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Registration of Temperate Quality Protein Maize (QPM) Lines BQPM9, BQPM10, BQPM11, BQPM12, BQPM13, BQPM14, BQPM15, BQPM16, and BQPM17

Hannah M. Worral
Iowa State University, hworral@iastate.edu

M. Paul Scott
United States Department of Agriculture, pscott@iastate.edu

Arnel R. Hallauer
Iowa State University, hallauer@iastate.edu

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Registration of Temperate Quality Protein Maize (QPM) Lines BQPM9, BQPM10, BQPM11, BQPM12, BQPM13, BQPM14, BQPM15, BQPM16, and BQPM17

Hannah M. Worral,* M. Paul Scott, and Arnel R. Hallauer

Abstract

The discovery of the *opaque2* (*o2*) mutation and *o2* modifier genes in maize (*Zea mays* L.) has resulted in the development of Quality Protein Maize (QPM) lines with increased lysine and tryptophan content. The QPM lines BQPM9 (Reg. No. GP-584, PI 671795), BQPM10 (Reg. No. GP-585, PI 671796), BQPM11 (Reg. No. GP-586, PI 671797), BQPM12 (Reg. No. GP-587, PI 671798), BQPM13 (Reg. No. GP-588, PI 671799), BQPM14 (Reg. No. GP-589, PI 671800), BQPM15 (Reg. No. GP-592, PI 673348), BQPM16 (Reg. No. GP-590, PI 671801), and BQPM17 (Reg. No. GP-591, PI 671802) were developed jointly by Iowa State University and the USDA-ARS to address the lack of QPM lines adapted to the US Corn Belt. These lines originated from crosses made between two QPM lines from the International Maize and Wheat Improvement Center (CIMMYT) (CLQ06901 and CLRQ00502) and six inbred lines released by Iowa State University (B91, B97, B98, B99, B100, and B113). Increased lysine and tryptophan content, characteristics associated with the presence of the *o2* mutation, and agronomic performance were used as selection criteria in the development of the nine BQPM lines released herein.

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5585 Guilford Rd., Madison, WI 53711 USA
*Corresponding author (hworral@iastate.edu)

The maize community has sought to improve the nutritional quality of maize (*Zea mays* L.), one of the world's staple food crops, for more than a century. While their deficiencies in maize have been documented for nearly 100 years, lysine and tryptophan continue to be limiting amino acids in the utilization of maize as a balanced source of protein for both human and animal consumption (Osborne and Mendel, 1914; Baker et al., 1969; Lewis et al., 1982). Various strategies have been applied effectively to the problem, including recurrent selection for higher amino acid concentrations (Choe et al., 1976; Scott et al., 2008), use of transgenic techniques to increase specific limiting amino acids (Lai and Messing, 2002; Huang et al., 2005; Houmard et al., 2007; Bicar et al., 2008; Tang et al., 2013), and supplementation of normal maize with soybean [*Glycine max* (L.) Merr.], synthetic methionine, and synthetic lysine. The latter is the simplest but also possibly the costliest option to achieve an optimal balance of amino acids in the diet. The cost reduction seen when Quality Protein Maize (QPM) is substituted for normal maize is appealing not only for large farms and corporations looking to minimize input expenses but also for small farms that may already rely on maize as the sole feed component (López-Pereira, 1993; Nyanamba et al., 2003). Similarly, QPM developed for human consumption has the potential to positively affect many countries where maize is a staple of the diet (Krivanek et al., 2007; Gunaratna et al., 2010).

Naturally occurring mutations, such as *opaque2* (*o2*) and *floury2* (*fl2*), decrease the tryptophan and lysine-poor zein (prolamine) protein fraction present in the endosperm of a mature kernel, resulting in proportionately higher levels of these limiting amino acids (Mertz et al., 1964; Nelson et al., 1965; Geetha et al., 1991; Munck, 1992; Habben et al., 1993). Following the discovery of *o2* and *fl2* in the 1960s, several nutritional studies were conducted to investigate the nutritional value of these mutants compared with normal maize (Beeson et al., 1966; Pickett, 1966; Cromwell et al., 1967). Despite the higher amino acid levels, the soft, chalky endosperm characteristic of these mutations was more susceptible to fungal

H.M. Worral and A.R. Hallauer, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011; M.P. Scott, USDA-ARS, Corn Insects and Crop Genetics Research, Iowa State Univ., Ames, IA 50011.

