

QTL ANALYSIS OF WESTERN CORN ROOTWORM RESISTANCE TRAITS IN MAIZE IBM POPULATION GROWN IN CONTINUOUS MAIZE

Andrija BRKIĆ^{1*}, Domagoj ŠIMIĆ^{1,2}, Antun JAMBROVIĆ^{1,2}, Zvonimir ZDUNIĆ^{1,2}, Tatjana LEDENČAN¹, Emilija RASPUDIĆ³, Mirjana BRMEŽ³, Josip BRKIĆ¹, Maja MAZUR¹, Vlatko GALIĆ¹

¹Agricultural Institute Osijek, Department for Breeding and Genetics of Maize, Osijek, Croatia

²Centre of Excellence for Biodiversity and Molecular Plant Breeding (CroP-BioDiv), Zagreb, Croatia

³Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Department for Plant Protection, Chair for Entomology and Nematology, Osijek, Croatia

Brkić A., D. Šimić, A. Jambrović, Z. Zdunić, T. Ledenčan, E. Raspudić, M. Brmež, J. Brkić, M. Mazur, V. Galić (2020). *QTL analysis of Western corn rootworm resistance traits in maize IBM population grown in continuous maize.*- Genetika, Vol 52, No.1, 137-148.

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte; WCR) is the most destructive maize pest in the USA, and one of the most dangerous pests in Europe. In just over a decade from the introduction it became one of the most serious pests in Croatia. Native resistant cultivars could be an efficient strategy for WCR control. Although research in WCR host plant resistance has continuously been conducted for over 70 years, no resistant maize hybrid is commercially available today. The aim of this study was to evaluate resistance traits in the maize IBM intermated recombinant inbred lines (IRILs), and detect QTLs associated with resistance to WCR herbivory. Total of 207 recombinant inbred lines were phenotyped for standard WCR resistance traits. Several associated QTLs were detected on chromosomes 1, 5, 6, 8, 9, and 10. QTLs on chromosomes 1 (6.41 % variance explained), and 6 (9.26 % variance explained) were mapped in regions harboring genes connected with plant defense against herbivory. Detected QTLs provide relevant guidelines for future research on WCR resistance in maize.

Keywords: QTL analysis, western corn rootworm, IBM population, maize

Abbreviations: IBM – Intermated B73×Mo17 (population); IRIL – IBM Recombinant Inbred Line; RD – Root Damage; RR – Root Regrowth; RS – Root Size; WCR – Western Corn Rootworm

Corresponding authors: Andrija Brkić, Agricultural Institute Osijek, Department for Breeding and Genetics of Maize, Južno predgrađe 17, Osijek 31000, Croatia, E-mail: andrija.brkic@poljin.hr, Telephone: +385 31 515 588

INTRODUCTION

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is the most destructive maize pest in the USA, and one of the most dangerous pests in Europe. Estimated management costs in the USA exceeded one billion US dollars (SAPPINGTON *et al.*, 2006). Both larvae and adults cause damage by feeding on maize plant. However, WCR larvae cause the most serious damages by feeding on roots (SUTTER *et al.*, 1990; SPIKE and TOLLEFSON, 1991; RIEDELL and REESE, 1999). Continuous maize yields less than maize under crop rotation (KURTZ *et al.*, 1984; CROOKSTON *et al.*, 1991). However, using WCR tolerant hybrids in continuous maize is a sustainable alternative in maize production because no additional treatments are needed. WCR native resistance research started in the 1930s in the US Corn Belt (BIGGER *et al.*, 1938), and since then wide array of techniques have been used in order to determine key factors that underline maize tolerance to this pest (HIBBARD *et al.*, 1999; KNUTSON *et al.*, 1999; IVEZIĆ *et al.*, 2006; PRISCHMANN *et al.*, 2007; TOLLEFSON, 2007; EL KHISHEN *et al.*, 2009; FLINT-GARCIA *et al.*, 2009; MARTON *et al.*, 2009; BOHN *et al.*, 2018). Some of the most common traits explored in WCR resistance research are root injury or root damage (RD), root regrowth (RR) and root size (RS). Nevertheless, to our knowledge, maize hybrid containing host plant resistance has not yet been developed. The mechanisms underlying insect resistance in maize are very complex and not yet fully understood, although recent progress in the research of maize resistance to herbivory provides some new insights (YAN *et al.*, 2012; WOUTERS *et al.*, 2016). Native or host plant resistance (HPR) is a natural defense of the plant against insects, which includes several different mechanisms (PAINTER, 1951; WISEMAN, 1985). BERNKLAU *et al.* (2010) reported antixenosis in maize against western corn rootworm larvae, which was first or one of the first known reports of the active defense mechanism of resistance to WCR, and at the same time the first known native resistant maize hybrid.

