Crop Breeding and Applied Biotechnology 4:163-170, 2004
Brazilian Society of Plant Breeding. Printed in Brazil

Evaluation of high quality protein maize (QPM) hybrids obtained by conversion of normal inbred lines

Jair Moura Duarte[1], Cleso Antônio Patto Pacheco[2*], Claudia Teixeira Guimarães[2], Paulo Evaristo de Oliveira Guimarães[2], and Edilson Paiva[2]

Received 20 August 2003

Accepted 13 June 2004

ABSTRACT - Two experimental QPM hybrids obtained by the partial conversion of three elite inbred lines by modified backcrossing were evaluated and compared to the normal versions of the same hybrids. Thirteen agronomic and 12 grain quality traits were evaluated. The hybrids were also analyzed by SSR markers in order to evaluate the genetic relationship among them. Results demonstrated that the converted QPM hybrids presented a better protein quality and a similar agronomic performance for most of the evaluated traits, including grain yield, in comparison to their normal versions. However, some of the deficiencies generally ascribed to QPM hybrids, such as a higher lodging percentage, thicker cobs, and shorter kernels were maintained up to this stage of the conversion process. In comparison to more recent elite hybrids, the normal versions as well as the converted QPM hybrids presented the worst performances in relation to the grain yield, thus indicating a loss of competitiveness.

Key-words: *Zea mays,* QPM, opaque-2, line conversion, backcrossing.

INTRODUCTION

Maize, although one of the world's most important food crops, has a restricted nutritional value for humans and other monogastric animals, since it is deficient in essential amino acids, especially lysine (Nelson 1969). Mertz et al. (1964) pointed out that the mutation *opaque-2* (o_2) practically doubled the lysine content in the maize endosperm. However, the negative effects brought on the physical properties of the endosperm and other important agronomic traits have limited its wide-spread use in the development of better nutritional quality maize.

The identification of modifier genes able to overcome the negative effects of the *opaque-2* mutation (Paez et al. 1969) gave rise to the development of *opaque-2* modified genotypes, designated as *Quality Protein Maize* or simply QPM (Gevers and Lake 1992, Villegas et al. 1992). QPM grains present the hardness and the vitrosity of normal genotypes, while the high lysine content of the *opaque-2* mutants is maintained.

The maize QPM breeding program is complex process since it requires the simultaneous manipulation of three genetic systems: the *opaque-2* gene, the endosperm modifier genes, and the genes that control the lysine content (Moro 1996). Actually, part of the attempts of these programs

[1]Syngenta Seeds Ltda, Rod BR 452, km 142, C.P. 585, 38400-974, Uberlândia, MG, Brasil
[2]Embrapa Milho e Sorgo, C.P. 151, 35701-970, Sete Lagoas, MG, Brasil. *E-mail: cleso@cnpms.embrapa.br

concentrate on the conversion of elite inbred lines, with the objective of obtaining QPM versions of normal hybrids with superior agronomic performance.

The method originally proposed to convert normal maize into QPM was composed by a backcross combination followed by recurrent selection in segregant populations (Vasal et al. 1980). This procedure allowed the development of a large number of QPM populations, although many generations are required to recover the recurrent parent genotype, and the selection of the QPM characteristics can be performed only in the segregating populations of each backcross generation.

More recently, Guimarães et al. (2000) proposed a modified backcross method that diminishes the time required to recover the recurrent parental genotype and offers the opportunity for the selection of QPM traits in all MBC generations (modified backcrossing), compared to the previous method. The procedure consists in the selection of heterozygous male parents (O_2o_2) BC_1F_1, BC_2F_1, BC_3F_1, BC_4F_1, BC_5F_1, and BC_6F_1, followed by crosses with homozygous females (o_2o_2) to obtain the MBC generations. Generation MBC_1F_1 is obtained by the selection of o_2 seeds with desirable QPM traits in the cross F_2 (o_2o_2) x BC_1F_1 (O_2o_2). MBC_2F_1 is obtained by the selection of o_2 seeds with desirable QPM trait in the population of MBC_1F_1 (o_2o_2) crossed with BC_2F_1 (O_2o_2). MBC_3F_1 is obtained by selecting seeds with desirable QPM traits in the cross MBC_2F_1 (o_2o_2) x BC_3F_1 (O_2o_2). The subsequent generations (MBC_4F_1, MBC_5F_1, and MBC_6F_1) are obtained in the same way. In each MBC generation it is possible to select o_2 seeds with desirable QPM traits that are visualized as endosperm modification. So, the modified backcross method has the potential to facilitate the development and release of QPM cultivars and hybrids, which can also be used in other programs to incorporate multiple seed traits controlled by recessive alleles and modifier genes (Guimarães et al. 2000).

