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RESOURCE ALLOCATION IN A MAIZE BREEDING PROGRAM FOR NATIVE RESISTANCE TO WESTERN CORN ROOTWORM

I. Brkić [(1)], A. Brkić [(1)], Marija Ivezić [(2)], Tatjana Ledenčan [(1)], A. Jambrović [(1)], Z. Zdunić [(1)], J. Brkić [(1)], Emilija Raspudić [(2)], D. Šimić [(1)]

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SUMMARY

The objective of this study was to determine the optimum allocation of the number of plants sampled per plot and number of locations and years required for screening maize genotypes for reduced root damage caused by western corn rootworm (WCR) larvae, major pest of maize in Croatia, Europe and in the USA. Field trials were conducted on two locations Eastern Croatia, a major maize production area with natural WCR occurrence under continuous maize growing conditions. The trials were set as an incomplete lattice block design in two replications in 2007, 2008 and 2009 including 128 genotypes from various maize gene-pools. Our results suggest that the effect of year and respective interactions including year were the most important factors in maize breeding programs for native resistance to WCR. Thus, screening germplasm for WCR resistance should be made in a multi-year experiment, but not necessarily as a multi-location experiment. Resource optimization should be done by reducing number of roots per plot to minimum 4 sampled plants due to small within-plot environmental variance.

Key-words: breeding, Diabrotica virgifera virgifera, maize, native resistance, root damage

INTRODUCTION

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (WCR) is a serious pest of maize in Croatia, and one of the major pests of maize in Europe and in the U.S.A. (Miller et al., 2005; Meinke et al., 2009; Spencer et al., 2009; Ivezić et al., 2011). Once detected, it is very difficult to eradicate and manage. The insect has shown an ability to evolve resistance to several control measures, including insecticides (Wright et al., 2000) and cultural practices (Levine et al., 2002). Recently, several biotech companies have developed transgenic maize cultivars by transferring insecticidal protein gene(s) from bacterium *Bacillus thuringiensis*, (*Bt*) into maize ('*Bt* maize'), as an alternative to chemical control and crop rotation for WCR control. However, *Bt* maize is viewed controversially in Europe and is not approved commercially for the growers in most European Union countries. Therefore, development of hybrids with certain level of host plant (native) resistance and tolerance would reduce the amount of insecticides and could be a sustainable alternative to transgenic approaches.

It was found that some maize inbred lines were WCR resistant (mostly tolerance). Studies in Missouri in 1997 and 1998 identified several crosses that had significantly less damage caused by WCR larvae (Hibbard et al., 1999). However, no maize cultivars with high levels of native resistance under moderate to high insect pressure were yet released. The reason for this is resource intensive maize breeding programs because of labor-consuming digging up the maize root and large scale field-based germplasm screening (Šimić et al., 2007; Ivezić et al., 2009). Therefore, it is mandatory that the available resources are spent in an optimum way. To our knowledge, no study regarding resource allocation in maize breeding for native WCR resistance exists in the literature. The objective of this study was to determine the optimum allocation of the number of plants sampled in a plot and environments required for screening maize

(1) DSc Ivan Brkić, Andrija Brkić, BSc, DSc Tatjana Ledenčan, DSc Antun Jambrović, DSc Zvonimir Zdunić, DSc Josip Brkić, DSc Domagoj Šimić (domagoj.simic@poljinos.hr) - Agricultural Institute Osijek, Južno predgrađe 17, HR-31103, Osijek, Croatia; (2) Prof. DSc Marija Ivezić, Prof. DSc Emilija Raspudić – Faculty of Agriculture, J.J. Strossmayer University of Osijek, K.P. Svačića 1d, HR-31000 Osijek, Croatia

genotypes for reduced root damage adapted to the climatic conditions of Eastern Croatia.