Jasmonates are phytohormones with multiple functions, including plant defense and reproduction (FARMER *et al.*, 2003; AVANCI *et al.*, 2010). Allene oxide cyclase is one of the most important enzymes in the jasmonic acid (JA) biosynthetic pathway. Gene encoding for this enzyme is allene oxide cyclase 1 (*aoc 1*) (HAMBERG, 1988; SCHALLER and STINTZI, 2009). Studies by YAN *et al.* (2012) and CHRISTENSEN *et al.* (2013) provided evidence for the significance of JA in maize insect defense. PECHAN *et al.* (2000) reported accumulation of 33-kD cysteine proteinase in maize leaves, which is an active defense mechanism of maize against various lepidopteran species, including corn earworm and European corn borer. Plant material used in the studies of WILLIAMS and DAVIS (1982) and WILLIAMS *et al.* (1990) was derived from Antigua germplasm – Mp704 and Mp708 lines, which was also used in isolating the maize insect resistance gene (*mir1*), encoding for 33-kD cystein proteinase (PECHAN *et al.*, 1999). In a study by LOPEZ *et al.* (2007), accumulation of maize insect resistance 1-cystein protease (Mir1-CP) was determined in various tissues of maize leaves (phloem), as well as the roots (xylem), as a response to lepidopteran larval herbivory. After the analysis of the Mp708 inbred line, CASTANO-DUQUE *et al.* (2017) confirmed its resistance to herbivory, induced by expression of herbivory defense genes (*rip1*, *mir1*), as well as high levels of JA.

QTL analysis of maize resistance to the WCR has not been the focus of many studies addressing WRC resistance. In the analysis of the genetic basis of WCR resistance, BOHN *et al.* (2018) reported that all chromosomes except chromosome 4 contained genomic regions

contributing to WCR resistance, and confirmed complex genetic structure of native resistance against WCR.

The aim of this study was to evaluate resistance traits in the maize IBM*Syn4* (Intermated B73xMo17 Synthetic 4) intermated recombinant inbred lines (IRILs), and detect QTLs associated with resistance to WCR herbivory.

MATERIAL AND METHODS

Plant material

Maize IBM*Syn4* population was developed by crossing the IBM parent lines (B73 and Mo17) followed by five generations of intermating and at least five generations of selfing (LEE *et al.*, 2002). In this study, 207 IBM IRILs were planted, along with the IBM parental components and 11 checks, comprising the 220 tested genotypes in total. Eight populations were used as checks: seven with known WCR tolerance – KAHLER *et al.* (1985); HIBBARD *et al.* (1999); HIBBARD *et al.* (2007), and one susceptible check developed from public inbreds. Three single cross hybrids of the Agricultural Institute Osijek (AIO) with known tolerance to WCR were added to complete the experimental genotype set.

The experiment was set as an incomplete randomized block design with two replicates and planted in 2015 and 2016 on the experimental field of Agricultural Institute Osijek in Osijek, Croatia (45°32'24"N, 18°44'00"E). WCR infestation was natural, however the field was maintained under continuous maize cropping for over 70 years prior to setting this trial, therefore the insect pressure was expected to be greater than in the fields under crop rotation (KISS *et al.*, 2005). Standard management practices for maize were applied at the trial location – the fertilization was carried out with non-limiting supplies of nutrients, and weed control was carried out when necessary. Each genotype was planted in one-row plots (plot size = 3.5 m²).

Phenotyping

Methods for the root evaluation included standard root rating field activities as well as using two different root evaluation scales. Four plant roots per genotype were excavated from the soil, tagged, transported, washed and cleaned with pressurized water and prepared for evaluation. Three main traits were selected for the evaluation – root damage, root regrowth, and root size. RD was evaluated with Iowa State University node injury scale (OLESON *et al.*, 2005), while RR and RS were evaluated with Eiben root size scale reversed (ROGERS *et al.*, 1975). Rate '0' in ISU node injury scale represents a root without any injury, while rate '3' represents three root nodes eaten up to five cm from the stalk. Rate '1' in Eiben scale represents a big root with high regrowth, while rate '6' represents a small root with low regrowth.

Monitoring of WCR adults was carried out from June to September with floral bait traps (Csalomon PALs traps) in both years at one trap site in the trial field. Yellow sticky plates were changed together with the bait every 20-25 days, and the beetle count was performed every seven days.

Genotyping

The IBM genetic map used in this study consisted of 2178 densely spaced, genetically mapped markers, mostly SSR and SNP. The total length of the map was 7090 cM with respect to

the increase in map size and density in IBM compared to regular F_2 map, as 1 cM in F_2 covers approximately 4 IBM cM (LEE *et al.*, 2002; FALQUE *et al.*, 2005). The average distance between adjacent markers was 3.2 IBM cM. The QTL results reported in this paper are in IBM cM on IBM2 Neighbors map.

