Biochemical and agronomic performance of quality protein maize hybrids adapted to temperate regions

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Abstract

Quality protein maize (QPM) is high lysine (tryptophan) maize with hard endosperm and good agronomic performance. QPM was developed primarily for utilization in tropical and sub-tropical regions where maize is a staple food. Its adaptation and cultivation in temperate areas is still not fully developed, although QPM could have merits for production and consumption in developed parts of the world, especially as animal feed. Maize Research Institute Zemun Polje has a program on developing QPM genotypes for growing in temperate regions. The objective of our research was initial screening of 72 hybrids (derived from crosses between QPM lines adapted to temperate environments and three commercial lines with standard kernel quality) for kernel modification, tryptophan and protein contents, quality index (QI) and grain yield. Five hybrids with high tryptophan content (0.071 to 0.081%) and yield at the level of standard hybrids (96 - 114%) were identified. Protein content ranged from 10 to 11.20%, similar to standard hybrids. QI was in the range from 0.71 to 0.74, which was better than in standard hybrids (0.57 – 0.62), but below the QPM threshold of 0.80. The percentage of good kernel modifications (type 1 and 2) was over 80% in three and over 74% in two hybrids. The results indicated the complexity of obtaining high yielding hybrids with high levels of essential amino-acids. Identified potential QPM hybrids have to be further evaluated for agronomic traits, but the results can be considered important in the context of limited information on QPM adapted to temperate environmental conditions.

Keywords: QPM, temperate climate, tryptophan, yield

Introduction

Maize, wheat and rice make up to 85% of global cereal production. They are often the only source of nutrition in developing and underdeveloped countries. In many parts of Latin America, Africa and Asia, maize is the major staple food and often the only source of protein. Additionally, 78% of total maize production goes into livestock feed in developed countries.

However, maize is deficient in several amino acids essential for monogastric animals with lysine the most limiting (Bhan et al, 2003). Cereal grains contain 1.5 to 2% lysine, while 5% is required for optimal human nutrition (Young et al, 1998). Consequently, maize has relatively low nutritional value for humans and monogastric animals.

The recessive *opaque2* (*o2*) mutation was found to increase lysine and tryptophan content in maize grains (Mertz et al, 1964). Increased concentrations of these two amino acids (their ratio is 4:1, respectively) in the grain endosperm can double the biological value of maize protein (Bressani, 1992). However, *o2* is associated with several undesirable traits. The *o2* maize kernels are dull and chalky, have 15-20% less grain weight and are more susceptible to several diseases and insects. Using conventional breeding methodologies, the CIMMYT (International Maize and Wheat Improvement Center, Mexico) interdisciplinary research team converted the floury soft endosperm kernels into harder types, increased grain yield potential to the level of the best maize hybrids, endowed the *o2* maize with disease and insect resistance, and increased utilization and storage qualities to those of superior maize materials (Vasal, 2001). The new, normal-looking, normal-tasting *o2* types were renamed Quality Protein Maize or QPM (Vivek et al, 2008).

The utility of QPM is manifold for both human nutrition and animal feed. Akuamoa-Boateng (2002) showed that children in Ghana who were fed with high lysine/tryptophan maize were healthier, had reduced stunting and better growth capabilities, compared with children fed with normal maize porridge. Bressani (1991) stated that people eating QPM had significantly higher nitrogen retention than those who ate normal maize, indicating that QPM protein is more „bioavailable“. Besides increased biological value, QPM has additional nutritional advantages, such as higher concentration of niacin (vitamin B3) and improved absorption of potassium (Graham et al, 1980) and carotene (De Bosque et al, 1988).

Although QPM was developed primarily for utilization in the regions where maize is the main staple food, it also may have merits for production and consumption in developed parts of the world, especially as animal feed. Projections of International Food

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Policy Research Institute (IFPRI) has indicated that between 1995 and 2020 there will be approximately over 30% increase in global demand for poultry and pork (www.ifpri.org). To exploit fully the genetic potential of monogastric animals, diets that are balanced in all nutritive elements are required. Utilization of QPM could substitute protein additives which are used in animal feed composites. A number of studies have proved the impact of QPM on the increase in body growth of poultry and pigs (Krivanek et al, 2007; Sofi et al, 2009). Mbuya et al (2011) showed a 50% increase in chicken body weight fed with QPM compared with normal maize.

