Aus dem Institut für
Pflanzenzüchtung, Saatgutforschung und Populationsgenetik
der Universität Hohenheim
Fachgebiet: Angewandte Genetik und Pflanzenzüchtung
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RESISTANCE BREEDING IN MAIZE (*ZEA MAYS* L.)

AGAINST THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HÜBNER)

AND THE USE OF DNA-MARKERS FOR MARKER-ASSISTED SELECTION

Dissertation
zur Erlangung des Grades eines Doktors der Agrarwissenschaften
vorgelegt der
Fakultät – Agrarwissenschaften
der Universität Hohenheim

von
Dipl.-Ing. sc. agr.
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aus
Mindelheim

2004

Die vorliegende Arbeit wurde am 27. Mai 2004 von der Fakultät Agrarwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften (Dr. sc. agr.)“ angenommen.

Tag der mündlichen Prüfung: 18. November 2004

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Contents

ABBREVIATIONS

15-A-DON	15-acetyl-deoxynivalenol
3-A-DON	3-acetyl-deoxynivalenol
ANT	date of anthesis
AU	absorption units
Bt	Bacillus thuringiensis
CDOM	cellulase digestibility of organic matter
CF	crude fiber
cM	centiMorgan
CP	crude protein
CPS	conventional phenotypic selection
CV	cross validation
CVAR	coefficient of variance
DIM	DIMBOA
DIMBOA	2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3-(4H)-one
DMC	dry matter content
DMCS	dry matter content of stover
DNDF	digestibility of neutral detergent fiber
DON	deoxynivalenol
DS	data set
ECB	European corn borer
ES	estimation set
EtOAc	ethylacetate
FER	ferulic acid
FUM	fuminosin
FUS-X	fusarenon-X
GCA	general combining ability
GYI	grain yield under infestation
GYP	grain yield under protection
h^2	heritability
Hb	Hübner
HPLC	high pressure liquid chromatography
HPR	host plant resistance
IVDOM	*in vitro* digestibility of organic matter
L	Linné
LOD	$\log_{10}$ of the likelihood odds ratio
LSD	least significant difference
LT	leaf toughness
MAS	marker assisted selection
MeOH	methanol
MON	moniliformin

NIRS	near infrared reflection spectroscopy
NIV	nivalenol
P	probability
p-CUM	*p*-cumaric acid
PDE	percentage of damaged ears
PHT	plant height
Q^2	genotypic variance explained by the QTL
QTL	quantitative trait loci
RE	relative efficiency
RFLP	restriction fragment length polymorphism
RGY	relative grain yield
RIL	recombinant inbred lines
SCA	specific combining ability
SD	stalk diameter
SDR	stalk damage ratings
spp	subspecies
SSR	simple sequence repeat
ST	stalk toughness
TC	testcross
TL	tunnel length
TS	test set

General Introduction

Maize (*Zea mays* L.) production has rapidly increased in Germany during the last decades. This development entails many maize-specific pests, headed by the European corn borer (ECB, *Ostrinia nubilalis* Hbn.), which reaches an alarming extent in Central Europe (Eder 2002, Langenbruch 2002). The ECB originally appeared in grain maize production regions of Southern Germany, but now it is also endemic north of Bonn and Cologne. As the name suggests, the insect originated from Europe, and was transferred to the U.S.A. in the late 19th century (http://www.ent.iastate.eu/pest/cornborer/intro/intro.html). In both Europe and North America it represents a severe pest, in addition to other stem borers or the corn root worm (*Diabrotica virgifera virgifera* LeConte). The ECB causes kernel yield reduction of up to 30% and reduces grain quality (Bohn et al. 1996). In silage maize, damage caused by ECB larvae feeding is not significant (Krützfeldt, Bavarian State Research Institute of Agronomy, personal communication 2003). Nevertheless, the demand for maize cultivars with an improved ECB resistance is high.

Occurrence and damage by the ECB

In Central Europe, the ECB occurs only univoltine, whereas in the U.S. Corn Belt up to four generations can occur (http://www.ent.iastate.edu/pest/cornborer/intro/intro.html). The first generation of ECB is characterized by sheath collar feeding. In contrast, the second generation of ECB damages the plants by stalk and ear shank tunneling, causing stalk breakage and ear loss (Guthrie et al. 1960). In Europe, the observed damage is comparable to that of the second generation in the U.S. Corn Belt. In Germany, the larvae occur at the pre-tasseling stage of maize in late June until early July, when moths of ECB deposit their eggs at the late whorl state before anthesis. After hatching, larvae migrate into the whorl or the tassel, where they feed on the epidermis and pollen up to the third instar stage. At this developmental stage, the larvae start to penetrate into the stalk for further development until the fifth instar stage. The main damage is caused by tunneling in the stalk and the ear shank. After overwintering in maize residues left in the field, pupation starts in spring (Hoffmann and Schmutterer 1983).

Plants damaged by ECB larvae feeding often show secondary infection with fungal diseases like *Fusarium* spp., *Asperigllus* spp., *Ustilago maydis*, and others (Schaafsma et al. 2002). The main problem for grain production is the infection with *Fusarium* spp., because not only ear and stalk rots are caused by these fungi, but they also produce mycotoxins often causing chronic or acute mycotoxicoses in livestock and humans (Logrieco et al. 2002). Immunosuppression, embryo abortion and deformation, swine endrogenic syndrome, porcine pulmonary edema and liver cancer in rats, as well as human esophageal cancer are reported to be related with the intake of mycotoxins (Reid et al. 1999). Depending on the *Fusarium* spp., a wide array of different mycotoxins is accumulated, comprising type B trichothecenes, nivalenol, fusarenon-X, fumonisin, and moniliformin. Therefore, the damage caused by the ECB larvae as well as the possibly linked accumulation of mycotoxins in the crop, emphasize the great importance of resistance against ECB larvae.

Control of ECB damage

Reduced tillage facilitating an undisturbed overwintering in maize residues in the field is in some regions responsible for the increasing occurrence of ECB larvae. Thus, crushing and plowing maize residues are the classical control methods. In addition to chemical (e.g., pyrethroids) and biological (*Trichogramma* parasites and *Bacillus thuringiensis*, *Bt*) control methods, natural host plant resistance (HPR) decreases the level of ECB infestation. Another very efficient way to reduce the damage caused by ECB larvae is the use of genetically modified maize hybrids carrying the *Cry*I*a* gene, encoding the *Bt* toxin. Currently, the use of genetically modified crops is very controversially discussed. Based on their monogenic inheritance and high efficacy of *Bt*-mediated resistance, it is likely that *Bt* resistant ECB larvae develop. In order to avoid or at least to slow down this development, the farmer has to apply sophisticated *Bt* management systems (Ostlie et al. 1997). Furthermore, the possible impact of transgenic plants on non-pest organisms and the dispersion of transgenic pollen in the ecosystem are not completely clarified. Even if the cultivation of transgene *Bt* cultivars is permitted in Germany and some new cultivars may officially be registered in 2005, it is questionable whether they will appeal to farmers and consumers.

Natural host plant resistance: possible resistance mechanisms

Compared with the monogenically inherited *Bt* resistance, HPR would offer considerable advantages. Because of its polygenic nature it might be more stable and the development of resistant insects is less likely. In areas with less severe occurrence of ECB larvae, maize cultivars with a relatively high level of HPR may provide a sufficient control. Moreover, the combination of HPR and the *Bt* gene could protect maize plants against *Bt* resistant larvae occurring in the field. Nevertheless, maize hybrids with HPR are difficult to develop, because HPR is governed by a multiple of genes.

HPR against the second generation of ECB larvae feeding is based on non-preference, antibiosis, and tolerance (Painter 1951). Non-preference is due to a lack of attractiveness of the host plant to the ECB moths as an egg deposit. Antibiosis decreases larval development and the number of larvae per plant, whereas tolerance is the ability of the maize plant to withstand the feeding of ECB larvae. In experiments with mandatory infestation with ECB larvae, like in the present study, only antibiosis and tolerance were evaluated.

The chemical and physiological background of HPR against stem boring insects was mainly evaluated using tropical and subtropical material as well as maize genotypes from the U.S. Corn Belt (Bergvinson et al. 1994a, 1994b, Bohn et al. 1996, Groh et al. 1998). Owing to the different feeding behavior of the ECB generations, no correlation was found between genetic control of resistance and resistance mechanisms against the first and second generation of ECB larvae (Cardinal et al. 2001). Nevertheless, it seems likely that resistance mechanisms against the first generation are of importance for leaf and pollen feeding in the first instars of the second generation.

In temperate maize germplasm, resistance against the first generation of ECB larvae is highly associated with the content of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3-(4H)-one (DIMBOA) concentrations, as well as the content of lignin and phenolic acids in leaf material (Bergvinson et al. 1994b). In addition, leaf toughness was associated with leaf feeding resistance (Bergvinson et al. 1994a, Groh et al. 1998). However, DIMBOA levels decrease with progressing plant development and, therefore, resistance against the second generation of ECB is mainly related to cell wall fortification and stalk toughness, as well as silage quality traits like digestibility (Viereck 1981, Buendgen et al. 1990, Buxton et al. 1996, Flint-Garcia et al. 2003).

Selection for ECB resistance with mandatory infestation trials is very time-consuming and cost-intensive even if it is the most accurate method to evaluate the level of resistance. Indirect selection based on specific plant constituents, tightly linked to ECB damage, such as lignin or phenolic acids and plant toughness, would be very advantageous for breeding progress. Furthermore, it would be important to know which agronomic characters or quality traits are negatively correlated with ECB resistance. It is difficult to improve these traits with the resistance of maize hybrids, because favorable alleles for both traits must be accumulated, which are supposedly often in repulsion phase linkage. Another positive aspect of HPR with different resistance mechanisms may be obtained with a horizontal resistance against more than one insect species or pathogen.

Marker-assisted selection

Conventional breeding strategies for improving ECB resistance in maize are often expensive and labor-intensive. Generally, several breeding cycles with mandatory infestation and evaluation of resistance are necessary to select the most promising genotypes. In some cases, the approach of marker-assisted selection (MAS) offers an effective method of selection without infestation trials. Guthrie et al. (1960) concluded that resistance to ECB was controlled by more genes. Therefore, in the first studies of resistance against leaf feeding of ECB larvae, translocation stocks were produced to identify chromosome arms carrying resistance factors (Scott et al. 1966, Onukogu et al. 1978). These studies indicated that ECB resistance was conditioned by many genes, and that recurrent selection in resistance breeding would be advantageous over backcrossing programs. During the last ten years, many QTL studies were conducted with various materials including tropical and subtropical populations (Schön et al. 1993, Groh et al. 1998, Khairallah et al. 1998, Jampatong et al. 2002, Krakowsky et al. 2002), as well as early-maturing European dent maize (Bohn et al. 2000). A common question of all studies was whether QTL regions were consistent across various populations, environments, and progeny types, e.g., lines *per se* and their testcrosses (TC). In maize breeding, the performance of a line in TC is more important than its performance *per se*. If TC performance could be predicted on the basis of line *per se* performance, the resource-demanding testcrosses of several types of progenies might not be necessary.

For a reliable application of MAS, the consistency of QTL regions across populations and progenies is important. In addition, a relative efficiency (proportion of genotypic variance explained by the respective QTL / heritability, *RE*) of MAS over conventional phenotypic selection (CPS) would be an indicator of a promising integration of MAS in breeding programs designed to improve resistance. For stalk damage ratings, Bohn et al. (2000) found a low *RE*. They suggested that MAS could only be superior over CPS when less-expensive marker techniques are available in contrast to cost-intensive mass rearing of ECB larvae. In contrast, Jampatong et al. (2002) proposed to set up a MAS program with prospective QTL regions for resistance against second generation of ECB in chromosome bins 5.05, 5.08, and 9.02. In their study, these QTL caused major genetic effects and were consistent across environments.