Statistical analysis

All statistical analyses were performed in R programming language and environment (R CORE TEAM, 2018). Variance components were estimated with linear mixed model solver implemented in *lme4* package (BATES *et al.*, 2014). In the mixed model, genotype, year, genotype x year interaction, replicate within year, and block within year were set as random effects. Heritabilities of traits were estimated on entry-mean basis (HALLAUER *et al.*, 2010) as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{G \times E}^2/nE + \sigma_e^2/r}$$

where σ_G^2 represents genotypic variance, $\sigma_{G \times E}^2$ genotype x environment interaction variance, σ_e^2 the residual variance and nE and r are number of environments and number of replicates respectively. Best linear unbiased predictions of genetic variance (σ_G^2) were used as phenotypes for QTL analysis. Genetic correlations were estimated with another mixed model solver allowing the specification of correlation structure among predictors implemented in *sommer* package (COVARRUBIAS-PAZARAN, 2016). The phenotypic coefficients of correlations were calculated using the R's own function *cor*.

QTL mapping

Inclusive composite interval mapping mapping was carried out in IciMapping software version 4.1 (MENG *et al.*, 2015) with BIP module, specially designed for mapping in biparental populations. In the first step, stepwise regression was run with forward selection and backward elimination of putative QTLs. The detected QTLs were then used as cofactors in composite interval mapping procedure to reduce the masking effects and to more precisely map the loci. Probability for inclusion in stepwise regression (PIN) was set to 0.001, and mapping step size was 1 cM. The threshold for declaring the significant QTL was calculated based on 1000 permutations, and the calculated threshold across all three phenotypes was 3.629. Peak LOD score in the region with significant phenotypic effect was considered a QTL, and the confidence intervals were estimated based on a one-unit LOD drop in forward and backward directions.

RESULTS AND DISCUSSION

Resistance traits and monitoring

Means of the RD estimates ranged from 0.15 to 1.31, RR from 1.25 to 5.88, and for RS from 1.00 to 6.00 in 2015. In 2016 means of the RD estimates ranged from 0.31 to 2.50, and for RR and RS from 1.00 to 6.00. Populations CRW8-1a and CRW8-2 showed lowest values among tolerant checks (less RD, higher RR and RS), scoring well below population means for all three traits, while susceptible check showed high values for RD and RR (Table 1). Tolerant checks CRW8-1a, CRW8-1b, CRW8-2, CRW3(C6), CRW2(C5), and LH51-CRW3 showed less root

damage (0.30-0.39) compared to the population mean and susceptible check, although values were comparable to both IBM parental lines. Several maize populations with known WCR resistance confirmed their resistance in this study. Other studies reported similar results with the same germplasm, namely PRISCHMANN *et al.* (2007) for CRW8-1 and CRW3(C6), EL KHISHEN *et al.* (2009) for CRW3(C6) and CRW2(C5), BRKIĆ *et al.* (2017) for CRW8-1a, CRW8-1b, CRW8-2, CRW3(C6), and LH51-CRW3.

Table 1. Means of the IBM population IRILs and checks for WCR attack along with variance components and heritabilities of root damage (RD), root regrowth (RR) and root size (RS) evaluated in two environments in continuous maize

	RD	RRG	RS
<i>IBM population</i>			
Mean	0.72 ± 0.02	3.85 ± 0.05	3.86 ± 0.05
<i>Susceptible check</i>			
B37xH84	0.88 ± 0.23	4.17 ± 0.44	3.08 ± 0.65
<i>Tolerant checks</i>			
CRW8-1A	0.30 ± 0.06	1.17 ± 0.08	2.17 ± 0.58
CRW8-1B	0.38 ± 0.07	2.75 ± 0.75	3.08 ± 0.22
CRW8-2	0.33 ± 0.07	1.92 ± 0.30	2.00 ± 0.50
CRW3	0.35 ± 0.06	2.42 ± 0.55	2.81 ± 0.10
CRW2(C5)	0.39 ± 0.07	2.25 ± 0.63	2.42 ± 0.74
NGSDCRW	0.56 ± 0.21	2.25 ± 0.50	2.25 ± 0.25
LH51-CRW3	0.34 ± 0.11	3.50 ± 0.00	2.42 ± 0.46
<i>Variance components</i>			
σ_g^2	0.0241	0.3023	0.2871
σ_{ge}^2	0.0555	0.0966	0.0259
σ_e^2	0.2001	0.9198	0.7952
H^2	0.24	0.52	0.58

Monitoring of the WCR beetles in 2015 showed two emergence peaks, one on June 28, and the other one on August 9 (data not shown). Total number of beetles was 192 (133 males and 59 females). Next season, total of 302 beetles were collected from the traps (232 males and 70 females), while the time pattern regarding emergence peaks was different – two peaks, one on July 25, and the other one on August 22.