Maize Research Institute Zemun Polje (MRI) has a breeding program for developing QPM lines and hybrids for growing in temperate regions. The objective of our study was initial screening of crosses between QPM lines developed for temperate regions and commercial lines with standard kernel quality. The idea was to test tryptophan increase in F_2 populations, since they segregate in 3:1 ratio for *opaque2* gene and one quarter of the kernels would be *o2o2*. The crosses were tested for 1) grain yield and moisture at the harvest, 2) tryptophan and protein contents and 3) quality index (QI), with the goal to select high tryptophan hybrids (with high grain yields) for further experiments.

Materials and Methods

Plant Material

Crosses between QPM inbred lines developed for temperate climate region (obtained from CIMMYT, created at Iowa State University by AR Hallauer) and lines with standard kernel quality were analyzed. A total of 72 hybrids, derived from 27 QPM and three MRI standard lines – ZPL-1 (L1), ZPL-2 (L2) and ZPL-3 (L3), were tested for yield and biochemical characteristics. ZPL-1 and ZPL-2 lines are of Lancaster origin, while ZPL-3 is of BSSS origin. The pedigree data for the QPM lines and the list of their crosses with commercial lines are given in Table 1.

Field trials

Seventy-two test-hybrids were planted in Zemun Polje in 2011. Field experiment was performed according to randomized complete block design (RCBD). Three standard kernel quality commercial hybrids (ZP 434, ZP 666, and ZP 735) were used as checks. The checks were planted per every 24 test-hybrids, making one trial (with three replications) of the field experiment. Plant density was 67.000 plants ha^{-1}. Each plot had two rows with 15 plants in each row. The first row was used for measurement of moisture and grain yield (t ha^{-1} with 14% moisture), and the second one for selfing. Selfed seed of each hybrid was used for biochemical analyses.

Kernel modifications

For each hybrid 200 kernels were scored accord-

Table 1 - The list of QPM lines, their pedigree data and commercial inbred lines they were crossed with.

QPM line	Pedigree	Crossed with commercial lines
QPM1	[B100 x CLQ 06901]-F2)-02-04-K1-B	ZPL1, ZPL2, ZPL3
QPM2	[B104 x CLQ 06901]-F2)-05-01-K1-B	ZPL1, ZPL2
QPM3	[B111 x CLQ 06901]-F2)-06-01-K1-B	ZPL3
QPM4	[B99 x CLQ 06901]-F2)-01-05-01-B	ZPL1, ZPL3
QPM5	[B99 x CLQ 06901]-F2)-07-01-01-B	ZPL1, ZPL2, ZPL3
QPM6	[B99 x CLRQ 00502]-F2)-02-02-K1-B	ZPL1, ZPL3
QPM7	[CLQ 06901 x B110]-F2)-02-03-K1-B	ZPL1, ZPL2
QPM8	[CLQ 06901 x B110]-F2)-04-03-K1-B	ZPL1, ZPL2, ZPL3
QPM9	[CLQ 06901 x B113]-F2)-01-01-K1-B	ZPL1, ZPL2, ZPL3
QPM10	[CLQ 06901 x B113]-F2)-01-05-K1-B	ZPL1, ZPL2, ZPL3
QPM11	[CLQ 06901 x B113]-F2)-01-05-K1-B	ZPL1, ZPL2, ZPL3
QPM12	[CLQ 06901 x B113]-F2)-02-01-K1-B	ZPL1, ZPL2, ZPL3
QPM13	[CLQ 06901 x B113]-F2)-02-04-K1-B	ZPL1, ZPL2
QPM14	[CLQ 06901 x B113]-F2)-03-02-K1-B	ZPL1, ZPL3
QPM15	[CLQ 06901 x B97]-F2)-02-03-01-B	ZPL1, ZPL3
QPM16	[CLQ 06901 x B97]-F2)-02-03-02-B	ZPL1, ZPL2, ZPL3
QPM17	[CLQ 06901 x B97]-F2)-02-03-03-B	ZPL1, ZPL2, ZPL3
QPM18	[CLQ 06901 x B97]-F2)-08-01-01-B	ZPL1, ZPL2, ZPL3
QPM19	[CLQ 06901 x B98]-F2)-01-02-01-B	ZPL1, ZPL2, ZPL3
QPM20	[CLQ 06901 x B98]-F2)-01-05-01-B	ZPL1, ZPL2, ZPL3
QPM21	[CLQ 06901 x B98]-F2)-04-01-01-B	ZPL1, ZPL2, ZPL3
QPM22	[CLRQ 00502 x B100]-F2)-03-02-K1-B	ZPL1, ZPL2, ZPL3
QPM23	[CLRQ 00502 x B109]-F2)-04-04-K1-B	ZPL1, ZPL2, ZPL3
QPM24	[CLRQ 00502 x B109]-F2)-04-05-K1-B	ZPL1, ZPL2, ZPL3
QPM25	[CLQ 06901 x B97]-F2)-03-04-01-B	ZPL1, ZPL2, ZPL3
QPM26	[CLQ 06901 x B98]-F2)-06-01-01-B	ZPL1, ZPL2, ZPL3
QPM27	[CLRQ 00502 x B100]-F2)-03-05-K1-B	ZPL1, ZPL2, ZPL3

