

**BREEDING APPROACHES IN SIMULTANEOUS SELECTION FOR
MULTIPLE STRESS TOLERANCE OF MAIZE IN TROPICAL
ENVIROMENTS**

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Maize is the principal crop and major staple food in the most countries of Sub-Saharan Africa. However, due to the influence of abiotic and biotic stress factors, maize production faces serious constraints. Among the agro-ecological conditions, the main constraints are: lack and poor distribution of rainfall; low soil fertility; diseases (maize streak virus, downy mildew, leaf blights, rusts, gray leaf spot, stem/cob rots) and pests (borers and storage pests). Among the socio-economic production constraints are: poor economy, serious shortage of trained manpower; insufficient management expertise, lack of use of improved varieties and poor cultivation practices. To develop desirable varieties, and thus consequently alleviate some of these constraints, appropriate breeding

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approaches and field-based methodologies in selection for multiple stress tolerance, were implemented. These approaches are mainly based on: a) Crossing selected genotypes with more desirable stress tolerant and other agronomic traits; b) Using the disease/pest spreader row method, combined with testing and selection of created progenies under strong to intermediate pressure of drought and low soil fertility in nurseries; and c) Evaluation of the varieties developed in multi-location trials under low and "normal" inputs. These approaches provide testing and selection of large number of progenies, which is required for simultaneous selection for multiple stress tolerance. Data obtained revealed that remarkable improvement of the traits under selection was achieved. Biggest progress was obtained in selection for maize streak virus and downy mildew resistance, flintiness and earliness. In the case of drought stress, statistical analyses revealed significant negative correlation between yield and anthesis-silking interval, and between yield and days to silk, but positive correlation between yield and grain weight per ear.

Key words: simultaneous selection, multiple stress factors, diseases, drought, earliness, grain texture, protein quality

INTRODUCTION

Maize is grown in all agro-ecological zones and is the principal crop and major staple food in many zones of Mozambique. There are 2.5 million agricultural family units, of which 1.9 million (78%) grow maize, with average maize area of about 0.7 to 0.9 hectares (MINAG, 1994). Maize production by the family sector occupied 39% of total arable land, or an area of 1.74 million hectares. It is estimated that the family sector occupies about 95% of the total maize area and produces 90% of the national maize crop. Grain yield of maize grown on peasant's farms has been about 0.6 t/ha (FAO, 1995), whereas in the 1995 season it ranged from 0.2 to 1.2 t/ha (DINA, 1995). Agriculture in Mozambique, like in many other Sub-Saharan Africa countries, is primarily small-scale, subsistence-level, and labor intensive. It is characterized by low use of external inputs, low to medium productivity, high diversity of products, and by a strategy of minimizing risks (KIEFT, 1993).

PROBLEM

Main constraints in maize production were identified (NUNES et al., 1985). Thus, among the agro-ecological conditions in southern Mozambique, main constraints are: a. Lack of rainfall; b. Diseases – maize streak virus (MSV) and downy mildew (DM); and c. Pests – borers and storage pests. In central and northern part of the country the main constraints are: a. Low soil fertility; b. Periodic droughts in low-land areas; c. Diseases – stem/ear rots, leaf blights and rusts at higher altitudes, maize streak virus (MSV) and DM in Manica and Sofala provinces; and d. Storage pests. Among the other production constraints are the

serious shortage of trained manpower, insufficient management expertise and poor cultivation practices, such as inadequate inter-cropping, poor soil preparation, poor irrigation techniques, poor weeding and poor plant practices.

Agricultural productivity is often hampered by poor infrastructure, which limits access to inputs and markets. Much of the existing and available maize germplasm is adapted to higher elevation and higher productivity environments, that are about 15 to 20 % of total arable land in Mozambique (DENIC, 1994). Similarly, existing maize production (crop management) technologies do not address the specific needs of the resource-poor family sector.

OBJECTIVES

Based on the constraints in maize production, main objective in this work is to develop, evaluate and select maize genotypes resistant or tolerant to principal biotic and abiotic factors, which is limiting production in principal maize areas. Thus among biotic factors attention is given to the selection for resistance to MSV (SR) and downy mildew (DMR), *Peronosclerospora sorghi*. In the case of abiotic factors attention is given to the selection to tolerance to drought (DT). In addition to this factor large part of the work is oriented to selection for agronomic traits earliness (E) and for hard type of grain texture (GT). Due to the fact that maize is staple food, attention is also given to creation of varieties with high nutritional value of proteins (QPM), with hard grain and resistant to principal diseases.

