Development of Biofortified Maize in Southern China

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Introduction

Maize is the staple food and main source of energy for millions of poor people living in developing countries including China (especially in southwestern China), India, and in many Africa and Latin America countries (IITA, 2009; Nuss and Tanumihardjo, 2010; Li et al., 2012). Though maize serves as a good source of carbohydrates, traditional cultivars do not provide a balanced diet when consumed in large quantities. Genetic variability exists in maize for further improving its nutritional and energy content for fulfilling more of the dietary requirements of humans and animals consuming the grain. This paper discusses gains that have been made in improving the nutritional and energy content of commercial maize cultivars developed in southern China, and provides an initial assessment of the impact for the farmers. The paper discusses the development and deployment of commercial high oil maize (HOM), quality protein maize (QPM) and Provitamin A (ProVA)-enriched maize hybrids in southern China.

Materials and methods

For the biofortification of high oil maize with improved levels of ProVitamin A, HOM maize hybrids Yunrui 8, Yunyou 19 and Yunyou 21 were used. The breeding program exploited heterosis and the xenia effect to develop the biofortified hybrids. For further biofortification of quality protein maize (QPM), the inbreds CML161 and CML171 were used. The temperate maize inbred line, Hp321-1 (provided by Prof. Jianbing Yan, Huazhong Agricultural University) carrying the favorable alleles *crtRB1-5'TE-2* and *crtRB1-3'TE-1* of the crtRB1 gene for high ProVA concentration, was used as the donor (male) parent. The breeding methods included foreground selection with functional markers and background selection with simple sequence repeat (SSR) markers.

Results and discussion

Genetic architecture of oil concentration and composition in maize kernels

With the use of high-density markers and some unique genetic resources, intensive QTL analyses were carried out to identify the loci or genes which are involved in the biosynthesis of maize kernel oil in several populations including IHO, Alexho single kernel synthetic and BHO. In total, about 50 QTL or genomic regions, including 25 regions with markers on 13 chromosome arms have been identified in the materials with the IHO genetic background (Glodman et al. 1994; Berker & Rocheford, 1995; Wassom et al. 2008). With the application of genome-wide association studies (GWAS), 74 genomic regions that are significantly associated with kernel oil concentration and composition were identified, among which 26 could explain up to 83 percent of the oil concentration variation (Li et al. 2013).

Agronomic practices and breeding methods developed for exploiting high oil maize

Improving agronomic practices including the proper application of fertilizers and optimizing plant density are important steps for increasing the grain yield of HOM (Welch, 1969; Wang et al. 2002). The physiological characteristics of the source and sink in the maize kernel have significant effects on the grain yield of HOM (Wu et al. 2005). In addition, the comprehensive use of three genetic effects (TEU) of pollen xenia, heterosis and male-sterile cytoplasm for hybrid production are very important components for improving kernel quality in HOM breeding programs (Song and Cong, 1998; Chen et al. 2001; Duan et al. 2000; Chen et al. 2003). The development of Lingaoyou 1 is a successful example for the utilization of the TEU model in maize production (Gao & Wang, 2002). Xenia has significant effect on the kernel development, which impacts kernel pericarp, mesocarp, kernel shape, color and chemical component, etc. (Song et al. 1991; Bulant & Gallais, 1998). Song et al. (1991) found the xenia effects in HOM and it showed obvious metroclinous inheritance in a series of reciprocal crosses of HOM and conventional maize. Heterotic grouping, especially when focused on classifying heterotic patterns, through the use of molecular technologies, provides many advantages for increasing breeding efficiency including the increase in grain yield of HOM (Stuber et al. 1992). Crossing between a male sterile, HOM hybrid, and a superior normal oil hybrid can effectively increase grain yield and improve oil concentration in a HOM breeding program (Duan et al. 2000; Ji et al. 2002). Among the three types of male sterility systems (C-, T- and S-type), the C-type male sterility can increase the yield by 10 percent, and is commonly used in maize production (Chen et al. 1977; Weider et al. 2009).