Abbreviations: CIMMYT, International Maize and Wheat Improvement Center; CL, commercial line; *fl2*, *floury2*; *o2*, *opaque2*; QPM, Quality Protein Maize.

ear rots, lower yielding, and unappealing to maize growers (Bjarnason and Vasal, 1992; Vasal, 2001; Ignjatovi-Micic et al., 2009). With the discovery of *o2* modifier genes, however, maize breeders were able to produce higher yielding, lysine-rich germplasm that lacked the characteristic opaque endosperm and is now designated QPM (Paez et al., 1969; Vasal et al., 1980; Mertz, 1992; Prasanna et al., 2001).

Despite progress in adapting these QPM lines to various environments, there is little documentation for QPM lines that are well-adapted to the US Corn Belt (Zarkadas et al., 2000; Bhatnagar et al., 2004; Ngaboyisonga et al., 2009). Our goal was to develop temperate QPM lines that were well-adapted to the US Corn Belt. We used two QPM lines (CLRQ00502 and CLQ06901) from the International Maize and Wheat Improvement Center (CIMMYT) as donors of *o2* and endosperm modifier genes and public inbred lines released by Iowa State University to develop temperate QPM lines BQPM9 (Reg. No. GP-584, PI 671795), BQPM10 (Reg. No. GP-585, PI 671796), BQPM11 (Reg. No. GP-586, PI 671797), BQPM12 (Reg. No. GP-587, PI 671798), BQPM13 (Reg. No. GP-588, PI 671799), BQPM14 (Reg. No. GP-589, PI 671800), BQPM15 (Reg. No. GP-592, PI 673348), BQPM16 (Reg. No. GP-590, PI 671801), and BQPM17 (Reg. No. GP-591, PI 671802).

Line Development

BQPM9, BQPM10, BQPM11, BQPM12, BQPM13, BQPM14, BQPM15, BQPM16, and BQPM17 are QPM lines adapted to the US Corn Belt derived from two QPM sources (CLQ06901 and CLRQ00502, developed at CIMMYT) and six Iowa inbred lines (B91, B97, B98, B99, B100, and B113) (Table 1; Russell, 1989; Hallauer et al., 1994, 1995, 1997, 1998, 2001). The QPM × Iowa inbred F_1 generation and one backcross to each Iowa inbred were conducted at CIMMYT in Mexico. Backcrosses were then planted at the Ames, IA, nursery in spring 2002 and self-pollinated. Subsequent generations (S_1–S_7) were planted ear-to-row each season in the nursery at Ames. Codominant simple-sequence repeat (SSR) markers *phi057* and *umc1066* were used to confirm the presence of the *opaque2* gene, and the presence of the *o2* modifiers was maintained by scoring kernels for percentage opacity on a light box (Babu et al., 2005). Only those kernels with opacity scores of 1 to 2 out of 5 (i.e., <25% opaque) were advanced to the next generation. Microbial amino acid assays were also used in some years to evaluate the lysine, methionine, and tryptophan concentrations of the selected kernels (Scott et al., 2004, 2009).

At the S_3 generation, a North Carolina design II mating design was used to produce hybrids to assess the combining ability of the developing temperate QPM lines (Comstock and Robinson, 1952); the results are presented in Scott et al. (2009). Yield trials of test cross hybrids were then conducted in 2007 and 2008 using two-row plots with 0.762-m spacing between rows and 4.57-m plot length (8.36-m^2 plot size) and planting densities similar to regional commercial production (~65,000 plants ha^{-1}). Planting occurred at two locations in 2007 (Crawfordsville and Carroll, IA) and three locations in 2008 (Ames, Crawfordsville, and Carroll, IA). Plants were evaluated for stalk and root lodging, grain yield, and moisture content. In addition, amino acid balance of the BQPM inbred lines per se was shown to be typical of QPM, with elevated levels of lysine and tryptophan (Scott et al., 2009).