MATERIALS AND METHODS

The breeding work was done at Experimental Research Station in Umbeluzi (30 km from Maputo). Breeding populations of normal (common) maize were created by crossing low-land tropical populations without DMR from International Center for Improvement of Maize and Wheat (CIMMYT), Mexico with DMR-SR commercial varieties Matuba or SEMOC 1, both originating from DMR-E-SR-W population from International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria. Group of entries with existing DMR background consisted of 11 DMR populations from IITA, 11 inbred lines extracted from Population 8072 DMR or from Population 8075 DMR, and 11 S3 DMR-SR lines from CIMMYT (Harare), was also included. This material at the season of evaluation was in C8 of selection for SR, earliness (E) and flintiness (F), and in C5 of selection for DMR.

In the case of QPM the donors of opaque-2 gene were Ghanaian line Entry 5 (E-5Q) or SEMOC S4 lines extracted from Pool 15 QPM (BC4) SR (SMLQ). Donors for DMR-SR were SEMOC S4 lines extracted from Matuba (MTL) or SEMOC inbred lines (SML) extracted from Population 8072DMR or Population 8075DMR. QPM material was in C3 of selection for SR and endosperm modification and in C1 of selection for DMR).

All materials were subjected to heavy disease infection in nurseries of DM and MSV. For evaluation of large number of breeding materials, method of spreader rows was recommended (WILLIAMS, 1984). To facilitate strong disease infection and increase number of entries for evaluation, the method of spreader rows (CARDWELL, 1994) was modified and combined with late and continuous planting (DENIC, 1996). Double plant density in the nurseries was maintained during 5 weeks after germination and then plants with DM were rough out. Number of diseased and healthy plants was recorded and % of plants with systemic DM disease was calculated. Evaluation of SR of QPM entries was done by CIMMYT, Harare, using artificial infestation with MSV vector from genus Cicada.

During anthesis the early-flowering plants from selected progenies with good aspect, DMR and SR are either self-pollinated or crossed by hand pollination. At harvest, ears with good aspect were selected. Preference in selection was given to the ears from early-flowering plants and to flint and semi-flint type of grain. In the case of QPM, kernels with good endosperm modification were selected using light tables.

RESULTS AND DISCUSSION

Screening for Multiple Stress Tolerance and Agronomic Traits in Normal Maize

Data from three groups of entries of normal maize, after C8 of selection for SR and C5 of selection DMR, show similar mean values of indices of SR and % of plants with DM symptoms (Table 1). In the case of earliness and grain texture, Group 2 (with selection for DMR initiated in C1) and Group 3 (with selection for DMR initiated in C3), exhibited earlier flowering and harder grain texture in comparison to the Group 1 (entries with existing DMR background). In the case of DMR, however the same two groups showed somewhat higher means and very high variation of % of plants with DM in comparison with Group 1. It should be pointed out that, in C1 of selection for DMR, means of % of plants with DM were 33.3 for Group 1, 53.5 for Group 2 and 75.6 for Group 3 (unpublished data). Difference in DMR between Group 2 and Group 3 illustrates importance of immediate selection after recombination.

Data across the groups of populations are shown in Table 2. Analyzing mean values of the traits, it is possible to see that in average there is good SR, DMR and semi flint grain texture. Further analyses of number and frequency distribution of FS families related to the studied traits under disease pressure show that 939 progenies (24.1 % of total) and 1,242 progenies (37.8 % of total) exhibited strong SR and DMR, respectively. The same data show that 447 progenies (14.6 %) belong to the group of very early maturity and 760 progenies (24.9 %) belong to the group of early maturity. The highest progress was achieved in the case of grain texture. It was found that 35.8 % (1,130 FS families) and 39.8 % (1,258 FS families) exhibited flint and semi flint grain texture, respectively.

Table 1. Indices of streak resistance (SR) % plants with downy mildew (DM), days to pollen shed and indices of grain type of FS families of three groups of populations of normal maize

Agronomic trait	Selection cycle	Group 1. With DMR background			
		Mean	S.D	C.V.%	Total No.
Indices of SR*	8	1.76	0.48	27.3	556
Plants with DM-%	5	29.3	6.32	21.6	798
Days to pollen shed	8	59.8	2.03	3.4	504
Indices of grain type**	8	2.37	0.58	24.5	680
Agronomic trait	Selection cycle	Group 2. DMR selection intd. in C1			
		Mean	S.D	C.V.%	Total No.
Indices of SR*	8	1.92	0.41	21.5	2.205
Plants with DM-%	5	33.1	16.91	51.1	1.667
Days to pollen shed	8	53.7	1.72	3.2	1.690
Indices of grain type**	8	1.76	0.19	27.7	1.751
Agronomic trait	Selection cycle	Group 3. DMR selection intd. in C3			
		Mean	S.D	C.V.%	Total No.
Indices of SR*	8	1.74	0.26	14.7	904
Plants with DM-%	5	38.2	23.8	62.3	821
Days to pollen shed	8	53.8	1.67	3.1	704
Indices of grain type**	8	1.93	0.33	17.1	726

