

**MAIZE GERmplasm OF EASTERN CROATIA WITH NATIVE RESISTANCE  
TO WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* LeConte)**

Andrija BRKIĆ<sup>1</sup>, Ivan BRKIĆ<sup>1</sup>, Antun JAMBROVIĆ<sup>1</sup>, Marija IVEZIĆ<sup>2</sup>, Emilija RASPUDIĆ<sup>2</sup>,  
Mirjana BRMEŽ<sup>2</sup>, Zvonimir ZDUNIĆ<sup>1</sup>, Tatjana LEDENČAN<sup>1</sup>, Josip BRKIĆ<sup>1</sup>, Monika  
MARKOVIĆ<sup>3</sup>, Goran KRIZMANIĆ<sup>4</sup>, Domagoj ŠIMIĆ<sup>1</sup>

- <sup>1</sup> Agricultural Institute Osijek, Department for Breeding and Genetics of Maize, Osijek, Croatia  
<sup>2</sup> Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Department for  
Plant Protection, Chair for Entomology and Nematology, Osijek, Croatia  
<sup>3</sup> Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Department for  
Plant Production, Chair for General Plant Production and Agricultural Melioration, Osijek,  
Croatia  
<sup>4</sup> Agricultural Institute Osijek, Department for Breeding and Genetics of Forage Crops, Osijek,  
Croatia

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 leconte*).*- Genetika, Vol 49, No.3, 1023-1034.

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte; WCR) is a serious maize pest in Croatia. The species was first registered in Europe in the early 1990s and since then became one of the most dangerous maize pests, especially in parts of Central and Southeast Europe. Larvae that feed on the maize roots cause the most serious damages in maize fields. Management of this pest is difficult and expensive, with possible serious impact on the environment. Native (or host-plant) resistance of maize against WCR could provide new economically and ecologically sustainable options in WCR management. Main goal of this study was to assess the variability of maize germplasm, correlations among resistance traits, and detect potential sources of resistance that could be used in breeding programs in order to develop hybrids with higher level of resistance against WCR. To our knowledge, the first native resistant hybrid is yet to be registered. Results showed great variability of estimated germplasm. Effect of the genotype was significant in all environments, as

---

*Corresponding author:* Andrija Brkić, Agricultural Institute Osijek, Department for Breeding and Genetics of Maize, Južno predgrađe 17, Osijek 31000, Croatia. Telephone: +385 31 515 588. E-mail: [andrija.brkic@poljinos.hr](mailto:andrija.brkic@poljinos.hr)

well as many interactions between genotype and the environment. Significant interactions emphasize the importance of the environment in WCR native resistance research. Significant positive correlations among all traits were detected. Several inbred lines were selected as a potentially useful germplasm for resistance breeding programs.

*Key words:* germplasm, maize, native resistance, root traits, western corn rootworm

## INTRODUCTION

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is the most destructive maize pest in United States (KRYSAN and MILLER, 1986), and one of the most dangerous maize pests in Croatia. It became an economically significant pest in the USA in the early 20th century (GILLETTE, 1912), and it was first registered in Europe in 1992 (BAČA, 1993). Since then this pest spread to many European countries, as far as Great Britain (CHEEK *et al.*, 2004) and Russia (EPPO, 2012). In the maize growing region of Eastern Croatia it can significantly affect grain yield, especially in continuous maize (IVEZIĆ and RASPUDIĆ, 2004). The most severe damages are caused by WCR larvae that feed on maize roots. Severely damaged plants are sensitive to lodging (LEVINE and OLOUMI-SADEGHI, 1991), which affects grain yield and increases the cost of maize production (GRAY, 2000; SAPPINGTON *et al.*, 2006). Development of maize resistant germplasm presents both economically and ecologically sustainable option in maize production areas of Eastern Croatia (it would reduce the cost of seed treatment and lower the impact of insecticides on the environment). Traditional resistance breeding programs (PAINTER, 1951) recognize three main mechanisms of pest resistance: 1) non-preference or antixenosis (insect avoids the plant as a result of plant's chemical or morphological defense); 2) antibiosis (plant has an active defense against insects by producing antibiotics); and 3) tolerance (plant does not stop the pest, it allows the presence of the pest without significant losses). Research on WCR native resistance (host-plant resistance) has been done in the USA (HIBBARD, 2007; TOLLEFSON, 2007; EL KHISHEN, 2009) and Europe (IVEZIĆ *et al.*, 2006a; ŠIMIĆ *et al.*, 2007; IVEZIĆ *et al.*, 2011), as the pest developed economically significant populations only in these two major regions. Research on WCR resistance started in the USA about 70 years ago and continues today. KNUTSON *et al.* (1999) compared screening techniques for WCR host-plant resistance. LARSEN (1999) conducted several studies in order to better understand complex mechanisms underlying the rootworm resistance. Research by IVEZIĆ *et al.* (2006a) showed some tolerant Croatian hybrids both in Croatia and USA. In search of an alternative for transgenic maize and soil insecticide treatment, field screening of maize germplasm for WCR resistance and tolerance was conducted by PRISCHMANN *et al.* (2007), whereas FLINT-GARCIA *et al.* (2009) studied relations of rootworm damage between inbred lines and their hybrids. To our knowledge, there are no reports of WCR infestations and economic damages at other continents except North America and Europe. However, despite considerable research in WCR resistance, no resistant hybrid was yet commercially released. Many studies (HIBBARD *et al.*, 1999; PRISCHMANN *et al.*, 2007; FLINT-GARCIA *et al.*, 2009; IVEZIĆ *et al.*, 2011; BRKIĆ *et al.*, 2014) detected populations, inbreds and test-crosses with certain level of resistance (tolerance), however what makes resistant hybrids difficult task to achieve is the fact that host plant resistance in maize is a complex, quantitative trait and its mechanisms are not yet fully explained. Research by PRISCHMANN *et al.* (2007), GRAY *et al.* (2009), and HESSEL (2014) confirm that there is more