Table 2 - Tryptophan content (%) of the 72 QPM x standard quality line crosses and standard hybrids.

Genotype	Parameter	Tryptophan content (%)			
		<0.06	0.060-0.069	0.70-0.74	≥0.075
QPM hybrids	Number of genotypes	14	35	15	8
	Range	0.046 - 0.059	0.060 - 0.065	0.070 - 0.074	0.076 - 0.088
	Average	0.056	0.064	0.071	0.080
Standard hybrids (checks)	Range	-	0.060-0.063	-	-
	Average	-	0.062	-	-

ing to Vivek et al (2008). Kernel modifications were visually assessed using light table, according to the scoring scale from 1 (completely translucent, with no opaqueness) to 5 (completely opaque). Modification score 2 was given to the kernel which was 25% opaque, while scores 3 and 4 were given to 50% and 75% opaque kernels, respectively.

Biochemical analysis

Sample preparation

Each hybrid was represented by 60 randomly chosen kernels from 10 competitive plants, divided into two samples consisting of 30 kernels each. Kernels were dried in a controlled oven at 65°C over night (16-18 hours), and milled in a Cyclone sample mill - Simmons Fastener, USA. The flour was defatted by hexane treatment for 4 hours in Soxhlet extractor.

Tryptophan content

Tryptophan content was determined using the colorimetric method of Nurit et al (2009). The color was developed in the reaction of flour hydrolysate (obtained by overnight digestion with papain solution at 65°C) with 2 ml of reagent containing 56 mg of Fe^{3+} dissolved in 1 l of glacial acetic acid and 2 ml of 15 M H_2SO_4. After incubation at 65°C for 15 minutes, absorbance was read at 560 nm. Tryptophan content was calculated using a standard calibration curve, developed with known amounts of tryptophan, ranging from zero to 30 μg ml^{-1}.

Protein content and Quality index

The protein content was determined by the standard Kjedahl method based on nitrogen determination as explained in Vivek et al (2008). The protein was estimated from the nitrogen value as: % protein = % nitrogen x 6.25 (conversion factor for maize)
Quality index (QI), defined as tryptophan to protein ratio in the sample, was calculated as: QI = 100 x (tryptophan content in the sample / protein content in the sample).

Statistical analysis

One factorial analysis of variance (ANOVA) for trials set up according to the RCB design was done for yield and biochemical traits, for each trial separately. Fisher's least significant difference (LSD) test was used to test the significance of differences between the observed hybrid means. Criterions for de-

Table 3 - Mean, mean square for genotypes (MS), standard deviation (SD), coefficient of variation (CV) and least significant difference (LSD) for the analyzed biochemical traits and grain yield of the 72 QPM x standard quality line hybrids and check hybrids.