The application of HOM in maize production

The United States is the leading country in HOM, and was the first country to begin breeding for this trait, Annually, in the U.S., HOM is planted in an area of more than 700,000 hectares. The HOM breeding program in China, was initiated relatively recently in the 1980s with some limitations: a narrow genetic base; vulnerability to diseases; and low yield. Significant progress has been achieved during the last decade, and currently HOM is grown in all maize production regions of China, where the planting area continues to grow. The environment in Yunnan province is highly favorable for germplasm improvement due to the fact that both tropical and temperate maize can flower and mature in Yunnan. We have also found significant genetic differences between temperate and tropical HOM germplasm (unpublished data). This difference can be exploited to develop high-yielding HOM by crossing temperate x tropical inbreds. As a result, several HOM hybrids including Yunrui 8, Yunyou 19 and Yunyou 21 have been released for use in southwestern China. Yunrui 8 has a yield potential up to 9848 kilograms per hectare (kg/ha) in varietal comparative testing trials, and is highly resistant to ear rot and head smut, as well as resistant to northern and southern leaf blights, based on national evaluations carried out by the Chinese Academy of Agricultural Sciences (CAAS). Since 2005, Yunrui 8 has occupied a cumulative area of 0.5 million hectares in southwestern China with a yield increase of 0.43 million tons, and an increased value of US $118 million. Therefore, it serves a very important role in the development of animal husbandry, and food safety for farmers living in the less-developed mountainous areas of the region.

The application of QPM in maize production

The QPM maize inbred lines CML161 and CML171, derived from the CIMMYT G25QPM, have been widely used in genetic research and in several breeding programs (Fan et al. 2001; Amiruzzaman et al. 2011; Setter et al. 2011; Fan et al. 2014). Some released hybrids such as HQ-2000 (released in Vietnam), INIA (released in Peru), Yunyou 78 (released in China), etc., have been developed using the inbred line CML161 as one of the QPM parents (Prasanna et al. 2001; Fan et al. 2006); others such as Yunrui 8, Yunyou 89, Qiandan 11, Quian 2609 (released in China), etc. have been developed using the inbred line CML171 as QPM parent (Cordova 2001; Zhu et al. 2003; Fan et al. 2014). These released hybrids have played a very important role in alleviating the malnutrition of children and have been used as an alternative feed ration for swine and poultry where conventional sources of lysine are from soybean meal or synthetic lysine (Lopez et al. 1992). Other QPM maize inbred lines such as YML32, CML140 and CML147 were used in southern China.

The QPM hybrids Yunrui 1 derived from YML32, Yunyou 19 derived from CML140, Yunrui 21 derived from CML147 have been widely planted in the maize production areas of southern China. Currently, the QPM maize hybrids have been planted on 0.03 million hectares (ha) per year.

## Transfer of high pro-vitamin A favorable alleles through marker assisted breeding

The high ProVA donor, Hp321-1 (provided by Prof. Jianbing Yan, Huazhong Agricultural University) and selectable markers for the favorable alleles *crtRB1-5'TE-2* and *crtRB1-3'TE-1,* were used in crosses to two important QPM lines, CML161 and CML171. The results in Table 1 show that the favorable alleles *crtRB1-5'TE-2* and *crtRB1-3'TE-1* for high ProVA concentration were transferred into the two QPM maize inbreds. The two favorable alleles *crtRB1-5'TE-2* and *crtRB1-3'TE-1* displayed a 1:1 Mendelian ratio in the F_1 and BC_1F_1 generation and segregated in the ratio of nearly 1:2:1 in the BC_2F_2.

