# Relations among western corn rootworm resistance traits and elements concentration in maize germplasm roots

## Odnosi između svojstava otpornosti na kukuruznu zlaticu i koncentracije elemenata u korijenu u germplazmi kukuruza

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# RELATIONS AMONG WESTERN CORN ROOTWORM RESISTANCE TRAITS AND ELEMENTS CONCENTRATION IN MAIZE GERMPLASM ROOTS

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## SUMMARY

***Western corn rootworm – WCR (Diabrotica virgifera virgifera LeConte) is an important maize pest in Croatia. Using native resistance of maize germplasm could reduce chemical treatments and other costs in maize production. Objectives of this study were: i) to assess variability of WCR resistance traits (root injury, regrowth and size) and concentrations of nine elements in roots of 128 maize genotypes, and ii) to determine correlations among the traits and ion concentrations. Results revealed high variability of maize genotypes for both WCR resistance traits and ion concentrations. Significant moderate negative correlations (>-0.4) were detected between root injury and boron as well as between root regrowth and iron, manganese and zinc concentrations in root. Consequently, ion concentration in maize roots might have an impact on WCR resistance research.***

***Key-words: western corn rootworm, root injury, ion concentration, maize germplasm***

## INTRODUCTION

Western corn rootworm – WCR (*Diabrotica virgifera virgifera* LeConte) is a major pest in USA and from early 1990s the pest spread substantially in Europe (Gray et al., 2009). Very soon after its discovery in 1995, WCR became one of the most important maize pests in Croatia. Significant efforts in research on native resistance of maize against WCR has been carried out in eastern Croatia (Ivezić et al., 2006, 2011; Šimić et al., 2007) for the purpose of identification of native resistance germplasm sources. Native resistant maize hybrids would reduce costs for growers, and represent a sustainable option in maize production.

The functional genomics of all ions (elements) called ionomics (Lahner et al., 2003), has been established recently, enabling measurement of concentrations of many elements simultaneously, which requires the application of high-throughput elemental analysis technologies. Accordingly, the root ionome of a maize plant represents its mineral and trace element content, and it is dependent on many factors including soil properties, several physiological processes and biotic environment including WCR. Furthermore, Boyd (2007) demonstrated that plant can be defended against some natural enemies by accumulating some elements sug-

gesting that defensive effects of elements in plants are more widespread than previously believed. To date, several studies characterized ionome of maize shoot, leaves and grain (e.g. Sorić et al., 2011; Šimić et al., 2012; Baxter et al., 2013; Mascher et al., 2014) but none dealt with maize root ionome in the context of WCR native resistance.

Objectives of this study were to assess variability of WCR resistance traits (root injury – RI, root regrowth – RR, and root size – RS), concentrations of nine elements in roots of 128 maize genotypes, and to determine correlations among the traits and ion concentrations.

## MATERIAL AND METHODS

Field trial was set out using rectangular (16×8) lattice incomplete block design with 128 genotypes planted in 2009 at one location (Osijek) in two replications. Genotype set consisted of 120 inbred lines from different heterotic groups developed at Agricultural Institute Osijek and 8 synthetic populations from USA (courtesy of Bruce

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Hibbard, ARS-USDA, University of Missouri). Genetic background of the genotypes was previously given by Brkić (2012). US populations already showed certain levels of resistance in US conditions (Hibbard et al., 2007; Prischmann et al., 2007).The research was carried out under natural WCR infestation conditions. Soil was eutric cambisol, the soil type of moderate fertility with no metal imbalances. Chemical properties of the soil corresponded to data presented by Šimić et al. (2009). Fertilizers were given according to usual requirements for high yielding maize, taking into account the soil characteristics and the previous cropping. No additional fertilizers with micronutrients were applied.