Trait	Statistical parameters	Trial		
		I	II	III
Protein	Mean	10.88	10.88	10.52
	MS	1.042***	1.716***	1.164 ***
	SD	0.73	0.94	0.76
	CV	0.77	0.74	0.62
	$LSD_{0.05}$	0.18	0.18	0.16
Tryptophan	Mean	0.062	0.069	0.070
	MS	0.68***	1.36***	0.625***
	SD	0.071	0.008	0.007
	CV	4.25	5.92	5.43
	$LSD_{0.05}$	0.006	0.009	0.008
QI	Mean	0.58	0.63	0.67
	MS	0.007***	0.004**	0.009***
	SD	0.06	0.05	0.07
	CV	4.32	3.04	5.68
	$LSD_{0.05}$	0.04	0.08	0.08
Yield	Mean	12.28	11.87	11.20
	MS	19.83***	11.82***	10.47***
	SD	3.16	2.43	2.29
	CV	9.385	11.88	11.33
	$LSD_{0.05}$	2.40	2.93	2.63

$LSD_{0.05}$ – least significant difference at 0.05 level; **,***- statistically significant at 0.01 and 0.001 level, respectively termining high tryptophan/yield hybrids were tryptophan content over 0.070% and that their grain yield was not statistically different from the check hybrids for grain yield. All statistical analyses were done in MSTAT-C software.

Results

Tryptophan content in F_2 kernels of the analyzed hybrids is presented in Table 2. Among all 72 QPM x standard lines, tryptophan content was in the range from 0.046 to 0.088%. Only eight hybrids (11.1%) had tryptophan values over the threshold set for QPM genotypes (≥0.075%). Another 15 hybrids (20.8%) had values near the limit, i.e. in the range from 0.070 to 0.074%. The average content of the three standard hybrids used as checks was 0.062%.

Results of ANOVA and LSD values for biochemical traits and grain yield (for each trial) are given in Table 3. ANOVA did not show significant difference between replications in any trial (data not shown), but significant difference between genotypes was determined for all traits at $P<0.001$, except for QI in trial II ($P<0.01$). Statistical analyses identified a total of five QPM hybrids with yield at the level (97 to 114%) of the standard hybrids (Table 4), which also had elevated/high tryptophan content. Protein content of these hybrids was in the range from 10 to 11.20 %, similar to the standard hybrids. QI was from 0.70 to 0.74, i.e. it was higher than in the standard hybrids. However, QI was below the QPM threshold of 0.80 for all five hybrids.

Yield of the remaining QPM hybrids was in the range of 31% to 85% of the standard hybrids. Average moisture of the five high tryptophan and yielding hybrids was in the range from 17.9 to 25% and of the standard hybrids from 14.4 to 22.7% (data not shown).

Difference (%) in biochemical components and grain yield values between the five QPM hybrids and standard hybrids is given in Figure 1. Corresponding standard hybrid values were calculated as average of the three check hybrids and given as 100% (1). Protein content was in the range from 94 to 109% of the standard hybrids. In all hybrids tryptophan content and QI were higher compared to standard hybrids - tryptophan from 15 to 27% and QI from 18 to 25%. Compared to check hybrids, QPM18 x L1 and QPM24 x L2 had higher yield for 14 and 11%, respectively, and yield of the remaining three hybrids was lower for 2 to 4%.

Percentages of kernel modifications for each of the five high tryptophan and high yielding hybrids are given in Table 5. Consistency between kernel modifications and the other analyzed traits could not be clearly defined. For example, the lowest percentage of good modifications was found in QPM17 x L1 (74%) and QPM6 x L1 (76%), which at the same time had the highest (0.081%) and the lowest (0.071%) tryptophan content, respectively. On the other hand, QPM10 x L2 had the highest percentage of good modifications (84%) and tryptophan content of 0.074%. All three hybrids had yield 2 to 3% lower than the standard hybrids. Average percent of kernel modifications for all QPM hybrids was 79% of types 1 and 2, 5% of type 3 and 16% of types 4 and 5. All standard hybrids kernels were translucent.

Discussion

The results presented in this paper are a part of an MRI project on development of QPM genotypes adapted for growing in temperate regions. The main problem with QPM hybrids is to achieve high levels of essential amino acids and high grain yield at the same time. It is well known that the *o2* gene has numerous negative pleiotropic effects and affects many traits of agronomic importance. One of the negative effects is decrease of yield due to soft endosperm. Most of the QPM hybrids analyzed in this paper had significantly lower yield, even up to 65% less than the standard hybrids. However, five genotypes were identified that had high tryptophan content and grain yield at the level of standard grain quality hybrids. Moisture content at harvest in these genotypes was also acceptable considering hybrid production, although it was, on the average, somewhat higher than in the standard hybrids, probably due to residual tropical germplasm in temperate QPM lines.

