ESTIMATING GENETIC PARAMETERS AND THE EFFICACY OF MOLECULAR BREEDING FOR RESISTANCE TO THE STALK BORER *ELDANA SACCHARINA*

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KEYWORDS: Marker-assisted Breeding, Eldana, Resistance, Heritability.

Abstract

BREEDING for resistance to the stalk borer *Eldana saccharina* is one of the goals of the SASRI variety improvement program being addressed through an integrated approach involving conventional and molecular breeding. The objectives of this study were: to evaluate a mass screening method to estimate eldana resistance at a family level; to estimate narrow-sense heritability of eldana resistance, as well as family repeatability of resistance; and to attempt an initial verification of the efficacy of molecular breeding for resistance. Seedlings from 36 bi-parental crosses were planted at high density and inoculated with eldana larvae at 6 months of age. Phenotypic data for eldana resistance of the parents of the crosses were available, and the parents had been characterised for eight previously identified marker loci that ascribe 37% of the phenotypic variation in resistance. After one month, plots were harvested, eldana damage was measured and subjected to appropriate analysis. Mean stalk length damaged was highly significant between families. Variance components estimated by REML gave a family repeatability of 0.90, indicating that the mass-screening method was highly effective in estimating eldana resistance on a family level. Heritability calculated from phenotypic mid-parent-offspring regression was estimated at 0.57 explaining 12.6% of the variation in resistance at the family level. Heritability estimated from molecular marker prediction was 0.82, explaining 19.2% of the variation in progeny resistance. Molecular markers have apparently been effective in breeding for eldana resistance. Using sets of markers exhibiting stronger association with resistance may further increase the efficiency of molecular breeding.

Introduction

The African sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is a major insect pest of sugarcane in South Africa, causing extensive damage especially when cane is under stress. Larvae enter the stalk about one week after hatching where they are protected from exposure, with the result that insecticidal control is variable and not widely practised. Host-plant resistance and cultural practices such as early harvesting have been, and will continue to be, the main methods of limiting damage caused by the insect.

Development of eldana resistant cultivars is one of the goals of the South African Sugarcane Research Institute's (SASRI) breeding program, and eldana damage is routinely measured in variety selection trials at SASRI's Gingindhlovu research station. In addition to eldana assessment in yield trials, all promising individuals are screened in eldana inoculation trials conducted under controlled conditions in a shade-house, and resistance ratings are assigned, based on statistical principles as described by Keeping (2006). The scale used is a modified version of the ISSCT-published scale (Hutchinson, 1968), where low values (1, 2, and 3) indicate resistance, and high values (7, 8, 9) indicate susceptibility.

Because of its economic importance and the scarcity of resistant genotypes in the breeding population, eldana resistance was identified as a trait to be improved by the development and application of molecular marker tools. Aspects of this work have been described in Butterfield *et al*. (2004) and Butterfield (2006). In brief, molecular markers (RFLP and AFLP), associated with resistance or susceptibility to eldana, were identified in a population of 78 genotypes through association analysis (Breseghello and Sorrels, 2006). A subset of four RFLP markers was selected to screen a further 53 genotypes used as parents in the breeding population (see Materials and Methods). If the association between DNA marker and phenotype has predictive value, progeny of crosses between parents are expected to vary in eldana resistance, dependent on the marker profiles of their parents, i.e., the presence or absence of markers associated with either resistance or susceptibility.

The broad aim of this study was to attempt to validate this hypothesis experimentally. As the routine method for shade-house inoculation screening of individual genotypes is limited in terms of the numbers of plants that can be assessed, a mass screening technique that previously had shown some promise (Keeping, unpublished data) was used. As mass screening has not been widely used at SASRI, the specific objectives of the trial were:

- To assess the effectiveness of the mass screening technique to estimate eldana resistance at the family level.
- To estimate the narrow-sense heritability of eldana resistance at the family level, as well as the family repeatability of resistance in mass screening trials.
- To empirically validate the efficacy of using molecular marker information to design cross combinations predicted to have increased resistance to eldana.