To assess the yield potential of the BQPM lines, an additional yield trial was initiated in 2009 following the same plot design as previously. In this trial, nine commercial checks and 39 BQPM lines were used as the female parent, and test crosses of those lines were made using three commercial, non-QPM inbred lines as male testers. The resulting test-cross lines were assessed for stalk and root lodging and the yield and moisture content of the grain (Table 2). Trials were conducted over a tristate area encompassing Iowa (Atlantic and Slater), Illinois (Mt. Pulaski), and Nebraska (York).

Characteristics

The nine BQPM lines herein released were selected on the basis of the presence of the *o2* and *o2* modifier genes and their agronomic performance when grown in the US Corn Belt. BQPM lines and commercial inbred checks were crossed to the same three testers so that BQPM hybrid performance could be compared with that of commercial hybrids. Across the nine BQPM lines released, there was an 11.5% ($p = 0.05$) drop in yield compared with the commercial checks. The top three yielding BQPM lines by tester were not significantly different in yield than the commercial checks ($p = 0.05$; Table 3). The average yield across the three testers for the BQPM lines was 6377.8 kg ha^{-1}, with BQPM9 hybrids having the highest average yield at

Table 1. Origin of each of the BQPM maize lines.

Line†	Pedigree‡	Recurrent parent	Origin	Source of QPM§
BQPM9	(B99 × CLQ 06901) × B99	B99(Hallauer et al., 1995)	Iowa Corn Borer Synthetic No 1 (R) C10	CLQ06901
BQPM10	(B99 × CLRQ 00502) × B99	B99 (Hallauer et al., 1995)	Iowa Corn Borer Synthetic No 1 (R) C10	CLRQ00502
BQPM11	(B100 × CLQ 06901) × B100	B100 (Hallauer et al., 1995)	(B85 × H99)H99	CLQ06901
BQPM12	(CLQ 06901 × B98) × B98	B98 (Hallauer et al., 1994)	Pioneer two-ear Composite (FR) C5	CLQ06901
BQPM13	(CLQ 06901 × B97) × B97	B97 (Hallauer et al., 1994)	Iowa Corn Borer Synthetic No 1 (R) C9	CLQ06901
BQPM14	(CLQ 06901 × B97) × B97	B97 (Hallauer et al., 1994)	Iowa Corn Borer Synthetic No 1 (R) C9	CLQ06901
BQPM15	(B91 × CLQ 06901) × B91	B91 (Russell, 1989)	Iowa Corn Borer Synthetic No 1 (R) C7	CLQ06901
BQPM16	(CLQ 06901 × B98) × B98	B98 (Hallauer et al., 1994)	Pioneer two-ear Composite (FR) C5	CLQ06901
BQPM17	(CLQ 06901 × B113) × B113	B113 (Hallauer et al., 2001)	Pioneer two-ear Composite (FR) C9	CLQ06901

† Lines developed in the state of Iowa are given names starting with B by convention.

‡ Pedigrees are shortened for simplicity.

§ The two Quality Protein Maize (QPM) donor lines have little genetic relationship to one another. CLRQ00502 comes from the subtropical population 502, and CLQ06901 was derived from Templado Amarillo QPM population 69. The latter has a flinty yellow kernel phenotype and intermediate maturity.

Table 2. Summary of agronomic traits for nine BQPM maize lines crossed to three testers at four (testers 1 and 3) and three (tester 2) locations. There were no significant differences between any of the BQPM lines for any of the agronomic traits at the 0.05 probability level.