MATERIAL AND METHODS

Field experiments were conducted on locations, Osijek and Karanac in Eastern Croatia, a major maize production area with natural WCR occurrence under continuous maize growing conditions. The trials were set as an incomplete lattice block design (Cochran and Cox, 1957) in two replications in 2007, 2008 and 2009. Genotypes (128) from various maize gene-pools were screened including inbred lines of Agricultural Institute Osijek, well known U.S. public lines, a highly susceptible hybrid of B37×H84 consisted of two public lines and seven synthetic populations from Missouri (courtesy of B.E. Hibbard, ARS-USDA) which express certain amount of resistance (tolerance) against *Diabrotica* (Prischmann et al., 2007): CRW-8-1a, CRW-8-1b, CRW-8-2, CRW3(S1)(C6), CRW2(C5), NGSDCRW(S2)C4, and LH51×CRW3(S1)C6. Iowa State University 0-3 Node Injury Scale (Oleson et al., 2005) was used to evaluate root damage ratios. Damage (injury) ratios from five individual plants of each plot were averaged to obtain plot mean for every maize genotype in each replication and location.

In the first step of the statistical analysis, individual ANOVA for lattice design (LD) (Cochran and Cox, 1957) was performed for every location and year (total of six trials). Effective mean square error (EMSE) for LD, relative efficiency (RE) for LD and repeatability (Rep) were calculated as intra-environment parameters of optimal setting. Details about these parameters were presented by Šimić et al., 2004 for maize stalk rot. Briefly, EMSE = (Intrablock MSE) $(1+k\mu)$ where k = block size, μ = correction factor for LD $[(MS_{block}$–Intrablock MSE)/ $k^2MS_{block})]$, MSE=means square error; RE = 100 [Block(adjusted)SS + Intrablock error SS] / $k(k^2 - 1)$ EMSE, where SS = sum of squares and *Rep* = σ^2_g / σ^2_g + EMSE, where σ^2_g= genotypic variance component. All variance components in this study were estimated according to Searle (1971).

We used heritability estimate (H) (Holland et al., 2003) and information on the relative magnitudes of genetic, genetic x environmental, and error variances to optimize allocation of resources. Adjusted means from individual experiments were combined in an across-environments analysis to obtain estimates of variance components: the genotypic variance σ^2_g, year variance σ^2_y, location variance σ^2_l, corresponding interaction variances (σ^2_{gl}, σ^2_{gy}, σ^2_{ly} σ^2_{gly}) and the residual variance σ^2_e. The residual variance included between-plot error variance, within-plot environmental variance $\sigma^2_{w\ (env)}$ and within-plot genetic variance with N=number of plants (roots) sampled per plot.

Heritability estimate in the combined analysis was calculated based on entry means:

$$H = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \frac{1}{L}\hat{\sigma}^2_{gl} + \frac{1}{Y}\hat{\sigma}^2_{gy} + \frac{1}{LY}\hat{\sigma}^2_{gly} + \frac{1}{LYRN}\hat{\sigma}^2_e + \frac{1}{LYRN}\hat{\sigma}^2_{w(env)}}$$

where L=number of locations, Y=number of years, R=number of replications.

Confidence intervals at the 95% probability level were calculated for H by the method proposed by Knapp and Bridges (1987). The PLABSTAT program package (Utz, 1995) was used for all statistical procedures. We made a simulation study based on the actual ANOVA data to detect optimal heritability by altering N and Y and the ratios $\sigma^2_g : \sigma^2_{gl} : \sigma^2_{gy} : \sigma^2_e : \sigma^2_{gly} : \sigma^2_{w\ (env)}$, when the variance component $\sigma^2_{w\ (env)}$ was two times larger than respective actual ones.