Variance component analysis

Non-zero variance components for all examined factors were observed. Lower estimate of genetic variance component found for RD resulted in lower estimated heritability compared to RR (0.52) and RS (0.58). Another factor contributing to the lower estimate of H^2 was higher estimated genotype x environment interaction observed for RD compared to RR and RS. Heritability estimates for root damage are usually lower than the estimates for other two traits, which were confirmed in this research – H^2 estimate for RD was 0.24, while for root regrowth and size estimates were 0.52 and 0.58 (Table 1). Study on the corn rootworm tolerance by

OWENS *et al.* (1974) yielded similar ratio of heritability estimates – lower estimate for the root damage (0.21), and higher estimates for the root regrowth (0.81) and size (0.70). In a study by ŠIMIĆ *et al.* (2007) repeatabilities for root regrowth were 0.89, while for root damage and size were significantly lower (0.17 and 0.21). In another study about WCR resistance genetics LARSEN (1999) reported heritability estimates of 0.55 for root damage and 0.54 for the root size comparable to results reported in our study.

Correlations

Positive genotypic and phenotypic correlations were detected among all traits (Table 2). Genotypic correlations RR-RS (0.787) and RD-RR were strongest, while among phenotypic correlations RS-RR showed strongest correlation (0.612). Possible causes of genetic correlations are pleiotropy or linkage (HALLAUER *et al.*, 2010). If the true pleiotropy is present, the QTL affecting both traits is expected. In our study, the QTL underlying the high estimates of genetic correlations between RD and RR, as well as between RR and RS were detected (Table 3). We assume the plausible cause of high genotypic correlation between RD and RR is linkage rather than true pleiotropy due to the differences in peak positions of the detected QTLs. Namely, the peaks of QTLs affecting RD and RR on chromosome 1 were detected on positions 403 and 410 respectively which are on the boundaries of the calculated confidence intervals. Congruent to these findings is the fact that the maize IBM population went through four successive generations of intermating, increasing its genetic map by breaking the linkage between proximal genetic regions (LEE *et al.*, 2002; FALQUE *et al.*, 2005). On the other hand, the QTL for RR and RS on chromosome 6, position 76 and 79 cM, falls within the calculated confidence intervals possibly indicating pleiotropy.

Table 2. Phenotypic (above diagonal) and genotypic (below diagonal) correlations among root damage (RD), root regrowth (RR) and root size (RS). All examined correlations were significantly different from 0 at $\alpha = 0.05$

Trait	RD	RR	RS
RD	-	0.131	0.339
RR	0.735	-	0.612
RS	0.401	0.787	-

QTL analysis

Several QTLs on chromosomes 1, 5, 6, 8, 9, and 10 were detected in the QTL analysis (Table 3, Figure 1). Seven QTL affecting RD were detected on chromosomes 1, 9, and 10 (five of them were detected on the chromosome 1 only), explaining between 4.77 and 16.83 % of genetic variance, with LOD scores ranging from 4.16 to 13.14. The detected QTL with flanking markers *umc2227* and *ufg13b* was found in the bin 1.04 of the chromosome 1 (361 cM), which is the same region where the gene allene oxide cyclase 1 (*aoc1*) was found (394 cM). Five QTLs affecting RR were detected on chromosomes 1, 6, 8, and 10, explaining between 5.29 and 19.08 % of the genetic variance, while five QTLs affecting RS were detected on chromosomes 1, 5 and 6, explaining from 9.26 to 17.38 % of the genetic variance. QTL detected with the markers *umc1656* and *sdg102c* was found in the bin 6.02 of the chromosome 6 (134 cM), which is the same region where genes *mir1* (127 cM) and *mir 2* (129 cM) are found.

Table 3. Results of inclusive composite interval mapping for root damage (RD), root regrowth (RR) and root size (RS)

Trait	Bin ^a	Position (cM)	Left flanking marker	Right flanking marker	LOD	R ^{2b}	Add. ^c	CI (cM) ^d
RD	1.04	361	umc2227	ufg13b	5.48	6.41	-	4
	1.05	403	asg3	csu3	4.35	5.39	-	4
	1.05	473	umc1395	umc1321	13.14	16.83	-	2
	1.06	535	bnlg1598	umc1123	11.84	14.85	-	4
	1.09	881	mmp195d	umc1082	6.31	7.79	-	5
	9.04	283	psr547	psr129a	4.16	4.77	+	5
	10.03	218	bnlg1712	AY112073	7.33	8.95	+	3
RRG	1.05	410	csu3	mmp61	12.41	19.08	-	7
	6.01	76	umc2312	bnlg1867	5.60	7.24	-	4
	6.06	388	umc38a	umc1912	4.04	5.29	-	5
	8.02	142	mmp166	chr117a	5.17	6.87	-	6
	10.04	266	jpsb527d	mzetc34	3.98	6.30	-	3
RS	1.05	451	umc1676	umc2231	8.55	9.74	-	5
	5.03	297	mmp58	bnlg1902	7.54	8.67	-	4
	5.06	489	umc1752	umc1941	6.90	8.24	-	3
	6.01	79	bnlg1867	mmp13	13.96	17.38	-	2
	6.02	134	umc1656	sdg102c	8.09	9.26	-	6