Materials and methods

Molecular markers and validation population

Molecular markers used for the study were chosen from a set of 275 RFLP polymorphisms scored across an association analysis population of 78 genotypes. The four markers, along with their effect on eldana rating and the probability value of the association estimated by multiple regression, are shown in Table 1. The markers explain 37.6% of the phenotypic variation in eldana resistance rating. Because of the direction of the ISSCT rating scale used, markers with negative effects are associated with resistance, and the marker with a positive effect is associated with susceptibility.

Table 1—Effect and significance level of markers associated with eldana rating.

Marker	Effect	P-value
Intercept	6.677	
PhoPhoD2	–1.337	0.001
GPrRecD3	1.360	0.025
Pho301 H2	–1.200	0.002
IsoRedD1	–0.971	0.014

The markers were scored across an additional 53 parent genotypes, and bi-parental crosses were made over two years between parents with known marker-type. Progeny are predicted to vary in eldana resistance, based on the segregation of resistance/susceptibility markers inherited from the parents. Seed from 36 families representing 42 different parents was selected for phenotypic eldana resistance screening. Twenty-two parents were represented once only, 13 parents occurred in two different crosses, three parents occurred in three and four crosses, and one parent was represented in five different crosses. From the phenotypic eldana ratings of the parents, eldana mid-parent ratings were calculated for all crosses.

The marker-type of the parents was expressed as a vector of 0's and 1's representing the presence or absence of the markers shown in Table 1. For four markers, there are $2^4 = 16$ possible marker-types. The eldana resistance ideotype will be represented by 1011 (i.e., the presence of the resistance markers (PhoPhoD2, Pho301H2 and IsoRedD1, and the absence of the susceptibility marker GprRecD3), with a predicted rating of 3.2.

The susceptible ideotype is represented by 0100, with a predicted rating of 8.0. The remaining 14 marker-types will vary in predicted rating between these limits. Cross vector analysis (CVA) was done for each of the 36 crosses by calculating the predicted segregation ratios in the progeny to the 16 marker-type classes based on the marker-vectors of the two parents. Markers were treated as 'single dose' in the parents – i.e., [1] x [0] will segregate 1:1, and [1] x [1] will segregate 1:3 in the progeny. Family mean eldana rating was calculated from the predicted rating for each marker-vector, weighted by the proportion of progeny segregating in each class.

Mass screening for eldana resistance

For the mass screening trial, seedling progeny from the 36 crosses were transplanted in August 2005 into 'airbricks' of six holes (2 x 3), each with a volume of approximately 640 mL. One seedling was planted into each hole, with a resultant planting density of 100 plants/m^2. The trial was laid out as a randomised complete block with three replications, with an additional row/column sub-blocking structure imposed. A single row of perimeter airbricks was placed as a guard row on all sides of each replication to reduce edge effects. Each plot consisted of 6 x 6 plants (i.e., 36 seedlings per plot).

The trial was established in a shade house with walls of 40% green shade cloth and transparent fibre-glass roofing. Each replication was placed on a base of 3-µ thick plastic sheeting to prevent penetration of seedling roots into the underlying soil base. Growing medium consisted of a 3:2:1 mixture of sieved and leached compost, river sand, and vermiculite.

Plants were fertilised monthly with Hygrotech® seedling mixture (25 g/25 L) and ammonium sulfate (250 g/25 L) or alternately 4:1:1 (44) N:P:K fertiliser. Due to the presence of thrips, plants were sprayed monthly with chlorpyriphos (2 mL/L) until water stressing.

During the first 4.5 months, seedlings were irrigated via overhead mist sprayers for 10 min/day, until the plants had adequate stalk material to support eldana. At this point, they were moisture stressed by reducing watering to 7 min/day (for 10 days), 5 min/day (for 5 days) and 3 min/day (for 5 days). Stressing increases the recovery and biomass of eldana from sugarcane (Atkinson and Nuss, 1969).