So far, only one European dent population has been analyzed for resistance against ECB (Bohn et al. 2000). For the assessment of MAS in maize resistance breeding, a further evaluation of European maize germplasm would be of interest, even though it was demonstrated that most of the QTL of several germplasm pools are located in common resistance clusters. Furthermore, studies about underlying resistance mechanisms are scarce and were only performed with U.S. or tropical maize material. Even though the present study can only be a further step in analyzing the physical and chemical background of resistance, it may provide a first basis to detect possible candidate genes involved in ECB resistance. The objectives of the present study were to:

(i) identify and characterize QTL for ECB resistance and agronomic traits in an early-maturing European dent population (Population A),

(ii) evaluate the consistency of QTL for line *per se* and TC performance in Population B,

(iii) investigate the consistency of QTL across two independent European dent populations (Populations A and B),

(iv) determine the resistance mechanisms in a subset of 20 extremely resistant or susceptible genotypes of Population B, and

(v) evaluate the relationship between ECB resistance and mycotoxin contamination caused by *Fusarium* spp. for a set of transgenic, isogenic, and commercial hybrids.

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Maydica 46 (2001): 195-205

QTL MAPPING FOR RESISTANCE TO EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HB.) IN EARLY MATURING EUROPEAN DENT MAIZE (*ZEA MAYS* L.) GERMPLASM AND COMPARISON OF GENOMIC REGIONS FOR RESISTANCE ACROSS TWO POPULATIONS OF F_3 FAMILIES

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Received August 29, 2001

ABSTRACT - The European corn borer (ECB, *Ostrinia nubilalis* Hübner) is a major pest of maize in central Europe. The objectives of our study were to (1) identify QTL for resistance to ECB, (2) estimate their genetic effects, and (3) investigate the consistency of QTL across two different populations. A total of 230 $F_{2:3}$ families derived from cross 1396A (resistant) × F478 (susceptible) were used for QTL analyses. Each $F_{2:3}$ family was evaluated for resistance traits tunnel length (TL), stalk damage ratings (SDR), and relative grain yield (RGY) using manual infestation with ECB larvae. The agronomic traits comprised grain yield under insecticide protection (GYP) and manual infestation (GYI), date of anthesis (ANT), dry matter content (DMC), and *in vitro* digestible organic matter (IVDOM) of stover. The field experiment was performed with two replications in two environments in 1995. Two QTL for SDR and two QTL for TL were detected explaining 24.7% and 26.0% of the genotypic variance (σ^2_G), respectively. For agronomic traits one to three QTL were found, explaining between 2.0% and 11.8% of σ^2_G. No common QTL for resistance traits were found across population 1396A×F478 and a second population of 230 $F_{2:3}$ families derived from cross D06 (resistant) × D408 (susceptible). Two QTL for IVDOM and DMC were in common among both populations. Due to the low consistency of QTL across populations, marker-assisted selection (MAS) is not recommended for improving ECB resistance in early maturing dent germplasm.

KEY WORDS: European corn borer; Host plant resistance; *Ostrinia nubilalis*; QTL; RFLP; Maize.

INTRODUCTION

The European Corn Borer (ECB) is a major pest of maize in Central Europe. Feeding of ECB larvae in stalks and ears causes reduced plant growth, broken stalks, and dropped ears, resulting in grain yield losses of up to 30% (MELCHINGER *et al.*, 1998a; BOHN *et al.*, 1999). Moths of the ECB hatch between the end of June and early July and deposit their eggs on the leaves of the maize plants. First- and second- instar larvae migrate into the whorl and feed on leaves and pollen. For further development the larvae penetrate the stalk and tunnel towards the basis of the stem (HOFFMANN *et al.*, 1983).

In the U.S. Cornbelt the ECB occurs bivoltine, whereas up to four generations were observed in the southern states of the USA. In contrast, only one generation of ECB is observed in Central Europe. Here, the occurrence as well as the feeding damage caused by the larvae is comparable to the 2nd ECB generation in the U.S. Cornbelt (http://www.ent.iastate.edu/pest/cornborer/intro/intro.html).

In addition to maize hybrids transformed with the *Bt* gene *CryIA* and the use of pesticides, natural host plant resistance decreases the level of ECB infestation and, therefore, protects maize production in affected areas. Natural host plant resistance against the second generation of ECB larvae feeding is based on non-preference, antibiosis, and tolerance. Non-preference is due to a lack of attractiveness of the host plant for the ECB moths to serve as an egg deposit. Antibiosis decreases larval development as well as the amount of larvae per plant. The level of antibiosis is assumed to be associated with lignin content, biogenic silicea, phenolic acids, and fiber content (BERGVINSON *et al.*, 1994). Tolerance is the ability of a maize plant to withstand the feeding of ECB larvae. The host plant resistance against ECB larvae feeding based on antibiosis is quantitatively inherited. Many studies evaluated the U.S. Cornbelt maize germplasm (SCHÖN *et al.*, 1993, OSTRANDER *et al.*, 1997, JAMPATONG *et al.*, 1998), but only a few experiments screened European maize germplasm for ECB resistance (SCHULZ *et al.*, 1997, MELCHINGER *et*

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al., 1998a). BOHN *et al*. (2000) performed an analysis of quantitative trait loci (QTL) in European dent germplasm and found eleven QTL, which explained about half of the genotypic variance for the traits tunnel length (TL) and stalk damage ratings (SDR). They suggested that marker-assisted selection (MAS) might be more efficient than conventional phenotypic selection for SDR, if costs of marker analysis are reduced, compared to the costs of mandatory manual infestation with ECB larvae. However, they pointed out that the usefulness of MAS depends on the consistency of QTL across populations.

The objectives of our study were to (1) map QTL conferring resistance against ECB larvae feeding in a population of F_3 families derived from a cross between a highly susceptible and a highly resistant maize inbred line originating from early maturing European dent germplasm, (2) estimate their genetic effects, and (3) investigate the consistency of QTL across populations derived from crosses between different inbred lines.

MATERIALS AND METHODS

Plant materials

The early maturing dent lines 1396A, developed by M. Menzi, FAP, Zürich-Reckenholz, Switzerland, and F478, developed by Institute National de la Recherche Agronomique (INRA), France, were chosen as parents. Inbred line 1396A is resistant and F478 is susceptible to ECB larvae feeding (MELCHINGER *et al*., 1998a). Both inbred lines were crossed to produce F_1 plants. During the summer season 1994, 230 F_2 plants derived from two randomly chosen F_1 individuals were selfed to produce 230 F_3 lines. For each F_3 line, 20 F_3 plants were selected to form an "immortalized F_2" (IF_2) population by randomly crossing ten F_3 plants as females with ten F_3 plants as males in the 1994 winter nursery. In the further course of this paper the IF_2 families are denoted as F_3 families. The second mapping population was produced in the same manner by crossing inbred lines D06 (resistant) × D408 (susceptible). For a detailed description see BOHN *et al*. (2000).

Field trials

The F_3 families were planted for phenotypic evaluation at Eckartsweier and Scherzheim in the summer season of 1995. Both experimental sites are located in the Upper Rhine Valley, one of the main maize growing regions of Germany. The experimental design at each site was a split plot with whole plots comprising the inbred lines. The treatments in two neighbouring one-row subplots were "insecticide protection" and "manual infestation". The experimental unit for screening of inbreds was a one-row plot with 25 plants, 5 m long, and a row spacing of 0.75 m. In the mapping experiment, one-row plots 4 m long, with 20 plants and a row spacing of 0.75 m, were used as experiment units. Trials were over-planted and later thinned to the final plant density of 8 plants m^{-2}. In the mapping experiment, 230 F_3 families and both parent inbreds as well as their F_1 and F_2 generation were evaluated in a 10 × 24 α-design with two replications in each environment. Parents were included as duplicate entries, whereas the F_1 and F_2 generation were included as triplicate entries.

European corn borer treatments

For evaluating the level of resistance against ECB, every plant of one subplot was manually infested. On average 20 neonate ECB larvae were applied three-times at weekly intervals for a total of about 60 larvae per plant. The other subplot was protected against natural infestation of ECB with an insecticide (FASTAC SC©) applied three times starting at the end of June in 10 to 14 d intervals. The time of manual infestation as well as protection was synchronized with the natural occurrence of ECB moths caught with a light-trap. The plants at this time varied from mid-whorl stage to tasseling or silking. Freshly hatched larvae were mixed with maize-cob grits and placed into the whorl or leaf collar of maize plants with dispensers (MIHM, 1983). Egg masses for the infestation were provided by Dr. P. Aupinel, INRA, Le Magneraud, France.

Evaluation of resistance and agronomic traits

The level of antibiosis against ECB larvae feeding was evaluated for TL and SDR before harvest. TL was measured in cm by splitting the stalks longitudinally below the primary ear node. SDR was evaluated based on a 1 to 9 rating scale (1 for intact plant, 9 for dropped ears or breakage below the ear) as described by HUDON and CHIANG (1991). For the agronomic traits ten plants from the center of the protected row and the ten plants that were manually infested with ECB larvae were hand-harvested from each subplot. Grain yield in $t \times ha^{-1}$ under protection (GYP) and under infestation (GYI) was adjusted to 15.5% grain moisture. The relative grain yield (RGY) in % was calculated as RGY = (GYI/GYP)×100. The date of anthesis (ANT) in days after sowing, *in vitro* digestible organic matter (IVDOM) of stover in %, plant height (PHT), and dry matter content (DMC) were recorded for plants from the insecticide-protected plots. IVDOM was determined by near-infrared reflectance spectroscopy according to the procedure described by DEGENHARDT (1996).

Marker analyses

For genomic DNA extraction, leaf material of each F_2 individual was lyophilized, ground to a fine powder and digested with restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, or *Bam*HI. A total of 152 maize DNA probes from the standard probe collection available at the University of Missouri, Columbia, was employed to screen the parents 1396A and F478 for polymorphisms. The resulting 80 polymorphic RFLP probes were applied to the F_2 population for segregation and linkage analysis.

Three mapped microsatellite (SSR) markers were analyzed. The sequences of these primers were obtained from the maize database and synthesized by Amersham Pharmacia Biotech (Freiburg, Germany). The polymerase chain reaction-amplification and MetaPhor gel-electrophoresis were performed as described by LÜBBERSTEDT *et al*. (1998).

Segregation and linkage analysis

Segregation of the genetic markers in each F_2 plant was checked for deviations from expected Mendelian segregation by standard χ^2 tests. A linkage map was created using RFLP and SSR marker data by applying the software package MAPMAKER (LANDER *et al*., 1987). For declaring significant linkage between two markers, a LOD ($\log_{10}$ of the likelihood odds ratio) threshold of 3.00 and a maximum recombinations frequency of 0.40 were

used. Genetic map distances between markers were estimated by recombination frequencies and transformed into centiMorgans (cM) using HALDANE's (1919) mapping function.

The level of heterozygosity (%) of the F_2 plants relative to the heterozygosity of the F_1 (=100%) was estimated by dividing the observed heterozygous marker loci by the total number of scorable marker loci in the respective plant. In the same way the percentage of the 1396A genome was calculated by dividing the sum of all 1396A marker alleles by twice the number of scorable marker loci in the respective plant.

For comparison of QTL locations across populations 1396A × F478 and D06 × D408, a combined linkage map was constructed. The position of markers not common to both populations were calculated relative to the position of the 38 common markers. Bin locations were designated by an X.Y code, where X is the linkage group containing the Bin and Y is the locations of the Bin within the linkage group (GARDINER *et al.*, 1993).

Statistical Analyses

The experiment at Eckartsweier had to be discarded due to extremely low temperatures in June that damaged plant development. Therefore, only the field trial in Scherzheim was evaluated. Analyses of variance were calculated for subplot means. Data of individual plants of each subplot were averaged to obtain a subplot mean for SDR and TL. Orthogonal contrasts among the F_3 families versus the midparent value ($\bar{P}$ = P1 + P2)/2) were calculated with Scheffe's tests by SAS procedure GLM (SAS Institute, 1996). Components of variance for the F_3 families were computed considering all effects in the statistical model as random. Estimates of variance components σ^2 (error variance) and σ^2_g (genotypic variance) were calculated as described by SEARLE (1971). Heritabilities (h^2) for F_3 families were computed on an a entry-mean basis. All calculations were performed with PLABSTAT (UTZ, 1991).