than one physiological mechanism underlying WCR host-plant resistance in maize, and that these mechanisms are bound to root traits (root injury, regrowth and size). Objective of this study was to assess the variability of maize germplasm, correlations among traits, and detect potential sources of resistance that could be used in breeding programs in order to develop hybrids with higher level of resistance against WCR, as well as to better understand native resistance of maize to WCR.

## MATERIALS AND METHODS

### *Experimental design*

Trials were conducted in three years (2007, 2008 and 2009) at two locations in Eastern Croatia - Osijek in Slavonija region (45° 33' 4" N, 18° 41' 38" E) and Karanac in Baranja region (45° 45' 38" N, 18° 41' 4" E). In each environment (location × year) trials had two replications and were set with incomplete block design ( $\alpha$ -design) (PATTERSON and WILLIAMS, 1976). Each replication had 8 incomplete blocks with 16 treatments (genotypes). All the genotypes were represented with single-row plots, 5 m long, 25 cm between hills and with 70 cm row spacing. WCR infection in the field was natural, and no crop rotation was performed in order to increase the insect pressure. Maize is grown continuously in the field in Osijek for more than 60 years, whereas the continuous maize growing took place in the field in Karanac for several years (the exact information is not available). Standard soil and crop management practices for maize production were applied in each environment. Trials are denoted as OS07, OS08, OS09, KA07, KA08 and KA09, each denotation representing one separate environment (e. g. OS07 represents the trial at Osijek location in 2007).

### *Germplasm*

Material comprised 119 inbred lines from different heterotic groups: 36 Iowa Dent (ID), 27 Lancaster-Ohio (LANC-OH), 28 Iowa Stiff Stalk (BSSS), 19 inbreds from single-cross (SC), 1 European flint (EFL), 5 sweet corn (SU), and 3 popcorn inbreds (PC), all developed at Agricultural Institute Osijek (AIO), as well as 8 populations (CRW) with various known levels of resistance to WCR (KAHLER, 1985; HIBBARD, 1999, 2007; PRISCHMANN *et al.*, 2007), including the hybrid B37×H84 developed from public inbreds, as a susceptible control (Tables 1 and 2). In all three years total of 127 genotypes was used, however in 2008 and 2009 the last genotype (LH51×CRW3(S1)C6) was planted twice (double entry). This set of inbreds developed at AIO has not been tested before in WCR resistance breeding research trials.

*Table 1. Germplasm divided in different heterotic pools (source of ID, LANC-OH = AIO)*

| Heterotic pool | Inbred lines  |
|----------------|---|
| ID             | Os 53-61, Os 10-58, Kr 742, Kr 757, TVA 4040-8, L 219, Os 2-48, ID 8320, Os 1-48, Kr 640, Kr 1184/2, Kr 879/63, Kr 704, Os-24, Os 29-48, Os-10, Os 1571, TVA 2177-8, ID 8551 2348, Os 2948-75, Os 5805, Os-MM, TVA 2340-8, Os-05, Os 14-48, Os 3-48, Os 4445 E, L 254, L 370, Kr 773, Kr 774, Os 23-48, Os 4445, Os 30-48, ID 8482, Os 5837 |
| LANC-OH        | Os 1-44 42-16, Os 821, Os 608, Os 42-16, DK 633/266, Os 6-2 L, Os 418, Os-OP, Os 135-88, Os 10-23, Os-C633, Os 2-87, Os 1-85, Os 133-02, Kr 720, Os 6-2, Os 6-2 24-48, Os 1-44, Mo 17 NS, Os 524, Os 5-23, To 240-3, Os 1-56, Os 213, Os 163-9, Os 1787, B 93   |