An important step of the conversion process is the evaluation of the converted hybrids. Traditionally, the evaluation is held in trials with replications, where the converted versions are compared to the original ones for a broad set of important traits. The process is considered successful when the hybrid performance is equal to or surpasses the isogenic counterpart, with the additional trait of interest that was introgressed. These comparative trials can also be carried out at intermediate stages of the process, thus serving as a preliminary evaluation of the conversion status under development.

This study aimed to evaluate the experimental QPM hybrids obtained by the partial conversion of three elite lines using the modified backcross method, in comparison to their normal versions and to the more recent elite hybrids.

MATERIAL AND METHODS

The experiment was established on an experimental area of Embrapa Maize and Sorghum, Sete Lagoas (lat 19° 27' S, long 44° 14' W, and altitude 730 m asl), State of Minas Gerais, where the plants were sown on 29/11/2001.

Two experimental QPM hybrids obtained by the partial conversion of three elite lines were evaluated by the modified backcross method (Guimarães et al. 2000), which were compared to their normal versions and to the more recent elite hybrids. The single cross HS200Q was obtained by the crossing of the QPM MBC_3F_1 generation of the converted lines L13 and L5. The cross between HS200Q hybrid and QPM MBC_3F_1 generation of the converted line L2 gave rise to the tri-way cross BR3123Q. Moreover, four recent elite hybrids of the Embrapa Maize and Sorghum, two normal and two QPM, were evaluated in the trial (Table 1).

The experimental design was that of randomized complete blocks, with six replications. Each plot consisted of two four-meter rows, spaced 0.90 m between rows, with 55000 plants ha^{-1}.

Table 1. Hybrids evaluated in a field trial of experimental QPM hybrids

Description	Hybrid	Cycle
HS200	Normal single-cross	Normal
HS200Q	QPM single-cross	Normal
BR3123	Normal tri-way cross	Early
BR3123Q	QPM tri-way cross	Early
97HT129Q	QPM tri-way cross	Normal
CMS00Q-03	QPM single cross	Early
BRS3060	Normal tri-way cross	Normal
BRS1001	Normal tingle cross	Early

Planting and harvest were carried out manually using the conventional planting system. The trials were fertilized with 400 kg ha^{-1} of 4-14-8 plus zinc and 100 kg ha^{-1} of urea were applied 40 days after planting date.

The following agronomic traits were evaluated: female flowering (FL): number of days from planting until 50% of the plants in the plot presented female inflorescence; plant height (PH): distance in centimeters between the soil surface beside the plant and the insertion of the male inflorescence with the flag leaf; ear height (EH): distance in centimeter from the soil surface beside the plant to the insertion of the superior ear;

lodging (LB): number of lodged and broken plants on the plot (data transformed to percentage in the stand); rotten ears (RE): number of ears with rotten kernels in the plot (data transformed to percentage in the stand); stem diameter (SD): stem thickness in cm evaluated at the first internode above the soil surface; ear length (EL): length of the hulled ear at harvest in cm; number of kernel rows (NK): number of kernel rows on the cob; ear diameter (ED): in cm measured in the middle of the ear; cob diameter (CD): in cm measured in the middle of the cob; grain length (GL): determined in cm by the formula: $GL = (ED - CD)/2$; plant population (POP): number of plants per hectare, obtained by the count of the number of plants (stand) of the plot at harvest. Grain yield (GY): grain weight of the plot, corrected to the water content of 14.5% in kg ha^{-1}. The traits PH, EH, SD, EL, NK, ED, and CD were evaluated in five representative plants of the plot