*Indices of SR 1 to 5; 1 being strong, 5 being very susceptible

** Indices of grain type 1 to 5; 1 being flint, 5 being dent

From this material, 720 DMRSR progenies were selected, planted in cold season (off season) and were subjected to intermediate drought stress. Data obtained in C2 of selection for drought tolerance are presented in Table 3. The average yield of trial was 3.0 tones per hectare, and represents about 40 % of yield of checks grown under good irrigation. The yield of the best yielders was 54 % higher than average trial yield, and 82 progenies (11.4 % out of 720) gave yield over 4 tones per hectare. There are no differences in days to pollen shed and days to silking between trial means and the means of the best yielders. This finding excludes possibility that top yielders are performing better due to the longer vegetation period. Significant negative correlation and regression was found between yield and days to silking. Significant negative correlation and regression was also found between yield and anthesis-silking interval (ASI) and between yield and grain weight per ear. 117 progenies (16.3 %) exhibited negative ASI and 144 progenies (20 %) gave over 100 g of grain per ear.

Table 2. Summary of data on indices of streak resistance (SR), % of plants with downy mildew (DM), days to pollen shed and indices of grain type (GT) of FS families of 3 groups of populations of normal maize

Trait	Parameter	Cycle	Mean	S.D	C.V %	No.of fmls
Indices of SR*	Interval value	8	1.85	0.39	21.2	3.903
Plants with DM %	Interval value	5	33.5	15.1	45	3.286
Days to pollen	Interval value	8	54.8	1.75	3.2	3.052
Indices of GT**	Interval value	8	1.93	0.45	23.1	3.157

Number (No) and frequency distribution (%) of FS families

No	%	No	%	No	%	No	%	No	%
	0 - 1.4		1.5 - 2.4		2.5 - 3.4		3.5 - 4.4		4.5 - 5.0
939	24.1	2424	62.1	515	13.2	25	0.6	0	0
	0 - 20		21 - 40		41 - 60		61 - 80		81 - 100
1242	37.8	983	29.9	647	19.7	335	10.2	79	2.4
	.50		51 - 53		54 - 56		57 - 59		60 - 65
447	14.6	760	24.9	933	30.6	588	19.3	324	10.6
	1.0 - 1.4		1.5 - 2.4		2.5 - 3.4		3.5 - 4.4		4.5 - 5.0
1130	35.8	1258	39.8	649	20.6	103	3.3	17	0.5

*Indices of SR 1 to 5; 1 being strong, 5 being very susceptible

** Indices of grain type 1 to 5; 1 being flint, 5 being dent

Table 3. Data on yield and some secondary traits on 720 selected DMRSR FS families subjected to intermediate drought stress in C2

Trait	Trail mean	Mean of 3 the best yielders	Best yielders vs trial mean	Correl. coeff	Regress. coeff	Probability
Yield t ha ⁻¹	3.0	4.61	1.54	-	-	-
Days to pollen	81.3	80.9	0.95	-0.381	-0.079	0.097
Days to silking	83.4	82.5	0.99	-0.562	-0.098	0.009
Anth-silk interval	2.1	1.55	0.74	-0.779	-0.460	<0.001
Ear per plant	0.9	1.01	1.12	0.357	0.441	0.133
Grain per ear-g	85.2	109	1.28	0.883	0.033	<0.001
Grain moist.%	17.8	18.3	1.03	241	0.102	0.305

Trait	Parameter	Number (No) and frequency distribution (%) of FS families									
		<2		2.0 - 2.9		3.0 - 3.9		>4.0		>4.0	
Yield t ha ⁻¹	Interval value	92	12.8	238	33.1	308	42.8	82	11.4	-	-
Days to Pollen	Interval value	80	11.1	149	20.7	247	34.4	156	21.7	87	12.1
Days to silking	Interval value	447	14.6	760	24.9	933	30.6	588	19.3	324	10.6
Anth-silk Interval	Interval value	1300	35.8	1258	39.8	649	20.6	103	3.3	17	0.5
Ear per Plant	Interval value	142	20.7	162	23.6	209	30.5	159	23.2	13	1.9
Grain per ear-g	Interval value	163	22.6	115	16	159	22.1	139	19.3	144	20
Grain moist.%	Interval value	86	11.9	180	25	161	22.4	126	17.5	167	23.2