Line	Grain			Lodging	
	Yield	Density	Moisture†	Stalk	Root
	kg ha^{-1}	kg m^{-3}	%		
BQPM9	6685.4	706.1	24.4	3.0	3.5
BQPM10‡	6564.1	716.2	24.0*	1.7	3.2
BQPM11	6460.8	708.3	26.9	2.6	0.8
BQPM12	6447.6	697.1	27.3	0.6	2.5
BQPM13	6398.6	704.9	26.9	2.4	8.0
BQPM14	6107.1	698.0	27.3	0.8	0.1
BQPM15‡	6078.5	687.3	25.2	1.4	2.2
BQPM16	6041.4	685.1	28.5*	0.1	1.7
BQPM17	6579.0	699.3	25.5	3.8	1.0
Mean§	6377.8	700.1	26.4	1.8	2.7
LSD (0.05)¶	782.3	55.2	4.3	8.6	7.9

† Means marked with an asterisk (*) are significantly different at the 0.05 probability level

‡ BQPM10 and BQPM15 were only crossed to two testers rather than three.

§ This mean was calculated from the nine BQPM lines and does not contain the nine commercial checks.

¶ 5% LSD calculated from the original 39 BQPM lines ($p < 0.05$).

6685.4 kg ha^{-1} and BQPM16 hybrids having the lowest at 6041.4 kg ha^{-1} (Table 2). Grain density, stalk lodging, and root lodging were not significantly different between the BQPM hybrids and their commercial line (CL) hybrid counterparts when averaged across the testers ($p < 0.05$). Percentage moisture varied across both CL and BPQM hybrids. The top-yielding BQPM hybrids for each tester had similar yields ($p < 0.05$) to hybrids derived from commercial stock and crossed to the same testers (Table

Table 3. Summary of agronomic traits for three testers crossed to nine commercial check inbred lines and the three highest yielding BQPM maize lines for each tester at four (testers 1 and 3) and three (tester 2) locations.

Line		Grain			Lodging	
Tester	Check†	Yield	Density	Moisture	Stalk	Root
		kg ha^{-1}	kg m^{-3}	%		
T1	CL Alfa	6795.3	691.0	24.1	0.8	0.2
	CL Bravo	7243.3	678.2	24.8	1.0	4.2
	CL Charlie	6806.0	678.2	27.8	0.4	0.8
	CL Delta	7931.4	683.4	27.4	1.2	3.1
	BQPM11‡	6981.6	708.3	26.9	2.6	0.8
T2	CL Echo	7440.4	706.6	25.2	0.0	1.4
	CL Delta	7358.0	701.4	26.0	0.0	0.0
	CL Foxtrot	7031.8	680.8	26.7	0.6	1.4
	BQPM17‡	6834.7	699.3	25.5	3.8	1.0
T3	CL Golf	6895.6	707.9	22.8	1.0	0.0
	CL Hotel	7203.8	706.6	25.6	0.6	0.0
	CL India	7375.9	716.9	21.1	1.5	0.0
	BQPM9‡	6634.0	706.1	24.4	3.0	3.5
	CL Mean	7208.1	695.1	25.2	0.7	1.1
	BQPM Mean§	6377.8	700.1	26.4	1.8	2.7
LSD (0.05)¶		788.0	54.9	4.2	8.52	7.8
Top BQPM mean#		6816.8	704.6	25.6	3.1	1.8
		%				
Checks vs. BQPM††		11.5*	NS‡‡	NS	NS	NS
Checks vs. top BQPM		NS	NS	NS	NS	NS

* Significant at the 0.05 probability level.

† Commercial inbred lines used as checks (CL) are coded to protect confidential business information.

‡ Highest yielding of the nine BQPM lines for the tester listed.

§ Calculated from Table 2.

¶ 5% LSD calculated from the original 39 BQPM lines by tester and the nine commercial checks by tester hybrid progeny ($p < 0.05$).

Calculated from the three highest yielding BQPM lines listed above.

†† Percentage difference between checks and BQPM.

‡‡ NS, not significant at the 0.05 probability level.

3). These results coincide with similar results reported in Atlin et al. (2011) concerning the agronomic performance of QPM lines versus contemporary non-QPM lines, which demonstrate that there is little to no disadvantage associated with QPM traits pertaining to yield and other important agronomic traits.