RESULTS AND DISCUSSION

In the individual ANOVAs for lattice design, the genotype effect was highly significant in all six trials (data not shown). The mean root damage ratios averaged over all 128 genotypes were 1.62 (Osijek 2007), 1.01 (Karanac 2007), 1.57 (Osijek 2008), 2.28 (Karanac 2008) and 1.44 in both Osijek 2009 and Karanac 2009. The means indicated that there was no considerable difference between the two locations for root damage. However, according to the estimates of three parameters of the intra-environment setting across the trials (Table 1), it was demonstrated that the Osijek location was better in quality due to lower EMSE estimates, and higher percentages of RE and Rep. The lowest EMSE and the highest RE and Rep were estimated in Osijek 2009. In a comparable study for stalk rot in Eastern Croatia (Šimić et al., 2004), EMSE estimates were much higher along with lower repeatabilites.

Table 1. Estimates of three parameters of intra-environment setting for root damage in maize at two locations in three years

Tablica 1. Vrijednosti tri procijenjena parametra unutarokolinskoga smještanja za oštećenje korijena kukuruza na dvije lokacije kroz tri godine

Year *Godina*	Osijek			Karanac		
	EMSE	RE (%)	Rep (%)	EMSE	RE (%)	Rep (%)
2007	0.085	159.1	38.2	0.084	131.4	36.4
2008	0.079	196.7	45.8	0.167	146.4	43.2
2009	0.039	436.7	75.1	0.096	110.0	39.3

EMSE = effective mean square error - *efektivna varijanca greške*; RE = relative efficiency - *relativna efikasnost*
Rep = repeatability - *ponovljivost*

Combined ANOVA revealed highly significant effects of year, genotype, as well as interaction effects of GY and GLY (Table 2). The effects of location and the GL interaction were not significant, as indicated in the individual ANOVAs. Notably largest variance had the year as main source of variation. Corresponding estimate of heritability was not small for inherently low-heritable trait of root damage caused by WCR larvae. Šimić et al. (2007) reported in a comparable study at environments in Croatia and USA about average heritability for root damage of just H=17.0. Heritability of 65.7 seems to be not overestimated since important sources of variation such as year, location, and within-plot environmental error variances were accounted for.

Table 2. Combined ANOVA for root damage scored in 128 maize genotypes on two locations in three years including within-plot environmental variance $\sigma^2_{w(env)}$ and heritability estimate (H) with confidence intervals at the 95% probability level

Tablica 2. Kombinirana ANOVA za oštećenje korijena kod 128 genotipova na dvije lokacije kroz tri godine uključujući okolinsku varijancu unutar parcelice $\sigma^2_{w(env)}$ i heritabilnost (H) s intervalima povjerenja na razini vjerojatnosti od 95%

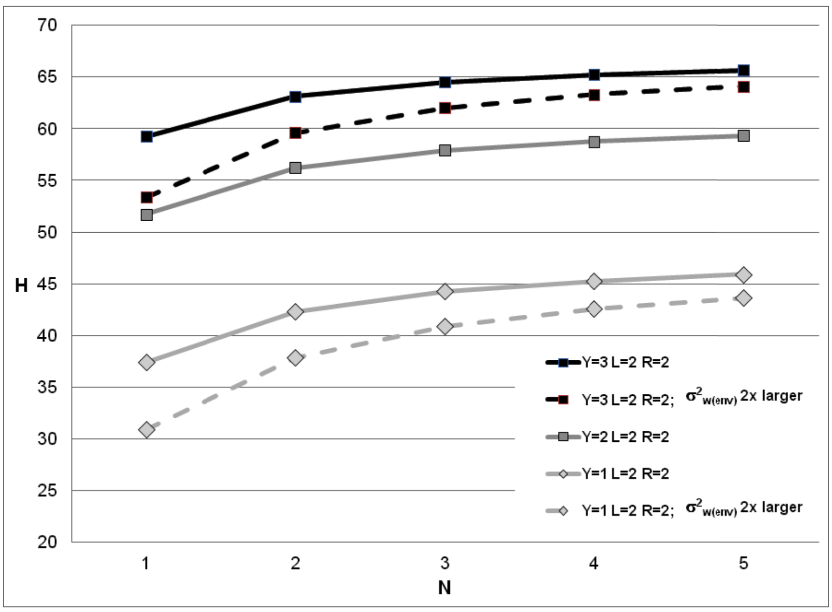
Source *Izvor*	**Degrees of freedom** *Stupnjevi slobode*	**Variance** *Varijanca*
Genotype (G)	127	0.20**
Location (L)	1	0.18ns
Year (Y)	2	26.59**
GL	127	0.08ns
GY	254	0.13**
GLY	253	0.08**
Within-plot environmental $\sigma^2_{w\ (env)}$		0.45**
H = 65.67 (55.82 - 71.34)		