For the evaluation of the grain quality traits, five representative plants of each plot were self-pollinated, in two replications, and the analyses performed in a sample of the kernels of these ears. This way, the pollen effect could be isolated. The grain quality analyses were carried out at the Bromatology Laboratory of Embrapa Maize and Sorghum, using the following traits: grain density (DEN): obtained from two samples of 200 grains, by the method described by Wessel-Beaver et al. (1984), with data expressed in g cm^{-3}; weight of one hundred seeds (WHS) in g; protein content of the grain (PCG): determined according to Pommer (1979) using the Micro-Kjeldahl method (AOAC 1970), with the results in percentage; tryptophan content in the protein (TRYP): determined by the colorimetric method described by Villegas et al. (1984), in percentage; tryptophan content in the grain (TRYG): obtained by the relation between the grain protein and the tryptophan protein contents; results in percentage; lysine content in the protein (LYSP): estimate by the regression equation: $y = 0.3601 + 4.074x$, where x = tryptophan content in the protein, determined according to Hernandez and Bates (1969); lysine content in the grain (LYSG): obtained by the relation between the protein grain and the lysine contents in the protein, results in percentage; protein yield (PY): protein yield in kg ha^{-1}, obtained from the grain protein content and the plot grain yield in kg ha^{-1}; tryptophan yield (TRY): tryptophan production in kg ha^{-1}, obtained from the tryptophan content in the grain and the grain yield in kg ha^{-1}; lysine yield (LY): lysine production in kg ha^{-1}, obtained from the lysine content in the grain and the grain yield in kg ha^{-1}. The oil content in the grain (OCG) was determined according to Silva (1981), with the results in percentage, and the oil yield (OY) was obtained from the oil content in the grain and the grain yield in kg ha^{-1}.

All agronomic and nutritional data were submitted to analysis of variance using the software Genes (Cruz 1987) and the means were compared by the Scott and Knott (1974) test at $P < 0.05$.

The genetic relationship among the hybrids was evaluated using SSR markers. Genomic DNA was extracted from a bulk of young leaves as described by Saghai-Maroof et al. (1984), and the SSR reactions according to Ninamango-Cárdenas et al. (2003), using 31 well distributed-primer pairs in the maize genome. The genetic similarity of each hybrid pair was estimated by the similarity coefficient of Dice (Dice 1945), which was represented by a dendrogram constructed using the UPGMA method with the program NTSYS (Rolf 2000).

RESULTS AND DISCUSSION

The source of variation for treatments was significant at $P < 0.05$ by the F test, for all evaluated agronomic traits, except for the plant population. Estimates of the experimental coefficients of variation varied from 1.34% to 42.62% for the traits female flowering and lodging percentage, respectively. In general, the coefficient of variation for all evaluated agronomic traits varied from low to medium, except for the traits lodging and rotten ears, indicating that the experiment was well-conducted and the traits were precisely evaluated (Scapim et al. 1995).

The mean of grain yield among the hybrids was 6282 kg ha^{-1}, significantly above the Minas Gerais State average with 4020 kg ha^{-1} in the harvest 2001/2002 (CONAB 2003). This shows a satisfactory yield potential of these hybrids when cultivated with an adequate technological management. According to the Scott-Knott test at $P < 0.05$, the single-cross hybrid BRS1001 with a mean yield of 8534 kg ha^{-1} was significantly superior to all others (Table 2).

The comparison between the converted QPM hybrids (HS200Q and BR3123Q) in relation to their normal versions showed that both versions had a similar performance in grain yield. Additionally, no statistical differences were observed between the hybrids BR3123 and BR3123Q, in the preliminary trial conducted in 12 locations of the central region of Brazil with QPM maize in 2001/2002. Paschoalick (1998) mentioned that QPM genotypes were around 10% less productive than the normal genotypes. However, the present results demonstrate that the QPM genotypes can be equally competitive in relation to the grain production when compared to their normal versions, without taking other factors into account.

Considering all evaluated agronomic traits, hybrids HS200 and HS200Q showed significant differences in the percentage of lodging and of rotten ears, in which the QPM version had an inferior performance, and presented a thicker cob and shorter kernels compared to its normal version.