Single samples for each of the nine BQPM lines were evaluated by the AOAC standard method for amino acid concentrations, and crude protein per line was determined via combustion analysis at the University of Missouri Experiment Station Chemistry Laboratory (Method 982.30 E(a,b,c), Horwitz, 2005a; Method 9990.03, Horwitz, 2005b) before release (Table 4). The results show a range of 0.28 to 0.51 for percentage (w/w) lysine and 0.06 to 0.09 for percentage (w/w) tryptophan. As described in Scott et al. (2009), ranges for normal inbred lines fall between 0.29 and 0.33% (w/w) lysine and 0.06% (w/w) tryptophan. In general, BQPM10 had the highest amino acid concentrations among the lines and also the highest crude protein content. BQPM14 had the lowest amino acid concentrations in general, which coincided with the lowest crude protein reading. Despite having the *o2* mutation, BQPM15 has demonstrated a relatively high methionine concentration in conjunction with a relatively low lysine concentration, which is not typical of a QPM line but may be of interest nonetheless.

Availability

Seed for each BQPM line in the amount of 50 kernels per line is available through the National Plant Germplasm System (NPGS) or by contacting Dr. Paul Scott at paul.scott@ars.usda.gov. We ask that proper recognition be given in the event that any of this germplasm is used in the development of a new cultivar, hybrid, or breeding line.

Conclusions

Since overcoming the negative pleiotropic effects of the *o2* mutation with *o2* modifiers, QPM has become a desirable crop to grow in terms of nutritive quality but continues to suffer from poorer yields relative to elite normal hybrids grown in the US Corn Belt. Temperate QPM lines BQPM9, BQPM10, BQPM11, BQPM12, BQPM13, BQPM14, BQPM15, BQPM16, and BQPM17 have higher lysine and tryptophan content than currently available inbred lines and a capacity for use in high-yielding hybrid maize production, making them good candidates for nutritive enhancement of feed corn.

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Table 4. Amino acid concentrations of the nine BQPM maize lines released.

Amino acid	BQPM9	BQPM10	BQPM11	BQPM12	BQPM13	BQPM14	BQPM15	BQPM16	BQPM17
Aspartic acid	0.96†	1.56	0.97	0.92	0.74	0.83	0.68	1.12	1.07
Threonine	0.39	0.46	0.38	0.45	0.37	0.35	0.40	0.44	0.42
Serine	0.43	0.51	0.40	0.51	0.45	0.39	0.51	0.52	0.47
Glutamic acid	1.74	2.24	1.63	2.40	1.84	1.51	2.25	2.41	1.94
Proline	0.94	1.18	0.93	1.24	0.94	0.79	1.13	1.15	1.03
Glycine	0.45	0.54	0.46	0.49	0.43	0.44	0.39	0.52	0.52
Alanine	0.64	0.79	0.58	0.85	0.69	0.55	0.86	0.80	0.69
Cysteine	0.27	0.34	0.25	0.30	0.24	0.24	0.25	0.29	0.31
Valine	0.52	0.63	0.53	0.59	0.51	0.48	0.53	0.59	0.56
Methionine	0.18	0.21	0.17	0.17	0.21	0.19	0.31	0.20	0.24
Isoleucine	0.33	0.39	0.32	0.39	0.33	0.29	0.40	0.39	0.37
Leucine	1.01	1.15	0.89	1.30	1.11	0.83	1.61	1.14	1.06
Tyrosine	0.30	0.35	0.27	0.40	0.30	0.26	0.39	0.35	0.30
Phenylalanine	0.43	0.49	0.40	0.53	0.45	0.37	0.58	0.50	0.46
Lysine	0.40	0.50	0.41	0.43	0.38	0.40	0.28	0.51	0.46
Histidine	0.39	0.46	0.39	0.44	0.36	0.36	0.34	0.43	0.41
Arginine	0.53	0.65	0.55	0.59	0.53	0.53	0.44	0.69	0.67
Tryptophan	0.07	0.09	0.06	0.08	0.06	0.07	0.06	0.09	0.09
Crude protein‡	11.27	13.93	10.77	13.02	10.97	9.93	11.55	13.61	12.46

† Values expressed as % (w/w) = g amino acid/100 g of sample.

‡ Percentage N × 6.25.

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