QTL Analyses

Analyses of QTL were performed with 230 F_3 families for which both complete molecular and phenotypic data were available. The method of composite interval mapping (CIM) was employed for QTL detection and estimation of QTL effects. A LOD threshold of 2.5 was chosen for declaring a putative QTL significant. QTL positions were determined at the LOD maxima in the regions under consideration. Cofactors were selected by stepwise regression. Putative QTL were examined for presence of digenic epistatic interactions. Estimates of the total genotypic variance explained by all QTL (σ^2_g) was obtained by the square of the partial correlation coefficient (R^2). The proportion of genotypic variance explained by the respective QTL (Q^2) was calculated as R^2_{adj}/h^2. Software package PLABQTL (UTZ, 1995) was used to perform all necessary computations.

QTL results obtained for cross 1396A × F478 were compared with QTL detected for cross D06 × D408 (BOHN *et al.* 2000) using means across both environments and phenotypic means from individual locations.

RESULTS

Marker analyses

Thirty-eight markers were in common across populations 1396A × F478 and D06 × D408 (Figure 1). The level of heterozygosity in F_2 plants of population 1396A × F478 were approximately normally distributed and varied from 25.8% to 77.0% with an average of 47.9% and a standard deviation of 9.6%. The percentage of parent F478 genome in F_2 plants ranged from 31.8% to 71.8% with an average of 50.3% and a standard deviation of 7.5%.

Large marker intervals (>50 cM) were observed on all chromosomes except on chromosomes 4, 8, and 10. Four marker intervals were detected with not significantly linked (LOD<3.0) flanking markers. The resulting partial linkage groups of a chromosome were combined according to published data obtained from the Maize Data Base (http://www.agron.missouri.edu/probes.html). All marker intervals with a LOD ≥ 3.0 span a distance of 1475 cM with an average marker distance of 18.9 cM.

Phenotypic data

Estimates of h^2 were low for resistance traits ($0.20 \leq \hat{h}^2 \leq 0.38$) and of moderate size for agronomic traits ($0.54 \leq \hat{h}^2 \leq 0.79$) (Table 1). The difference between the overall mean of F_3 families and the midparent value was not significant for the resistance traits. The phenotypic mean of the bulked F_2 individuals was significantly different ($P < 0.05$) from the midparent value for GYP but not for the other traits. The F_1 progenies showed significant midparent heterosis ($P < 0.05$) for the agronomic traits GYP, GYI, and PHT. The distribution of the phenotypic means of the F_3 families for TL, SDR, and RGY followed approximately a Gaussian distribution (Figure 2). Transgressive segregation to both sides of the distribution was observed for RGY, whereas transgressive segregation only to the negative side was observed for SDR and TL.

Correlations among resistance traits in F_3 families were highly significant ($P < 0.01$) but of small magnitude (Table 2). RGY was negatively correlated with TL and SDR, whereas TL and SDR were positively correlated. Correlations among agronomic traits and between resistance and agronomic traits were moderate to low. SDR was negatively associated with GYI and ANT, but positively correlated with DMC. The correlation between the percentage of resistant parent 1396A genome and agronomic traits was positive for PHT and ANT, but negative for DMC. Correlation coefficients observed between heterozygosity and agronomic traits as well as between heterozygosity and resistance traits were not significant (data not shown).

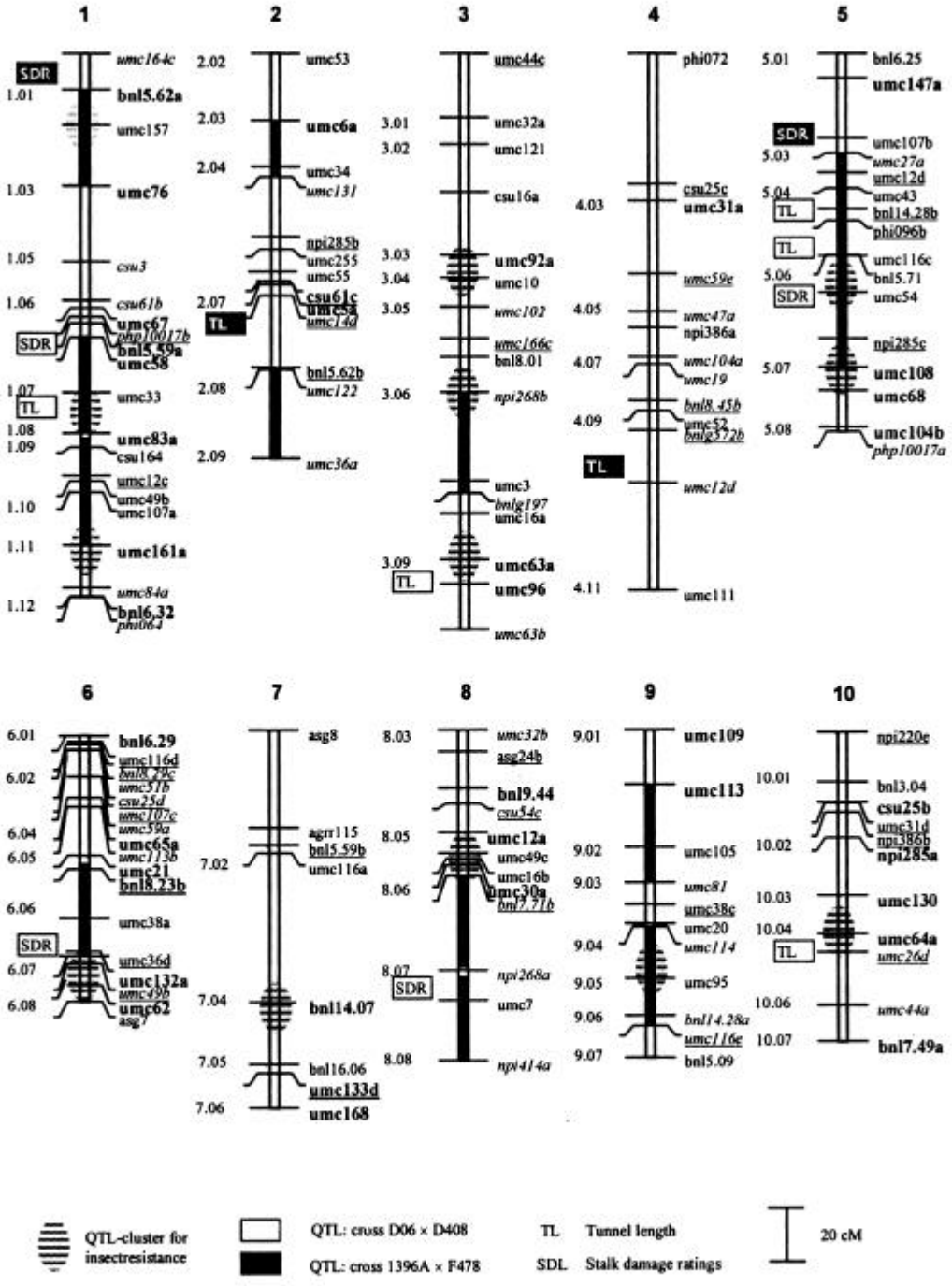

FIGURE 1 - Combined RFLP and SSR linkage map of maize based on 230 F_2 individuals from crosses 1396A × F478 and D06 × D408 as well as QTL detected for resistance against European corn borer. The map shows 132 RFLP and five SSR marker loci with 38 markers in common across both populations. The short arm of each chromosome is shown towards the top of the figure. Underlined marker loci indicate new loci detected with the respective molecular marker. Bold marker loci are common to both mapping populations, whereas loci in italics are unique for cross 1396A × F478, loci in roman are unique for cross D06 × D408. QTL positions are indicated by boxes. Large marker intervals >50cM in cross 1396A × F478 are indicated by black boxes within the chromosome.

TABLE 1 - *Means of inbred lines 1396A and F478, the F_1 and F_2 generations, and 230 F_3 families, as well as heritabilities for resistance and agronomic traits.*

Generation	Entries	Resistance traits			Agronomic traits					
		SDR‡	TL	RGY	GYP	GYI	PHT	ANT	DMC	IVDOM
		1-9 scale	cm	%	t ha^{-1}	t ha^{-1}	cm	days	%	%
Means†										
1396A	2	1.57 ± 0.25	2.71 ± 0.76	91.50 ± 12.02	4.81 ± 0.91	4.29 ± 0.49	174.58 ± 7.54	84.80 ± 0.57	53.68 ± 1.03	54.87 ± 2.04
F478	2	4.44 ± 0.58	6.12 ± 0.62	44.00 ± 7.07	2.83 ± 0.23	1.27 ± 0.16	89.84 ± 1.45	76.42 ± 0.67	57.94 ± 1.06	56.14 ± 0.50
$\bar{P}$††	4	3.01 ± 1.70a	4.42 ± 2.04a	67.75 ± 28.58a	3.82 ± 1.15a	2.78 ± 1.77a	132.21 ± 49.13	80.61 ± 4.85ab	55.81 ± 2.61a	55.50 ± 1.42a
F_1	3	3.05 ± 0.36a	4.38 ± 0.53a	97.00 ± 7.21a	8.41 ± 0.25b	8.21 ± 0.48b	184.34 ± 0.51b	75.28 ± 0.40b	58.16 ± 0.67a	56.76 ± 0.65a
F_2	3	3.69 ± 0.41a	4.90 ± 1.64a	74.33 ± 25.73a	6.06 ± 0.56c	4.42 ± 1.13a	160.34 ± 3.80ab	76.26 ± 0.60b	58.11 ± 0.21a	56.76 ± 1.75a
F_3	230	3.34 ± 0.77a	5.16 ± 1.66a	69.57 ± 17.66a	4.51 ± 0.94a	3.12 ± 1.02a	152.28 ± 6.30a	80.53 ± 2.50a	56.20 ± 2.73a	56.75 ± 1.28a
Heritability		0.35	0.20	0.38	0.63	0.71	0.79	0.72	0.54	0.54

a-c Mean values with different letters are significantly different at the 0.05 probability level.
† Standard errors are attached.
†† $\bar{P}$ = mean of 1396A and F478.
‡ SDR = stalk damage ratings, TL = tunnel length, RGY = relative grain yield (GYI/GYP)*100, GYP = grain yield of protected subplots, GYI = grain yield in infested subplots, PHT = plant height, ANT = date of anthesis, DMC = dry matter content, IVDOM = *in vitro* digestible organic matter of stover.

QTL analyses

Two putative QTL for SDR (chromosomes 1 and 5) explained in a simultaneous fit 24.7% of σ_g^2 (Table 3). The resistance allele of the QTL on chromosome 1 originated from the susceptible parent. Two putative QTL for TL (chromosomes 2 and 4) explained 26.0 % of σ_g^2. The allele on chromosome 4 also originated from the susceptible parent. The putative QTL on chromosome 2 for TL is at the same position as one QTL for DMC. All four resistance QTL showed additive gene action. No QTL for RGY yield was detected. Estimated digenic epistatic effects among putative QTL were not significant for all traits.