In early July plants (roots) were manually pulled out of the soil, tagged, transported to water station and washed clean. After washing three main root traits were estimated – root injury with 0-3 Iowa State University node injury scale (Oleson et al., 2005), and root regrowth and size with 1-6 Eiben reversed scale (Rogers et al., 1975). Ion concentrations of nine elements in roots (boron - B, cadmium - Cd, copper - Cu, iron - Fe, magnesium - Mg, manganese - Mn, phosphorus - P, strontium - Sr, zinc - Zn) were determined by inductively coupled plasma – optical emission spectrometry

(ICP-OES) technique (Optima 2100 DV by PerkinElmer). Microwave digestion of root samples ($HNO_3$ and $H_2O_2$ added) was performed with Mars Extraction microwave oven in the laboratory of the Research Institute for Soil Science and Agricultural Chemistry of Hungarian Academy of Science in Budapest, Hungary (Zarcinas et al., 1987; Sorić et al., 2011). Ion concentrations were expressed on root dry matter basis.

Analysis of variance, coefficients of variation, repeatability estimates for lattice designs and correlation coefficients were calculated using PLABSTAT program package (Utz, 1995).

## RESULTS AND DISCUSSION

Analysis of variance showed that all sources of variation were highly significant (P<0.01) for all three WCR resistant traits, except effect of replication on root injury, which was not significant (Table 1). Previous study by Brkić (2012) showed the same variability estimates, where only the effect of replication for root injury was not significant. Sources of variation for root ion concentrations were also highly significant, except effect of replication for Mg concentration, which was significant (P<0.05, Table 2).

### Table 1. ANOVA for WCR resistance traits
*Tablica 1. Analiza varijance za svojstva otpornosti na kukuruznu zlaticu*

| Source of variation<br>*Izvor variranja* | Df<br>*Ss* | F-value / *F-vrijednost* | | |
|---|---|---|---|---|
| | | Root injury (RI)<br>*Oštećenje korijena* | Root regrowth (RR)<br>*Sekundarni porast korijena* | Root size (RI)<br>*Veličina korijena* |
| **Replication**<br>*Ponavljanje* | 1 | 0.07 | 85.63** | 39.47** |
| **Genotype**<br>*Genotip* | 127 | 7.24** | 2.56** | 2.72** |
| **Block**<br>*Blok* | 14 | 35.49** | 5.07** | 5.45** |

**, * Significant at 0.01, 0.05 probability level, respectively - *Statistički značajno na razini vjerojatnosti 0,01, odnosno 0,05*

### Table 2. ANOVA for root ion concentrations
*Tablica 2. Analiza varijance za koncentracije iona u korijenu*

| Source of variation<br>*Izvor variranja* | F-value / *F-vrijednost* | | | | |
|---|---|---|---|---|---|
| | B | Cd | Cu | Fe | K |
| **Replication** / *Ponavljanje* | 14.30** | 130.23** | 38.13** | 15.79** | 8.66** |
| **Genotype** / *Genotip* | 3.29** | 4.27** | 2.29** | 2.31** | 3.90** |
| **Block** / *Blok* | 3.85** | 2.69** | 5.33** | 5.12** | 4.19** |
| **Source of variation**<br>*Izvor variranja* | F-value / *F-vrijednost* | | | | |
| | Mg | Mn | P | Sr | Zn |
| **Replication** / *Ponavljanje* | 4.18* | 9.14** | 28.12** | 90.41** | 43.56** |
| **Genotype** / *Genotip* | 2.43** | 2.21** | 5.89** | 2.36** | 3.83** |
| **Block** / *Blok* | 3.27** | 4.50** | 5.65** | 2.49** | 16.06** |

**, * Significant at 0.01, 0.05 probability level, respectively - *Statistički značajno na razini vjerojatnosti 0,01, odnosno 0,05*

Among all traits, iron, manganese and boron concentration had the highest coefficients of variation (Fe=56.0%, Mn=53.5%, B=47.6%) (Table 3). Higher repeatabilities of root injury (75.1%), phosphorus concentration (70.5%) and zinc concentration (70.4%) indicate that these traits can be reliably identified in maize genotypes (Table 3). In a comparable study by Šimić et al. (2007) repeatability estimates for root injury were lower (16.6%), while estimates for root regrowth were much higher (89.3%), under both artificial and natural infestation treatments.