Screening for disease resistance and endosperm modification in QPM

Breeding work on QPM in Mozambique was initiated in 1998. Three cycles of selection for SR, grain texture and endosperm modification, and one cycle of selection for DMR were completed. Data on SR were recorded on 419 S1 and S2 lines from four populations created by the program and variety Sussuma, which originated from commercial SR variety Obatanpa (Table 4). Mean values of indices of SR of three experimental populations, with incorporated genes conferring SR and DMR, indicate better SR than the commercial variety. Number and frequency distribution of lines from the same populations with strong SR clearly demonstrate better SR than the commercial variety.

Table 4. Mean, number (No) and frequency distribution (%) of selected QPM population related to resistance to MSV (C3) and downy mildew (C1)

Entry No.	Population	Stage	Mean of score	Total No of lines
<i>Indices of maize streak virus - Intervals</i>				
1	Matuba lines/LSMQ lines	S2 lines	2,7	79
2	Matuba lines/Entry 5Q	S2 lines	2,3	78
3	SML/LSMQ x MTL/LSMQ	S1 lines	2,3	9
4	LSMQ/PI 15 EWFQ	S1 lines	3,5	68
5	Sussuma (Obatanpa)	S1 lines	3,2	185
	Mean or total	S1 & S2	2,8	419
<i>% of plants with downy mildew -Intervals</i>				
1	Matuba lines/LSMQ lines	S2 lines	72,5	74
2	Matuba lines/Entry 5Q	S2 lines	63,3	72
3	SML/LSMQ x MTL/LSMQ	S1 lines	56	9
	Mean or total	S1 & S2	63,9	155

continue

Number (No) and frequency distribution (%) of lines

No	%	No	%	No	%	No	%	No	%
	<i>0 - 1.4</i>		<i>1.5 - 2.4</i>		<i>2.5 - 3.4</i>		<i>3.5 - 4.4</i>		<i>4.5 - 5.0</i>
24	30,4	38	48,1	8	9,9	7	8,9	2	2,5
18	23,1	40	51,3	14	17,9	4	5,1	2	2,6
2	22,2	4	44,4	3	33,3	0	0	0	0
3	4,4	11	16,2	26	38,2	18	26,5	10	14,7
0	0	57	30,8	81	43,8	42	22,7	5	2,7
47	11,2	150	35,8	132	31,5	71	16,9	19	4,5
	<i>0 -20</i>		<i>21 - 40</i>		<i>41 - 60</i>		<i>61 - 80</i>		<i>81 - 100</i>
7	9,5	6	8,1	15	20,3	12	16,2	34	45,9
6	8,3	10	13,9	19	26,4	14	19,4	23	31,9
0	0	2	22,2	2	22,2	5	55,6	0	0
13	8,4	18	11,6	36	23,2	31	20	57	36,8

Data on % of plants with DM, though still high with mean of 63.9 %, illustrate some DMR in comparison with susceptible checks, which are reaching 95 to 100 % of diseased plants (data not shown). Big difference between SR and DMR of the same material is large part due to the difference in number of selections for SR (C3) and for DMR (C1). Perhaps, one part of the difference might be ascribed also to the difference in number of genes conferring resistance of these two diseases. It is believed that DMR is controlled at least with two major genes.

These data are part of the extensive program on screening for disease resistance and endosperm modification in QPM, which in C1 of selection for DMR included 38 experimental populations with 933 progenies (Table 5). Here again, in average relatively high % of plants with DM was recorded (64.5 %). Different types of progenies showed some kind of distinctive classes of DMR with class intervals of 10 %. Larger class interval of 20 % is between FS families and BC families. Highest DM susceptibility of BC families can be ascribed to the influence of back cross with susceptible QPM pollen parent.