For GYP and GYI one QTL was detected at the same Bin location (4.09) explaining 2.0% and 4.8% of σ_g^2, respectively. For PHT two putative QTL explaining 11.8% of σ_g^2 were found. One putative QTL for DMC on chromosome 2 explaining 8.5% of σ_g^2 and three putative QTL for IVDOM on chromosomes 1, 3, and 8 explaining 10.5% of σ_g^2 were detected. No QTL for ANT was revealed. All putative

TABLE 2 - *Phenotypic correlation coefficients among resistance traits and agronomic traits in a population of 230 F_3 families derived from cross 1396A × F478.*

	Resistance traits		Agronomic traits					
	TL†	RGY	GYP	GYI	PHT	ANT	DMC	IVDOM
SDR	0.31**	−0.37**	−0.03	−0.28**	−0.12	−0.26**	0.36**	−0.07
TL		−0.19**	0.11	−0.06	−0.01	−0.03	0.17	−0.00
RGY			0.13	0.76**	0.23**	0.23**	−0.24**	0.07
GYP				0.70**	0.41**	−0.18**	0.28**	−0.12
GYI					0.44**	0.05	0.00	−0.01
PHT						0.24**	−0.11	0.03
ANT							−0.58**	0.26**
DMC								−0.25**

** Phenotypic correlation was significant at at the 0.01 probability level.
† SDR = stalk damage ratings, TL = tunnel length, RGY = relative grain yield (GYI/GYP)*100, GYP = grain yield of protected subplots, GYI = grain yield in infested subplots, PHT = plant height, ANT = date of anthesis, DMC = dry matter content, IVDOM = *in vitro* digestible organic matter of stover.

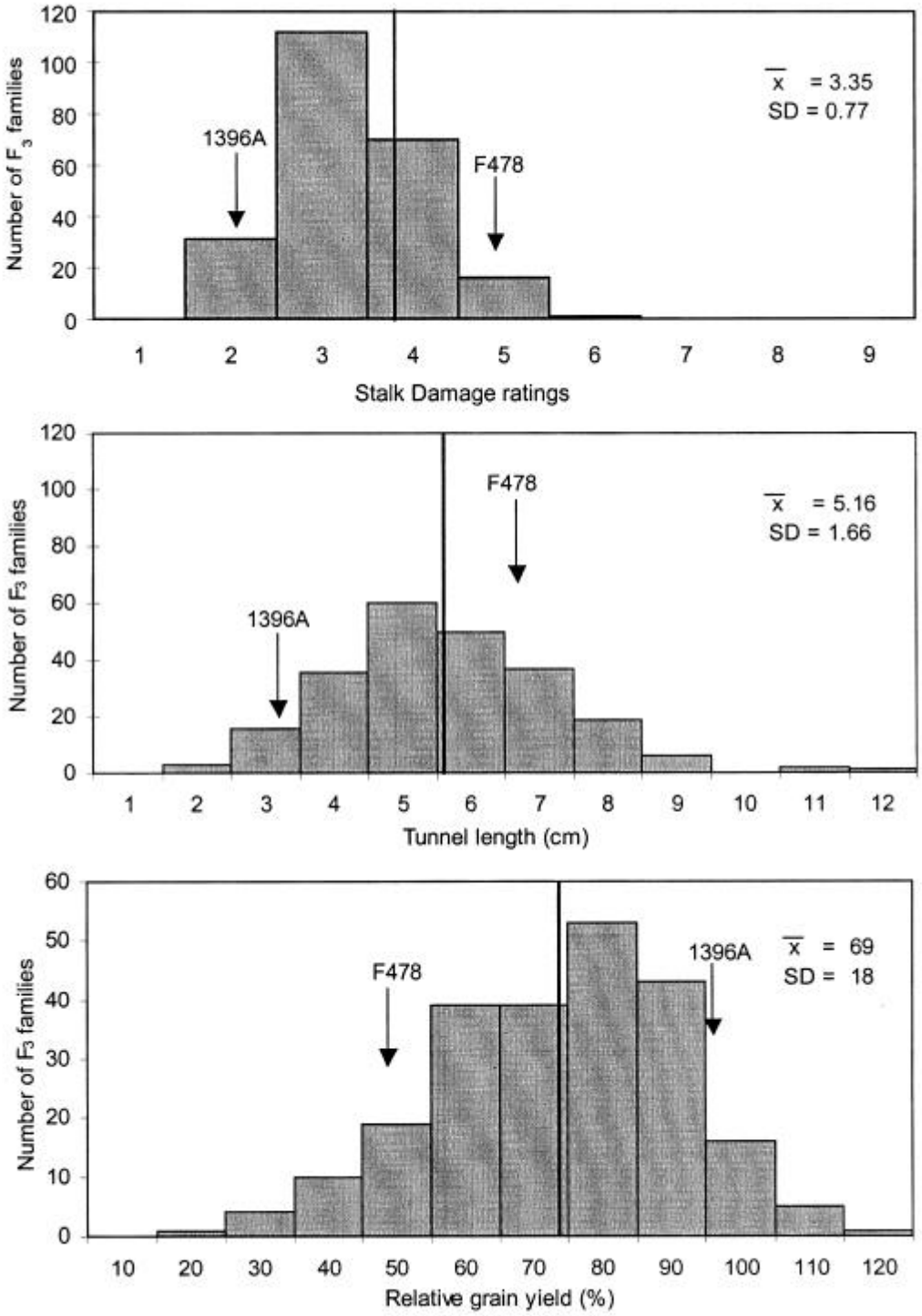

FIGURE 2 - Histograms for stalk damage ratings, tunnel length, and relative grain yield, for means of 230 F_3 families derived from the cross 1396A × F478. Arrows indicate the means of parental lines, $\bar{x}$, solid line, respectively = grand mean for all 230 F_3 families, SD = standard deviation of all F_3 families.

TABLE 3 – *Parameters associated with putative QTL for stalk damage ratings, tunnel length, grain yield under protection, grain yield under infestation, plant height, dry matter content, and IVDOM. Parameters were estimated from phenotypic data of 230 F_3 families from cross 1396A × F478 evaluated at one location in 1995.*

	Marker interval		Genetic effect††					
Bin†	**Position cM**	**left marker**	**right marker**	**LOD**	**Add.**	**Dom.**	**Gene action‡**	**R^2‡‡**	**Q^2§**
Stalk damage ratings					1-9 scale				
1.01	1	*umc164*	*bnl5.62*	2.53	–0.27	NS	A	5.6	
5.03	76	*umc27a*	*umc108*	3.53	0.36	NS	A	6.5	**24.7**
Tunnel length					cm				
2.07	82	*umc5a*	*umc14d*	3.91	0.52	NS	A	7.5	
4.09	108	*bnlg572b*	*umc12d*	4.06	–0.42	NS	A	9.2	**26.0**
Grain yield under protection					t ha^{-1}				
4.09	130	*bnlg572b*	*umc12d*	2.72	–0.25	NS	A	6.3	**2.0**
Grain yield under infestation					t ha^{-1}				
4.09	130	*bnlg572b*	*umc12d*	3.66	–0.32	0.80	OD	8.4	**4.8**
Plant height					cm				
3.05	66	*umc166c*	*npi268b*	5.26	NS	26.70	OD	10.0	
9.06	170	*bnl14.28.a*	*umc116c*	3.63	–2.83	NS	A	11.7	**11.8**
Dry matter content					%				
2.07	80	*umc5a*	*umc14*	3.40	0.95	NS	A	6.6	**8.5**
IVDOM§§					%				
1.11	292	*umc161a*	*umc84a*	2.78	–0.25	NS	A	5.4	
3.07	134	*bnlg197*	*umc63a*	3.49	0.35	NS	A	6.8	
8.03	42	*bnl9.44*	*csu54c*	2.91	–0.46	NS	A	5.7	**10.5**

† Bin locations are designated by an X.Y code, where X is the linkage group containing the Bin and Y is the location of the Bin within the linkage group (GARDINER *et al.*, 1993).

†† Genetic effects were estimated in a simultaneous fit using multiple regression.

‡ A = additive gene action, ($|d_k|/|a_k| < 0.2$) or d_k was not significantly different from zero, PD = partial dominance ($0.2 < |d_k|/|a_k| < 0.8$), D = dominance ($0.8 < |d_k|/|a_k| < 1.2$), OD = overdominance ($|d_k|/|a_k| > 1.2$).

‡‡ R^2 = Proportion of phenotypic variance explained by the respective QTL.

§ Q^2 = proportion of genotypic variance calculated as: R^2_{adj} / heritability.

§§ IVDOM = *in vitro* digestible organic matter of stover in %.

QTL displayed additive gene action except for the GYI and PHT QTL, which showed overdominance.

For resistance traits, we found no common QTL across populations 1396A × F478 and D06 × D408. For agronomic traits one common QTL for IVDOM on chromosome 8 and one common QTL for DMC on chromosome 2 were detected. Using phenotypic data of cross D06 × D408 from single locations for QTL analyses two common QTL for SDR and one common QTL for TL were detected across locations (Table 4). The analysis employing means across environments detected one QTL for SDR and two QTL for TL, which could not be detected in the single location analyses.

DISCUSSION

QTL detection in cross 1396A ×F478

In the present QTL study, the number of detected QTL for resistance traits and agronomic characteristics was considerably smaller than in previous studies (SCHÖN *et al.* 1993, BEAVIS, 1994, LÜBBERSTEDT *et al.*, 1998, BOHN *et al.*, 2000). The lack of identified QTL can be explained by the low power of QTL detection realized in this experiment. The power of QTL detection is a function of the size of the mapping population and the heritability of the traits under consideration (UTZ *et al.*, 1994, MELCHINGER *et al.*, 1998b).

In this study, we tested 230 genotypes for their

TABLE 4 - *List of detected QTL for resistance traits in crosses 1396A × F478 and D06 × D408. QTL analyses were performed on F_3 family means obtained for an individual location and across locations.*

Cross 1396A × F478			Cross D06 × D408								
Scherzheim			Scherzheim			Eckartsweier			Across Locations		
Bin Loc†	LOD	R^2††	Bin Loc†	LOD	R^2	Bin Loc†	LOD	R^2	Bin Loc†	LOD	R^2
Stalk damage rating											
1.01	2.53	4.9									
									1.05	2.84	5.6
			2.05	2.63	2.5						
5.03	3.53	7.7									
			5.06	3.23	3.8				**5.05**	2.89	5.7
						5.07	3.60	6.6	**5.07**	3.31	6.5
			6.07	6.00	7.9	**6.08**	4.06	10.9	**6.07**	7.13	13.5
						7.05	2.56	2.3			
						8.03	4.28	4.3	**8.05**	5.52	10.6
Q^2‡		24.7			16.0			31.5			51.5
Tunnel length											
						1.07	4.87	5.3	**1.07/08**	4.19	6.6
2.07	3.91	7.5									
			3.09	6.10	4.1				**3.09**	4.26	6.3
4.09	4.06	9.2									
									5.03	3.15	5.4
						5.04	3.03	0.6	**5.05**	3.63	3.5
			6.03	3.75	2.9						
			6.05	3.65	5.0	**6.07**	3.30	5.9			
			8.05	3.00	2.9						
									9.03	3.49	7.4
									10.08	2.81	8.1
Q^2		26.0			28.5			26.8			54.8

† Bin locations are designated by an X.Y code, where X is the linkage group containing the Bin and Y is the location of the Bin within the linkage group (GARDINER *et al.*, 1993)

†† R^2 = proportion of phenotypic variance explained by the respective QTL.

‡ Q^2 = proportion of genotypic variance by the respective QTL calculated as: R^2_{mdj} / heritability.

resistance against ECB larvae feeding. In contrast to the majority of published QTL studies, this is a considerably larger population. However, the number of detected QTL is higher in other studies. BOHN *et al.* (2000) also employed 230 genotypes but found 11 QTL for resistance traits TL and SDR. CARDINAL *et al.* (2001) detected nine QTL for ECB tunneling with 200 genotypes. Therefore, other factors than solely the population size might be responsible for the low number of revealed QTL.

Due to low temperatures during early development of the maize plants at Eckartsweier, only data from Scherzheim were analyzed. This resulted in lower heritability estimates that were also upwardly biased due to genotype × environment interactions. In addition, if we assume QTL × environment interactions to be a major cause of variation between genotypes it might be very likely that QTL remained undetected. However, QTL × environment interactions were found to be low for ECB resistance traits, even though genotype × environment interactions were highly significant (GROH *et al.*, 1998b, BOHN *et al.*, 1997). In order to determine QTL × environment interactions with high precision, a large number of locations ($N > 10$) would be necessary (UTZ *et al.* 2000).