^a number before the decimal is a linkage group followed by the bin number

^b percent of genotypic variance explained

^c Additive action of male parent allele

^d in forward and backward direction

Two of the QTLs found in this study for RD and RS were found in the same regions as the two genes coding for the insect resistance proteins (*aoc1* in the bin 1.04 of the chromosome 1, and *mir1* in the bin 6.02 of the chromosome 6). Gene *aoc1* codes for allene oxide cyclase, an enzyme included in JA biosynthesis pathways, and JA synthesis is a crucial plant mechanism in insect resistance (YAN *et al.*, 2012; CHRISTENSEN *et al.*, 2013). Peak position of the QTL found in the bin 6.02 was mapped in the proximity of two insect resistance genes *mir1* and *mir2*. Genes of the *mir* family encode for synthesis of proteins in maize tissues which represent an active defense mechanism against insect herbivory – lepidoptera such as European corn borer, corn earworm, fall armyworm, southwestern corn borer and other (PECHAN *et al.*, 2000; LOPEZ *et al.*, 2007), and against beetles such as WCR (CASTANO-DUQUE *et al.*, 2017). In accordance with findings of BOHN *et al.* (2006) for the root traits, for all three resistance traits QTLs were found on chromosome 1 – five QTLs for RD, one for RR, and one for RS (totally four in the bin 1.05), making chromosome 1 a QTL hotspot. QTLs found on chromosomes 1 and 6 might provide relevant guidelines for future QTL analyses as they are found in genomic regions harboring relevant genes included in insect-resistance.

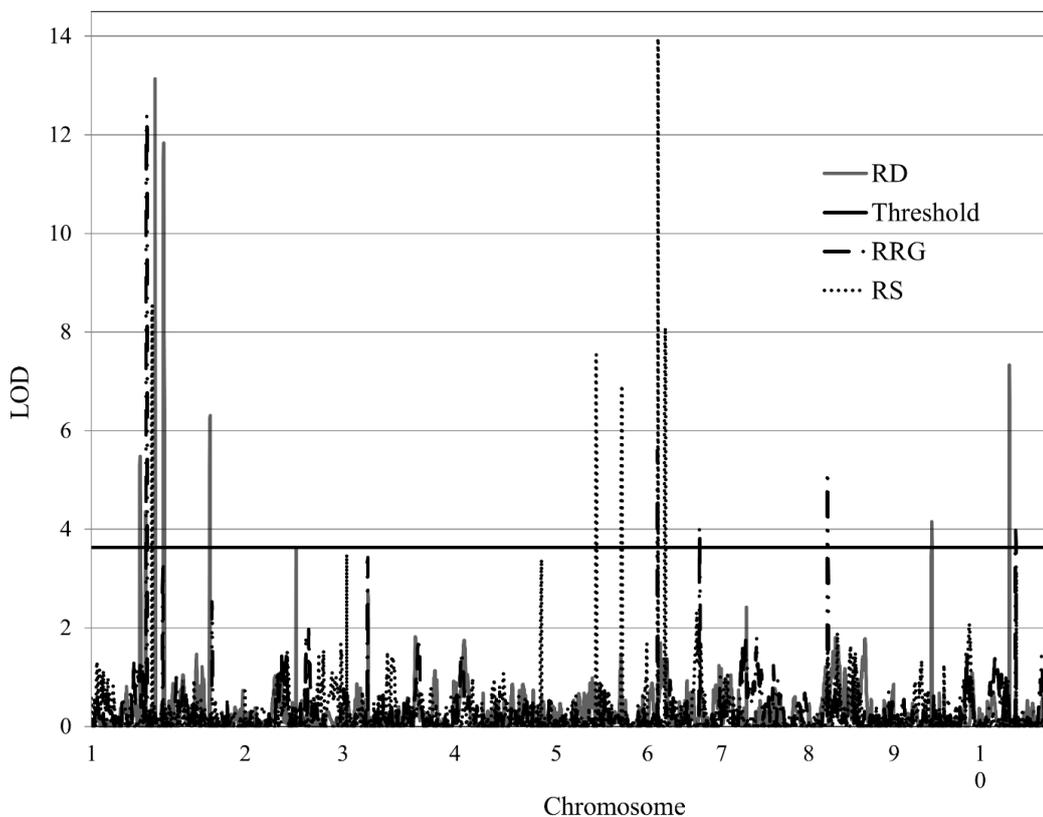


Figure 1. Results of inclusive composite interval mapping for root damage (RD), root regrowth (RR) and root size (RS). The calculated LOD threshold based on 1000 permutations was 3.629.

In order to better understand complex mechanism of insect resistance in maize, more studies using both QTL mapping and different methods of targeting insect resistance are needed.

ACKNOWLEDGMENTS

This research was funded by the EU project “Biodiversity and Molecular Plant Breeding”, grant number KK.01.1.1.01.0005, of the Centre of Excellence for Biodiversity and Molecular Plant Breeding (Crop-BioDiv), Zagreb, Croatia.

