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BREEDING SUGARCANE FOR LEAF SCALD RESISTANCE: A GENETIC STUDY

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ABSTRACT

Six sugarcane genotypes were combined in 15 bi-parental crosses, following a balanced diallel type II design. The objective was to obtain a better understanding of the genetic variance of sugarcane resistance to Leaf Scald (LS). The progeny, and the parental clones, were evaluated in randomized block design with three replications of seventy plants per plot. Plots were inoculated with LS when harvested in plant cane and the ratoon was evaluated for symptoms. Results show a high broad-sense heritability ($h^2_b = 0.98$) and moderate narrow-sense heritability ($h^2_n = 0.57$). Analysis of the genetic variance showed significant general combining ability (GCA) as well as specific combining ability (SCA). The genotypes SP80-1520, SP80-185 and TCP86-3361 showed the highest GCA values while SP80-185 and SP82-3386 showed the highest SCA values. The use of LS susceptible parents that possess other favorable traits in sugarcane breeding programs cannot be ruled out. The SCA demonstrated by some parental genotypes means that resistant seedlings might be obtained from the crosses with these parents.

INTRODUCTION

Leaf scald is one of the most important sugarcane diseases in Brazil and in the world (Rott and Davis, 2000). It may cause high yield losses and even completely destroy crops of susceptible varieties within a few years. The disease is caused by the bacterium, *Xanthomonas albilineans,* and is disseminated by cutting implements and by wind-blown rain (Comstock, 2001).

The most important control method is to plant resistant varieties. During the 1990s, many countries reported an increase in the occurrence of leaf scald, including United States (Louisiana and Florida) (Davis et al., 1994, Grisham et al., 1994 and Klett and Rott, 1994). Investigating the leaf scald effects on the yield of seven varieties during a 3-year crop, there was a 12 – 21% yield loss for two of the varieties studied (Rott et al., 1995). The intensity of symptoms also varied between varieties with symptoms being predominantly in the plant cane of variety R570 and the ratoon of B69566. In this study, the number of symptomatic stalks was always less than the number of stalks colonized by the bacteria, resulting in diseased yet asymptomatic plants.

Few papers have been published on the genetic variability or it heritability. Bell (1932, 1933 and 1935) investigated the resistance of progeny from nine crosses and showed that the

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frequency of diseased plants was higher when the cross involved susceptible parents. Similar results were obtained by Hughes et al. (1968 and 1970), who estimated a broad-sense heritability of 0.97 for leaf scald resistance.

Aiming to improve our knowledge on the genetics of leaf scald resistance, we investigated the segregation of resistance of progeny of crosses involving genotypes with different degrees of resistance. Estimates of narrow and broad-sense heritabilities were obtained, as well as detailed estimates of general and specific combining abilities.

MATERIALS AND METHODS

During the 1998 hybridization program at the COPERSUCAR experiment station in Camamu, Brazil, six genotypes (SP80-1520, SP80-185, SP81-3386, SP82-3384, SP84-2711 and TCP86-3361) were crossed in 15 bi-parental combinations. To avoid self-pollination, all flowers utilized as female were emasculated (Machado Jr. et al., 1995). From each cross and also from each parent, 210 plants were planted in August and transplanted to the field in October 1998, at the COPERSUCAR experimental station in Piracicaba, Sao Paulo, Brazil (except for the cross between SP84-2711 and TCP86-3361 which failed to produce any plants). Field plots were five rows by 6 m, with 1.4 m space between rows and 0.46 m space between plants within rows. A randomized complete block design was used, with three replicates and 70 plants in each plot.

Eight months after planting, plots were ratooned and inoculated at the same time with *Xanthomonas albilineans* using cane knives previously dipped into cane juice obtained from sugarcane stalks showing severe leaf scald symptoms. After cutting, the inoculum was also sprayed over freshly cut surfaces of the stubble. Nine months after inoculation seedlings were inspected for leaf scald symptoms (March 2000), according to the method proposed by Rott and Davis (2000). The stalks were divided in five groups according to the symptoms: (1) one or two streaks in the leaf; (2) more than two streaks in the leaf; (3) partial or complete chlorosis; (4) necrotic sections in the leaf and (5) death stalks or side shoots. The disease severity (S) for each plant was obtained according to the equation:

$$S = 100\ (1\ NS_1+2\ NS_2+3\ NS_3+4\ NS_4+5\ NS_5)\ /\ (5\ NS_T)$$

where $NS_{1,2...5}$ corresponds to the number of stalks in each group of symptoms, and NS_T is the total number of stalks of the seedling. Once the severity had been determined the values were converted into grades, from 1 to 9, according to the following scale:

Grade	Severity
1	0.0
2	0.1 – 2.0
3	2.1 – 5.0
4	5.1 – 10.0
5	10.1 – 15.0
6	15.1 – 20.0
7	20.1 – 30.0
8	30.1 – 50.0
9	> 50.0

The leaf scald reaction data (percentage of plants with symptoms - LS%) were transformed using square root (x+0.5). The analyses of variance to estimate the effects of both general (GCA) and specific combining ability (SCA) for leaf scald reaction were performed according to the balanced diallel proposed by Griffing (1956), method II. With this method, both F_1 hybrid combinations and their respective parents are included. The genotypes utilized as parents in this work were taken at random from the total population, which allowed the values estimated for GCA and SCA to be considered as random effects. The proportion of the variation explained by GCA in relation to the SCA was estimated according to Baker (1978). The broad and narrow-sense heritability estimates, based on analysis of variance and parent offspring regression, were estimated as described by Cruz and Regazzi (1997).