The ProVA concentrations of the resulting populations were tested using a colorimetric assay, with the results presented in Figure 1. The mean ProVA concentration of the for the CML161 BC_2F_3 generation at BMRS and YMRS locations were 5.38 ug g^{-1} and 5.69 ug g^{-1} with the across location mean of 5.54 ug g^{-1}, which was significantly higher than that of recurrent parent with the ProVA concentrations of 1.61 ug g^{-1} and 1.72 ug g^{-1} and across location mean of 1.67 ug g^{-1}, respectively (p<0.01). Similar results were obtained for the CML171 BC_2F_3 generation with a mean ProVA concentration of 7.58 ug g^{-1} and 8.81 ug g^{-1} with an across location mean of 8.20 ug g^{-1}, which was significantly higher than that of recurrent parent with the ProVA concentrations of 1.80 ug g^{-1} and 1.90 ug g^{-1} with an across location mean of 1.85 ug g^{-1}, respectively (P<0.01). The results indicated that converted QPM inbred lines of CML161 and CML171 obtained through backcrossing had mean ProVA concentrations 3.32 and 4.43 fold higher after foreground and background selection using molecular markers. Generally, the level of ProVA at the YMRS location tended to higher than that of BMRS. However, it was not significant (p<0.05). The results indicated that ProVA concentrations of maize might be affected by the interaction of genotype and environment, but is mainly controlled by genetic factors. The converted QPM lines can be used for developing new QPM hybrids biofortified in ProVA, for improving the nutritional benefits of the grain for human and animal consumption.

Table 1. Foreground selection for BC_1F_1, BC_2F_1 and BC_2F_2 generations in the CML161 and CML171 populations using functional markers.

Inbred	Generation	Location[1]	Replicate	Total plants	Plant with homozygous allele	Plant with heterozygous allele	Plant without target allele	P value (X^2 test)
	BC_1F_1	-	-	597	290	-	307	0.4866
	BC_2F_1	-	-	779	410	-	369	0.1418
CML161		BMRS	1	737	187	374	176	0.4161
	BC_2F_2		2	691	153	364	174	
		YMRS	1	1268	322	588	358	0.0001
			2	1184	307	541	336	
	BC_1F_1	-	-	462	218	-	244	0.2264
	BC_2F_1	-	-	1055	497	-	558	0.0604
CML171		BMRS	1	702	162	391	149	0.0002
	BC_2F_2		2	852	201	466	185	
		YMRS	1	1401	358	655	388	0.0226
			2	1374	373	660	341	

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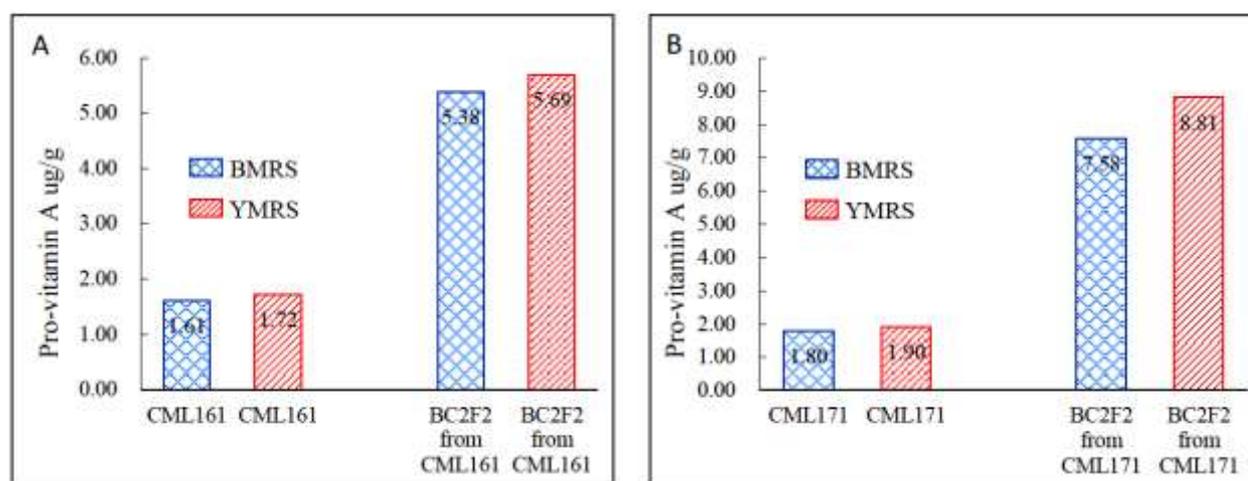
Figure 1. The increase in ProVA concentrations in the QPM maize though molecular marker assisted foreground and background selections; (A) CML161 population; (B) CML171 population.

