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Combining Ability of Inbred Lines Derived from Quality Protein Maize Populations

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ABSTRACT

Quality protein maize (QPM) with improved kernel quality over o_2o_2 soft genotypes was developed by introducing modifier genes and selecting for a hard, vitreous endosperm in o_2o_2 germplasm at the International Maize and Wheat Research Center (CIMMYT). Three QPM populations developed by CIMMYT were used in the research. S_0-plants of preferred morphological characters were self-pollinated to produce S_1 until S_3 lines. Ten S_3 inbred lines were examined for the opaque-2 gene using the phi057 marker and a diallel cross was made. The experiment was conducted in a 7×7 simple lattice design at the National Corn and Sorghum Research Center, Thailand. The results showed that 10 inbred lines were detected with polymorphism the same as opaque-2 maize (o_2o_2) but were different from non-opaque-2 maize. The protein content in endosperm of these inbred lines ranged from 7.76 to 8.61% while those of opaque-2 and non-opaque-2 maize contained about 8.45 and 8.73%, respectively. However, the protein content of inbred lines, the diallel cross and check variety were not significantly different. Means of grain yield (t ha^{-1}) were 4.48 for the diallel cross (F_1), 1.97 for inbred lines, 3.30 for opaque-2 and 6.61 for non-opaque-2 hybrid check. However, grain yield of the best diallel cross was 6.10 t ha^{-1} which was not significantly different from the non-opaque-2 hybrid. Protein contents were not significant among the diallel cross, opaque-2 and non- opaque-2 hybrids. Tryptophan content in endosperm of the diallel cross was higher than non-opaque-2 hybrids. Inbred P10 had the best combination of GCA effects for grain yield. Moreover, inbred lines, P1, P7, P8 and P9 also gave positive GCA effects.

Key words: QPM, tryptophan content in endosperm, protein, molecular marker , combining ability

INTRODUCTION

Although maize is mainly considered as a carbohydrate source, it is also an important protein source because of its considerably total protein yield per hectare. Grain protein quantity in ordinary maize is low (80-110 g kg^{-1}) and of poor quality because it has low levels of the amino acids, lysine and tryptophan (Bjarnason and Vasal, 1992). The discovery of the recessive Opaque-2 (o_2) mutant gene increases the proportion of the better-balanced proteins in the endosperm of maize kernel. The most significant effect of the o_2o_2 genotype is the increased content of the amino

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acids, lysine and tryptophan, and reduced leucine levels, in maize endosperm protein (Mertz *et al*., 1964). Yet, it expresses negative pleiotropic effects on the grain quality such as lower density, susceptibility to pests and diseases and a floury appearance (Vasal, 2001).

The International Maize and Wheat Research Center (CIMMYT) has developed quality protein maize (QPM) that improves kernel quality characteristics over o_2o_2 soft genotypes, by introducing modifier genes and selecting for a hard, vitreous endosperm in o_2o_2 germplasm (Vasal, 2001). The discovery of modifier genes creates new hope for the utilization of opaque-2 maize. In maize kernels, modifier genes restore the endosperm to various degrees of normality. Several studies have shown that modified endosperm is quantitatively inherited (Sriwatanapongse *et al*., 1974; Motto, 1979; Ortega and Bates, 1983; Wessel-Beaver *et al*., 1985). The *opaque-2* is a recessive gene located on chromosome 7 and the modifiers behave as a multigenic trait. Conventional breeding procedures have been used to convert commercial lines to QPM but the procedure is tedious and time consuming. With the development and access to reliable PCR-based allele specific markers such as simple sequence repeat (SSR), DNA marker-assisted selection (MAS) became an attractive option for detecting simple inherited traits in the newly developed cultivars with higher yield potential (Ribaut and Hoisington, 1998; Pixley and Bjarnason, 2002; Babu *et al*., 2005). The previous and recent studies have reported yields of QPM hybrids competitive with the best locally available normal-endosperm cultivars for many tropical sites. It, therefore, would be interesting to grow hybrid varieties of QPM in Thailand. The objectives of this study were: (i) to improve inbred lines for quality protein maize (QPM) using marker-assisted selection (MAS), (ii) to estimate GCA and SCA effects for grain yield, tryptophan and protein content and agronomic traits of QPM inbred lines.