In addition, the screening for polymorphism between parental lines revealed large proportions of monomorphic chromosomal regions. Regions on chromosomes 1, 5, 8, and 9 that contained important ECB resistance QTL in other mapping populations (SCHÖN *et al.*, 1993, BOHN *et al.*, 2000) were monomorphic in this study, making it impossible to identify further QTL for SDR and TL. However, the parental lines 1396A and F478 were significantly

($P < 0.01$) different for the resistance traits TL and SDR indicating that other important resistance QTL remained undetected.

Resistance traits

Both parental lines contributed QTL alleles for ECB resistance and susceptibility. However, for TL and SDR transgressive segregation among F_3 families was observed only towards susceptibility. Sampling effects may explain the lack of genotypes with an increased level of ECB resistance.

All QTL for TL and SDR showed additive gene action confirming earlier 'translocation studies on ECB resistance (JENNINGS *et al.*, 1974). This result is also in accordance with BOHN *et al.* (2000), who mainly detected QTL with additive effects for TL. However, due to the large error of dominance effects and the low power of QTL detection, it is not possible to draw firm conclusions about the importance of dominance in the inheritance of ECB resistance in the cross 1396A × F478.

In agreement with earlier studies (RUSSELL, 1994), SDR was significantly ($P < 0.01$) negatively correlated with ANT but significantly ($P < 0.01$) positively associated with DMC. These findings were substantiated by common QTL positions for TL and DMC. HUDON *et al.* (1991) explained these associations with the improved stalk quality of late maturing germplasm at harvesting time. Pleiotropy and linkage in repulsion phase are possible reasons for the observed associations between ECB resistance and maturity. Linkage in repulsion phase makes it difficult to combine the alleles for early flowering and high level of resistance against ECB larvae feeding in one genotype using conventional breeding techniques. Many breeding cycles with artificial infestation would be necessary to identify recombinant genotypes. However, the mean of ANT in the mapping population 1396A × F478 was six days earlier than in population D06 × D408, even though the difference between the mean SDR values of both populations was only marginal. This indicates that it may be possible to select early maturing genotypes with an increased ECB resistance level from cross 1396A × F478. BOHN *et al.* (2000) also found with graphical genotyping some individuals in cross D06 × D408, which combined early flowering with a high level of resistance.

Resistance mechanisms

BERGVINSON *et al.* (1994) and OSTRANDER *et al.* (1997) detected significant relationships between cell wall components, *i.e.*, neutral detergent fibre, acid detergent fibre, and lignin, cell-protein content and ECB resistance. In the present study, a QTL for SDR and the *bm3* locus (http://www.agron.missouri.edu/maps.html) were located in adjacent intervals on chromosome 5. *bm3* is involved in lignin production and results in the brown midrib phenotype with a lower content of lignin than the wild type. Because lignin is a major component in cell-wall fortification, a relationship between the level of resistance against ECB larvae feeding and the *bm3* gene was proposed (BOHN *et al.*, 1996). Other studies also showed an association between high levels of resistance against tropical stem borers and increased leaf toughness and cell-wall fortification (GROH *et al.*, 1998a).

Comparison across two populations

Common QTL for resistance traits TL and SDR across populations 1396A × F478 and D06 × D408 were not found. In addition, only poor agreement with other studies (BEAVIS *et al.*, 1994, BOHN *et al.*, 1997, GROH *et al.*, 1998a, JAMPATONG *et al.*, 1998) were observed. Two of the detected QTL, one for TL in Bin 2.07 and one for SDR in Bin 1.01, were mapped in a cross between U.S. Corn Belt inbred lines B73 and B52 (SCHÖN *et al.*, 1993). In an independent population derived from cross B73 × B52, CARDINAL *et al.* (2001) found marker locus *npi104* (Bin 5.04) to be linked with a QTL for TL. Marker *npi104* also mapped to Bin 4.09, a Bin location carrying one of our QTL detected for TL. A possible translocation event might explain the correspondence between both genomic regions. In contrast to the resistance traits, two common QTL for the agronomic traits IVDOM and DMC were found on chromosomes 2 and 8, respectively.

Previous studies also demonstrated a low consistency of QTL for insect resistance. BOHN *et al.* (1997) found only three common genomic regions for resistance to southwestern corn borer (SWCB) across the two $F_{2:3}$ populations CML131 × CML67 and Ki3 × CML139. GROH *et al.* (1998a) compared these $F_{2:3}$ lines with their corresponding recombinant inbred lines (RILs). They showed that across the RIL populations two out of nine QTL for leaf feeding damage caused by SWCB larvae feeding were in common. GROH *et al.* (1998a) concluded that the detected QTL regions are population specific. In addition, a rather low consistency across the $F_{2:3}$ lines and the RILs was observed. They explained these findings with different sample sizes in

each population and dominance effects of some QTL in the $F_{2:3}$ lines, which prevented their detection in populations of RILs. Additional reasons for the low consistency across both populations might be the high level of monomorphism in 1396A × F478, sampling effects, and the possible low power of QTL detection in both populations.

Conclusions for MAS

BOHN *et al.* (2000) pointed out that MAS for insect resistance could be superior to conventional selection, if the relative efficiency ($RE = \sqrt{Q^2/b^2}$) of MAS over conventional selection is higher than 1, and the detected QTL are consistent across populations. In the present study *RE*, for SDR was 0.7 and for TL 1.3. This is in good agreement with results obtained for population D06 × D408 (BOHN *et al.*, 2000). However, the consistency of QTL for resistance traits across both populations was inadequate to justify the initiation of a MAS breeding program. Furthermore, the low consistency across populations in this study and between populations of $F_{2:3}$ lines and RILs as it was observed by GROH *et al.* (1998) rather supports alternative selection methods like recurrent selection. An early study of PENNY *et al.* (1967) showed that three cycles of recurrent selection were sufficient to substantially increase the level of ECB resistance. Based on recent screening studies (MELCHINGER et al, 1998a; SCHULZ *et al.*, 1997), it should be possible to identify early maturing maize lines that combine promising high levels of ECB resistance with superior general combining ability for yield in order to initiate a recurrent selection program.

ACKNOWLEDGEMENTS - This research was supported by grants form the German Ministry of Education and Research (BMBF) and the Gemeinschaft zur Förderung der Privaten Deutschen Pflanzenzüchtung e.V. (GFP). Special thanks to Fa. Südwestdeutsche Saatzucht Dr. R. Späth, Rastatt, for providing a field test location at Scherzheim. The authors gratefully acknowledge the skilled technical assistance of F. Mauch, A. Karg, and S. Pluskat for conducting the field trials as well as E. Kokai-Kota for the excellent technical assistance in the laboratory work.

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Theor Appl Genet (2004) 108:1545–1554
DOI 10.1007/s00122-003-1579-3

ORIGINAL PAPER

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QTL mapping for European corn borer resistance (*Ostrinia nubilalis* Hb.), agronomic and forage quality traits of testcross progenies in early-maturing European maize (*Zea mays* L.) germplasm

Received: 16 October 2003 / Accepted: 12 December 2003 / Published online: 9 March 2004

Abstract In hybrid breeding the performance of lines in hybrid combinations is more important than their performance per se. Little information is available on the correlation between individual line and testcross (TC) performances for the resistance to European corn borer (ECB, *Ostrinia nubilalis* Hb.) in maize (*Zea mays* L.). Marker assisted selection (MAS) will be successful only if quantitative trait loci (QTL) found in F_2 derived lines for ECB resistance are still expressed in hybrid combinations. The objectives of our study were: (1) to identify and characterize QTL for ECB resistance as well as agronomic and forage quality traits in a population of testcrossed $F_{2:3}$ families; (2) to evaluate the consistency of QTL for per se and TC performances; and (3) to determine the association between per se and TC performances of $F_{2:3}$ lines for these traits. Two hundred and four $F_{2:3}$ lines were derived from the cross between maize lines D06 (resistant) and D408 (susceptible). These lines were crossed to D171 and the TC progenies were evaluated for ECB resistance and agronomic performance in two locations in 2000 and 2001. Using these TC progenies, six QTL for stalk damage rating (SDR) were found. These QTL explained 27.4% of the genotypic variance in a simultaneous fit. Three QTL for SDR were detected consistently for per se and TC performance. Phenotypic and genotypic correlations were low for per se and TC performance for SDR. Correlations between SDR and quality traits were not significant. Based on these results, we conclude that MAS will not be an efficient method for improving SDR. However, new molecular tools might provide the opportunity to use QTL data as a first step to identify genes involved in ECB resistance. Efficient MAS procedures might then be based on markers designed to trace and to combine specific genes and their alleles in elite maize breeding germplasm.

Communicated by G. Wenzel

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Introduction

The European corn borer (ECB, *Ostrinia nubilalis* Hb.) is a pest of maize (*Zea mays* L.) with growing importance in European maize production. In contrast to the U.S. corn belt, where the ECB has up to four generations, only one generation is observed in Central Europe. Leaf feeding and stem tunneling by ECB larvae reduce plant growth and cause stalk lodging and ear dropping, resulting in severe yield losses of up to 30% (Bohn et al. 2000).

Chromosomal regions affecting ECB resistance in the resistant U.S. inbred B52 were first identified using translocation stocks (Onukogu et al. 1978). B52 and the resistant inbreds DE811 and Mo47 were also investigated in QTL studies in crosses with susceptible inbreds B73 and Mo17 (Lee 1993; Schön et al. 1993; Jampatong et al. 2002). In agreement with the translocation study, the QTLs with the largest effects on ECB resistance were detected on chromosomes 1 and 2. In early maturing European dent maize, QTL found for tunnel length and stalk damage ratings explained approximately one half of the genotypic variance. However, agreement of QTL results across the different mapping populations and the resistance traits measured was low. This low consistency was explained by the partly different genetic basis of the

different resistance traits, as indicated by the low to moderate correlation between these traits, and the low power of QTL detection (Melchinger et al. 1998). In addition, the populations used might differ for the set of segregating resistance gene alleles and their epistatic interactions (Stuber 1995).

Even though the consistency across these studies was low, simulation experiments showed that most QTL detected in F_2 populations are not likely to be false positives (Beavis 1994) and might be used for marker assisted selection. However, after 10 years of genetic studies using molecular markers to identify a large set of putative QTL, the question of whether these can be used for marker assisted selection to improve ECB resistance in maize is unanswered. A few studies concluded that MAS might be promising (Jampatong et al. 2002; Flint-Garcia et al. 2003), whereas other studies pointed out that the use of MAS would not result in increased selection gains based on relative efficiency calculations (Bohn et al. 2000; Papst et al. 2001). However, in order to finally judge the prospects of MAS to improve ECB resistance in maize, information on the correlation between per se and testcross (TC) performance for ECB resistance is needed.

In hybrid breeding programs the performance of maize lines in hybrid combinations is more important than their performance per se. Little information is available on the correlation between line per se and hybrid performance for ECB resistance in temperate maize germplasm. Correlations between per se and TC performance were high for stalk damage ratings, but low for tunnel length and yield reduction caused by ECB larvae feeding (Kreps et al. 1998). In tropical maize the association between per se and TC performance was low for corn borer resistance (Thome et al. 1992; Groh et al. 1998) and the consistency of QTL mapped for per se and TC performance was poor. These results suggest that MAS can only be successfully employed to improve ECB resistance in maize if QTL identified in lines per se are expressed in hybrid combinations. Therefore, the objectives of our study were: (1) to identify and characterize QTL for ECB resistance, as well as agronomic and forage quality traits, in a population of testcrossed $F_{2:3}$ families derived from a cross between two early-maturing European dent lines; (2) to evaluate the consistency of QTL for per se and TC performances; and (3) to determine the association between per se and TC performances of $F_{2:3}$ lines for these traits.

Materials and methods

Plant materials

Dent lines D06 (ECB resistant) and D408 (ECB susceptible) were crossed to produce 230 $F_{2:3}$ families; these families were evaluated for per se performance and used for QTL mapping as described in detail by Bohn et al. (2000). Out of this set of $F_{2:3}$ families, 204 families were testcrossed by crossing ten randomly chosen plants per family with D171, a susceptible line from the flint pool. Seeds were harvested from all ten plants and bulked to evaluate TC progenies.