Table 2. Germplasm divided in different heterotic pools (source of BSSS, SC, EFL, PC, SU = AIO; source of CRW = NCRPI, SDSU AES, USDA-ARS Columbia-MO, public inbreds)

| Heterotic pool | Inbred lines   |
|----------------|--|
| BSSS           | Os 87-24, Os 84-28, Os 2-56, Os 3-56, Os 431-89, Os 33, Os-DW, DK 377, LH 132, LH 119, Kr 1120/43, 645 Kr 146/527, Os 438-95 M-Bc, Kr 1081/23, Kr 1081/30, Os 10-64, Os 10-75, Os 1-95, Os 4275/13, B 104, B109, Os 438-95, B 73, Os 2253, 645 Kr 438-95, Os 438-95 MP, B 84, Os 441 95 645 Kr |
| SC             | TVA 501 227, Os 9906, TVA 3818-8, TVA 1970-8, Os 8718/1, Kr 244, Os-MP, Os-M418, Os 6-58 24-48, Os 1767/99, Os (6-58 P 796) 6-58, Os 522/99, Os 5982 SLOP, Os 542, Os 541, Os 606-2982, Os 606-8020, MONA, Os 2222   |
| EFL            | Os 1252/99   |
| PC             | Os 29/96, Os 31/96, Os 605 p.c.  |
| SU             | Os 7035 T, Os 7046 T, Os 7065-01, Os 254 su, Os 255 su   |
| CRW            | CRW8-1a, CRW8-1b, CRW8-2, CRW3(S1)C6, CRW2(C5), NGSDCRW1(S2)C4, LH51×CRW3(S1)C6  |

(NCRPI = North Central Regional Plant Introduction Station; SDSU AES = South Dakota State University Agricultural Experiment Station; USDA-ARS = United States Department of Agriculture-Agricultural Research Service)

### Evaluation methods

Methods for root evaluation were given in detail by IVEZIĆ *et al.* (2006a). Three traits associated with WCR resistance (root injury – RI, root regrowth – RR, and root size – RS) were evaluated with different scales, root injury with Iowa State University (ISU) 0-3 Node Injury Scale (OLESON *et al.*, 2005), root regrowth and root size with reversed Eiben 1-6 Scale (ROGERS *et al.*, 1975). ISU 0-3 Node Injury Scale is based on the number of plant nodes eaten by WCR (e. g. rating '0' means no injury, while rating '3' means all three nodes eaten). Reversed Eiben 1-6 Scale is based on secondary roots developed by the plant as a defense mechanism against root weakening caused by WCR, as well as on the root size (e. g. rating '1' denotes rich secondary roots formation and big root, while rating '6' denotes poor secondary roots formation and small root).

### Monitoring of adults

Monitoring is an important part of WCR research. Distribution of WCR adults in the field during the season represents a valuable information for both researchers and producers of maize (catches of five or more adults per yellow sticky trap in one day indicate potential problems with WCR the following year). In this research in all three seasons PALs traps with a floral bait (Csal♀m♂N<sup>®</sup>, Plant Protection Institute, MTA ATK, Budapest) were used for monitoring WCR adults in the field (post-emergence period of the WCR life cycle). Traps were replaced at each monitoring site in the field every 20-25 days. Monitoring of the environment KA09 was not performed due to technical difficulties.

### Data analysis

Data from each environment were first analysed separately, and combined ANOVA (COCHRAN and COX, 1957) was performed afterwards. Heritability of three traits was calculated in combined ANOVA based on entry means (Figure 1; HALLAUER and MIRANDA, 1988), where

$\sigma_g^2$  is the genotypic variance,  $\sigma_{ge}^2$  variance of genotype  $\times$  environment interaction,  $\sigma^2$  the pooled error variance, E is the number of environments, and R is the number of replications. Correlation coefficients among traits were also calculated. Statistical analysis was performed using Plabstat software package (UTZ, 1995).

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{1}{E}\sigma_{ge}^2 + \frac{1}{ER}\sigma^2}$$

Figure 1. Heritability calculation formula

## RESULTS AND DISCUSSION

### *Monitoring of WCR adults*

Trap monitoring data showed that the highest insect pressure was at Karanac location in 2008, with 23.64 WCR adults per site per day (Table 3). Higher population density of the pest suggests more damage to maize crops, however interaction among maize genotypes, WCR adults and the environment is very complex, and in some cases higher pressure does not indicate higher damages (e.g. environment OS07 with only 2.82 adults per site per day showed average root injury rating of 1.62, while environment OS09 with 12.81 adults per site per day showed average root injury rating of 1.44). In a study about WCR populations in the continuous maize, using the same PALs traps IVEZIĆ *et al.* (2006b) reported 6.3 adults per site per day at Duboševica location, which is only about 20 km from Karanac location. As a result of larger WCR populations in Karanac it is assumed that WCR was probably introduced to Baranja region couple years earlier compared to Slavonija region.