MATERIALS AND METHODS

Plant materials

Three QPM populations developed by the International Maize and Wheat Research Center (CIMMYT), namely Pop61C_1 (Tropical maize Early Flowering Yellow Flint; TEYF), Pop62C_6 (Tropical maize Late Flowering White Flint; TLWF) and Pop65C_6 (Tropical maize Late Flowering Yellow Flint; TLYF) were obtained from the Nakhon Sawan Field Crop Research Center, Nakhon Sawan Province, Thailand. The seeds were sown at the National Corn and Sorghum Research Center, Kasetsart University, Nakhon Ratchasima Province, in March 2003. S_0-plants of preferred morphological characters, i.e., early flowering, short anthesis-silking interval (ASI), healthy plants and the other desirable agronomic traits were selected. All selected plants were self-pollinated to produce S_1 lines. Selected S_1 lines from each population were sown in September, 2003. Ten selected plants from each S_1 line were self-pollinated to produce S_2 lines. S_2 lines were sown in May, 2004 and selected S_2 plants were self-pollinated to produce S_3 lines.

Inbred polymorphism analysis for opaque-2 gene

Ten inbred lines (S_3) derived from QPM populations with yellow and white grains were checked for the opaque-2 gene using a phi057 marker (Jompuk *et al*, 2006). Inbred lines P1-P4 were derived from Pop65C_6, P5-P8 from Pop61C_1 and P9-P10 from Pop62C_6. DNA was extracted from 150-250 mg fresh leaves of each inbred using the method described by Agrawal *et al.* (1992). Simple sequence repeat (SSR) marker, phi057 (KU-VECTOR Custom DNA Laboratory, Kasetsart University) was used to detect the *opaque-2* plants. This marker gave amplification product of about 140 - 160 bp (Chin *et al*., 1996).

Amplification was done in 20 µl reaction mixture containing 1x reaction buffer, 10% glycerol, 2.5 mM $MgCl_2$, 150 µl of mixed dNTP, 0.3 µM of each primer, 1 U of Taq DNA polymerase and 50 ng of genomic DNA. Amplifications were performed in a thermocycler programmed for the first denaturation step of 1 min at 94°C, followed by 30 cycles of 30 s at 94°C, 1 min at 58-60°C, 1 min at 72°C and a final extension of 5 min at 72°C. For the phi057 marker, the amplified fragments were separated on a 6% polyacrylamide denaturing gel in 1xTBE buffer and silver-stained.

Diallel cross

These ten inbred lines were made a diallel cross using fixed model method IV (Griffing, 1956) in May, 2005 at the National Corn and Sorghum Research Center, Nakhon Ratchasima Province. Forty five combinations (F_1) were formed. At harvesting, the healthy and well pollinated ears were harvested. The diallel cross yield trial consisted of 45 F_1 hybrids, two commercial hybrids and two QPM populations as check varieties. The experiment was conducted in a 7×7 simple lattice design, with two replications in October, 2005 at the same research center. Each plot consisted of two 5-meter rows with 75 cm between rows and 25 cm between plants within row. Basal fertilizer 15-15-15 was applied at the rate of 312 kg ha^{-1} before planting. Atrazine mixed with Pendimethalin, a pre-emergence herbicide, was used at the rate of 4 kg ha^{-1} and 4 l ha^{-1}, respectively. After 2 weeks, plants were thinned to 1 plant $hill^{-1}$ or a population size of 53,331 plants ha^{-1}. At the 4^{th} week, 312 kg ha^{-1} of ammonium sulfate was topdressed. Agronomic traits, such as 'days to silking' (the number of days from planting until 50% of the plants show silks), 'days to anthesis' (the number of days from planting until 50% of the plants shedding of the pollens), plant height (distance in centimeters from the ground to the top of tassel), ear height (distance in centimeters from the ground level to the main ear-bearing node), grain moisture content at harvesting (using a moisture tester) and grain yield (combine-harvested grain weight expressed in ton per hectare and adjusted to 15 percent standard moisture content), were collected.