At the end of the stress period, the replicates were inoculated sequentially with eldana eggs at a rate of 200 eggs/brick. Methods of inoculation followed those of Keeping (2006). Briefly, small pieces of tissue paper each bearing batches of approx. 100 eggs were placed behind a lower leaf sheath of two central plants per brick.

Care was taken not to drown emerging neonates through overhead watering; instead, daily manual watering at ground level ensured that plants received sufficient water to maintain water stress at four green leaves per plant (Keeping, 2006). The infestation in each replicate was allowed to develop over a period (approx. 35 days) required to accumulate 521 day-degrees (developmental threshold temperature = 10°C), equivalent to the sixth larval instar/pupal stage. Hence, the trial assessed infestation damage from a single generation of borers.

Replicates were harvested sequentially in the same order as their inoculation. All plants were removed from each brick and dissected to record the following data: total larval and pupal numbers per plant, length of stalk damaged per plant, and number of internodes damaged per plant. Only measures of stalk length damaged were used for further analyses.

Statistical analysis

Measurements of stalk length damaged were transformed ($\log_e$) and subjected to Restricted Maximum Likelihood (REML) analysis in Genstat 9.1 using two statistical models. Model 1 included Family as a fixed effect to estimate family means, and Model 2 included family as a random effect to estimate variance components.

Row/column sub-blocks did not improve the efficiency of the analysis, so replication was treated as a random effect in both models. Family mean stalk damage was converted to a rating using the SASRI method (Keeping, 2006), i.e., the trial average damage was given a rating of 5, and individual families were rated relative to their deviation from the this, in units of 1 standard error of the difference between means.

Family repeatability in the mass screening method was estimated as:

$$\frac{Vf}{Vf + \frac{1}{n_r}Vr + \frac{1}{n_e}Ve}$$

with V_f, V_c and V_e = the variance due to family, replication and error, n_r = number of replications, and n_e = number of individuals per family. (Wilkinson, 1977).

Heritability of family mean based on phenotype was estimated by the regression of eldana mid-parent rating on family observed rating (Falconer, 1981). Heritability based on molecular marker information was estimated by the regression of CVA-predicted rating on family observed rating.

Results

Mean **s**talk length damaged (Log_e transformed) varied from 1.8 to 3.0 between families, with a standard error of mean difference of 0.104. This translated to geometric means varying from 6.3 to 20.1 centimetres length damaged. When converted to a rating based on deviation from the average, family average rating varied from 3.0 to 8.0. Variance components estimated from REML analysis are shown in Table 2.

Table 2—Variance components derived from REML analysis with family and replication as random effects.

Term	Variance	S.E.
Family	0.061	0.0158
Replication	0.003	0.0039
Residual	0.599	0.0147

From the variance components, repeatability of family means is estimated as $R = 0.90$. This indicates that the mass screening method used is highly effective at discriminating differences in eldana resistance at the family level.

Family mid-parent values ranged from 3.0 to 5.5, while family CVA prediction based on molecular marker profile ranged from 4.1 to 6.4. Results from the estimation of heritability from the regression of observed progeny mean resistance on either phenotypic mid-parent rating, or CVA marker prediction are shown in Table 3.

Family heritability for eldana resistance estimated conventionally from parent phenotype was moderate, at $h^2 = 0.56$. Variation in mid-parent rating explained 12.6% of the variation in progeny mean rating. As is often the case with heritability estimates, the standard error was high, at 44% of the h^2 value.

Table 3—Results from parent-offspring regression analysis for heritability estimation.

	Mid-parent	CVA prediction
h^2	0.56	0.82
S.E.	0.25	0.28
r^2	0.126	0.192
P-value	0.031	0.007

Family heritability estimated from molecular marker data was high, at $h^2 = 0.82$, explaining 19.2% of the variation in observed progeny rating. On a percentage basis, the standard error was slightly lower than that based on mid-parent value, at 35%. Progressive regression analysis was done to compare the statistical significance of the two regression models (data not shown). Due to the high standard errors of the regression coefficients, the difference between the models was not significant.

