. Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol Breed. 26, 163–175.
- Wang F.H., Wang G.X., Li X.Y., Huang J.L., Zheng J.K. 2008. Heredity, physiology and mapping of a chlorophyll content gene of rice (*Oryza sativa* L.). J. Plant Physiol. 165, 324–330.
- Wang S., Basten C.J., Zeng Z.B. 2011. Windows QTL Cartographer 2.5. Releigh, NC. Department of Statistics, North Carolina State University.
- Xue D., Chen M., Zhou M., Chen S., Mao Y., Zhang G. 2008. QTL analysis of flag leaf in barley (*Hordeum vulgare* L.) for morphological trait and chlorophyll content. J. Zhejiang Univ. Sci. B 9(12), 938–943.
- Yang D.L., Jing R.L., Chang X.P., Li W. 2007. Quantitative trait *loci* mapping for chlorophyll fluorescence and associated traits in wheat (*Triticum aestivum* L.). J. Integr. Plant Biol. 49, 646–654.
- Yin Z., Meng F., Song H., He X., Xu X., Yu D. 2010. Mapping quantitative trait *loci* associated with chlorophyll and fluorescence parameters in soybean (*Glycinie max* (L.) Merr.). Planta 231, 875–885.

Zhang Z.B., Xu P., Jia J.Z., Zhou R.H. 2010. Quantitative trait *loci* for leaf chlorophyll fluorescence traits in wheat. Aust. J. Crop Sci. 4, 571–579.

Abstract. The objective of the study was to identify QTLs for the chlorophyll content (Chc) and the maximum photochemical activity of PSII (F_v/F_m) in rye. RIL population of DS2 × RXL10 hybrid cross consisting of 70 individuals of F_7 generation constituted as experimental material. The obtained results were subjected to statistical analysis, and significant differences in the diversity of both traits in parental phenotypes and individuals of mapping population, were shown. No statistically significant correlation between Chc and F_v/F_m was observed, and calculated broad sense heritabilities (H_B) were estimated at 56 and 53% respectively. With LOD score ≥ 2.0 , 19 QTL regions were established, including 9 for the content of chlorophyll and 10 for a total photochemical efficiency of PSII. They were mainly localized in the distal or centromeric regions of 1R, 3R, 5R, 6R, 7R chromosomes. On 1R, 5R and 6R common regions for QTLs were found: F_v/F_m1 and F_v/F_m5 (1R), *Chc8* and F_v/F_m9 (5R) as well as F_v/F_m3 and F_v/F_m10 (6R). The resulting QTLs provide preliminary knowledge on hereditary basis of chlorophyll content and maximum photochemical activity of PSII in rye.