Field trials

Experiments with manual infestation of ECB larvae and those under protection (insecticide application without infestation) were conducted in Pulling and Frankendorf in the summer seasons of 2000 and 2001. Forage quality traits were evaluated in Frankendorf and Straubing in 2001. All experimental sites are located in southeastern Germany and are characterized by increasing ECB population densities over the past 5 years (Zellner, Landesanstalt für Landwirtschaft Freising, personal communication). Each year-location combination was treated as an environment in the subsequent statistical analyses. A total of 210 TC entries were evaluated, including the 204 $F_{2:3}$ families and duplicate entries of the parental lines, and the F_1 hybrid as duplicate entries. The experimental design was a $21\times10\alpha$-design with two replications at all locations. The experimental unit was a two-row plot with 50 plants, 4 m long, and a row spacing of 0.75 m. Trials were over-planted and later thinned to a final plant density of 8 plants/m^2. The first and the last two plants of each row were eliminated before grain harvest. These plants were used for forage quality analyses in 2001.

ECB treatment

An average number of 20 neonate ECB larvae were applied three times at weekly intervals for a total of about 60 larvae per plant. Freshly hatched larvae were mixed with maize-cob grits and placed into the whorl or leaf collar of maize plants with special dispensers (Mihm 1983). The manual infestation was synchronized with the natural occurrence of ECB moths between the end of June and mid-July. The plant development stages varied at infestation time from mid-whorl stage to tasseling or silking. Egg masses for manual infestation were provided by Dr. P. Aupinel, INRA, Le Mangeraud, France. The insecticide-protected whole plots were treated with FASTAC SC applied twice starting at the end of June in 10- to 14-day intervals.

Evaluation of resistance and agronomic traits

Resistance against ECB larvae feeding was determined using stalk damage ratings (SDR) based on a 1–9 rating scale (1 for intact plants, 9 for dropped ears or breakage below the ear) as described by Hudon and Chiang (1991). Grain yield, in tonnes per hectare, under protection (GYP) and manual infestation (GYI) was adjusted to 15.5% grain moisture, and the relative grain yield reduction (RGY) was calculated as (GYI/GYP)×100. The date of anthesis (ANT), in days after sowing, plant height (PHT), in centimeters, and dry matter concentration (DMC), as a percentage, were recorded for plants from the insecticide-protected plots. The four plants, not harvested for grain yield in the insecticide protected plots, were hand-harvested without ears at the end of September to determine dry matter concentration of stover (DMCS), in per cent, cellulose-digestible organic matter (CDOM) (De Boever et al. 1986), concentration of crude protein (CP) (Kjeldhal 1883), concentration of crude fiber (CF) (Naumann and Bassler 1998), water-soluble carbohydrates (WSC) (Luff and Schoorl 1929), in vitro digestible organic matter (IVDOM) (Tilley and Terry 1963), and digestibility of neutral detergent fiber (DNDF) (Dolstra and Medema 1990), in g kg^{-1} 10^{-1}. All forage quality traits were analyzed by near-infrared reflectance spectroscopy using calibrations provided by KWS Saat AG, Germany.

Marker and linkage analyses

Details on the marker analyses and the linkage map development were presented by Bohn et al. (2000). Briefly, a total of 230 F_2 plants were genotyped for 93 RFLP and two SSR marker loci. The linkage map was constructed using MAPMAKER3.0b (Lander et al. 1987) software. Linkage between two markers was declared significant in two-point analyses if the LOD score ($\log_{10}$ of the

likelihood odds ratio) exceeded a threshold of 3.0. After determining linkage groups and the correct linear arrangement of marker loci along the linkage groups, recombination frequencies between marker loci were estimated by multi-point analyses and transformed into centiMorgans (cM) by Haldane's mapping function (Haldane 1919).

Statistical analyses

Analyses of variance were performed on field data from each experiment within each environment. Adjusted entry means and effective error mean squares were used to compute the combined analyses of variance and covariance across environments for experiments with and without ECB infestation. The sums of squares for entries (210 *df*) were subdivided into the variation among TC progenies of the $F_{2:3}$ families (203 *df*) and the orthogonal contrasts among the means TC progenies of the $F_{2:3}$ families versus the midparental value and P1 versus P2. Components of variance for the TC progenies were computed considering all effects in the statistical model as random. Estimates of the genotypic variance (σ^2_g), genotype×environment interaction variance (σ^2_{gl}), error variance (σ^2), and phenotypic variance (σ^2_p) and their standard errors were calculated as described by Searle (1971). Heritabilities (h^2) for TC progenies were computed on an entry-mean basis and confidence intervals on $\hat{h}^2$ were estimated according to Knapp et al. (1985). Phenotypic ($\hat{r}_p$) and genetic ($\hat{r}_g$) correlation coefficients were calculated among resistance and agronomic traits by applying standard methods (Mode and Robinson 1959). PLABSTAT (Utz 2001) and SAS (SAS Institute 1996) software packages were used for all calculations.

QTL analyses

QTL analyses were performed on the subset of 201 TC progenies for which both molecular and phenotypic data were available. Composite interval mapping (CIM) was employed for QTL detection and estimation of QTL effects. A LOD threshold of 2.5 was chosen for declaring a putative QTL significant, ensuring a comparison-wise error rate of $P<0.0036$ and an experiment-wise error rate of $P<0.30$. Estimates of QTL positions were obtained at the position, where the LOD score reached its maximum in the region under consideration. All putative QTL were examined for presence of digenic epistatic effects and QTL × environment interactions. The proportion of the phenotypic variance explained by all QTL was determined by the adjusted coefficient of determination of regression (R^2_{adj}) fitting a model including all detected QTL. The proportion of the genotypic variance explained by all QTL for one trait (p) was calculated as $p = R^2_{adj}/h^2$.

Five-fold cross validation (CV/G) was performed for the $F_{2:3}$ lines per se and their TC progenies following procedures described by Utz et al. (2000) and Bohn et al. (2001). The whole data set (DS) containing the entry means across environments for each mapping population was randomly split into k=5 disjoint subsets. Four subsets were combined to form the estimation set (ES) for QTL detection and estimation of genetic effects. The remaining subset formed the test set (TS) in which predictions derived from ES were tested for their validity by correlating predicted and observed data. By permuting the subsets, five different CV/G runs are possible for a five-fold CV/G. Subsets were formed randomly 200 times, yielding a total of 1,000 replicated CV/G runs. Following Utz et al. (2000), the proportion of the genotypic variance explained by the detected QTL in TS ($\hat{p}_{TS.ES}$) was calculated from the adjusted squared correlation coefficient between the phenotypic entry means observed in TS (Y_{TS}) and the predicted genotypic values ($Q_{TS.ES}$) on the basis of results derived from ES, divided by the heritability of the trait under study:

$$\hat{p}_{TS.ES} = \frac{R^2_{adj}(Y_{TS}, Q_{TS.ES})}{\hat{h}^2}.$$

Using a LOD threshold of 2.5, each CV/G run yielded different estimates for the number of QTL, their location, and genetic effects in the ES. Estimates of p in ES and TS were calculated as the median $\tilde{p}$ over all replicated CV/G runs. The average number of QTL was determined as the mean across replicated CV/G runs. The median additive genetic effect $\tilde{a}_{ES}$ was calculated for each scanned chromosomal position. For each $\tilde{a}_{ES}$, the corresponding additive effect from TS ($\hat{a}_{TS.ES}$) was determined by multiple regression based on the map positions of all QTL detected in ES and the marker genotypes of the $F_{2:3}$ family in TS at the respective flanking marker loci, according to described procedures (Haley and Knott 1992; Utz and Melchinger 1996). Subsequently, the median $\tilde{a}_{TS.ES}$ was calculated across all CV/G runs for a given position.

Relative efficiency of MAS

The relative efficiency (RE) of MAS over conventional phenotypic selection was calculated according to the formula of Lande and Thompson (1990). Assumed molecular-marker scores were recorded without errors, and selection intensities of MAS and CPS were equal. If selection is only performed on marker loci, the efficiency was calculated as $RE = \sqrt{p/h^2}$. If phenotypic and molecular data were combined, the *RE* was calculated by using the formula $RE_c = \sqrt{p/h^2 + (1-p)^2/(1-ph^2)}$.

Correlation between TC progenies and $F_{2:3}$ families

Associations between per se and TC performance of $F_{2:3}$ families were determined using the following three methods. Firstly, phenotypic correlations ($\hat{r}_p$) were estimated on entry-mean basis for per se and TC performance of the 204 $F_{2:3}$ families. Because per se and TC performance was not evaluated in the same locations, genotypic correlation ($\hat{r}_g$) was calculated as $\hat{r}_g = MP/E\sqrt{\hat{\sigma}^2_{gT} \times \hat{\sigma}^2_{gL}}$, where MP is the mean product of entry means for per se and TC performance, E is the number of environments, $\hat{\sigma}^2_{gT}$ is the estimated genotypic variance for TC performance, and $\hat{\sigma}^2_{gL}$ is the estimated genotypic variance for the per se performance. PLABSTAT (Utz 2001) was used to perform the necessary calculations. Secondly, the correlation coefficient between LOD profiles (r_{LOD}) determined in the mapping experiments for per se and TC performance was calculated. Significance of this correlation was determined by employing a permutation test (Keightley and Knott 1999). The data were permuted 1,000 times by randomizing the order of chromosomes independently for each mapping experiment. The reordered chromosomes were lined up and the correlation between profiles was calculated under the null hypothesis that LOD profiles were not correlated. All computations were performed using the software program CORRESP (Utz 2002). Thirdly, multiple regression was used to determine the combined effect of the QTL positions identified for per se perfor- mance in TC progenies. The pro-portion of σ^2_g explained by these chromosomal regions for TC performance was calculated as $p_{TL} = R^2_{adj}(Y_{TC}, Q_{TC.per\ se})/h^2$, where $R^2_{adj}(Y_{TC}, Q_{TC.per\ se})$ is the adjusted squared correlation coefficient between the phenotypic entry means observed in TC progenies (Y_{TC}) and the predicted genotypic values ($Q_{TC.per\ se}$) on the basis of QTL results derived from the per se performance and h^2 is the heritability for TC performance of the trait under study. All calculations were performed with PLABQTL (Utz and Melchinger 1996) software.

Results

Phenotypic data

TC performance of $F_{2:3}$ families

TC progeny means of parental lines D06 and D408, their F_1 hybrid, and the population TC mean were not significantly different for all traits (Table 1). Genotypic variances were highly significant ($P<0.01$) for all traits and σ^2_{ge} estimates were significant ($P<0.01$) only for SDR, GYP, DMC, and quality trait DNDF (data not shown for DNDF). Heritabilities were low for RGY, GYP and quality traits DMCS, CP, and DNDF ($\hat{h}^2 \leq 0.45$) (data for quality traits not shown) but of moderate size for all other traits ($0.50 < \hat{h}^2 < 0.71$).

QTL for per se performance

Here, we report QTL for ECB resistance and agronomic traits obtained by using five-fold cross validation. Corresponding results found without cross validation were presented in a previous paper (Bohn et al. 2000).

Resistance traits

For SDR two QTL on chromosome1 and one each on chromosomes 6 and 8 were detected. These four QTL explained 27.6% of $\hat{\sigma}^2_g$ (Table 2). QTL detection frequencies ranged from 19.4% to 100%. For tunnel length two QTL were found on chromosome 5 and one each on chromosomes 1, 2, 3, 7, 8, and 10. These QTL explained in a simultaneous fit 10.6% of $\hat{\sigma}^2_g$. The QTL detection frequencies varied between 19.6% and 79.5%.

Agronomic traits

A total of 32 QTL were found for agronomic traits GYP (3 QTL), GYI (2 QTL), PHT (7 QTL), ANT (10 QTL), and DMC (10 QTL) (data not shown). Most of them showed additive gene action. The proportion of $\hat{\sigma}^2_g$ explained by the QTL detected for each trait ranged from 17.3% to 27.0 %. Quantitative trait loci found for grain yield under insecticide protection and ECB infestation explained less than 3% of $\hat{\sigma}^2_g$. Five out of 32 QTL were detected in more than 97% of all cross validation runs for each trait. The remaining QTL were found with frequencies between 13.0% and 94.6 %.