Received, September 27th, 2019

Accepted February 18th, 2020

REFERENCES

- AVANCI, N.C., D.D., LUCHE, G.H., GOLDMAN, M.H.S., GOLDMAN (2010): Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. *Genetics and Molecular Research*, 9 (1): 484-505.
- BATES, D., M., MÄCHLER, B., BOLKER, S., WALKER (2014): Fitting Linear Mixed-Effects Models using lme4, 67.
- BERNKLAU, E.J., B.E., HIBBARD, L.B., BJOSTAD (2010): Antixenosis in maize reduces feeding by western corn rootworm larvae (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, 103(6): 2052-2060.
- BIGGER, J.H., J.R., HOLBERT, W.P., FLINT, A.L., LANG (1938): Resistance of certain corn hybrids to attack of southern corn rootworm. *Journal of Economic Entomology*, 31: 102-107.
- BOHN, M.O., J., NOVAIS, R., FONSECA, R., TUBEROSA, T.E., GRIFT (2006): Genetic evaluation of root complexity in maize. *Acta Agronomica Hungarica*, 54(3):291-303.
- BOHN, M.O., J.J., MARROQUIN, S., FLINT-GARCIA, K., DASHIELL, D.B., WILMOT, B.E., HIBBARD (2018): Quantitative trait loci mapping of western corn rootworm (Coleoptera: Chrysomelidae) host plant resistance in two populations of doubled haploid lines in maize (*Zea mays* L.). *Journal of Economic Entomology*, 111(1): 435-444.
- BRKIĆ, A., I., BRKIĆ, A., JAMBROVIĆ, M., IVEZIĆ, E., RASPUDIĆ, M., BRMEŽ, Z., ZDUNIĆ, T., LEDENČAN, J., BRKIĆ, M., MARKOVIĆ, G., KRIZMANIĆ, D., ŠIMIĆ (2017): Maize germplasm of Eastern Croatia with native resistance to western corn rootworm (*Diabrotica virgifera virgifera* Le Conte). *Genetika*, 49(3): 1023-1034.
- CASTANO-DUQUE, L., K.W., LOADES, J.F., TOOKER, K.M., BROWN, W.P., WILLIAMS, D.S., LUTHE (2017): A maize inbred exhibits resistance against western corn rootworm, *Diabrotica virgifera virgifera*. *Journal of Chemical Ecology*, 43:1109-1123.
- CHRISTENSEN, S.A., A., NEMCHENKO, E., BORREGO, I., MURRAY, I.S., SOBHY, L., BOSAK, S., DEBLASIO, M., ERB, C.A., ROBERT, K.A., VAUGHN, C., HERRFURTH, J., TUMLINSON, I., FEUSSNER, D., JACKSON, T.C.J., TURLINGS, J., ENGELBERTH, C., NANSEN, R., MEELEV, M.V., KOLOMIETS (2013): The maize lipoxygenase, ZmLOX10, mediates green leaf volatile, jasmonate and herbivore-induced plant volatile production for defense against insect attack. *Plant Journal*, 74: 59-73.
- COVARRUBIAS-PAZARAN, G. (2016): Genome-Assisted Prediction of Quantitative Traits Using the R Package *sommer*. *PLoS ONE*, 11(6): e0156744.
- CROOKSTON, R.K., J.E., KURLE, P.J., COPLAND, J.H., FORD, W.E., LUESCHEN (1991): Rotational cropping sequence affects yield of corn and soybean. *Agronomy Journal*, 83: 108-113.
- EL KHISHEN, A.A., M.O., BOHN, D.A., PRISCHMANN-VOLDSETH, K.E., DASHIELL, B.W., FRENCH, B.E., HIBBARD (2009): Native resistance to western corn rootworm (Coleoptera: Chrysomelidae) larval feeding: characterization and mechanisms. *Journal of Economic Entomology*, 102(6): 2350-2359.
- FALQUE, M., L., DÉCOUSET, D., DERVINS, A.M., JACOB, J., JOETS, J.P., MARTINANT, X., RAFFOUX, N., RIBIÈRE, C., RIDEL, D., SAMSON, A., CHARCOSSET, A., MURIGNEUX (2005): Linkage mapping of 1454 new maize candidate gene loci. *Genetics*, 170: 1957-1966.
- FARMER, E.E., E., ALMÉRAS, V., KRISHNAMURTHY (2003): Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Current Opinion in Plant Biology*, 6(4): 372-378.
- FLINT-GARCIA, S.A., K.E., DASHIELL, D.A., PRISCHMANN, M.O., BOHN, B.E., HIBBARD (2009): Conventional screening overlooks resistance sources: Rootworm damage of diverse inbred lines and their B73 hybrids is unrelated. *Journal of Economic Entomology*, 102(3): 1317-1324.
- HALLAUER, A.R., M.J., CARENA, J.B., MIRANDA FILHO (2010): *Quantitative genetics in maize breeding*. Springer, New York, USA, 500p.
- HAMBERG, M. (1988) Biosynthesis of 12-oxo-10,15(Z)-phytodienoic acid: Identification of allene oxide cyclase. *Biochemical and Biophysical Research Communications*, 156(1):543-550.