Grain yield at the level of standard hybrids in QPM germplasm can be attributed to the effect of the modifier genes (Opm), which convert soft endosperm of *o2* to the vitreous phenotype of wild type maize (Holding et al, 2008). There are multiple, unlinked

Table 4 - Protein and tryptophan contents, quality index (QI) and grain yield of the five identified high tryptophan/yield hybrids.

Trial	Genotype	Protein	Tryptophan	QI	Yield
I	QPM6 x L1	10.21^{k}	0.071^{a}	0.70^{a}	14.43^{abc}
	QPM10 x L2	10.00^{l}	0.074^{a}	0.74^{a}	14.24^{bcde}
	Standard (average)[1]	$10.65^{b,hi,l}$	$0.062^{bcdef,cdefg,defg}$	$0.59^{cde,fg,gh}$	$14.89^{ab,bcd,bcde}$
II	QPM24x L2	11.06^{ef}	0.079^{bc}	0.71^{ab}	15.72^{a}
	Standard (average)	$10.11^{d,h,k}$	$0.062^{fghi,ghij,hij}$	$0.62^{cdefgh,fgh,h}$	$14.20^{a,abc,cdef}$
III	QPM17 x L1	11.20^{cd}	0.081^{a}	0.72^{ab}	13.10^{abcd}
	QPM18 x L1	10.27^{g}	0.073^{abc}	0.71^{abc}	15.29^{a}
	Standard (average)	$10.92^{ab,e,h}$	$0.064^{def,ef,fg}$	$0.57^{ef,f,f}$	$13.38^{abc,abcd,abcd}$

[1] Standard values are given as the average of three check hybrids for each trial and trait; a-l - values in each column with the same letter are not significantly different ($LSD_{0.05}$) and are given for each standard hybrid (separated by commas)

Table 5 - Percentages of kernel modifications for the five hybrids with high tryptophan content and high grain yield.

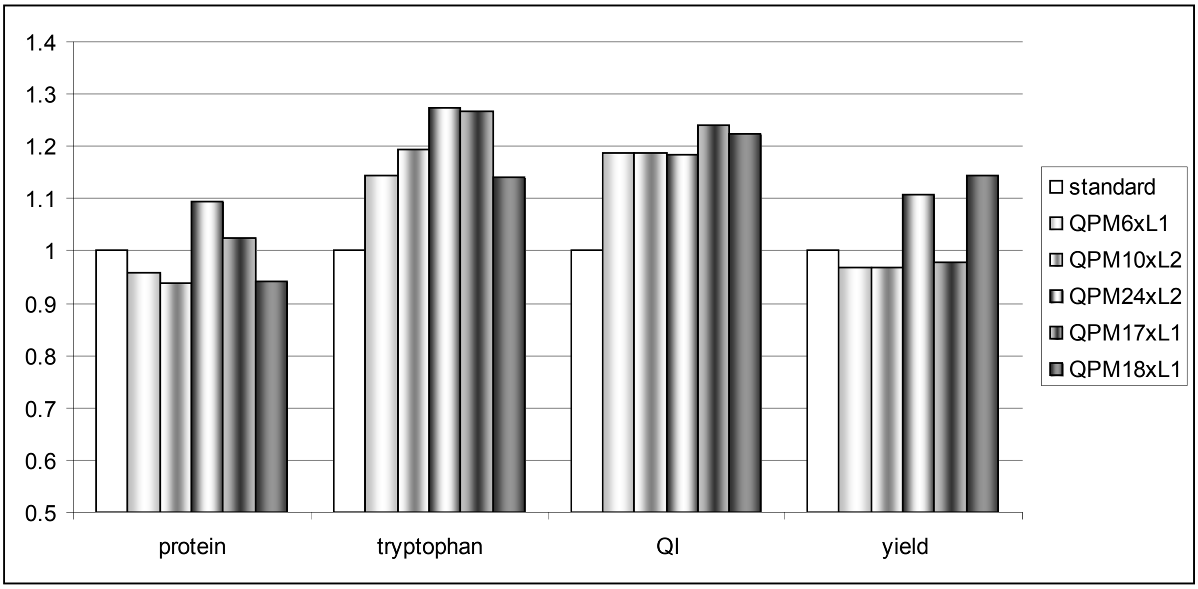
Genotype	Kernel modifications (%) Good modifications (types 1 and 2)	Medium modifications (type 3)	Bad modifications (types 4 and 5)
QPM6 x L1	76	0	24
QPM10 x L2	84	6	10
QPM17 x L1	74	3	23
QPM18 x L1	83	6	11
QPM24 x L2	80	9	11

