

APPROACHES IN BREEDING FOR HIGH QUALITY PROTEIN MAIZE

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Denić M., P. Chaque, P. Fato, C. Senete, D. Mariote and W. Haag
(2008): *Approaches in breeding for high quality protein maize* -Genetika,
Vol. 40, No. 3, 237 - 247.

Maize is the principal crop and major staple food in the most African and South American countries. The main problem in human nutrition in developing countries, and in livestock feed in developed countries, is insufficient production and poor quality of cereal proteins. In the case of maize, due to the very low content of essential amino acids lysine and tryptophan in grain endosperm, biological value is very low, which is main limiting factor of common maize in human nutrition and feeding of monogastric animals. Quality protein maize (QPM) can help in solving of this problem. Maize production also faces serious constraints caused by agro-ecological conditions and poor socio-economic situation. To alleviate the effect of the constraints, selected genotypes with more desirable traits

and appropriate field-plot techniques to create multiple-stress conditions, were used. It was found that, in downy mildew nursery distance up to 35 m from spreader plot is providing sufficient down load of spores for plant infection, provided that the testing breeding materials are planted towards to down-stream direction of the dominant wind. Using these breeding approaches large number of early, white and flint synthetics, composites and inbred lines were created with resistance or tolerance to downy mildew (DMR), maize streak virus (SR) and drought (DT). Created genotypes exhibited very good kernel modification and yield potential under low and normal inputs. In the case of synthetics and composites, besides tolerance to multiple stress factors, they were competing in yield with local QPM and normal maize checks. In the case of created inbred lines high combining ability was exhibited both in non-conventional and conventional maize hybrids. Trial data revealed that in the most cases the best entries were over-yielding the best checks.

Key words: maize, quality protein, multiple resistance, synthetics, lines, hybrids

INTRODUCTION

Agriculture is the most important sector in the countries of sub-Saharan economies. Maize is the principal crop and major staple food in the most African and South American countries. The main problem in human nutrition in developing countries and in livestock feed in developed countries is insufficient production and poor quality of cereal proteins. Maize proteins in livestock feed are very important since they participate up to 80 % in the diet. However, due to the very low content of essential amino acids lysine and tryptophan in grain endosperm, biological value is very low, which is main limiting factor of common maize in feeding of monogastric animals, including human beings. Therefore increased production of high quality protein maize (QPM) (MERTZ *et al.*, 1964; CROMWELL *et al.*, 1967; MISOVIC *et al.*, 1969; EGGUM *et al.*, 1983; BRESSANI, 1975) is essential for the improvement of the nutritional value of the daily diet. However, maize production faces serious constraints caused by agro-ecological conditions and poor socio-economic situation. Among the agro-ecological conditions, the main constraints are: lack of rainfall; low soil fertility; poor weed control; diseases and pests (maize streak virus and downy mildew, borers and storage pests); and stem/cob rots, leaf blights, gray leaf spot and rusts. To alleviate many of these constraints, appropriate field-based breeding methodologies to select for multiple stress tolerance were implemented to develop desirable varieties. These approaches are mainly based on: a) Crossing selected genetic resources, developed by the local program, the International Maize and Wheat Improvement Center (CIMMYT) and the International Institute of Tropical Agriculture (IITA), with more desirable stress tolerant traits; b) Using the disease/pest spreader row method, combined with testing and selection of created

genotypes (progenies) under strong to intermediate pressure of multiple stress factors in nurseries; and c) Evaluation of the varieties developed in multi-location trials under low and “normal” inputs.

MATERIALS AND METHODS

To create desirable varieties and hybrids, QPM 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 downy mildew resistance (DMR) and maize streak virus resistance (SR) were SEMOC S4 lines extracted from Matuba (MTL) or SEMOC inbred lines (SML) extracted from Population 8072DMR or Population 8075DMR.