Tryptophan and total protein analysis

For tryptophan and total protein analysis, twenty five seeds from each hybrid were collected and soaked in distilled water for 25 min before removing pericarps and embryos. The endosperms were air-dried overnight and grounded (to approximately 0.5 mm) in a cyclone mill and wrapped in a commercial filtered-paper envelope to defat with 100% hexane in a Soxhlet-type continuous extractor. The defatted ground samples were analyzed for tryptophan content using a spectrophotometer as described by Villegas and Mertz (1971) and the protein content was measured using the microkjeldahl method (Bailey, 1967).

Statistical analyses

Data from the diallel experiment were analyzed according to the lattice design using a 7×7 simple lattice design for all characteristics by R computer program (R Development Core Team, 2006). Significances of hybrid, general combining ability (GCA), and specific combining ability (SCA) mean squares were estimated with F-tests. GCA effects of the parents and SCA effects of the crosses were estimated following Griffing's Method IV, for diallel analysis (Griffing, 1956).

RESULTS AND DISCUSSION

Inbred polymorphism analysis for the *opaque-2* gene

With the primer phi057, the amplified products of 10 inbred lines were detected with polymorphism similar to opaque-2 but differed from non-opaque-2 maize (sw1). The protein content in endosperm of these inbred lines ranged

from 7.76 to 8.61% while opaque-2 and non-opaque-2 maize contained 8.45 and 8.73%, respectively. However, the protein contents among all tested maize were not significantly different (Figure 1). The results agreed with Jompuk *et al*. (2006) and Vasal (1994) who indicated that the protein content in maize endosperm was the same for normal and opaque-2 maize. But tryptophan content in protein of opaque-2 almost doubled in amount when compared to normal maize (Prasanna *et al*., 2001). The phi057 marker could detect amplified products of 160 bp in non-opaque-2 and 170 bp fragments in opaque-2 (Babu *et al*., 2005; Jompuk *et al*., 2006). The phi057 is a co-dominant marker and could detect homozygous dominant (O_2O_2), heterozygous (O_2o_2) and homozygous recessive (o_2o_2) plants separately which is useful for marker-assisted selection for the *opaque-2* gene (Ribaut and Hoisington, 1998). These results showed that 10 inbred lines were opaque-2, which was different from the normal maize.

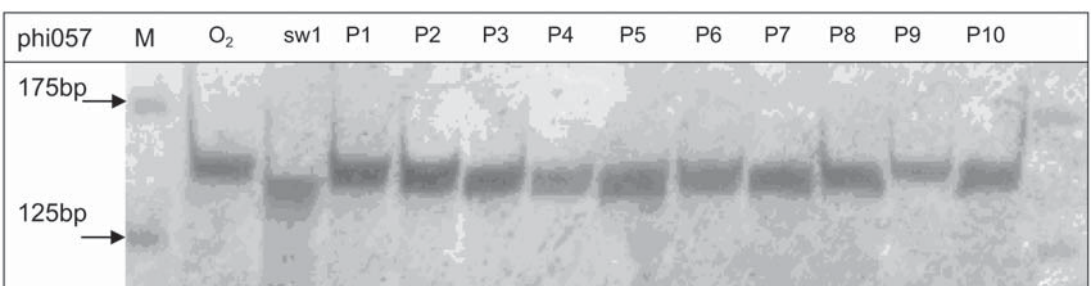
Analysis of variance

Significant differences between treatments were observed for grain yield, tryptophan content in protein, ear height, plant height, days to male and female flowering except protein content, moisture content and shelling. Significant differences between general combining ability effects (GCA effects) were observed only for grain yield, whereas specific combining ability effects (SCA effect) were significantly different for both grain yield and male flowering (Table 1).