Effectiveness of the marker-assisted backcross procedure

In the BC_1F_1 generation, the recovery rate of plants with *crtRB1-5'TE-2* and *crtRB1-3'TE-1* alleles were 77.4 percent and 84.5 percent in the CML 161 and CML 171 populations, respectively (Table 2). In the BC_2F_2 generation, the recovery rate of plants with *crtRB1-5'TE-2* and *crtRB1-3'TE-1* alleles were 89.9 percent and 92.1 percent in the CML 161 and CML 171 populations, respectively. The results from both populations showed that the individuals of two consecutive backcross generations using molecular marker assisted selection had recovered to a similar homozygous level of the recurrent parent.

A commercial high ProVA maize hybrid for Southwestern China

Following several years of collaborative work with China Agricultural University, the high ProVA hybrid YR 506, with a mean ProVA content above >15 ug/g, has been released for use in southwestern China. In multi-locational regional varietal trials carried out in 2011-2013, the mean yield was 10,032 kg/ha with an 8% yield increase compared to local commercial check hybrids.

Table 2. Background selection for BC_1F_1 and BC_2F_2 generations of CML161 and CML171 populations using SSR markers.

Generation	Population	Allele of *crtRB1*	No. of plant	No. of polymorphic marker	Range of recovery rate %	Average recovery rate %
BC_1F_1	CML161	*crtRB1-5'TE-2* & *crtRB1-3'TE-1*	276	98	63.8-90.8	77.4
	CML171	*crtRB1-5'TE-2* & *crtRB1-3'TE-1*	210	89	75.8-95.5	84.5
BC_2F_2	CML161	*crtRB1-5'TE-2*	187	98	81.9-87.2	84.8
		crtRB1-5'TE-2 & *crtRB1-3'TE-1*	374	98	85.1-94.7	89.9
		crtRB1-3'TE-1	176	98	84.0-96.8	90.4
	CML171	*crtRB1-5'TE-2*	162	89	80.9-90.4	84.8
		crtRB1-5'TE-2 & *crtRB1-3'TE-1*	391	89	88.3-96.8	92.1
		crtRB1-3'TE-1	149	89	90.4-97.9	93.6

References

Amiruzzaman M, Islam M A, Pixley K V, et al. Heterosis and combining ability of CIMMYT's tropical × subtropical quality protein maize germplasm. International Journal of Agricultural Sustainability, 2011, 3:76-81.

Berke T, Rocheford T R. Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Science, 1995, 35: 1542-1549.

Bulant C, Gallais A. Xenia effects in maize with normal endosperm: I. Importance and stability. Crop Science, 1998, 38: 1517-1525.

Chen S J, Song T M. Identification haploid with high oil xenia effect in maize. Acta Agron Sin, 2003, 29(4): 587-590. (in Chinese with abstract in English)

Chen S J. From the success of high oil corn to the innovation of corn breeding. Journal of Agricultural Science and Technology, 2001, 3(1): 59-63. (in Chinese with abstract in English)

Chen W C, Luo F H, Ji L Y. Some genetic aspects of the C-type cytoplasmic male-sterility in maize and its use in breeding. Acta Agromonica Sinica, 1979, 4: 21-28. (in Chinese with abstract in English)

Cordova H. Quality protein maize: improved nutrition and livelihoods for the poor. Maize Research Highlights 1999-2000, CIMMYT pp 26.

Duan M X, Song T M, Fan H W, et al. Utilizing genetic markers mixing-pollento estimate heterosis value and xenia effect value of high oil corn. Journal of China Agricultural University, 2000, 5 (3): 45-50. (in Chinese with abstract in English)

Fan X M, Chen H M, Tan J, et al. Analysis of yield combining ability of subtropical tropical quality protein maize inbred line and temperate normal maize inbred line. Journal of Maize Science, 2006, 14:12-15. (in Chinese with English abstract)

Fan X M, Tan J, Huang B H, et al. Analyses of combining ability and heterotic group of yellow grain quality protein maize inbreeds. Hereditas 2001, 23:547-552.