Based on the experience of field –plot techniques, the following strategies were used:

1. Late and continuous planting in hot season and early planting in cold (off) season
2. Avoid application of fungicides and insecticides.
3. All materials were subjected to heavy disease infection in nurseries of downy mildew (DM), maize streak virus (MSV) and stem borers. 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 described by CARDWELL *et al.*, 1994) and modified by DENIC, 1996 was used as follows:
 - Select seed of strong (robust) variety susceptible to DM.
 - Treat seed with 5 % chlorine for 1 min. and then wash with tap water.
 - Pre-germinate treated seed during 72 hrs.
 - Harvest DM diseased leaves at late afternoon.
 - In plastic box make layer of diseased leaves.
 - Place layer of seedlings over the layer of DM diseased leaves.
 - Keep over-night plastic boxes at 20^o C.
 - Next morning plant seedlings in moist soil in inner sides of lateral irrigation canals of the nursery.
 - After 10 days replant the seed of the same variety within rows of previously planted seedlings.
 - After 14 days plant material to be evaluated and selected.

Screening for drought tolerance (DT) was done in the cold season with controlled irrigation as described by BANZIGER *et al.* (2000). Conditions created in the nursery contribute to the increase of the load of disease spores or vectors (*Cicadulina sp.*) of maize streak virus, as well as to the high scores of stem borer attacks recorded at the harvest. 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 in the entries and susceptible check and % of plants with systemic DM disease was calculated.

During anthesis, selected early-flowering plants from selected progenies with good aspect, DMR and SR are, either self-pollinated to create inbred lines (using pedigree method) or crossed by hand pollination to create synthetics (using recurrent full-sib selection). In later phase of selection several composites were formed by crossing selected synthetics. At harvest, ears with good aspect were selected. Preference in selection was given to the ears from early-flowering plants, flint and semi-flint type of grain and kernels with good endosperm modification.

RESULTS AND DISCUSSION

Combining advantages of field-based screening methodology and indicated breeding approaches, synthetics, composites and inbred lines were created. Part of the results on frequency distribution of FS families, BC families and S1 and S2 lines, related to SR and DMR at initial stage of selection, were published earlier (DENIC *et al.*, 2007). Here we are reporting more on details of field plot techniques (which are essential for simultaneous selection for multiple stress tolerance) and on materials at pre-release stage of testing and selection.

Data presented in Table 1 show influence of distance from spreader plot of downy mildew on disease incidence in susceptible check. Data obtained show that up to 35 m meters of distance from disease spreader plot incidence is very high, ranging from 89.8 to 93.6 % of plants with systemic DM symptoms (% PDM). At distance of 49 m from spreader plot (which is only 14 m more) the incidence dropped down to 34.7 % PDM (which is 2.6 times less compare to that of 35 m). The same data also show difference of 13.5 % of plants with disease between 1st section of the plot (28.5 %PDM) and 3rd section of the plot (42.0 %PDM). This indicates that there is an influence of the direction of blowing of dominant wind toward the testing plot, meaning that dominant wind was not blowing straight towards the testing plot under the angle of 90⁰.

Table 1. Influence of distance from downy mildew spreader plot on disease incidence

Sections Distance meters	1st Section 0 - 42.4 m				2nd Section 42.4 - 84.8 m				3rd Section 84.8 - 127.2 m				Mean of % with DM
	Number of plants	Without DM	With DM	% with D.M.	Number of plants	Without DM	With DM	% with D.M.	Number of plants	Without DM	With DM	% with D.M.	
7.0	484	27	457	94.4	417	37	380	91.0	579	48	531	91.7	92.4
21.0	536	48	488	91.0	503	23	480	95.5	528	26	502	95.0	93.6
35.0	506	78	428	84.6	442	20	422	95.7	523	45	478	89.2	89.8
49.0	411	294	117	28.5	376	250	126	33.6	263	154	109	42.0	34.7

To test the influence of dominant wind on spreading disease, additional data were collected from another plot of downy mildew nursery using spreader rows. Data presented in Table 2 show influence of dominant wind position on DM incidence using spreader rows. Thus, disease incidence in the plot at wind up-stream position was 22.6 % DM infected plants, whereas in the plot at wind down-stream

position disease incidence was 78.1 % of DM infected plants, which is 3.5 times higher than that at wind up-stream position.