- HIBBARD, B.E., L.L., DARRAH, B.D., BARRY (1999): Combining ability of resistance leads and identification of a new resistance source for western corn rootworm (Col.: Chrysomelidae) larvae in corn. *Maydica*, 44: 133-139.
- HIBBARD, B.E., D.B., WILMOT, S.A., FLINT-GARCIA, L.L., DARRAH (2007): Registration of the maize germplasm CRW3(S1)C6 with resistance to western corn rootworm. *Journal of Plant Registrations*, 1(2):151-152.
- IVEZIĆ, M., J.J., TOLLEFSON, E., RASPUDIĆ, I., BRKIĆ, M., BRMEŽ, B.E., HIBBARD (2006): Evaluation of corn hybrids for tolerance to corn rootworm (*Diabrotica virgifera virgifera* LeConte) larval feeding. *Cereal Research Communications*, 34: 1101-1107.
- KAHLER, A.L., R.E., TELKAMP, L.H., PENNY, T.F., BRANSON, P.J., FITZGERALD (1985): Registration of NGSDCRW1(S2)C4 maize germplasm. *Crop Science*, 25: 202.
- KISS, J., J., KOMÁROMI, K., BAYAR, C.R., EDWARDS, I., HATALA-ZSELLÉR (2005): Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and the crop rotation systems in Europe. In: Vidal S, Kuhlmann U, Edwards CR (eds) *Western corn rootworm: Ecology and management*, CABI Publishing, Cambridge, MA, p. 189-220.
- KNUTSON, R.J., B.E., HIBBARD, B.D., BARRY, V.A., SMITH, L.L., DARRAH (1999): Comparison of screening techniques for western corn rootworm (Coleoptera: Chrysomelidae) host-plant resistance. *Journal of Economic Entomology*, 92(3): 714-722.
- KURZ, L.T., L.V., BOONE, T.R., PECK, R.G., HOEFT (1984): Crop rotations for efficient nitrogen use. In Hauck RD (ed) *Nitrogen in Crop Production*, American Society of Agronomy, Madison, WI, 295.
- LARSEN, J.S. (1999): The genetic variation and genetics of the resistance of maize (*Zea mays* L.) to the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). PhD School of Graduate Studies and Research, University of Ottawa, Ottawa-Carleton Institute of Biology.
- LEE, M., N., SHAROPOVA, W.D., BEAVIS, D., GRANT, M., KATT, D., BLAIR, A., HALLAUER (2002): Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Molecular Biology*, 48(5-6): 453-61.
- LOPEZ, L., A., CAMAS, R., SHIVAJI, A., ANKALA, P., WILLIAMS, D.S., LUTHE (2007): Mir1-CP, a novel defense cysteine protease accumulates in maize vascular tissues in response to herbivory. *Planta*, 226: 517-527.
- MARTON, L.C., C., SZÓKE, J., PINTÉR, E., BODNÁR (2009): Studies on the tolerance of maize hybrids to western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Maydica*, 54: 217-220.
- MENG, L., H., LI, L., ZHANG, J., WANG (2015): QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop Journal*, 3(3): 269-283.
- OLESON, J.D., Y.L., PARK, T.M., NOWATZKI, J.J., TOLLEFSON (2005): Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, 98: 1-8.
- OWENS, J.C., D.C., PETERS, A.R., HALLAUER (1974): Corn rootworm tolerance in maize. *Environmental Entomology*, 3: 767-772.
- PAINTER, R.H. (1951): *Insect Resistance in Crop Plants*. Macmillan, New York.
- PECHAN, T., B.H., JIANG, D.S., STECKLER, L., YE, L., LIN, D.S., LUTHE, W.P., WILLIAMS (1999): cDNA clones encoding cysteine proteinases from corn (*Zea mays* L.) callus. *Plant Molecular Biology*, 40, 111-119.
- PECHAN, T., L., YE, Y., CHANG, A., MITRA, L., LIN, F.M., DAVIS, W.P., WILLIAMS, D.S., LUTHE (2000): A Unique 33-kD Cysteine Proteinase Accumulates in Response to Larval Feeding in Maize Genotypes Resistant to Fall Armyworm and Other Lepidoptera. *Plant Cell*, 12: 1031-1040.
- PRISCHMANN, D.A., K.E., DASHIELL, D.J., SCHNEIDER, B.E., HIBBARD (2007): Field screening maize germplasm for resistance and tolerance to western corn rootworm (Col.: Chrysomelidae). *Journal of Applied Entomology*, 131(6): 406-415.
- R CORE TEAM (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