**Table 3. Mean, coefficient of variation and repeatability of WCR resistance traits and root ion concentrations**

*Tablica 3. Srednje vrijednosti, koeficijent varijacije i ponovljivost za svojstva otpornosti na kukuruznu zlaticu i koncentracije iona u korijenu*

| Trait / *Svojstvo* | Mean ± SE <br> *Srednja vrijednost i standardna pogreška* | C.V. (%) <br> *Koeficijent varijacije (%)* | Repeatability (%) <br> *Ponovljivost (%)* |
|---|---|---|---|
| **Injury** / *Oštećenje* | 1.44 ± 0.14 | 13.8 | 75.1 |
| **Regrowth** / *Sekundarni porast* | 3.97 ± 0.46 | 16.5 | 43.3 |
| **Size** / *Veličina* | 3.63 ± 0.39 | 15.0 | 45.8 |
| **B** (mg/kg) | 4.80 ± 1.62 | 47.6 | 53.0 |
| **Cd** (mg/kg) | 0.25 ± 0.04 | 20.2 | 61.9 |
| **Cu** (mg/kg) | 10.99 ± 1.73 | 22.3 | 38.7 |
| **Fe** (mg/kg) | 3518.24 ± 1393.83 | 56.0 | 39.1 |
| **Mg** (mg/kg) | 1581.59 ± 221.90 | 19.8 | 41.5 |
| **Mn** (mg/kg) | 93.47 ± 35.38 | 53.5 | 37.4 |
| **P** (mg/kg) | 1760.15 ± 107.93 | 8.7 | 70.5 |
| **Sr** (mg/kg) | 5.61 ± 0.63 | 15.8 | 40.5 |
| **Zn** (mg/kg) | 17.40 ± 2.23 | 16.7 | 70.4 |

Results show significant positive and negative correlations among elements. The highest correlation was between Mn and Fe (0.99), as well as between Mg and Fe (0.88), Mn and Mg (0.88), Sr and Mn (0.80), Sr and Fe (0.77), Zn and Mn (0.77), Sr and Mg (0.76), Zn and Fe (0.76), Zn and Sr (0.71), and Zn and Mg (0.70) (Table 4). Significant positive and negative phenotypic correlations between elements in maize ear-leaf were reported by Sorić et al. (2011) (Fe-Cu, Mg-Cu, Mg-Fe, Mg-K, Mn-Fe, Mn-Mg, Sr-Cu, Sr-K, Sr-Mg and Sr-Mn), and Zdunić et al. (2014) (Fe-Cu, Mn-Fe, Mn-K, Mn-Mg, Sr-Fe, Sr-K, Sr-Mg and Sr-Mn). Another study by Baxter et al. (2013) showed significant correlations of elements in maize grain (Fe-Zn, K-P, Mn-P, Mg-P, Cu-K, Mn-Zn, P-Zn, Mg-Zn and others). Neither of these studies included root ionomics.

Significant and positive correlations were determined among WCR resistance traits as well. Highly significant and negative correlations were found out between WCR resistance traits and root mineral concentrations, such as between root injury and B (-0.41), Cd (-0.23) and Sr (-0.26), as well as between root regrowth and Cd (-0.27), Fe (-0.46), Mg (-0.33), Mn (-0.44), Sr (-0.36) and Zn (-0.41) (Table 4). Association between WCR root traits and different root ion concentrations suggests that further research should be conducted in order to better understand the correlation between root ionomics and WCR resistance. In this paper, root size did not have any significant correlations with root elemental concentrations.