Table 2. Mean values and Scott-Knott test for the agronomic traits of the hybrids in a field trial of experimental QPM hybrids

Hybrids	Traits *						
	FL[1]	PH[2]	EH[3]	LB[4]	RE[5]	SD[6]	EL[7]
HS200	66 a	199 b	113 b	1.86 c	0.00 c	1.63 a	14.6 b
HS200Q	66 a	196 b	107 b	6.01 b	5.69 b	1.59 a	15.6 b
BR3123	63 c	201 b	120 b	5.98 b	7.17 a	1.51 b	16.2 b
BR3123Q	63 c	208 b	129 a	12.12 a	8.91 a	1.65 a	15.9 b
97HT129Q	63 c	228 a	119 b	6.92 b	5.69 b	1.50 b	17.7 a
CMS00Q-03	63 c	223 a	132 a	6.71 b	4.99 b	1.50 b	16.3 b
BRS3060	65 b	223 a	116 b	9.68 a	0.00 c	1.61 a	16.7 a
BRS1001	63 c	218 a	137 a	2.55 c	4.54 b	1.56 b	18.0 a
Mean	64	212	121	6.48	4.62	1.56	16.4
CV (%)	1.34	4.82	8.82	42.62	42.30	6.04	7.06

Hybrids	Traits *					
	NK[8]	ED[9]	CD[10]	GL[11]	POP[12]	GY[13]
HS200	15 c	4.3 c	2.3 c	0.99 b	56250 a	4764 e
HS200Q	15 c	4.4 c	2.6 b	0.90 c	55324 a	4555 e
BR3123	16 b	4.4 c	2.7 b	0.89 c	53009 a	5355 d
BR3123Q	18 a	4.8 a	2.9 a	0.95 c	54861 a	5614 d
97HT129Q	16 b	4.7 b	2.9 a	0.90 c	54398 a	6702 c
CMS00Q-03	14 c	4.6 b	2.8 a	0.92 c	54630 a	7233 b
BRS3060	13 d	4.6 b	2.5 c	1.07 a	53703 a	7501 b
BRS1001	13 d	4.6 b	2.6 b	0.98 b	53009 a	8534 a
Mean	15	4.5	2.6	0.95	54600	6282
CV (%)	6.98	3.55	5.19	6.32	4.21	7.56

[1]Female flowering (days); [2]Plant height (cm); [3]Ear height (cm); [4]Lodging (%); [5]Rotten ears (%); [6]Stem diameter (cm); [7]Ear length (cm); [8]Number of kernel rows; [9]Ear diameter (cm); [10]Cob diameter (cm); [11]Kernel length (cm); [12]Plant population per ha; [13]Grain yield in kg ha^{-1}
* Means followed by the same letter are not statistically different at $P < 0.05$ by the Scott-Knott test

Similarly, the QPM hybrid BR3123Q showed higher ear height, percentage of lodging and cob diameter when compared to the normal hybrid BR3123. In relation to the traits stem diameter, number of kernel rows, and ear diameter, the QPM version presented a superior performance. In a maize production system, high lodging percentage is an extremely undesirable trait, since it reduces the final grain yield and quality. In this trial, although there were no significant differences for grain yield between the normal and QPM versions, the greater lodging percentage of the QPM hybrids may reduce their competitiveness in relation to normal ones.

It is important to point out that the QPM versions evaluated in this trial were obtained from the generation MBC_3F_1, whose theoretical estimate of percentage of recurrent genotype recovery is 84.38%. So, the differences observed between the normal and the QPM hybrid versions for the agronomic traits are in the expected range. However, higher lodging percentage, thicker cobs, and shorter kernels are difficulties frequently faced in maize QPM breeding programs (Guimarães et al. 1994b, Pacheco et al. 1999, Paschoalick 1998). These problems were not solved so far by this conversion, enforcing the possible pleiotropic or linkage effects of the o_2 allele with these negative traits.

In QPM breeding programs, special attention is given to the grain quality traits, besides all the traditionally evaluated agronomic parameters. By the F test at $P < 0.05$, there were significant differences for all grain quality traits evaluated in the trial, except for the oil content in the kernel and the oil yield.

The grain density of the converted QPM hybrids was lower than of their normal versions, but the grain density of the other elite QPM hybrids were similar to the normal ones, except for hybrid BRS1001, which presented the highest density in the trial (Table 3). Low density is generally associated to a low grain yield, and is a further indication for opacity and for a lack of endosperm modification. Guimarães et al. (1994a) verified that among 22 QPM hybrids classified according to the endosperm modification, the most opaque kernels were 4% less dense and 11% less heavy in relation to the more vitreous. In this trial, although the scale of endosperm modification was not directly evaluated, the difference in density between the QPM and normal versions is a strong indicator that the applied conversion process was not able to maintain the degree of endosperm modification in the converted versions. This trait was also inferior to other QPM hybrids evaluated in the trial that were developed by other breeding strategies. These results indicate that one of the advantages proposed by the modified backcross method (Guimarães et al. 2000) was not attained, at least up to the evaluated conversion phase.