Fan X M, Zhang Y D, Yao W H, et al. Reciprocal diallel crosses impact combining ability, variance estimation, and heterotic group classification. Crop Science, 2014, 54:89-97.

Gao G L, Wang X D. The model on multipurpose utilization of three genetic effects in high oil maize. Journal of Shanxi Teacher's University Natural Science Edition, 2002, 16(4): 68-70. (in Chinese with abstract in English)

Goldman I L, Rocheford T R, Dudley J W. Molecular marker associated with maize kernel oil concentration in an Illinois high protein × Illinois low protein cross. Crop Science, 1994, 34: 908-915.

International Institute of Tropical Agriculture (IITA), 2009. Maize Production, Consumption and Harvesting. Retrieved from: www.iita.org, (Accessed on: 9th September, 2011).

Ji L Y, Hu Y M Li Y L, et al. Studies on the recross heterosis effect of male-sterile single-hybrids in corn. Agricultural Sciences in China, 2002, 1(5): 491-497. (in Chinese with abstract in English)

Li H, Peng Z Y, Yang X H, et al. Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nature Genetics, 2013, 45: 43-51.

Li J, Lammerts van Bueren E T, Jiggins J, et al. Farmers' adoption of maize (*Zea mays* L.) hybrids and the persistence of landraces in Southwest China: implications for policy and breeding. Genetic Resources and Crop Evolution, 2012, 59:1147-1160.

López-Pereira M A. The economics of quality protein maize as an animal feed: Case studies of Brazil and El Salvador, 1992, CIMMYT, Mexico, DF.

Nuss E T, Tanumihardjo S A. Maize: A paramount staple crop in the context of global nutrition. Comprehensive Reviews in Food Science and Food Safety, 2010, 9:417-436.

Prasanna B M, Vasal S K, Kassahun B, et al. Quality protein maize. Current Science, 2001, 81:1308-1319.

Setter T L, Yan J, Warburton M, et al. Genetic association mapping identifies single nucleotide polymorphisms in genes that affect abscisic acid levels in maize floral tissues during drought. Journal of Experimental Botany, 2011, 62:701-716.

Song T M, Cong Z J. Utilizing cytoplasmic male sterility, single hybrid re-cross heterosis and xenia effects to produce high yield and high-oil commercial corn. Beijing: China Agricultural Scientech Press, 1998: 216-221. (in Chinese)

Song T M, Wu H P, Dai S H, et al. Genetic effects of corn kernel oil controlling genes in reciprocal crosses. Journal of China Agricultural University, 1991, 17(1): 15-22. (in Chinese with abstract in English)

Stuber C W, Lincoln S E, Wolff D W, et al. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics, 1992, 132: 823-839.

Wang P, Wang W D, Wang Q X. Effect of plant density on yield and grain oil content in high oil corn 298. Journal of Maize Sciences, 2002, 10(2): 49-52. (in Chinese with abstract in English)

Wassom J J, Mikkelineni V, Bohn M O, et al. QTL for fatty acid composition of maize kernel oil in Illinois high oil 9 B73 backcross-derived lines. Crop Science, 2008, 48: 69-78.

Weider C, Stamp P, Christov N, et al. Stability of cytoplasmic male sterility in maize under different environmental conditions. Crop Science, 2009, 49: 77-84.

Welth L E. Effects of N, P, and K on the percent and yield of oil in corn. Agronomy Journal, 1969, 61 (6): 890-891.

Wu Z F, Wang K J, Dong S T, et al. Physiological characteristics of source and sink in high oil corn I. The Grain yield of high oil corn limited by leaf source Capacity. Acta Agronomica Sinica. 2005, 31(3): 283-288. (in Chinese with abstract in English)

Zhu Y F, Chen Z H, Gao X. The application of exotic maize germplasm in Guizhou. Seeds, 2003, (4):67-68. (in Chinese with English abstract).