Table 5. Means of % of plants with DM, variation, number (No) and frequency distribution (%) of progenies related to DMR of QPM in C1 of selection for DMR

Group of plant progenies	Number (No) and frequency distribution (%) of progenies related to DMR									
	No	%	No	%	No	%	No	%	No	%
	0 - 20		21 - 40		41 - 60		61 - 80		81 - 100	
FS families	14	6,8	9	4,4	32	15,6	48	23,4	102	49,8
BC families	0	0	0	0	2	2,0	13	12,7	87	85,3
S1 lines	40	14,1	71	25,0	59	20,8	60	21,1	54	19,0
S2 lines	55	16,1	56	16,4	61	17,8	48	14,0	122	35,7
Acr.all prgns.	109	11,7	136	14,6	154	16,5	169	18,1	365	39,1

Group of	Group mean	Standard deviation		Coefficient of variation		Total
		Between Populat.	Within Populat.	Between Populat.	Within Populat.	
Plant progenies	plants with DM - %	Between Populat.	Within Populat.	Between Populat.	Within Populat.	number of progenies
FS families	70,5	18,5	21,1	26,2	32,7	205
BC families	89,4	6,7	7,8	7,5	8,9	102
S1 lines	50,8	3,9	26,3	7,7	51,6	284
S2 lines	61,8	16,1	28,8	26,1	50,5	342
Acr.all prgns.	64,5	10,6	21,2	16,5	37,8	933

Analyses on number and frequency distribution of progenies related to DMR show that, across all groups of progenies, 11.7 % of progenies (109 progenies) exhibited strong DMR (Table 5). Lower means of % of diseased plants of progeny groups (S1 and S2 lines) were followed by higher frequency distribution of DMR progenies, and vice versa, higher means of progeny groups (BC and FS families) were followed by absence (BC families) or low frequency distribution of DMR progenies (FS families).

The same data consistently show larger variation in DMR within populations (i.e. between the progenies within the same populations), than between the populations. Similar results were obtained also with normal maize (data not shown). This variation makes selection for disease resistance more efficient.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results obtained in this work the following conclusions can be drawn:

1. Using adaptable low land tropical germplasm with DMR and SR background can be improved disease resistance of susceptible populations, both in normal maize and QPM.
2. The method of spreader rows, combined with control of moisture and soil fertility stress, is suitable for screening of large number of entries, with possibility to include more stress factors.
3. It was found large variation of DMR, SR, grain texture and earliness. Satisfactory fractions of progenies with improved traits under selection were found.
4. Early selection for DMR under disease pressure is recommended after introgression.
5. FS and S2 recurrent selection, combined with phenotypic selection, is suitable for participatory maize breeding, i. e. early testing of breeding material including small-scale farmers.

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**OPLEMENJIVACKI PRISTUPI U SIMULTANOJ SELEKCIJI NA
OTPORNOST PREMA VISE FAKTORA STRESA U TROPSKIM
USLOVIMA**

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Izvod

Kukuruz je glavni usev i osnovna komponenta u ishrani ljudi u većini zemalja Afrike ispod Sahare. Međutim, zbog uticaja abiotskih i biotskih stres faktora, proizvodnja kukuruza je limitirana u velikoj meri. Među agro-ekološkim uslovima glavni činioci su; nedovoljna količina i loš raspored padavina, neplodna zemljišta, bolesti (crtičavost lista, plamenjača, pegavost lišća, rdja, trulež stabljike i korena) i štetočine (busaci i štetočine skladista). Među socio-ekonomske faktore ograničavanja proizvodnje kukuruza su: siromašna ekonomija, nedovoljno stručnih kadrova i neobučenost radne snage, loše agrotehničke mere i odsustvo korišćenja novih sorata. Da bi se stvorile odgovarajuće sorte, i time umanjilo delovanje većeg broja stres faktora, primenjeni su određeni pristupi u odobiranju roditeljskih parova i metoda rada u laboratorijskim i poljskim uslovima. Ovi pristupi se uglavnom zasnovani na: a) Ukrštanje odabranih genotipova sa više poželjnih agronomskih osobina, a posebno onih koje utiču na otpornost prema faktorima stresa, b) Kombinovano korišćenje metoda zaraženih redova sa bolestima i štetočinama i metoda testiranja i odabiranja kreiranih genotipova u uslovima suše i niske plodnosti zemljišta. Ovakvi pristupi omogućavaju testiranje i odabiranje velikog broja potomstava, koje je neophodno u simultanoj selekciji na otpornost prema više faktora stresa. Dobiveni podaci pokazuju da je dobiveno značajno poboljšanje osobina koje su bile predmet selekcije. Najveće poboljšanje osobina je postignuto na otpornost prema crtichavosti lišća i plamenjače, tvrdoći zrna i ranostasnosti. U slučaju otpornosti na sušu, statistički podaci pokazuju negativnu korelaciju između prinosa i svilanja i intervala između prašenja polena i svilanja, ali i značajnu pozitivnu korelaciju između prinosa i težine zrna po klipju.

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