The contents of tryptophan and lysine in kernel and protein were superior for all QPM hybrids in relation to normal hybrids (Table 3). These contents are comparable to the results of other evaluations of normal and QPM hybrids (Paschoalick 1998, Pixley and Bjarnason 1994, Pixley and

Table 3. Mean values and Scott-Knott test for the grain quality traits of the hybrids in a field trial of experimental QPM hybrids

Hybrids	Traits *					
	DEN[1]	WHS[2]	PCG[3]	TRYG[4]	TRYP[5]	LYSG[6]
HS200	1.25 b	17.6 b	9.48 a	0.07 b	0.73 b	0.31 b
HS200Q	1.13 d	19.1 b	9.74 a	0.11 a	1.17 a	0.50 a
BR3123	1.26 b	20.3 b	8.76 a	0.07 b	0.76 b	0.30 b
BR3123Q	1.15 c	21.9 b	9.01 a	0.11 a	1.25 a	0.50 a
97HT129Q	1.26 b	29.7 a	8.97 a	0.11 a	1.17 a	0.46 a
CMS00Q-03	1.26 b	33.6 a	8.61 a	0.13 a	1.45 a	0.54 a
BRS3060	1.26 b	32.1 a	8.36 a	0.06 b	0.77 b	0.29 b
BRS1001	1.29 a	19.4 b	10.68 a	0.08 b	0.74 b	0.36 b
Mean	1.23	24.2	9.20	0.09	1.01	0.41
CV (%)	0.81	10.30	5.56	10.28	10.49	9.82
Hybrids	**Traits ***					
	LYSP[7]	PY[8]	TRY[9]	LY[10]	OCG[11]	OY[12]
HS200	3.32 b	423.51 c	2.90 d	13.81 c	2.88 a	128.60 a
HS200Q	5.12 a	414.93 c	4.69 c	21.33 b	4.33 a	185.69 a
BR3123	3.45 b	459.80 c	3.42 d	15.76 c	3.86 a	202.65 a
BR3123Q	5.44 a	481.78 c	5.84 b	26.27 a	4.12 a	221.27 a
97HT129Q	5.12 a	581.80 b	6.81 b	29.46 a	3.34 a	215.18 a
CMS00Q-03	6.28 a	587.81 b	8.53 a	36.84 a	3.88 a	264.87 a
BRS3060	3.47 b	645.15 b	4.67 c	22.14 b	2.86 a	221.72 a
BRS1001	3.39 b	947.91 a	7.10 b	31.95 a	3.60 a	319.52 a
Mean	4.45	567.83	5.49	24.69	3.61	219.94
CV (%)	9.56	9.73	10.62	9.18	16.50	16.75

[1]Grain density (g cm^{-3}); [2]Weight of 100 seeds (g); [3]Protein content in the grain (%); [4]Tryptophan content in the grain (%);[5]Tryptophan content in the protein (%); [6]Lysine content in the grain (%); [7]Lysine content in the protein (%); [8]Protein yield in kg ha^{-1}; [9]Tryptophan yield in kg ha^{-1}; [10]Lysine yield in kg ha^{-1}; [11]Oil content in the grain (%); [12]Oil yield in kg ha^{-1}

* Means followed by the same letter are not statistically different at $P < 0.05$ by the Scott-Knott test

Bjarnason 2002, Zarkadas et al. 2000). In average, the tryptophan and lysine protein contents of the evaluated QPM hybrids were 68% and 62% superior in comparison to the normal hybrids, respectively. The superiority of the QPM hybrids for these nutritional traits indicates that the conversion process kept the lysine and tryptophan contents of the donor QPM lines at a high level, despite no laboratory technique was applied during the selection process to analyze these contents. For the production of tryptophan and lysine per area, the superiority of the converted QPM hybrids to the normal versions was maintained. However, once this trait is influenced by the grain yield, the normal elite hybrid BRS1001 presented an equivalent high lysine yield when compared to other converted and elite QPM hybrids. These results support the arguments that the aggregated values for QPM hybrids in the maize production chain will gain economic importance only if the other agronomic traits, mainly grain yield, attain comparable levels to normal elite hybrids available on the market.