Discussion

This is the first empirical validation of the effect of marker-assisted breeding on the efficiency of variety improvement in sugarcane. Although many theoretical studies have been published comparing the efficiency of marker-assisted selection (MAS) to phenotypic selection (e.g., Lande and Thompson, 1990, Moreau *et al.*, 2000), few validation studies have been reported. In one of the few published studies, Yousef and Juvik (2001) compared MAS and phenotypic selection (PS) in breeding for a number of kernel traits in sweet corn. Out of 52 paired comparisons, MAS gave higher gains than PS in 38 comparisons, while PS was better than MAS in 4 cases. On average, MAS resulted in an average gain of 10.9%, compared to a gain of 6.1% for phenotypic selection. No attempt was made to estimate the difference in genetic parameters between MAS and phenotypic selection.

The mass screening technique used in this trial was highly effective at discriminating among the average eldana resistance of different families. This could potentially become an additional tool in the recurrent mass selection breeding strategy for eldana resistance, as a reasonably large number of families can be screened. Resistant families can be identified, and large numbers of progeny planted in the field for phenotypic evaluation; not only for eldana resistance but also for agronomic traits such as yield and sucrose content.

Although the differences in heritability for eldana resistance in this study were not statistically significant, the prediction of progeny performance based on molecular markers was substantially better than that based on parent phenotype. Standard errors of heritability estimates are notoriously large (Falconer, 1981) unless the regression is based on very large numbers. An additional contributing factor to the lack of significance was that the range in mid-parent ratings and CVA predicted ratings was quite small. This was because highly susceptible genotypes are routinely excluded from the parental pool. Estimates of genetic parameters may have been increased by the inclusion of highly susceptible parents among the families used, but this would be an artificial situation compared to the breeding strategy used in practice. Another potential criticism of the design used to estimate heritability is the un-equal representation of parents across the 36 families. This inequality occurs in practical breeding programs however, and the purpose of this study was to compare the efficiency of conventional *versus* molecular breeding in this particular population, and not to generalise the results to other potential populations.

This study has provided an initial empirical validation of the usefulness of molecular markers in sugarcane breeding. Since this study was initiated, additional molecular markers ascribing up to 60% of the phenotypic variation in eldana resistance rating have been identified (Butterfield, 2006).

These markers are currently being screened across a population of 100 genotypes selected as parents to be used in crossing during 2007. We anticipate that designing desirable cross combinations among these parents based on CVA prediction will lead to further increases in the efficiency of breeding sugarcane for resistance to eldana.

Acknowledgements

We thank Andrew Govender, Sipho Zuma and the SASRI field teams for their assistance in all practical aspects of the trial, as well as Jay Maharaj and Reggie Dhrumaraj from the Plant Breeding Glasshouse for assistance in crossing. The SASRI Insect Rearing Unit produced the eldana eggs. Stuart Rutherford, Lucy Thokoane and Julie Richards provided RFLP data on the marker discovery population and the parent genotypes used in crossing.

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ESTIMATIONS DES PARAMÈTRES GÉNÉTIQUES ET DE L'EFFICATITÉ DE LA SÉLECTION ASSISTÉE PAR MARQUEURS MOLÉCULAIRES POUR LA RÉSISTANCE AU FOREUR DE TIGE *ELDANA SACCHARINA*

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MOTS CLÉS: La Sélection Assistée par Marqueurs, Eldana, Résistance, Héritabilité.