Table 1 Testcross means of parental lines D06 and D408, their F_1 generation, and 204 $F_{2:3}$ families, as well as estimates of variance components and heritabilities for resistance, agronomic, and quality traits evaluated at two locations in 2000 and 2001

Generation	Entries (no.)	Resistance traits		Agronomic traits				Quality traits	
		SDR (1–9 scale)[a]	RGY (%)	GYP (t ha^{-1})	PHT (cm)	ANT (days)	DMC (%)	CF (g kg^{-1}10^{-1})	IVDOM (g kg^{-1}10^{-1})
Testcross means[b]									
D06	2	3.39±0.36	94.4±0.8	7.73±0.17	274.9±3.6	83.4±0.8	69.3±0.02	33.48±0.08	66.6±1.16
D408	2	4.30±0.04	87.3±4.4	8.36±0.18	270.3±0.7	83.8±0.9	67.1±0.22	30.41±1.01	72.44±2.37
$\bar{P}$[c]	4	3.84±0.57	90.8±4.9	8.05±0.39	272.6±3.4	83.6±0.7	68.2±1.29	31.94±1.87	69.52±3.70
F_1	2	3.97±0.06	88.3±5.1	8.18±0.01	271.5±2.6	81.9±1.0	68.0±0.35	31.94±0.13	68.98±0.38
$F_{2:3}$	204	3.34±0.77	89.1±5.1	8.11±0.42	273.4±7.7	83.1±1.2	68.0±0.85	32.00±0.89	69.19±1.74
Variance components									
σ^2_g		0.08±0.17**	8.93±3.01**	0.76±0.19**	41.53±5.90**	0.94±0.14**	0.47±0.08**	0.43±0.09**	1.74±0.33**
σ^2_{gl}		0.04±0.17**	1.77±4.16	0.55±0.21**	2.93±4.02	0.11±0.10	0.22±0.06**	0.07±0.09	0.33±0.33
σ^2_{ge}		0.68±0.56**	5.60±4.70**	0.36±0.30**	67.64 ±55.36**	13.71±11.20**	0.16±0.13**	4.11±3.36**	24.32±19.87**
Heritability									
$\hat{h}^2$		0.50	0.35	0.45	0.71	0.69	0.65	0.53	0.55
90% C.I. on $\hat{h}^2$		(0.34; 0.63)	(0.14; 0.59)	(0.28; 0.59)	(0.62; 0.78)	(0.59; 0.77)	(0.53; 0.73)	(0.39; 0.64)	(0.42; 0.66)

** Variance component was significant at the 0.01 probability level.
[a] SDR = stalk damage ratings, RGY = relative grain yield, GYP = grain yield under protection, PHT = plant height, ANT = days to anthesis, DMC = dry matter content, CF = content of crude fiber, IVDOM = in vitro digestible organic matter.
[b] Standard errors are attached.
[c] $\bar{P}$ = mean of parental testcross hybrids.

Table 2 Position of detected QTL and their respective additive effects for stalk damage ratings (*SDR*) and tunnel length (*TL*) determined using the whole data set ($\hat{a}$) or 200 five-fold cross validation runs ($\hat{a}_{TS.ES}$). Parameters were estimated from phenotypic per se data of 210 $F_{2:3}$ families derived from the cross D06×D408 evaluated at two locations in 1995

Bin[b]	Position (cM)	$\hat{a}$[a]	$\hat{a}_{TS.ES}$			$\hat{p}$[d]
			Median	(10; 90) Percentile	Frequency (%)[c]	
Stalk damage ratings		1–9 scale				
1.02	46	–0.21	–0.08	(–0.11; –0.07)	19.4	
1.06	166	0.27	0.24	(0.23; 0.24)	88.6	
6.07	144	0.27	0.25	(0.23; 0.25)	84.0	
8.05	58	–0.26	–0.27	(–0.28; –0.26)	100.0	27.6
Tunnel length		cm				
1.07	202	0.54	0.36	(0.35; 0.40)	79.2	
2.04	110	–0.70	0.36	(–0.40 ;–0.25)	19.6	
3.09	304	0.32	0.28	(0.27; 0.31)	79.5	
5.03	80	–0.74	–0.21	(–0.27; –0.20)	36.9	
5.04	102	0.80	0.26	(0.23; 0.33)	27.3	
7.05	102	0.32	–0.01	(–0.04; 0.03)	27.5	
8.05	58	–0.55	–0.23	(–0.23; –0.17)	50.3	
10.06	148	0.86	0.46	(0.42; 0.54)	29.1	10.6

[a] Median and percentiles were calculated based on 200 five fold CV/G runs.
[b] Bin locations are designated by an X.Y code, where X is the linkage group containing the Bin and Y is the location of the Bin within the linkage group (Gardiner et al. 1993).
[c] Frequency of QTL detection across 200 five fold CV/G runs.
[d] $\hat{p}$ = proportion of genotypic variance explained by detected QTL calculated as R^2_{adj} / heritability in 200 cross validation runs (for heritability and results without cross validation see Bohn et al. 2000).

Table 3 Position of detected QTL and their respective additive effects for stalk damage ratings (*SDR*), content of crude fiber (*CF*), in vitro digestibility of organic matter (*IVDOM*) determined using the whole data set ($\hat{a}$) or 200 five-fold cross validation runs ($\hat{a}_{TS.ES}$). Parameters were estimated from phenotypic data of TC progenies of 204 $F_{2:3}$ families derived form the cross D06×D408 evaluated at two locations in the years 2000 and 2001; bold letters indicate common QTL positions across per se and TC evaluations

Bin[b]	Position (cM)	$\hat{a}$[a]	$\hat{a}_{TS.ES}$			$\hat{p}$[d]
			Median	(10; 90) Percentile	Frequency (%)[c]	
Stalk damage ratings		1–9 scale				
1.01	**25**	**–0.31**	–0.26	(-0.26; - 0.24)	**91.7**	
3.09	315	0.20	0.14	(0.13; 0.15)	62.1	
6.06	**110**	**0.59**	0.53	(0.52; 0.54)	**86.0**	
7.04	180	0.25	0.06	(0.04; 0.08)	41.0	
8.04	**30**	**–0.29**	**–0.17**	(–0.18; –0.16)	**66.7**	
10.04	160	0.37	0.11	(0.10; 0.13)	54.5	**27.4**
Crude fiber		g kg^{-1} 10^{-1}				
3.01	20	0.35	0.45	(0.37; 0.46)	24.1	
6.05	80	–0.42	–0.48	(–0.54; –0.48)	63.7	
9.02	28	0.29	0.28	(0.25; 0.32)	14.6	**4.7**
In vitro digestibility of organic matter		g kg^{-1} 10^{-1}				
8.06	76	0.65	0.55	(0.52; 0.56)	69.8	
9.01	26	–0.47	–0.60	(–0.66; –0.53)	47.9	**3.3**

[a] Median and percentiles were calculated based on 200 five fold CV/G runs.
[b] Bin locations are designated by an X.Y code, where X is the linkage group containing the Bin and Y is the location of the Bin within the linkage group (Gardiner et al. 1993).
[c] Frequency of QTL detection across 200 five fold CV/G runs.
[d] $\hat{p}$ = proportion of genotypic variance explained by detected QTL calculated as R^2_{adj} / heritability in 200 cross validation runs.

QTL for TC performance

Resistance traits

Six QTL for SDR on chromosomes 1, 3, 6, 7, 8, and 10 were found, explaining between 6.7% and 13.4% of $\hat{\sigma}^2_p$ (Table 3). In the 1,000 CV/G runs, the mean number of detected QTL was 4.4 for SDR, which explained 27.4% of $\hat{\sigma}^2_g$ in a simultaneous fit. The frequencies of QTL detection in cross validation varied from 41.0% to 91.7%. Except for QTL in bins 1.01 and 8.04, the resistance allele originated from the resistant parent D06. No QTL for RGY was detected.

Agronomic traits

A total of 16 QTL were found for GYP (4 QTL), GYI (3 QTL), PHT (4 QTL), ANT (3 QTL), and DMC (2 QTL) (data not shown). One QTL for PHT accounted for 17.7% of $\hat{\sigma}^2_p$, whereas the other QTL explained between 3.8% and 13.6% of $\hat{\sigma}^2_p$. Averaged across cross validation runs,

Table 4 Phenotypic ($\hat{r}_p$) and genotypic ($\hat{r}_g$, below the diagonal) correlation coefficients among resistance and agronomic traits calculated in a population of 204 testcrossed $F_{2:3}$ families derived from the cross D06×D408

	Resistance traits		Agronomic traits				
	SDR	RGY	GYP	GYI	PHT	ANT	DMC
SDR		−0.44**	−0.03	−0.41**	−0.29**	−0.32**	0.18**
RGY	−0.84^{++}		−0.32**	0.63**	0.16*	0.13	−0.08
GYP	−0.03	0.01		0.52**	0.27**	0.25**	0.01
GYI	−0.58^{++}	0.69^{++}	0.46^{++}		0.38**	0.31**	−0.07
PHT	−0.44^{++}	0.36^{++}	0.27^{++}	0.46^{++}		0.43**	−0.14*
ANT	−0.50^{++}	0.23^{+}	0.54^{++}	0.53^{++}	0.58^{++}		−0.37**
DMC	0.30^{++}	−0.09	−0.07	−0.13^{+}	−0.18^{+}	−0.45^{++}	

*,** Phenotypic correlation was significant at the 0.05 and 0.01 probability levels, respectively.
$^{+}$, $^{++}$Genotypic correlation exceeded once or twice its standard error, respectively.
[a] SDR = stalk damage ratings, RGY = relative grain yield, GYP = grain yield under protection, GYI = grain yield under infestation, PHT = plant height, ANT = date of anthesis, DMC = dry matter content.

QTL explained between 3.3% (DMC) and 18.1% (PHT) of $\hat{\sigma}_g^2$ in a simultaneous fit. One QTL each for GYP (bin 5.07), GYI (bin 3.08), and PHT (bin 9.02) and two for ANT (bins 3.03 and 8.03) were detected in more than 97% of the 1,000 cross validation runs. QTL×environment interactions were significant (P<0.05) for ANT, DMC, and GYP.

Quality traits

Twenty-two QTL were detected for all evaluated forage quality traits (2 QTL for DNDF, IVDOM; 3 QTL for CF, DMCS; 4 QTL for CDOM, CP, and WSC; data not shown, for CF and IVDOM see Table 3). Each QTL explained between 5.7% and 10.7% of $\hat{\sigma}_p^2$ and between 3.3% and 8.0% of $\hat{\sigma}_g^2$ in a simultaneous fit. The frequency of QTL detection varied between 14.6% (CF, bin 9.02) and 89.6% (WSC, bin 10.06) of the cross validation runs. QTL×environment interactions were significant (P<0.05) for DNDF.

Correlations between resistance and agronomic traits

The genotypic and phenotypic correlation coefficients between RGY and SDR were highly significant (P<0.01) and negative (Table 4). Associations between agronomic and resistance traits were moderate to low. Stalk damage ratings were negatively associated with GYI, PHT, and ANT but positively correlated with DMC. Significant but low genotypic correlations (r_g >−0.27) were found between SDR and quality traits CDOM, DNDF, IVDOM, and WSC (data not shown).

Correlations between per se and TC performance

Phenotypic correlations between per se and TC performance of $F_{2:3}$ families were highly significant (P<0.01) and of moderate size for SDR, PHT, ANT, and DMC (Table 5). Corresponding genotypic correlations were moderate to high for all traits except RGY. The correlation between LOD profiles (r_{LOD}) for per se and TC performance showed significant (P<0.01) values only for SDR, PHT, and ANT. QTL identified for per se performance explained between 23% and 27% of σ_g^2 for TC performance for SDR, PHT, and ANT. Estimates of p_{TL} were practically zero for all other traits.