All hybrids in the trial presented a similar performance for protein and oil content in the kernel and the oil production per area (Table 3), while Paschoalick (1998) found superior oil content in the kernels of the QPM genotypes.

Besides the agronomic traits and the grain quality, the hybrids under study were also analyzed with SSR markers in order to investigate the genetic relationship among them. A total of 73 alleles were obtained from 31 SSR primer pairs, which were polymorphic in at least one of the hybrids. Greatest genetic similarities were observed for the normal and QPM versions of the hybrids BR3123 and HS200, with the respective values 0.91 and 0.90 (Figure 1). These results evidence that, although strongly related, some differences are still found between the normal and QPM versions of the converted hybrids, as also demonstrated by the agronomic and nutritional data. The high genetic similarity between the hybrids HS200 and BR3123 (0.74), highlighted by the molecular markers, reflects the parentage degree since BR3123 has HS200 as female parental hybrid. Furthermore, hybrid BRS1001 was genetically the most diverse from the other hybrids, suggesting its origin from a different germplasm, and maybe its superior performance in grain yield.

The efficiency of an inbred line conversion strategy is a determinant factor to measure the success of a breeding program. This process has to be quick and dynamic to allow that both versions of an elite hybrid can be released at the same level of competitiveness and importance, but sharing different sectors of the market. In this trial, both versions of the converted hybrids were competitive for grain yield, which is a positive aspect of the conversion program, but they were the worst compared to the more recent elite hybrids. Considering these aspects, and since backcrossing is a conservative breeding method, it is imperative that the conversion process be carried out quickly and efficiently. Additionally, backcross generations with different degrees of recurrent genotype recovery can be used as germplasm sources for the extraction of new inbred lines, representing a promising strategy to speed up the inbred line recycling. So, the combination of conversion and recycling of maize inbred lines complements the objectives of the QPM breeding program and has a great potential to bring forth superior germplasm and hybrids.

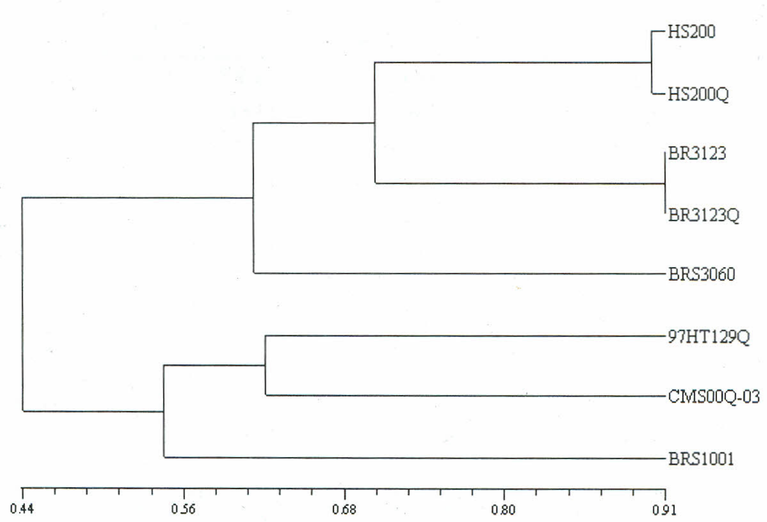

Figure 1. UPGMA dendrogram representing the genetic similarities among the hybrids

CONCLUSIONS

The QPM hybrids obtained by the partial conversion of normal inbred lines presented a better protein quality and a similar performance of most of the agronomic traits, including grain yield, when compared to the original versions. However, some of the deficiencies normally associated to QPM hybrids, such as a higher percentage of lodging, thicker cobs, and shorter kernels, were maintained up to this stage of the process. Even showing similar grain yield performance, both versions of the converted hybrids were less competitive in relation to the more recent normal and QPM elite hybrids.