Table 3. Seasonal monitoring of WCR adults

| Year                          | 2007.  |         | 2008.   |         | 2009.   |
|-------------------------------|--------|---------|---------|---------|---------|
| Location                      | Osijek | Karanac | Osijek  | Karanac | Osijek  |
| Monitoring sites in the field | 3      | 3       | 6       | 6       | 1       |
| Total number of adults        | 949    | 1990    | 7097    | 17730   | 1422    |
| Adults per site               | 316.33 | 663.33  | 1182.83 | 2955.00 | 1422.00 |
| Adults per site per day       | 2.82   | 4.99    | 9.46    | 23.64   | 12.81   |

### *Variability*

The results showed great variability of evaluated genotypes in each environment, which was expected with such diverse background of used germplasm collected from different sources. One-way ANOVA revealed significant effects of genotypes for all three traits in each environment except in KA09 for root regrowth and size. Effect of replication was very significant in KA07, KA08 for root injury, in OS07, OS08, OS09, KA09 for root regrowth, and in KA08, OS09 for root size, while the effect of block was very significant in all environments for root injury, in OS08, KA08, OS09 for root regrowth and in all environments except KA09 for root size (Table 4). In a similar study by ŠIMIĆ *et al.* (2007) the effect of genotypes was significant in all tested environments in the USA and at some in Croatia; root regrowth was the only trait that had consistently non-significant effects of replication and significant effect of

genotype in all environments. IVEZIĆ *et al.* (2009) reported non-significant effect of the genotype across environments for root injury and root size, while for root regrowth effect of genotype was significant. Various results in different studies (MARTON *et al.*, 2009; IVEZIĆ *et al.*, 2011; BRKIĆ, 2012) underline the importance of the environment in WCR research.

*Table 4. Effect of genotype, replication and block in all environments*

| Effect of genotype |    |    |    | Effect of replication |    |    |    | Effect of block |    |    |    |
|--------------------|----|----|----|-----------------------|----|----|----|-----------------|----|----|----|
| Environment        | RI | RR | RS | Environment           | RI | RR | RS | Environment     | RI | RR | RS |
| OS07               | ** | ** | ** | OS07                  | ns | ** | ns | OS07            | ** | ns | ** |
| KA07               | ** | ** | ** | KA07                  | ** | ns | ns | KA07            | ** | ns | ** |
| OS08               | ** | ** | ** | OS08                  | ns | ** | ns | OS08            | ** | ** | ** |
| KA08               | ** | ** | ** | KA08                  | ** | ns | ** | KA08            | ** | ** | ** |
| OS09               | ** | ** | ** | OS09                  | ns | ** | ** | OS09            | ** | ** | ** |
| KA09               | ** | *  | ns | KA09                  | ns | ** | ns | KA09            | ** | ns | ns |

\*\*, \* Significant at 0.01, 0.05 probability level, respectively

### ***G × E interaction***

Mean values of genotypes ranged in different environments from 1.07 (Os 1-56) to 1.94 (Os-05) for root injury, from 2.25 (CRW-8-2) to 4.98 (Os 31/96) for root regrowth, and from 2.45 (CRW-8-2) to 4.78 (Os-24) for root size (data not shown). Means of all traits varied significantly depending on the environment. Environment KA07 had the lowest mean root injury value, environment OS07 had the lowest mean root regrowth value, while the environment KA09 had the lowest mean root size value. Environment KA08 had the highest mean value with all three traits (Table 5). Very poor ratings in the environment KA08 can be attributed to higher insect pressure in Baranja region in 2008; WCR trap monitoring revealed highly increased number of adult WCR at Karanac location in 2008 maize growing season (BRKIĆ, 2012; Table 3). After three-way ANOVA (location, year, genotype) the results showed significant effects of the location, year and genotype for all three traits, except the effect of the location on root injury (Table 6). Interaction between year and location was significant for all traits, while the interaction between genotype and location was not significant. Interaction between genotype and year was significant for root injury and regrowth. Finally interaction among genotype, year and location was significant for all three traits. In the research by IVEZIĆ *et al.* (2009) genotypes tested for WCR native resistance showed significant differences among years for all three traits.

*Table 5. Means of 127 genotypes in all environments*

| Environment | Injury | Regrowth | Size |
|-------------|--------|----------|------|
| 1 (OS07)    | 1.62   | 2.76     | 3.35 |
| 2 (KA07)    | 1.02   | 3.75     | 3.81 |
| 3 (OS08)    | 1.57   | 3.70     | 3.85 |
| 4 (KA08)    | 2.26   | 4.20     | 4.38 |
| 5 (OS09)    | 1.44   | 3.97     | 3.63 |
| 6 (KA09)    | 1.43   | 4.05     | 3.21 |
| Mean        | 1.56   | 3.74     | 3.71 |
| LSD 0.05    | 0.08   | 0.18     | 0.15 |