Table 2. Influence of dominant wind position on downy mildew incidence using spreader rows

	Total number of plants	Infected plants	Uninfected plants	% of infected plants	S.E.
<i>Plot at wind up-stream position</i>					
1st rep	603	144	459	23,9	
2nd rep	553	117	436	21,2	
Total/Mean	1156	261	895	22,6	1,35
<i>Plot at wind down-stream position</i>					
1st rep	1112	889	223	79,9	
2nd rep	1013	772	241	76,2	
Total/Mean	2125	1661	464	78,1	1,86

Distance between the plots 70 m, with 20 spreader rows in between.

Data which illustrate the influence of selection for DMR of FS families and S3/S4 lines (originated from synthetics) under strong disease pressure created by spreader rows method are presented in Table 3. Data obtained with FS families show lower disease incidence in cycle 2 (C 2) as compared to the same in cycle 1 (C 1). In average, in C2 and C 1 disease incidence was 60.3 and 82.3 in % of susceptible check, respectively, meaning one increase of 22 % per cycle. In the case of inbred lines, in average, in cycle 1 and cycle 2 were found infected plants 76.2 and 49.5 in % of susceptible check, respectively, meaning that difference between cycle 1 and cycle 2 was 26.7 in % of susceptible check.

Table 3. Difference in disease incidence between cycle 1 and cycle 2 of selection for DMR in FS families and S₃/S₄ lines

Variety	Origin	FS families				S ₃ /S ₄ lines				Cycle difference	
		% of plants with DM		In % of check		% of plants with DM		In % of check		FS fmls	Lines
		Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2		
Synthetic 1Q	MTL/LSMQ	79,1	52,7	92,4	57,2	71,8	24,2	85,9	68,0	35,2	17,9
Synthetic 2Q	MTL/E-5	81,1	51,9	91,5	52,9	63,9	28,1	84,2	59,8	38,6	24,4
Synthetic 3Q	SML/E-5	74,9	66,7	86,4	76,8	73,0	24,4	86,3	78,8	9,6	7,5
Synthetic 4Q	SML/LSMQ	43,3	33,8	56,1	54,3	38,3	14,4	58,1	19,5	1,8	38,6
Synthetic 5Q	MTL/E-5/SML/E-5	70,7	41,2	81,2	64,4	53,6	19,5	76,4	36,4	16,8	40,0
Synthetic 6Q	SML/LSMQ/MTL/LSMQ	80,7	49,5	86,7	56,1	48,0	25,9	66,0	34,3	30,6	31,7
Mean		70,5	50,9	82,3	60,3	58,1	22,8	76,2	49,5	22,0	26,7

One of the very important agronomic characteristic of QPM is kernel modification, which is controlled by endosperm modifier genes. Data presented in Table 4 show frequency distribution of kernels with different degrees of modification in QPM synthetics and commercial variety Sussuma. Based on degree of kernel modification they are grouped in 5 classes, where class 1 is complete modification and class 5 is without modification and makes endosperm very soft. Therefore modification of endosperm is actually contributing to the harder grain texture and consequently contributing to the reduction of negative effects of epistasis of homozygous recessive allele of opaque-2 gene. These data show that, in average, highest frequency distribution of modified kernels belongs to class 2 (38,5 %), which is the same case for commercial QPM variety Sussuma (44,7 %). If desirable modifications (class 1 and class 2) are joined than the frequency distribution of the two classes is 67.7 %. Based on these data it is possible to see that the best kernel

modification was in Synthetic 1Q (79.7 %), Synthetic 4Q (71 %), Synthetic 6Q (76.7 %) and in Sussuma (77.6 %). In the case of undesirable modification (class 5), in average, only 1.9 % of kernels were without modification.

Table 4. Kernel modification of synthetics and Sussuma as % of total number of analyzed kernels

Variety	Nmbr of anld kernels	Class 1	Class 2	Class 3	Class 4	Class 5	Classes 1+2	Classes 4+5
Synthetic 1 Q	17074	30,33	49,40	9,70	2,70	0,73	79,73	3,43
Synthetic 2 Q	14571	16,90	37,00	36,10	8,50	1,50	53,90	10,00
Synthetic 3 Q	8900	22,20	37,50	24,40	13,60	2,30	59,70	15,90
Synthetic 4 Q	27863	48,60	22,40	10,20	14,40	4,40	71,00	18,80
Synthetic 5 Q	13327	26,40	38,90	20,10	13,70	0,81	65,30	14,51
Synthetic 6 Q	21103	30,50	45,80	16,20	5,90	1,50	76,30	7,40
Total and means	102838	29,16	38,50	19,45	9,80	1,87	67,66	11,67
Sussuma	2808	32,90	44,70	12,30	10,10	0,04	77,60	10,14