** significant at the 0.01 probability level according to F-test; ns - not significant

According to the relative magnitude of variance components, the most important is the year variance followed by the within-plot environmental variance $\sigma^2_{w(env)}$. In order to find out optimum ratios of variances reflected in H, we made a simulation study based on the actual ANOVA data (Figure 1). It indicates that the optimal heritability may be obtained when number of roots sampled per plot was 4, particularly if $\sigma^2_{w(env)}$ was two times larger approaching to the actual H. If $\sigma^2_{w\ (env)}$ were two times larger than actual one in a three-year trial, H estimate would be considerably lower approaching to H for root damage estimated in a two year trial when N=1.

Figure 1. Effects of number of years (Y) and number of plants (roots) sampled per plot (N) on the heritability estimate (H) for root damage caused by WCR larvae when a trial had two replications per location (R) and grown at two locations (L). Cases when within-plot environmental error ($\sigma^2_{w\ (env)}$) was two times larger, are marked with dashed lines

Slika 1. Učinci broja godina (Y) i broja biljaka (korijena) iskopanih po parceli (N) na heritabilnost (H) za oštećenje korijena nastaloga zbog larvi kukuruzne zlatice kada se pokus sastoji od dva ponavljanja po lokaciji (R) i dvije lokacije (L). Slučajevi kada je $\sigma^2_{w\ (env)}$ dva puta veća, označena je isprekidanom crtom



CONCLUSION

Our results suggest that the effect of year and respective interactions including year were the most important factors in maize breeding programs for native resistance to WCR. Thus, screening germplasm for WCR resistance should be made in a multi-year experiment, but not necessarily as a multi-location experiment. Resource optimization should be done by reducing number of roots per plot to minimum 4 sampled plants due to small within-plot environmental variance.

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ALOKACIJA RESURSA U PROGRAMU OPLEMENJIVANJA KUKURUZA ZA PRIRODNU OTPORNOST NA KUKURUZNU ZLATICU

SAŽETAK

Cilj ovoga rada bio je odrediti optimalnu alokaciju broja biljaka za uzorak po parcelici, kao i broj lokacija i godina potrebnih za procjenu genotipova kukuruza za oštećenje korijena, nastaloga zbog larvi kukuruzne zlatice (WCR), glavnoga štetnika na kukuruzu u Hrvatskoj, Europi i SAD-u. Poljski su pokusi provedeni na dvije lokacije u Istočnoj Hrvatskoj, gdje je učestala pojava WCR u monokulturi. Pokusi su postavljeni prema nepotpunome bloknome planu (lattice) u dva ponavljanja u 2007., 2008. i 2009. godini, uključujući 128 genotipova kukuruza različitoga genetskoga podrijetla. Rezultati našega istraživanja ukazuju na veliku važnost faktora godine i interakcija koje uključuju godinu za programe oplemenjivanja kukuruza za prirodnu otpornost na WCR. Stoga je procjenu germplazme za otpornost na WCR potrebno učiniti u višegodišnjim pokusima, koji se ne moraju nužno provesti na više lokacija. Optimizacija resursa trebala bi uključivati i smanjenje broja biljaka po parcelici na minimum od 4 biljke za uzorak, zbog relativno male okolinske varijance unutar parcelice.

Ključne riječi: oplemenjivanje, Diabrotica virgifera virgifera, kukuruz, prirodna otpornost, oštećenje korijena

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