Table 6. Three-way ANOVA for locations, years and genotypes and their interactions

| Source of variation        | Degrees of freedom | Variance |          |          |
|----------------------------|--------------------|----------|----------|----------|
|                            |                    | Injury   | Regrowth | Size     |
| Location                   | 1                  | 0.18 ns  | 50.56 ** | 7.24 **  |
| Year                       | 2                  | 26.59 ** | 43.84 ** | 33.61 ** |
| Genotype                   | 127                | 0.20 **  | 2.23 **  | 1.53 **  |
| Year × Location            | 2                  | 27.85 ** | 12.62 ** | 17.52 ** |
| Genotype × Location        | 127                | 0.08 ns  | 0.51 ns  | 0.40 ns  |
| Genotype × Year            | 254                | 0.13 **  | 0.66 *   | 0.39 ns  |
| Genotype × Year × Location | 253                | 0.08 **  | 0.50 **  | 0.35 **  |

\*\*, \* Significant at 0.01, 0.05 probability level, respectively

### Heritability

Heritability of the genotypes had the highest estimates for root size (75.39), and root regrowth (74.45), while heritability estimate for root injury was lower (50.35). In a comparable research of WCR root traits and elements concentration in maize roots by BRKIĆ *et al.* (2015) repeatability estimates ranged from 43.30 (root regrowth), and 45.80 (root size) to 75.10 (root injury). In a study about genetics of maize WCR resistance given by LARSEN (1999), with 270  $F_{2,3}$  families developed for genotyping and phenotyping, heritability estimates were similar for root injury (55.00) and root size (54.00). ŠIMIĆ *et al.* (2007) reported high repeatability estimates for root regrowth (89.30), while estimates for root injury (16.60) and root size (20.50) were much lower.

### Correlations

Table 7. Correlations among resistance traits in all environments

|      |          |         |          |
|------|----------|---------|----------|
| OS07 | Regrowth | -0.04   | -        |
|      | Size     | 0.12    | 0.55 **  |
|      | Injury   |         | Regrowth |
| KA07 | Regrowth | 0.08    | -        |
|      | Size     | 0.09    | 0.51 **  |
|      | Injury   |         | Regrowth |
| OS08 | Regrowth | 0.10    | -        |
|      | Size     | 0.26 ** | 0.64 **  |
|      | Injury   |         | Regrowth |
| KA08 | Regrowth | 0.50 ** | -        |
|      | Size     | 0.50 ** | 0.71 **  |
|      | Injury   |         | Regrowth |
| OS09 | Regrowth | 0.20 ** | -        |
|      | Size     | 0.20 ** | 0.58 **  |
|      | Injury   |         | Regrowth |
| KA09 | Regrowth | 0.24 ** | -        |
|      | Size     | 0.27 ** | 0.35 **  |
|      | Injury   |         | Regrowth |

\*\*, \* Significant at 0.01, 0.05 probability level, respectively

Very significant positive correlations (ranging from 0.20 to 0.71) were detected in all environments (Table 7). Correlation between root injury and root regrowth was very significant

in three environments (KA08, OS09, KA09), correlation between root injury and root size was significant in four environments (OS08, KA08, OS09, KA09), while correlation between root regrowth and root size was very significant in all environments. These results are consistent with the results from BRKIĆ *et al.* (2015). Their study detected significant and positive correlations among all three resistance traits. In a similar study, IVEZIĆ *et al.* (2006a) reported high, positive correlation ( $r=0.847$ ) between root regrowth and root weight in grams, which indicates that root size is also positively correlated to root weight and presumably to root regrowth (secondary roots increase the size of the root, and larger roots comprise more weight).

### ***Inbred line selection***

Several inbred lines with various levels of resistance (tolerance) have been selected after data analysis of all environments (Tables 8 and 9). Rating thresholds for resistance traits were arbitrary (1.25 for root injury, and 2.80 for root regrowth and size). In the whole genotype set 7% of inbreds scored ratings lower than threshold for root injury and root regrowth, while for root size percentage was slightly lower (6%). Genotypes with lowest root injury ratings were inbred lines Os 1-56 and Os 6-2 L, populations CRW8-1b, CRW3(S1)C6, as well as inbred lines Kr 774, Os 1787, Os 1571, Os 6-2, and population CRW8-2. Populations and inbreds with lowest root regrowth ratings were CRW8-2, CRW8-1b, CRW8-1a, MONA, TVA 3818-8, Os 6-2, B 104, CRW3(S1)C6, and Os 2253, while populations CRW8-1a, CRW8-2, CRW8-1b, LH51×CRW3(S1)C6, and inbreds TVA 501 227, TVA 3818-8, Os 1767/99, and Os 2222 showed lowest root size ratings of all estimated genotypes. Inbreds that showed the lowest root injury ratings (primarily Os 1-56 and Os 6-2 L) have never been used in WCR resistance breeding programs before. Thus they represent interesting and valuable genetic material for different types of WCR resistance research (including testcross performance evaluation, ionomic root analysis, QTL mapping and array-based genotyping), as well as promising target populations for new cycles of selection in WCR resistance breeding. Low ratings of resistance traits in five Missouri populations were consistent with research by HIBBARD *et al.* (2007), PRISCHMANN *et al.* (2007), EL KHISHEN *et al.* (2009), where these populations were used as resistance sources. Interestingly, the population NGSDCRW1(S2)C4 with moderate resistance to WCR, registered by KAHLER *et al.* (1985), did not score ratings below the threshold.