- RIEDEL, W.E., R.N., REESE (1999): Maize morphology and shoot CO₂ assimilation after root damage by western corn rootworm larvae. *Crop Science*, 39: 1332-1340.
- ROGERS, R.R., J.C., OWENS, J.J., TOLLEFSON, J.F., WITKOWSKI (1975): Evaluation of commercial corn hybrids for tolerance to corn rootworms. *Environmental Entomology*, 4: 920-922.
- SCHALLER, A., A., STINTZI (2009): Enzymes in jasmonate biosynthesis – Structure, function, regulation. *Phytochemistry*, 70: 1532–1538.
- SPIKE, B.P., J.J., TOLLEFSON (1991): Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. *Journal of Economic Entomology*, 84: 1585-1590.
- SUTTER, G.R., J.R., FISHER, N.C., ELLIOTT, T.F., BRANSON (1990): Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, 83: 2414-2420.
- ŠIMIĆ, D., M., IVEZIĆ, I., BRKIĆ, E., RASPUDIĆ, M., BRMEŽ, I., MAJIĆ, A., BRKIĆ, T., LEDENČAN, J.J., TOLLEFSON, B.E., HIBBARD (2007): Environmental and genotypic effects for western corn rootworm tolerance traits in American and European maize trials. *Maydica*, 52: 425-430.
- TOLLEFSON, J.J. (2007): Evaluating maize for resistance to *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). *Maydica*, 52: 311-318.
- WILLIAMS, W.P., F.M., DAVIS (1982): Registration of Mp704 germplasm line of maize. *Crop Science*, 22: 1269-1270.
- WILLIAMS, W.P., F.M., DAVIS, G.L., WINDHAM (1990): Registration of Mp708 germplasm line of maize. *Crop Science*, 30: 757.
- WISEMAN, B.R. (1985): Types and mechanisms of host plant resistance to insect attack. *International Journal of Tropical Insect Science*, 6(3): 239-242.
- WOUTERS, F.C., B., BLANCHETTE, J., GERSHENZON, D.G., VASSÃO (2016): Plant defense and herbivore counter-defense: benzoxazinoids and insect herbivores. *Phytochemistry Reviews*, 15: 1127–1151.
- YAN, Y., S., CHRISTENSEN, T., ISAKEIT, J., ENGELBERTH, R., MEELEV, A., HAYWARD, R.J., NEIL EMERY, M.V., KOLOMIETS (2012): Disruption of OPR7 and OPR8 reveals the versatile functions of jasmonic acid in maize development and defense. *Plant Cell*, 24: 1420-1436.

QTL ANALIZA ZA OTPORNOST NA KUKURUZNU ZLATICU KOD IBM POPULACIJE KUKURUZA GAJENE U MONOKULTURI

Andrija BRKIĆ^{1*}, Domagoj ŠIMIĆ^{1,2}, Antun JAMBROVIĆ^{1,2}, Zvonimir ZDUNIĆ^{1,2}, Tatjana LEDENČAN¹, Emilija RASPUDIĆ³, Mirjana BRMEŽ³, Josip BRKIĆ¹, Maja MAZUR¹, Vlatko GALIĆ¹

¹Poljoprivredni institut Osijek, Departman za oplemenjivanje i genetiku kukuruza, Osijek, Hrvatska

²Centar Izvrsnosti za biodiverzitet i Molekularno Oplemenjivanje Biljaka (CroP-BioDiv), Zagreb, Hrvatska

³Josip Juraj Strossmayer Univezitet u Osijeku, Fakultet za agrobiotehničke nauke Osijek Departman za zaštitu biljaka, Šef entomologije i nematologije, Osijek, Hrvatska

Izvod

Kukuruzna zlatica (*Diabrotica virgifera virgifera* LeConte; WCR) je najrazornija štetočina kukuruza u SAD-u i jedna od najopasnijih štetočina u Evropi. Za nešto više od decenije od introdukcije postala je jedna od najozbiljnijih štetočina u Hrvatskoj. Domaći otporni kultivari mogli bi biti efikasna strategija za kontrolu WCR-a. Iako se istraživanje otpornosti biljaka domaćina na WCR kontinuirano sprovodi više od 70 godina, danas nijedan otporan hibrid kukuruza nije dostupan u prodaji. Cilj ovog izučavanja bio je proceniti osobine otpornosti u rekombinantnim inbred linijama kukuruza IBM (IRILs) i otkriti QTL-ove povezane sa rezistentnošću na kukuruznu zlaticu. Fenotipizirano je 207 rekombinantnih inbred linija za standardne karakteristike otpornosti na WCR. Detektovano je nekoliko povezanih QTL-ova na hromozomima 1, 5, 6, 8, 9 i 10. QTL-ovi na hromozomima 1 i 6 su mapirani u regionima koji sadrže gene povezane sa odbranom biljke protiv biljojeda. Otkriveni QTL-ovi pružaju relevantne smernice za buduća istraživanja otpornosti kukuruza na zlaticu (WCR).

Primljeno 24.IX.2019.

Odobreno 18. II. 2020