Table 5. Grain yield of QPM synthetics, composites and normal maize checks

Entry	Year 1 st	Year 2 nd	Year 3 rd	Mean	RGY ¹	Standard error
QS7705	4,51	2,53		3,52	1,22	0,99
SUSSUMA	3,42	2,98	3,71	3,37	1,17	0,22
MANICASR	4,11	2,60		3,36	1,17	0,75
SYN6QC4	3,99	2,38	3,57	3,31	1,15	0,49
MATUBA	3,83	2,61		3,22	1,12	0,61
SYN4QC4	3,84	2,46	3,00	3,10	1,08	0,40
COMP6QC2		2,70	3,08	2,89	1,00	0,19
SYN1QC4	3,65	2,57	2,40	2,87	1,00	0,39
COMP2QC2		1,50	3,69	2,60	0,90	1,09
COMP3QC2		2,32	2,88	2,60	0,90	0,28
SYN2QC4	2,96	2,12	2,66	2,58	0,90	0,25
SYN5QC4	3,40	2,08	2,21	2,56	0,89	0,42
1517SYN99Q	3,98	0,98		2,48	0,86	1,50
COMP1QC2		1,93	2,92	2,43	0,84	0,50
SYN3QC4	3,00	1,82	2,21	2,34	0,81	0,35
MEAN	3,70	2,24	2,94	2,88	1,00	0,81

¹Relative grain yield = ratio between yield and trial mean of yield.

Regarding the yield performance of QPM synthetics, composites and standard maize checks data are presented in Table 5. The best average yield of 3.52 t/ha (for period of 2 years) was obtained with QPM hybrid QS 7705 from South Africa, followed by Sussuma (3.37 t/ha), Manica SR standard maize check (3.36 t/ha), Synthetic 6QC4 (3.31 t/ha), Matuba standard maize check (3.22 t/ha) and Synthetic 4QC4 (3.1 t/ha). Generally, these data show that QPM synthetics and composites are competing with commercial varieties of standard maize. Low yields can be ascribed to relatively low dosage of fertilizer application and drought. Due to poor socio-economic situation in majority of Sub-Saharan countries, many small scale farmers

do not have capacity to buy fertilizers and pesticides. Therefore, in breeding objectives in this region must be given attention to multiple stress tolerance to low soil fertility, drought and diseases and insect pests. Thus principal objective becomes risk avoiding, rather than high yield potential which small-scale farmer cannot utilize.

However, in the case of private sector, with better economic conditions (though is holding only about 5 % of total arable land and participates with 10 % in maize production) situation is the other way around. Therefore, strategy in breeding is to create varieties that will perform better under both, poor and optimal conditions.

Data summarized in Table 6 show grain yields of 4 groups of top-crosses, namely:

1. Group with 4 sub-groups (each sub-group of 40 entries) of top-crosses between S₃/S₄ lines extracted from 4 synthetics and Sussuma;
2. Group of top-crosses between S₄ lines extracted from CIMMYT CNSLQEGL progenitor and Sussuma;
3. Group of top-crosses between Sussuma S₁ lines and CIMMYT best yielding hybrid;
4. Group of top-crosses between Sussuma S₁ lines and Population 62 SRQ.

Table 6. Summary of grain yield of groups of QPM top-crosses between S₃/S₄ lines from synthetics