*Table 8. Most resistant/tolerant genotypes selected by the means of resistance traits across all environments*

| Genotype    | Heterotic group | RI < 1.25 | RR < 2.80 | RS < 2.80 |
|-------------|-----------------|-----------|-----------|-----------|
| Os 1571     | ID              | 1.23      | 4.30      | 3.80      |
| TVA 501 227 | SC              | 1.59      | 4.17      | 2.63      |
| Kr 774      | ID              | 1.21      | 3.42      | 2.88      |
| Os 6-2 L    | LANC-OH         | 1.17      | 3.48      | 4.10      |
| Os 1787     | LANC-OH         | 1.22      | 3.82      | 3.83      |
| Os 6-2      | LANC-OH         | 1.24      | 2.70      | 3.38      |
| Os 1-56     | LANC-OH         | 1.07      | 3.50      | 3.37      |
| TVA 3818-8  | SC              | 1.50      | 2.68      | 2.68      |
| B 104       | BSSS            | 1.45      | 2.72      | 3.46      |
| LSD 0.05    |                 | 0.36      | 0.85      | 0.70      |

Table 9. Most resistant/tolerant genotypes selected by the means of resistance traits across all environments

| Genotype        | Heterotic group | RI < 1.25 | RR < 2.80 | RS < 2.80 |
|-----------------|-----------------|-----------|-----------|-----------|
| Os 2253         | BSSS            | 1.44      | 2.77      | 3.67      |
| Os 1767/99      | SC              | 1.71      | 3.28      | 2.71      |
| MONA            | SC              | 1.49      | 2.62      | 3.19      |
| Os 2222         | SC              | 1.72      | 3.07      | 2.73      |
| CRW8-1a         | CRW             | 1.26      | 2.58      | 2.40      |
| CRW8-1b         | CRW             | 1.19      | 2.47      | 2.57      |
| CRW8-2          | CRW             | 1.24      | 2.25      | 2.45      |
| CRW3(S1)C6      | CRW             | 1.19      | 2.75      | 2.93      |
| LH51×CRW3(S1)C6 | CRW             | 1.34      | 3.48      | 2.60      |
| LSD 0.05        |                 | 0.36      | 0.85      | 0.70      |

### CONCLUSIONS

The results showed great variability of evaluated genotypes, as well as significant effects of genotypes for all three traits in each environment except in KA09 for root regrowth and size. Variability of the germplasm used in this research was expected given the fact that it was collected from various pools and various sources. Multifactorial analysis revealed significant G×E and G×L×Y interactions for all traits. These results underline the importance of genotype and the environment in WCR research. Additional and more extensive research is needed to better understand all the interactions among maize genotypes, environments and WCR populations, as well as complex underlying mechanisms of maize WCR resistance. New approaches may include evaluation of testcrosses, QTL analysis, genomic approaches and other. High heritability estimates for root regrowth and size indicate that maize genotypes can be reliably identified for both traits. Correlations among traits were significant and positive (correlation between root regrowth and root size was significant in all environments). Inbred lines containing resistance or tolerance to WCR have the potential of transferring that resistance to hybrids. However, in hybrid development except resistance traits maize breeders have to deal with the yield, stability and many other polygenic traits, therefore development of resistant hybrids is a continuous and lengthy process. In this study, total of 18 inbred lines with certain level of resistance were identified and selected, and will be used in further research. Inbred lines Os 1-56, Os 6-2L, MONA, Os 3818-8, Os 501 227 and other selected lines represent a very valuable material for future WCR research and resistance breeding programs, especially since those lines were not previously tested in WCR resistance trials.

### ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Education of the Republic of Croatia, grant number 079-0730463-2708.