Avaliação de híbridos de milho de alta qualidade protéica (QPM) obtidos pela conversão de linhagens elites normais

RESUMO - *Dois híbridos experimentais QPM obtidos a partir da conversão parcial de três linhagens elites, utilizando a metodologia dos retrocruzamentos modificados, foram avaliados em comparação com as versões normais destes mesmos híbridos. Foram avaliadas 13 características agronômicas e 12 características de qualidade dos grãos. Tais híbridos foram também analisados por marcadores SSRs, avaliando-se o relacionamento genético entre eles. Os resultados evidenciaram que os híbridos QPM apresentaram uma melhor qualidade protéica, e comportamento semelhante para a maioria das características agronômicas avaliadas, incluindo a produtividade de grãos, quando comparados às suas versões normais. Entretanto, algumas das deficiências geralmente associadas aos QPM, como uma maior porcentagem de acamamento e quebramento, sabugos mais grossos e grãos mais curtos, mantiveram-se até esta fase do processo. Quando comparados aos outros híbridos utilizados como testemunhas, tanto os híbridos normais como os QPM apresentaram os piores desempenhos em termos de produtividade de grãos, indicando, dessa forma, uma perda de competitividade em relação aos híbridos mais recentes.*

Palavras-chave: *Zea mays*, QPM, opaco-2, conversão de linhagens, retrocruzamento.

REFERENCES

Association of Official Analytical Chemists - AOAC (1970) **Official methods of analysis of the Association of Official Analytical Chemists**. AOAC, Washington, 1015p.

Companhia Nacional de Abastecimento - CONAB (2003) Intenção de plantio da safra 2002/03. Brasília. http://www.conab.gov.br/politica-agricola/safra/avalia.html.

Cruz CD (1997) **Programa Genes: aplicativo computacional em genética e estatística**. Editora UFV, Viçosa, 442p.

Dice L (1945) Measures of the amount of ecological association between species. **Ecology 26**: 297-302.

Gevers HO and Lake JK (1992) Development of modified *opaque2* maize in South Africa. In: Mertz ET (ed.) **Quality protein maize**. The American Society of Cereal Chemists, St. Paul, p. 49-78.

Guimarães PEO, Gama EEG, Pacheco CAP and Santos MX (1994a) Obtenção de linhagens QPM vítreas and estáveis. In: **Relatório técnico anual do Centro Nacional de Pesquisa de Milho and Sorgo 1992-1993**. EMBRAPA/CNPMS, Sete Lagoas, p. 220.

Guimarães PEO, Lopes MA, Gama EEG, Santos MX, Parentoni SN, Paes MC, Vieira Junior PA, Silva AE, Paiva E, Correia LA and Pacheco CAP (1994b) Quality protein maize improvement at the National Maize and Sorghum Research Center. In: **Proceedings of the International Symposium on Quality Protein Maize**. EMBRAPA/CNPMS, Sete Lagoas, p. 185-204.

Guimarães PEO, Pacheco CAP and Lopes MA (2000) **Processo de introdução de características genéticas expressadas em sementes e controladas por um gene recessivo e seus modificadores**. Patente: Privilégio e Inovação. n. PI 00046, "Processo de introdução de característica". 14 de setembro de 2000 (Depósito).

Hernandez HH and Batez LS (1969) **A modified method for rapid tryptophan analysis of maize**. CYMMIT, México, 7p. (Research Bulletin 13).

Mertz ET, Bates LS and Nelson OE (1964) Mutant gene that changes the protein composition and increases the lysine content of maize endosperm. **Science 145**: 279-280.

Moro GL (1996) **The biochemical genetics of quality protein maize**. PhD Thesis, University of Arizona, Tucson, 187p.

Nelson OE (1969) Genetic modification of protein quality in plants. **Advances in Agronomy 21**: 171-194.

Ninamango-Cárdenas FE, Guimarães CT, Martins PR, Parentoni SN, Carneiro NP, Lopes MA, Moro JR and Paiva E (2003) Mapping QTLs for aluminum tolerance in maize. **Euphytica 130**: 223-232.

Pacheco CAP, Guimarães PEO, Parentoni SN, Lopes MA, Santos MX, Gama EEG, Vasconcelos MJV, Correia LA and Meirelles WF (1999) O desenvolvimento de milho de alta qualidade nutricional no Brasil. In: **Memórias 28ª Reunión Latinoamericana Del Maiz**. CIMMYT, México, p. 13-26.