Locations Groups of Entries	Umbeluzi					Sussundenga				
	Trial mean	Best entry	Best check	B.E ¹ / _{T.M} ²	B.E./B.C ³	Trial mean	Best entry	Best check	B.E./T.M.	B.E./B.C.
Grain yield in tones per hectare										
[2mtl+2e5+1lsmq]S ₄ x Sussuma	4,07	5,60	4,43	1,38	1,20	2,41	4,59	2,69	1,90	1,70
[sml/lsmq]S ₄ x Sussuma	5,33	8,49	4,98	1,59	1,70	3,40	6,55	3,52	1,93	1,86
[mtl/e5/sml/e5]S ₃ x Sussuma	4,30	5,60	2,20	1,30	2,55	2,60	4,78	2,83	1,84	1,69
[sml/lsmq//mtl/lsmq]S ₃ x Sussuma	5,19	7,16	6,51	1,38	1,10	3,01	5,68	4,40	1,89	1,29
CNSLQEGL-S ₄ x Sussuma	3,40	3,95	3,13	1,16	1,26	2,91	5,65	4,36	1,94	1,30
Sussuma S ₁ x [CML 144/CML 159]F1	3,69	5,64	2,38	1,53	2,37	3,82	7,94	3,50	2,08	2,29
Sussuma S ₁ x Pop 62 SR Q	4,30	6,41	3,00	1,49	2,14	4,59	7,82	5,74	1,70	1,36
CIMMYT Zimbabwe Hybrids	7,17	8,45	6,16	1,18	1,37	7,17	8,45	6,16	1,18	1,37

¹Best entry; ²Trial mean; ³Best check

For the comparison of non-conventional top-cross hybrids with conventional QPM hybrids it is included also summarized CIMMYT trial with conventional hybrids. In the case of Group 1, with 4 sub-groups of top-crosses, at Umbeluzi, the highest average yield of the trial (5.33 t/ha) and the yield of the best entry (8.49 t/ha) was found in [sml/lsmq]S₄ x Sussuma sub-group of top-crosses. Another sub-group of top-crosses of Group 1, which exhibited high trial mean value (5.19 t/ha) and high yield of the best entry (7.16 t/ha, was [sml/lsmq//mtl/lsmq]S₃ x Sussuma. At Sussundenga, the same sub-groups also exhibited highest yields of trial mean and of the best entry. Thus, the yields of the best entries were 6.55 t/ha and 5.68 t/ha of top-crosses of sub-group and the highest trial mean yields were [sml/lsmq]S₄ x Sussuma and [sml/lsmq//mtl/lsmq]S₃ x Sussuma), respectively. If these data on the performance of lines (extracted from QPM synthetics by inbreeding/pedigree

procedure) are compared with the performance of the progenitor QPM synthetics (formed by recurrent FS selection, see Tab.5), it is obvious that the same synthetics were also performing better compare to the other synthetics. This finding illustrates the importance of selection of progenitor for creation of open pollinating varieties and hybrids. These data also show that created early non-conventional hybrids, under sub-Saharan conditions, are competing with conventional hybrids (CIMMYT Zimbabwe).

Table 7. Grain yield of the best three-way QPM hybrids of CIMMYT female and IIAM male parents

Treatment	Female parent	Male parent	Yield t/ha	Location
<i>IIAM male parent</i>				
1	VH054417	Synthetic 2QFS	5.3	Sussund.
2	VH054418	Synthetic 3QFS	7.15	Angonia
3	VH054450	Synthetic 6QS6-1-7	7.20	Angonia
4	VH054452	Composite 1/1Q	6.05	Angonia
5	VH054470	Composite 1/1Q	6.20	Angonia
6	VH054473	Synthetic 6QFS9/04	5.93	Angonia
7	VH054479	Synthetic 6QS6-1-31	6.37	Angonia
8	VH054518	Synthetic 6QS6-1-22	6.00	Sussundenga
<i>CIMMYT male parent</i>				
1	CZH03033	[CML389/CML176]-B-29-2-2-B*3	6.91	Angonia
2	CZH03030	MAS[MSR/312]-117-2-2-1-B*4	5.20	Sussundenga
3	CZH03009	CML 142	6.30	Sussundenga
4	CZH03009	CML 142	7.47	Angonia

Further testing of formed genotypes in three-way QPM hybrids, formed by crossing CIMMYT single cross hybrids with IIAM male composites, synthetics and inbred lines, gave also very good results. Data presented in Table 7 show that, among the best 12 male combiners with CIMMYT female hybrids, 8 were from IIAM program and 4 were from CIMMYT program. Details on these results were reported elsewhere (CHAUQUE *et al.*, 2007).