RESULTS AND DISCUSSION

Disease Ratings of progeny

A large proportion of the plants studied did not show visual symptoms of the disease (Table 1), which suggests one of three possibilities: 1) high frequency of resistant plants, 2) high frequency of plants with latent infection or 3) high frequency of escapes from the inoculation process. The parents SP81-3386 and SP82-3384, as well as the progeny resulting form the cross between them showed the highest number of symptomatic plants, with more than 20% of plants with visible symptoms. The parents SP80-1520, SP80-185 and TCP86-3361 did not show any symptoms and the progenies originating from crosses between them also had the lowest frequency of diseased plants. Egan (1974), using a stubble inoculation method, obtained values ranging from 2% to 88% of individuals with symptoms in seven bi-parental progenies in Australia. This author reported that repeating the inoculation of the asymptomatic plants did not change the frequency of symptomatic plants.

The distribution of symptomatic plants in the different severity grades did not vary between cross of resistant by susceptible parents (SP80-1520 x SP81-3386), susceptible by intermediate parents (SP81-3386 x SP81-3384) or even just intermediate parents (SP81-3384 x SP84-2711). The low levels of disease precluded a detailed study on the segregation of disease severity and the susceptibility of the parents crossed. For future studies, a different evaluation method should be used and the trials could be rated over a number of ratoon crops. The inoculated plants could also be screened for the presence of the bacteria in the laboratory to rate plants for latent infection.

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Table 1. The percentage of symptomatic plants in each grade for each family and for their parents.

Female	Male	Leaf scald grade								
		1	2	3	4	5	6	7	8	9
SP80-1520	SP80-185	99			1					
SP80-1520	SP81-3386	82		2	2	3	1	3	2	5
SP80-1520	SP82-3384	95			1	1		2	1	
SP80-1520	SP84-2711	98			1					1
SP80-1520	TCP86-3361	93	1	1	2	1		1	1	
SP80-185	SP81-3386	93		1	1	1	2	1	1	
SP80-185	SP82-3384	89		2	2	1	1	1	2	2
SP80-185	SP84-2711	94		2	1	1	1	1		
SP80-185	TCP86-3361	97			1	1			1	
SP81-3386	SP82-3384	66	1	1	8	2	5	2	7	8
SP84-2711	SP81-3386	81		1	5	3	2	2	3	3
SP84-2711	SP82-3384	80		1	4	1	3	4	4	3
TCP86-3361	SP81-3386	80		1	5	2	2	3	2	5
TCP86-3361	SP82-3384	88		1	1	2	2	2	2	2
Family mean		*88*		*1*	*3*	*1*	*1*	*2*	*2*	*2*
SP80-1520		100								
SP80-185		100								
SP81-3386		21			5	4	10	10	20	30
SP82-3384		73		4	9	10	2	1	1	0
SP84-2711		90		3	4	2	1			
TCP86-3361		100								
Parent means		*81*		*1*	*3*	*3*	*2*	*2*	*3*	*5*

Analysis of variance

Mean square values from the combined analysis of variance on the percentage of plants with leaf scald symptoms (LS%) are presented on Table 2. The GCA and SCA components, based on mean values, showed that both dominant and additive genetic variance were significant (p<0.01) (Table 2).

The data also showed that additive genetic variance (0.87) was the most important factor for this trait. The narrow-sense heritability estimate (h_n^2), based on genetic and environmental components in the analysis of variance, was moderate (0.57), and indicated that the selection of resistant parents for crosses will result in moderate genetic gains in the progeny. The high value for the estimate of the broad-sense heritability (h_b^2 = 0.98), also based on the analysis of variance indicates that resistant families can be identified in the early stages of selection. In other words, the family selection will be more efficient than the selection of parents. Similar results for h_b^2 have been reported by Hughes et al. (1970). The results from this study suggest that the combination of selecting less susceptible parents and discarding the susceptible plants or even families during the first selection stages will give the most rapid progress for breeding for leaf scald resistance.

Table 2. Mean square values and genetic estimates for percentage of plants with leaf scald symptoms (LS%), after transformation of data with square root (x + 0.5), of a diallel cross with six sugarcane genotypes.

Source	DF	LS%	
Blocks	2	0.4549	
Treatments	19	12.3790	**
GCA	5	41.2910	**
SCA	14	2.0532	**
Error	38	0.2795	

Mean: 3.35; CV error : 15.80%.

** Significant at 1% level (p<0.01).

The majority of genotypes studied displayed significant additive genetic effect (p<0.05) (Table 3). Based on GCA values, the genotypes SP80-1520, SP80-185 and TCP86-3361 should be selected for use in crosses aimed at producing resistant progenies, while the genotypes SP81-3386 and SP82-3384 should be avoided.