Combining ability analysis

Grain yield

Mean grain yield (t ha^{-1}) was 4.48 for the diallel cross (F_1), 1.97 for inbred lines, 3.30 for opaque-2 and 6.61 for non-opaque-2 check (Table 2). By contrast comparison, grain yield was significantly different between F_1 and opaque-2, F_1 and non opaque-2, and opaque-2 and non opaque-2. There were also significant differences between the diallel cross (Table 1). GCA and SCA effects of these traits were significantly different. The mean grain yield, GCA effects and SCA effects of the diallel cross are shown in Table 3. The ratio of GCA: SCA (2.32*) was significantly different at a probability of 0.05, which showed that additive gene effects played an important role for this trait. These results agreed with Sriwatanapongse *et al*. (1974) who indicated that additive gene effects had more important role on grain yield of opaque-2 maize than dominant gene effects.



1/ = non-significant differences

2/** = highly significant differences, $LSD_{0.01}$= 0.132

Figure 1 Polymorphism of 10 inbred lines (s_3), opaque-2 (o_2) and non-opaque-2 (sw1) using phi057 on 6% polyacrylamide and the percentage of protein and tryptophan content in protein.

Table 1 Mean squares of grain yield and other traits of F_1-hybrid; a comparison between F_1-hybrid and QPM populations and single cross (commercial), trial conducted at the National Corn and Sorghum Research Center in early 2005 rainy season.

Source of variation	d.f	MS								
		Grain Yield (ton ha^{-1})	Protein (%)	Tryptophan in protein (%)	Moisture content (%)	Shelling (ratio)	Ear height (cm)	Plant height (cm)	Day to anthesis (d)	Day to silking (d)
Replications	1	0.092^{NS}	0.168^{NS}	0.003^{NS}	12.715	0.024	0.2^{NS}	864.1**	18.9**	1.0^{NS}
Treatments	48	2.255**	0.939^{NS}	0.023*	1.433^{NS}	0.008^{NS}	167.4**	23736.5**	5.8**	6.0**
F_1-hybrid (F_1)	44	1.292**	-	0.017^{NS}	-	-	146.4*	256.0**	4.7**	5.2*
F_1-vs opaque-2	1	5.296**	-	0.001^{NS}	-	-	355.2*	7849.4**	0.8^{NS}	0.1^{NS}
F_1 vs non-opaque-2	1	26.419**	-	0.378**	-	-	1187.8**	4578.8**	63.0**	58.2**
Opaque-2. vs non-opaque-2	1	28.125**	-	0.207**	-	-	140.3^{NS}	154.9^{NS}	40.5**	32.0**
GCA	9	1.541**	-	-	-	-	204.8**	308.4**	6.9**	6.5**
SCA	35	0.664**	-	-	-	-	39.4^{NS}	81.6^{NS}	1.20**	1.6^{NS}
Blocks/reps. (adj)	12	0.098	0.875	0.005	0.858	0.006	88.422	48.175	5.224	5.973
Error	36	0.211	0.522	0.012	1.020	0.005	70.236	113.559	0.971	1.842
Total	97									
CV (%)		10.126	9.66	12.59	12.09	9.40	13.15	7.41	1.89	2.57
LSD: 0.05		0.932	-	0.20	-	-	17.43	19.82	2.19	2.98
: 0.01		1.125	-	0.27	-	-	23.37	26.45	2.94	3.99

- = no determine, ^{NS} = non significant differences, * = significant differences, ** = highly significant differences

Table 2 Means of yield and other traits of F_1-hybrid, inbred lines, opaque-2 and single cross hybrids