Table 5 Phenotypic ($\hat{r}_p$) and genotypic ($\hat{r}_g$) correlation coefficients between per se and TC performance of 204 $F_{2:3}$ families of cross D06×D408 as well as correlation coefficients between LOD profiles (r_{LOD}) and the proportion of the genotypic variance in TC performance explained by QTL detected for per se performance (p_{TL})

Trait[a]	$\hat{r}_p$	$\hat{r}_g$	r_{LOD}[b]	p_{TL}
SDR	0.33**	0.62^{++}	0.27*	23.1
RGY	0.09	0.30^{+}	0.10	–[c]
GYP	0.16*	0.30^{++}	−0.16	2.0
GYI	0.17*	0.27^{++}	0.12	2.1
ANT	0.56**	0.74^{++}	0.34*	26.7
PHT	0.69**	1.00^{++}	0.44**	27.0
DMC	0.47**	0.63^{++}	0.06	1.9

*,** Phenotypic correlation was significant at the 0.05 and 0.01 probability levels, respectively.
$^{+}$, $^{++}$Genotypic correlation exceeded once or twice its standard error, respectively.
[a] SDR = stalk damage ratings, RGY = relative grain yield, GYP = grain yield under protection, GYI = grain yield under infestation, PHT = plant height, ANT = days to anthesis, DMC = dry matter content.
[b] Correlation coefficients of LOD profiles for resistance and agronomic traits across testcross progenies and $P_{2:3}$ families.
[c] No QTL was detected for RGY.

Discussion

Consistency of QTL for per se and TC performance

The prime goal of maize breeding is to identify new lines with superior performance in hybrid combinations. Selection of potential parents is based on their general combining ability (GCA) to increase the probability of finding superior hybrid combinations. This is necessary, because performance of lines per se does not provide an adequate measure of their value in hybrid combinations for most traits of agronomic importance (Hallauer 1990).

The predictive value of GCA depends on the relative importance of GCA and the specific combining ability (SCA) for the trait under study. Based on these relationships, it seems logical to use QTL detected for TC performance or QTL found for per se performance with consistent expression in TC for identifying potential hybrid parents using MAS. However, QTL for TC performance might be tester-specific, resulting in the need to develop several QTL populations with different testers. In addition, the use of QTL for per se performance in MAS is attractive, because this information is available at least two generations earlier than is TC performance.

Therefore, the detection of QTL contributing to the GCA of a line for a specific trait and the consistency of QTL across line per se and TC evaluations, as well as between testers, are of central importance for developing a successful MAS program. The level of consistency depends on the power of QTL detection in per se and TC evaluations, the type of gene action displayed by the genes involved in the inheritance of the trait under study, and the specific allelic effects of the chosen tester. The power of detecting QTL for per se and TC performance is a function of the size of the QTL effects, the heritability of the trait under study, and the size of the mapping populations. In addition, the power of detecting the same QTL in per se and in TC evaluations is the product of the power of QTL detection in the separate studies. Therefore, a QTL will only be consistently detected in per se and TC evaluations, if the power of QTL detection is high in both studies.

In per se evaluations of $F_{2:3}$ families most likely QTL with additive effects will be found, because only a quarter of the dominance effects present in the F_1 generation can be detected in a population of $F_{2:3}$ families. In contrast, the average effect of a gene substitution is determined in TC evaluations. The average effect of a gene substitution is a function of additive and dominance effects as well as allele frequencies in the tester. The latter shows the influence of the tester allele. If the tester allele is dominant or partially dominant over the alleles of the two parental lines, it may mask the effect of the QTL allele segregating in the mapping population and hence this QTL is not detected for TC performance.

For TC performance, we detected six QTL for SDR explaining an average of one quarter of the genotypic variance across cross-validation runs. Three of these were in adjacent chromosomal bins (on chromosomes 1, 6, and 8) to QTL for SDR per se performance. In agreement with the above outlined expectations, QTL for per se performance displaying overdominance were not detected for TC performance, if their additive effect was not of considerable size, such as for the QTL for SDR in chromosomal bin 1.06. In addition, QTL alleles of the tester may be dominant over the alleles of the parental lines. These effects and the decreased genotypic variance displayed by testcrossed $F_{2:3}$ families reduced the power of QTL detection using TC progenies and resulted in a low consistency of QTL across per se and TC evaluations. Despite these disadvantages of TC progenies in QTL detection, three QTL were found for TC performance that remained undetected for per se performance. One possible explanation for this result could be the use of a highly susceptible tester. This tester was selected based on its high level of susceptibility to ECB larvae feeding combined with otherwise good agronomic performance (Schulz et al. 1997). In the case of a susceptible tester, specific interactions of the tester and segregating alleles facilitate the detection of new QTL (Kreps et al. 1998). In addition, this finding might be due to the low power of QTL detection in the per se and TC evaluations as a result of the relatively small population size (N<210), the low to moderate heritabilities for the evaluated ECB resistance traits, and the fact that per se and TC evaluations were performed in different environments.

Similar results were reported by Groh et al. (1998), who evaluated two tropical maize populations of recombinant inbred lines for their resistance against tropical stem borer species (*Diatraea* spp.) in per se and TC evaluations. Based on the mostly additive gene action found in early generations of both RIL populations (Bohn et al. 1996), it was expected to find several common resistance genes between per se and TC evaluations. But the reported consistency was low. The authors explained their findings by the low power of QTL detection in the TC progenies and the evaluation of both progeny types in different environments.

In a set of 16 European flint and 24 dent lines, correlations between per se and TC performance were high for stalk damage ratings, but low for tunnel length and yield reduction caused by larvae feeding (Kreps et al. 1998). In tropical maize the association between per se and TC performance was low for corn borer resistance (Thome et al. 1992). In accordance with these studies, we found low but significant phenotypic and genotypic correlation coefficients between per se and TC performance. However, based on the size of these correlations, it was not surprising that only half of the QTL detected in $F_{2:3}$ lines per se were rediscovered using their TC pro-genies.

In most ECB QTL studies, the majority of QTL associated with resistance showed additive effects and only to a minor extent dominance (Bohn et al. 2000; Papst et al. 2001; Krakowsky et al. 2002). Therefore, tight correlations between per se and TC evaluations were expected. However, as already state above, QTL with additive effects will be preferentially detected in populations of $F_{2:3}$ families, resulting in an underestimation of dominance involved in the inheritance of ECB resistance and in an overestimation of the association between per se and TC performance. In addition, predictions were based on QTL that often explained less than 50% of the genetic variance for the evaluated ECB resistance trait.

Clustering of QTL for insect resistance

The QTL regions for SDR detected only for TC performance in our study were located in adjacent intervals known to carry QTL for tunnel length observed for per se

performance (chromosomes 3, 7, and 10). All QTL found for TC performance were located adjacent to QTL regions detected for stem borer resistance in other temperate and tropical maize populations (Schön et al. 1993; Groh et al. 1998; Khairallah et al. 1998; Bohn et al. 2000; Papst et al. 2001; Jampatong et al. 2002; Krakowsky et al. 2002). A compilation of all known QTL positions based on their bin location showed that QTL for stem borer resistance were not randomly distributed across the maize genome but occur in clusters on chromosome 1, 5, and 9. This information is a possible starting point to determine candidate genes involved in the inheritance of stem borer resistance. Previous findings suggested cell wall fortification caused by increased lignin content as one putative resistance mechanism (Buendgen et al. 1990; Bergvinson et al. 1996). Known genes of the lignin biosynthesis pathway are located in the stem borer resistance gene clusters. It might be possible to substantiate the hypothesis that lignin is an important factor in stem borer resistance by applying new molecular tools to determine the association between allelic variation at candidate gene loci and a specific phenotype (McMullen et al. 1998; Buckler and Thornsberry 2002).

Correlations between traits

We determined tissue digestibility characteristics to test the hypothesis that cell wall fortification is one possible resistance mechanism. In our study no significant association was found between digestibility traits (CDOM, IVDOM, DNDF) and SDR. This is in good agreement with Kreps et al. (1998), who evaluated a set of 41 inbred lines for their per se and TC performance and reported no significant phenotypic correlation between in vitro digestibility of organic matter (IVDOM) and ECB resistance. Two possible reasons might account for the lack of association between digestibility traits and ECB resistance. First, the physical properties of a lignin polymer largely depend on its monolignol subunit composition. In maize, genes are known that directly influence lignin content in cell walls and its subunit structure. Specific alleles at these loci may cause the production of lignin with a subunit composition in cell walls that result in an increased IVDOM without compromising cell wall strength. These genes might also improve digestibility traits without increased SDR. Next to cell wall fortification and reduced forage quality, high concentrations of foliar phenolic acids are assumed to increase resistance to insect herbivores by causing oxidative stress in the midgut of insects (McMullen et al. 1998). Phenols are oxidized to quinones, which bind amino acids and proteins reducing their nutrional value and/or bioavailability and thus inhibiting larval development (Felton et al. 1989; Duffey and Felton 1991; McMullen et al. 1998). However, tobacco plants overexpressing a key gene involved in phenolic production did not exhibit higher levels of resistance against *Heiothis virescens* (Johnson and Felton 2001).

We found a negative association between SDR and ANT and a positive association between SDR and DMC in testcrosses in accordance with previous studies (Groh et al. 1998; Bohn et al. 2000; Magg et al. 2001). Bohn et al. (2000) found $F_{2:3}$ family genotypes that combined early flowering with a high level of ECB resistance. They conjectured, by examining graphical genotypes, that the correlation between ANT and ECB resistance was mainly caused by tight linkage instead of pleiotropy. The $F_{2:3}$ families, which combined early flowering with a high level of ECB resistance for per se performance, also showed this trait combination for TC performance.

Prospects of MAS for ECB resistance

The main goal of QTL mapping is the identification of chromosomal regions involved in the inheritance of economically important quantitative traits as a starting point for MAS. In the case of improving ECB resistance, costs for mass rearing of larvae and manual infestation are high and h^2 of ECB resistance traits are low. Therefore, marker-based technologies could offer a more efficient way to develop new genotypes with improved ECB resistance. In our companion study, the relative efficiency of MAS over conventional phenotypic selection was 0.87 for SDR indicating that conventional phenotypic selection is more efficient than MAS (Bohn et al. 2001). Even with low relative efficiencies (RE<1) MAS may be competitive over conventional phenotypic selection, if cost-effective PCR-based marker systems are available and costs of artificial infestation are high. However, the effectiveness of MAS strongly depends on the accuracy of QTL mapping results. Here, we reanalyzed data reported by Bohn et al. (2001) using CV. QTL for SDR explained 27.6% of σ_g^2 resulting in a low relative efficiency of MAS over conventional phenotypic selection (RE=0.47). If MAS and conventional phenotypic selection were combined, values of RE approached 1.05. This result suggested only a small gain in selection response employing MAS in selection programs for improving ECB resistance.

What are possible alternative approaches to utilize QTL information for stem borer resistance gathered in multiple studies over the last 10 years? A first step to utilize this wealth of information would be the performance of a meta-analysis (Goffinet et al. 2000) to confirm the hypothesis that QTL for stem borer resistance occur in clusters. These clusters might be large and will contain hundreds of genes, but based on knowledge about putative resistance mechanisms it might be possible to identify the underlying biochemical pathways and respective candidate genes. Their effect on stem borer resistance might be tested with association studies. Association studies might also provide plant breeders with information about the allelic variation that can be exploited for each resistance gene. In this case, MAS will be based on gene sequences

that allow tracing and combining candidate genes and their specific alleles directly.

Acknowledgements This research was supported by grants from the Bavarian State Ministry for Agriculture and Forestry. We are grateful to K. Fickler and S. Götze for technical assistance with field trials. The authors gratefully acknowledge the skilled technical assistance of E. Kokai-Kota for the excellent laboratory work. The support of KWS SAAT AG, Germany, for NIRS calibration of forage quality traits is greatly appreciated.

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