Résumé

LA SÉLECTION pour la résistance au foreur de tige *Eldana saccharina*, par une approche intégrée comprenant la sélection conventionnelle et moléculaire, est un des objectifs du programme d'amélioration variétale du SASRI. Les objectifs de cette étude consistaient en: une évaluation de la méthode de criblage en masse pour estimer la résistance à *Eldana* au sein de la famille; une estimation de l'héritabilité au sens strict de la résistance à l'*Eldana*, ainsi que la répétabilité de la résistance au niveau de la famille; une vérification de l'efficacité de la sélection assistée par marqueurs moléculaires pour la résistance à l'*Eldana.* Des plantules provenant de 36 croisements bi-parentaux ont été plantées à forte densité et au bout de six mois elles ont été inoculées avec des larves d'*Eldana*. Les données phénotypiques des parents pour la résistance à l'*Eldana* étaient disponibles et elles ont été caractérisées par huit marqueurs identifiés au préalable, expliquant jusqu'à 37% de la variation phénotypique pour la résistance au foreur. Après un mois, les parcelles ont été récoltées et les dégâts causés par *Eldana* ont été notés et les données soumises à des analyses appropriées. La différence entre la longueur moyenne des tiges endommagées entre les familles était fortement significative. Les composantes de la variance estimées par REML ont montré une répétabilité de 0,90 au sein des familles, indiquant que la méthode de criblage en masse était très efficace pour estimer la résistance à l'*Eldana* au niveau de la famille. L'héritabilité estimée à partir de la méthode de régression phénotypique parents-descendants de 0,57 expliquait jusqu'à 12,6% de la variation de la résistance au niveau de la famille. L'héritabilité estimée à partir des données moléculaires était de 0,82, expliquant 19,2% de la variation de la résistance chez les descendants. Les marqueurs moléculaires ont apparemment été efficaces pour la sélection de la résistance à l'*Eldana*. En employant un ensemble de marqueurs montrant une forte association avec la résistance à l'*Eldana*, l'efficacité de la sélection par marqueurs moléculaires peut être améliorée.

ESTIMACIÓN DE PARÁMETROS GENÉTICOS Y DE LA EFICACIA DEL MEJORAMIENTO MOLECULAR PARA RESISTENCIA AL BARRENADOR DEL TALLO *ELDANA SACCHARINA*

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PALABRAS CLAVE: Mejoramiento Asistido por Marcadores, Eldana, Resistencia, Heredabilidad.

Resumen

EL MEJORAMIENTO por resistencia al barrenador del tallo *Eldana saccharina* es uno de los objetivos del programa de mejoramiento genético de SASRI que está orientado hacia una investigación integral que involucra el mejoramiento convencional y el molecular. Los objetivos de este estudio fueron: 1.- Evaluar el método de selección masal para estimar la resistencia al barrenador a nivel de la familia. 2.- Estimar la heredabilidad en el sentido estrecho de la resistencia al barrenador así

como también la repetibilidad de la familia a la resistencia. 3.- Verificar la eficacia del mejoramiento molecular a la resistencia. Se sembraron plántulas de 36 cruzamientos biparentales en alta densidad e inoculadas con larvas de *Eldana* a los 6 meses de edad. Se dispuso de los datos fenotípicos de la resistencia a *Eldana* de los padres de los cruzamientos. Estos padres habían sido caracterizados para ocho locus de marcadores identificados previamente, responsables por el 37% de la variación fenotípica de la resistencia. Después de un mes, se cosecharon las parcelas, se midió el daño causado por *Eldana* y se analizó oportunamente. El promedio del daño en el tallo fue altamente significativo entre familias. Los componentes de varianza estimados por REML dieron una repetibilidad familiar de 0.90 indicando que el método de selección masal fue muy efectivo para estimar la resistencia a *Eldana* a nivel familiar. La heredabilidad calculada por medio de la regresión fenotípica entre los padres y su descendencia se estimó en 0.57 que explica el 12.6% de la variación de la resistencia a nivel familiar. La heredabilidad estimada por medio del marcador fue de 0.82 que explica el 19.2% de la variación de la resistencia en la progenie. Aparentemente, los marcadores moleculares han sido efectivos en el mejoramiento para la resistencia a *Eldana*. El uso de marcadores que presentan una asociación fuerte con la resistencia puede incrementar en el futuro la eficiencia del mejoramiento molecular.