**Table 4. Correlation coefficients of WCR resistance traits and root ion concentrations**

*Tablica 4. Korelacijski koeficijenti za svojstva otpornosti na kukuruznu zlaticu i koncentracije iona u korijenu*

| | RI | RR | RS | B | Cd | Cu | Fe | Mg | Mn | P | Sr |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RR** | 0.20* | | | | | | | | | | |
| **RS** | 0.20* | 0.58** | | | | | | | | | |
| **B** | -0.41** | 0.10 | 0.00 | | | | | | | | |
| **Cd** | -0.23** | -0.27** | -0.02 | 0.13 | | | | | | | |
| **Cu** | 0.00 | -0.16 | 0.07 | -0.12 | 0.25** | | | | | | |
| **Fe** | -0.12 | -0.46** | -0.11 | -0.16 | 0.17 | 0.51** | | | | | |
| **Mg** | -0.04 | -0.33** | 0.02 | -0.12 | 0.24** | 0.51** | 0.88** | | | | |
| **Mn** | -0.13 | -0.44** | -0.12 | -0.18* | 0.17 | 0.50** | 0.99** | 0.88** | | | |
| **P** | -0.20* | -0.01 | -0.15 | 0.19* | -0.07 | 0.03 | -0.26** | -0.19* | -0.24** | | |
| **Sr** | -0.26** | -0.36** | -0.09 | -0.03 | 0.30** | 0.40** | 0.77** | 0.76** | 0.80** | -0.15 | |
| **Zn** | -0.18* | -0.41** | -0.09 | -0.11 | 0.20* | 0.47** | 0.76** | 0.70** | 0.77** | -0.13 | 0.71** |

**, * Significant correlation at 0.01, 0.05 probability level, respectively - *Statistički značajna korelacija na razini vjerojatnosti 0,01, odnosno 0,05*; RI = root injury/ *oštećenje korijena* RR = root regrowth/ *sekundarni porast korijena*, RS = root size/ *veličina korijena*

## CONCLUSION

Maize genotypes assessed in this research showed high variability for both WCR resistance traits and ion concentration in roots. Also, correlation coefficients of assessed traits showed several significant correlations, particularly correlations between WCR resistance traits (root injury and root regrowth) and some ion concentrations. Therefore, concentration of elements in maize root might have a greater role in WCR resistance research – additional studies are needed in order to better understand physiological mechanisms behind the influence of elemental concentrations on WCR resistance traits.

## ACKNOWLEDGEMENTS

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# ODNOSI IZMEĐU SVOJSTAVA OTPORNOSTI NA KUKURUZNU ZLATICU I KONCENTRACIJE ELEMENATA U KORIJENU U GERMPLAZMI KUKURUZA

**SAŽETAK**

*Kukuruzna zlatica (Diabrotica virgifera virgifera LeConte) važan je štetnik kukuruza u Hrvatskoj. Korištenje prirodne otpornosti u germplazmi kukuruza moglo bi smanjiti troškove kemijskoga tretiranja, kao i ostale troškove pri proizvodnji kukuruza. Glavni ciljevi ovoga rada bili su: i) procijeniti varijabilnost svojstava otpornosti na kukuruznu zlaticu (oštećenje, sekundarni porast i veličina korijena) i koncentracije devet elemenata u korijenu 128 genotipova kukuruza te ii) utvrditi korelacije između svojstava otpornosti i koncentracije iona. Rezultati su pokazali veliku varijabilnost ispitivanih genotipova i za svojstva otpornosti na kukuruznu zlaticu i za koncentracije iona u korijenu. Značajne negativne korelacije (>-0.4) utvrđene su između oštećenja korijena i bora, kao i između sekundarnoga porasta i željeza, mangana i cinka. Koncentracija iona u korijenu kukuruza mogla bi imati veći utjecaj u istraživanju otpornosti kukuruza na kukuruznu zlaticu.*

*Ključne riječi: kukuruzna zlatica, oštećenje korijena, koncentracija iona, germplazma kukuruza*