Opm in QPM and their nature and mode of action are unknown. Additive, dominant, semi-dominant and recessive gene action was postulated for different genetic backgrounds. The results of recent experiments suggest that several different genes and metabolic pathways can contribute to the formation of vitreous endosperm in QPM (Wu et al, 2010; Holding et al, 2011). In this work a total of 27 QPM lines were used, but only five produced hard endosperm kernels in hybrid genotypes with high tryptophan content and good grain yield. Also, only two of three standard lines used were components of these hybrids. Furthermore, different segregation ratios for endosperm hardness found among the five QPM hybrids indicate different modes of gene action. A 3:1 ratio found in two hybrids could probably be attributed to one dominant/recessive gene, with possibly some other putative genes having minor effects in the given genetic background. It is obvious that the segregation ratio found in the remaining three hybrids points out to some other, yet unclear, type of gene action. All these results support the assumption that the action of modifier genes occurs in a complex manner.

Tryptophan content and QI were high, although the threshold set for QPM (Vivek et al, 2008) was attained for tryptophan only in two out of five identified hybrids. However, their values were higher compared to standard hybrids for approximately 20%. These increases can define the five hybrids as potential quality protein maize. Protein content was at the level of standard hybrids. For final validation they have to be tested in field trials at several locations, and in different years.

The effect of genotype by environment interaction can influence some of the traits analyzed in this study. The *o2* gene exhibits not only pleiotropic effects but also distinct modes of inheritance for various traits, e.g., the nearly recessive single gene inheritance for lysine content whereas incomplete dominance for protein content. Moreover, the expression of the *o2* gene is conditioned by environment and this magnitude varies depending on the trait. The degree is relatively small for lysine/tryptophan content while large for protein (Lou et al, 2005). It was shown that tryptophan level of QPM under stress and optimum conditions was higher than the tryptophan level of normal maize under optimum conditions (CIMMYT, 2003; Mosisa, 2005). Similarly, it was reported that protein

Figure 1 - Protein and tryptophan contents, quality index (QI) and grain yield of the potential QPM hybrids given as % of the standard hybrids. Corresponding standard hybrid values were calculated as average of the three check hybrids and are given as 1 (100%).



quality is more stable than protein content and endosperm modification of QPM varieties across environments (Pixley and Bjarnason, 2002; Zaidi et al, 2008).

Taking into account stability of protein quality and tryptophan content, this experiment was performed only at one location with the goal to identify hybrids with high values for these two quality components. Considering that yield significantly depends on environmental conditions and stress environments produce high genotype by environment interaction (Banziger et al, 2000; Banziger and Diallo, 2004), and that quality traits also depend on environment, field trials on several different locations will be performed for the five hybrids to test their stability. However, the initial screening has identified the potential QPM hybrids and thus significantly decreased the number of hybrids for further experiments.

QPM varieties have been released in more than 23 developing countries for large scale cultivation on the area over 2.5 million ha (Akande and Lamidi, 2006; Sofi et al, 2009). However, their adaptation and cultivation in temperate areas is still insignificant, although in these regions quality protein maize could be beneficial primarily for animal feed, but also in human nutrition. The results presented in this paper indicate the complexity of obtaining high yielding hybrids with high levels of essential amino-acids, even when crossing QPM lines with standard kernel quality. The genotypes identified as potential QPM hybrids have to be further checked for agronomic traits. Nevertheless, the results can be considered important in the context of limited information on QPM adapted to temperate environmental conditions.

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