Received, February 23<sup>th</sup>, 2017

Accepted July 18<sup>th</sup>, 2017

## REFERENCES

- BAČA, F. (1993): New member of the harmful entomofauna of Yugoslavia *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). IWGO News Letter, 13 (1-2): 21-22.
- BRKIĆ, A. (2012): Genotypic variability of native resistance to western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in maize germplasm. PhD Thesis. Josip Juraj Strossmayer University of Osijek, Croatia.
- BRKIĆ, A., I. BRKIĆ, M. IVEZIĆ, E. RASPUDIĆ, M. KOVAČEVIĆ, D. ŠIMIĆ (2014): Integration of western corn rootworm native resistant germplasm in maize breeding program. In: Proceedings of 49th Croatian and 9th international symposium on agriculture, Dubrovnik. Faculty of Agriculture in Osijek pp. 209-213.
- BRKIĆ, A., I. BRKIĆ, E. RASPUDIĆ, M. BRMEŽ, J. BRKIĆ, D. ŠIMIĆ (2015): Relations among western corn rootworm resistance traits and elements concentration in maize germplasm roots. Poljoprivreda, 21 (Vol.1): 3-7.
- CHEEK, S., R.H.A. BAKER, R.J.C. CANNON, A. MACLEOD, E. AGALLOU, P. BARTLETT (2004): First finding of the western corn rootworm in the UK. IWGO News Letter, 25 (1): 21.
- COCHRAN, W.G., G.M. COX (1957): Experimental Designs (2nd ed). John Wiley/Sons, New York.
- EL KHISHEN, A.A., M.O. BOHN, D.A. PRISCHMANN-VOLDSETH, K.E. DASHIELL, FRENCH, B.W., B.E. HIBBARD (2009): Native resistance to western corn rootworm (Coleoptera: Chrysomelidae) larval feeding: characterization and mechanisms. J. Eco. Entomol., 102 (6): 2350-2359.
- EPPO (2012): Reporting service, No. 01, Paris, 2012. Retrieved 2012 January 1 from (<http://archives.eppo.int/EPPOreporting/2012/Rse-1201.pdf>).
- 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. J. Eco. Entom., 102 (3): 1317-1324.
- GILLETTE, C.P. (1912): *Diabrotica virgifera* as a corn rootworm. J. Eco. Entom., 5: 364-366.
- GRAY, M.E. (2000): Prescriptive use of transgenic hybrids for corn rootworms: an ominous cloud on the horizon? In: Proceedings of the Illinois Crop Protection Technology Conference 2000, University of Illinois Extension, Urbana-Champaign, IL pp. 97-103.
- GRAY, M.E., T.W. SAPPINGTON, N.J. MILLER, J. MOESER, M.O. BOHN (2009): Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Ann. Rev. Entom., 54: 303-321.
- HALLAUER, A.R., J.B.F.O. MIRANDA (1988): Quantitative Genetics in Maize Breeding. Iowa State University Press (2nd ed), Ames, Iowa.
- HESSEL, D.A. (2014): Deciphering the genetic architecture of native resistance and tolerance to western corn rootworm larval feeding. PhD Thesis, Iowa State University. Paper 13874.
- 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. WILLMOT, S.A. FLINT-GARCIA, L.L. DARRAH (2007): Registration of the maize germplasm CRW3(S1)C6 with resistance to western corn rootworm. J. Plant Reg., Vol. 1, No. 2, September 2007.
- IVEZIĆ, M., E. RASPUDIĆ (2004): Ekonomski značajni štetnici kukuruza na području Istočne Hrvatske [Economically significant maize pests in Eastern Croatia]. Razprave, dissertationes XLV-1, Slovenska akademija znanosti inugetnosti, Ljubljana, Slovenia pp. 88-98.
- IVEZIĆ, M., J.J. TOLLEFSON, E. RASPUDIĆ, I. BRKIĆ, M. BRMEŽ, B.E. HIBBARD (2006a): Evaluation of corn hybrids for tolerance to corn rootworm (*Diabrotica virgifera virgifera* LeConte) larval feeding. Cer. Res. Comm., 34: 11101-11107.
- IVEZIĆ, M., I. MAJČIĆ, E. RASPUDIĆ, M. BRMEŽ, B. PRAKATUR (2006b): Značaj kukuruzne zlatice u ponovljenom uzgoju kukuruza. Poljoprivreda (Osijek), 12, 1: 35-40.