Table 3. General combining ability (GCA) estimates for leaf scald symptoms (LS%), after transformation of data with square root (x + 0.5), studied in a diallel cross involving six genotypes.

Genotype	GCA	
SP80-1520	-1.128	*
SP80-185	-1.270	*
SP81-3386	2.060	*
SP82-3384	1.076	*
SP84-2711	-0.131	
TCP86-3361	-0.606	*
$\hat{s}^2_{gi}$	0.0291	

$\hat{s}^2_{gi}$ = variance estimate for the GCA effects.

* Significant at 5% level (p<0.05,t test).

Estimates for specific combining ability (SCA) are presented on Table 4. A small value (non significant) for SCA indicates that the hybrids obtained from 2 genotypes have their performance estimated on the basis of the GCA of each genotype, whereas significant values indicate the combinations may be better or worse than the one expected based on the SCA (Cruz and Regazzi, 1997).

From the GCA analysis, it can be noted that the genotypes SP80-185, SP80-1520 and TCP86-3361 were those with the lowest percentage of leaf scald in their progeny. The cross between SP80-1520 and TCP86-3361 presented a significant (p<0.05) and unfavorable SCA

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(1.146), that is it had a higher frequency of susceptible plants in the progeny than would be predicted from the resistance of the parents. The cross between SP80-185 and SP82-3386, which had favorable and unfavorable GCA, respectively, had a favorable and significant estimate for SCA (-1.423). These results indicate an interaction on the values for GCA and SCA, given the susceptible progenies obtained from parents chosen based on their GCA. In summary, one can see that the selection of resistant parents did not necessarily result in a higher percentage of resistant progenies.

Table 4. Specific combining ability estimates (SCA) for leaf scald symptoms (LS%) after transformation of data with square root (x + 0.05), studied in a diallel cross involving six genotypes.

Female	Male	SCA
SP80-1520	SP80-1520	-0.346
SP80-1520	SP80-185	0.174
SP80-1520	SP81-3386	0.070
SP80-1520	SP82-3384	-0.335
SP80-1520	SP84-2711	-0.362
SP80-1520	TCP86-3361	1.146 *
SP80-185	SP80-185	-0.062
SP80-185	SP81-3386	-1.423 *
SP80-185	SP82-3384	0.267
SP80-185	SP84-2711	0.516
SP80-185	TCP86-3361	0.590
SP81-3386	SP81-3386	1.472 *
SP81-3386	SP82-3384	-0.563
SP81-3386	SP84-2711	-0.816
SP81-3386	TCP86-3361	-0.211
SP82-3384	SP82-3384	-0.252
SP82-3384	SP84-2711	0.454
SP82-3384	TCP86-3361	0.681
SP84-2711	SP84-2711	0.104
TCP86-3361	TCP86-3361	-1.103 *
$\hat{s}^2_{sij}$		0.2196
$\hat{s}^2_{sii}$		0.1497

* Significant at 5% level (p<0.05,t test).

$\hat{s}^2_{sij}$ = estimate value for the variance effects of SCA among the F_1 families.

$\hat{s}^2_{sii}$ = estimate of the variance of the SCA among parents.

The high values of SCA in some crosses resulted in only a moderate estimate of narrow-sense heritability (0.57), decreasing consequently the efficiency of parent selection. On the other hand, the selection of resistant individuals or families were efficient, take in account the high estimate for broad-sense heritability (0.98), this is in good agreement with the one obtained by

Hughes et al. (1968), cited by Egan (1974), who found a value of 0.97 on the analysis of progenies obtained from nine bi-parental crosses.

Parent-offspring regression analysis

The estimate for the narrow-sense heritability ($\hat{h}_n^2$), obtained from the parent-offspring regression analysis of mean percentage of leaf scald, was 0.58. This value is similar to that obtained from the analysis of variance, confirming again that the selection of resistant parents has an intermediate efficacy on the generation of resistant progenies. The moderate narrow-sense heritability means that screening plants for resistance needs to be continued during the clonal selection process. Superior parents that are susceptible to leaf scald should not be discarded. Due to the values for SCA, a proportion of the family resulting from crosses with these susceptible parents can provide some resistant progeny or clones.

CONCLUSIONS

- The leaf scald inoculation process led to a high percentage of asymptomatic plants (either escapes or latent infections), precluding a more detailed study between the segregation of disease severity and the susceptibility of the parents crossed.
- The estimate of broad-sense heritability for leaf scald resistance was high and the estimate of narrow-sense heritability was intermediate. Breeding for resistance to leaf scald should combine selection of resistant parents and screening for resistant individuals or families.
- Susceptible parents with favorable agronomic traits should not be discarded, since a proportion of the progeny resulting from such crosses will be resistant, given the SCA effects, and new favorable resistant progenies could be found.
- Based on GCA data, the best genotypes were SP80-1520. SP80-185 and TCP86-3361, whereas based on SCA data, the best cross was SP80-185 and SP81-3386.

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