To compare performance of QPM and common maize, data obtained at 7 locations across the country, are given in Table 8. These data show large variation in grain yield at different locations, which is caused mainly by agro-climatic conditions. Generally, QPM entries performed better at Mozambican low-land areas and normal maize performed better at higher altitudes. The reason for this might be, at least partly, ascribed to the influence of breeding and testing under low-land areas, since created genotypes belong to the group of early varieties and hybrids.

Table 8. Grain yield of QPM and normal maize varieties at different locations

Locations	Umbeluzi	Chokwe	Sussundenga	Nampula	Namialo	Angonia	Lichinga
	Grain yield in tones per hectare						
QPM	3,74	5,05	3,71	5,54	2,17	5,03	2,31
Normal maize	2,59	4,33	2,04	5,16	1,82	6,04	2,97
Ratio (N/QPM)	1,44	1,17	1,82	1,07	1,19	0,83	0,78

CONCLUSION

To compare performance of QPM and common maize, data obtained at 7 locations across the country, are given in Table 8. These data show large variation in grain yield at different locations, which is caused mainly by agro-climatic conditions. Generally, QPM entries performed better at Mozambican low-land areas and normal maize performed better at higher altitudes. The reason for this might be, at least partly, ascribed to the influence of breeding and testing under low-land areas, since created genotypes belong to the group of early varieties and hybrids.

Received September 26th, 2008

Accepted November 10th, 2008

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PRISTUPI U OPLEMENJIVANJU KUKURUZA SA VISOKIM KVALITETOM PROTEINA

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²SG 2000, Maputo, Mozambique

I z v o d

Kukuruz je osnovni usev i glavna hrana u vecini zemalja Afrike i Juzne Amerike. Glavni problem u ishrani ljudi uzemljama u razvoju i ishrani domacih zivotinja u razvijenim zemljama je nedovoljna proizvodnja i los kvalitet biljnih proteina u zitaricama. U slucaju standardnog tipa kukuruza sadrzaj lizina i triptofana je veoma nizak sto dovodi do niske bioloske vrednosti (niska hranljiva vrednost i svarljivost proteina). Zbog toga se pristupilo stvaranju sorata i linija kukuruza visokog kvaliteta proteina sa tvrdim zrnom putem koriscenja opaque-2 gena i gena modifikatora endosperma. Proizvodnja kukuruza se takodje suocava sa losim agroekoloskim uslovima i teskom socio-ekonomskom situacijom u zemljama u razvoju. Da bi se ublazilo delovanje abiotskih (susa, neplodnost zemljista) i biotskih (bolesti i stetocine) faktora, neophodno je stvaranje sorata i hibrida otpornih na ciniocce koji ogranicavaju proizvodnju kukuruza. Zbog toga su u procesu stvaranja novih genotipova izvrsen izbor genotipova sa vecim brojem pozelnih osobina na koje se vrsi oplemenjivanje. Istivremeno su na eksperimentalnim parcelama (maticnjak i sortni ogledi) stvoreni visestruki stres uslovi (jednovremeno delovanje suse, bolesti i stetocina) koji omogucuju strogu selekciju na otpornost ili tolerantnost na navedene uslove. Istrazivanja su pokazala da na odstojanju do 35 m od parcele zarazene kukuruznom plamenjacom postoji dovoljno inokuluma za testiranje selekcionog materijala u maticnjaku pod uslovom da dominantni vetar u toku sporulacije duva upravo na maticnjak. Kombinovanjem seleksionih pristupa u izboru pocetnog materijala i tehnika u poljskim uslovima stvoren je veliki broj ranih sorata (sintetici, kompositi), inbred linija i hibrida tvrdog zrna otpornih ili tolerantnih prema kukuruznoj plamenjaci, virozi crticavost lisca i susi. Stvoreni genotipovi su ispoljili dobru modifikaciju endosperma i potencijal rodnosti pod losim i normalnim uslovima gajenja. Stvorene sorte su, pored visestruke otpornosti na stresne uslove, bile rodne kao standardni kukuruz. Takodje, stvorene inbred linije su ispoljile visoku kombinacionu sposobnost u nekonvencionalnim i konvencionalnim hibridima. Ogledi u poljskim uslovima su pokazali da najrodniji hibridi nadmasuju po prinosu najbolje komercijalne hibride standardnog tipa kukuruza.

Primljeno 26. IX. 2008.

Odobreno 10. XI. 2008.