- IVEZIĆ, M., E. RASPUDIĆ, M. BRMEŽ, I. MAJIĆ, D. DŽOIĆ, A. BRKIĆ (2009): Maize tolerance to western corn rootworm larval feeding: screening through five years of investigation. *Agriculturae Conspectus Scientificus*, 74 (No.4): 291-295.
- IVEZIĆ, M., E. RASPUDIĆ, I. MAJIĆ, J.J. TOLLEFSON, M. BRMEŽ, A. SARAJLIĆ, A. BRKIĆ (2011): Root compensation of seven maize hybrids due to western corn rootworm (*Diabrotica virgifera virgifera* LeConte) larval injury. *Bulg. J. Agric. Sci.*, 17 (No1): 107-115.
- KAHLER, A.L., R.E. TELKAMP, L.H. PENNY, T.F. BRANSON, P.J. FITZGERALD (1985): Registration of NGSDCRW1(S2)C4 maize germplasm. *Crop Sci.*, 25:202.
- 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. *J. Econ. Entom.*, 92(3): 714-722.
- KRYSAN, J.L., T.A. MILLER (1986): *Methods for study of pest Diabrotica*. Springer, New York, USA
- 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 Thesis, School of Graduate Studies and Research, University of Ottawa.
- LEVINE, E., H. OLOUMI-SADEGHI (1991): Management of diabroticite rootworms in corn. *Ann. Rev. Entom.*, 36: 229-255.
- MARTON, C.L., C. SZŐKE, J. PINTER (2009): Studies on the tolerance of maize hybrids to western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Maydica*, 54: 217-220.
- 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). *J. Econ. Entom.*, 98: 1-8.
- PAINTER, R.H. (1951): *Insect resistance in crop plants*. Macmillan, New York.
- PATTERSON, H.N., E.R. WILLIAMS (1976): A new class of resolvable incomplete block designs. *Biometrika*, 63: 83-92.
- 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*). *J. App. Entom.*, 131 (6): 406-415.
- ROGERS, R.R., J.C. OWENS, J.J. TOLLEFSON, J.F. WITKOWSKI (1975): Evaluation of commercial corn hybrids for tolerance to corn rootworms. *Environ. Entom.*, 4: 920-922.
- SAPPINGTON, T.W., B.D. SIEGFRIED, T. GUILLEMAUD (2006): Coordinated *Diabrotica* genetics research: accelerating progress on an urgent insect pest problem. *Am. Entomol.*, 52: 90-97.
- 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.
- UTZ, H.F. (1995): PLABSTAT Version M. Ein Computerprogramm zur statistischen Analyse von pflanzenzüchterischen Experimenten [Computer program for statistical analysis of plant breeding trials]. Selbstverlag Universität Hohenheim, Stuttgart.

**IZVORI OTPORNOSTI NA KUKURUZNU ZLATICU (*Diabrotica virgifera virgifera* LeConte) U GERMPLAZMI KUKURUZA ISTOČNE HRVATSKE**

Andrija BRKIĆ<sup>1</sup>, Ivan BRKIĆ<sup>1</sup>, Antun JAMBROVIĆ<sup>1</sup>, Marija IVEZIĆ<sup>2</sup>, Emilija RASPUDIĆ<sup>2</sup>,  
Mirjana BRMEŽ<sup>2</sup>, Zvonimir ZDUNIĆ<sup>1</sup>, Tatjana LEDENČAN<sup>1</sup>, Josip BRKIĆ<sup>1</sup>, Monika  
MARKOVIĆ<sup>3</sup>, Goran KRIZMANIĆ<sup>4</sup>, Domagoj ŠIMIĆ<sup>1</sup>

<sup>1</sup>Poljoprivredni institut Osijek, Odjel za oplemenjivanje i genetiku kukuruza, Osijek, Hrvatska

<sup>2</sup>Sveučilište Josipa Jurja Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku, Zavod  
za zaštitu bilja, Osijek, Hrvatska

<sup>3</sup>Sveučilište Josipa Jurja Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku, Zavod  
za bilinogojstvo, Osijek, Hrvatska

<sup>4</sup>Poljoprivredni institut Osijek, Odjel za oplemenjivanje i genetiku krmnog bilja, Osijek,  
Hrvatska

Izvod

Kukuruzna zlatica (*Diabrotica virgifera virgifera* LeConte) je jedan od glavnih štetočina kukuruza u Hrvatskoj. Otkrivena je u Evropi ranih devedesetih godina prošloga veka i relativno brzo postala jedna od najopasnijih štetočina kukuruza u srednjoj i južnoj Evropi. Larve koje se hrane na korenu kukuruza uzrokuju najznačajnije štete u usevima kukuruza. Suzbijanje ovog insekta je složeno i skupo, te može negativno uticati na okolinu. Prirodna otpornost kukuruza na kukuruznu zlaticu mogla bi pružiti nove ekonomski i ekološki održive opcije suzbijanja. Glavni cilj ovoga istraživanja bio je ispitati varijabilnost germplazme kukuruza, korelacije među svojstvima i odrediti potencijalne izvore otpornosti koji bi se mogli koristiti u oplemenjivačkim programima kako bi se stvorili hibridi s višim nivoom otpornosti na kukuruznu zlaticu. Koliko je poznato iz dosadašnje literature, do danas nije priznat nijedan otporni hibrid. Rezultati su pokazali veliku varijabilnost ispitivane germplazme. Efekti genotipa bili su značajni u svim okolinama, kao i mnoge interakcije između genotipa i okoline. Značajne interakcije naglašavaju važnost okoline u ovakvim istraživanjima. Ustanovljene su značajne pozitivne korelacije između svih svojstava. Nekoliko inbred linija odabrano je kao potencijalno važna germplazma za programe oplemenjivanja na otpornost.

Primljeno 23.II.2017.

Odobreno 18. VII. 2017.