Maize	Mean ± sd[1/]								
	Yield (ton ha^{-1})	Protein (%)	Tryptophan in protein (%)	Moisture content (%)	Shelling (ratio) (cm)	Ear height (cm)	Plant height (d)	Days to anthesis (d)	Days to silking
F_1-hybird	4.48±0.92	7.84±0.91	0.81±0.12	8.17±1.13	0.78±0.08	64±10.50	130±13.66	57±1.89	57±2.01
Inbreds	1.97±0.36	7.88±0.69	0.73±0.07	7.5±1.33	0.8±0.04	58±13.05	117±13.05	53±2.59	51±2.63
Opaque-2[2/]	3.30±0.07	8.18±0.17	0.82±0.02	8.70±1.97	0.83±0.02	74±4.54	175±7.38	57±1.73	57±1.63
Non-opaque-2[3/]	6.61±0.01	7.76±0.80	0.42±0.21	8.05±0.80	0.84±0.04	78±10.09	161±9.37	61±0.41	61±0.75

[1/] = Standard deviation
[2/] = Population of quality protein maize
[3/] = Single cross hybrids

Grain yield of the diallel cross ranged from 2.45 to 6.10 t ha^{-1} (Table 3). The highest grain yield was the cross of P9 × P4 where these inbred lines were extracted from different populations. On the other hand, the lowest grain yield was the cross of P6 × P5 where these inbred lines came from the same population. Inbred P10 had the best combination of GCA effects for grain yield. Moreover, inbred lines, P1, P7, P8 and P9 also gave positive GCA effects. Therefore, P9 and P10 could be used as tester lines for the quality protein maize program or the positive GCA effect lines could be used as the lines to form new quality protein maize populations. Grain yield of the diallel cross was significantly different from the commercial hybrids (single cross hybrid varieties) (Table 1 and 2). However, yields of the top ten crosses (>5.22 kg ha^{-1}) were not different from the commercial hybrid checks. Grain yield results indicated that a single cross hybrid of the opaque-2 gene with vitreous endosperm could possibly be grown in Thailand. QPM is likely to gain wider acceptance if hybrids are produced agronomic performance similar to normal hybrids and retain an enhanced nutritional quality (Babu *et al*., 2005). Moreover, the previous and recent studies have reported yields of CIMMYT QPM hybrids that are competitive with the best locally available normal-endosperm cultivars for many tropical sites (Bjarnason and Vasal, 1992; Pixley and Bjarnason, 1993 and 2002). Data from (non-CIMMYT) QPM trials in Brazil, Ghana, Guatemala and South Africa also documented similar yields of QPM relative to the test of available normal-endosperm maize checks (Mertz, 1992). QPM hybrids had several advantages over the open-pollinated QPM varieties such as more uniform and stable endosperm modification, and less monitoring for ensuring protein quality in seed production. There was an increasing number of elite exotic QPM inbreds being developed outside Thailand. Therefore, characterization and selection for adaptation of these tropical yellow QPM inbreds

could enhance protein quality, increase genetic variability for quality, improve productivity and be a source of valuable genes for abiotic and biotic stress resistances. Besides, if these inbred lines could not adapt to the environments, the backcross method could be used to transfer the opaque-2 and marker-assisted selection could be applied for this trait (Jompuk *et al*., 2006).

Protein and tryptophan content

The protein contents in endosperm of the diallel cross did not significantly differ among themselves and from the check varieties (Table 1). The protein content ranged from 6.15 to 9.18% for diallel cross (Table 4). Average protein content was 7.84% for diallel cross, 8.18% for opaque-2 and 7.76% for non opaque-2. There was no significant difference between general and specific combining ability of protein content in endosperm. Tryptophan content in maize endosperm was not significantly different between the diallel cross and opaque-2, and average tryptophan contents were 0.81 and 0.82% of protein of the diallel cross and opaque-2, respectively (Table 2). Tryptophan content was higher in the diallel cross (0.81%) than non-opaque-2 maize (0.42%) (Table 1 and 2). These results showed that QPM varieties had almost double the amount of tryptophan compared

Table 3 Means yield of F_1-hybrid in t ha^{-1} (below the diagonal), general combining ability (on the diagonal line) and specific combining ability (above the diagonal) in the diallel cross

Parents	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
P1	**0.29**	-1.26	0.63	0.93	1.11	-0.97	-0.65	0.20	-0.54	0.37
P2	3.08	**-0.44**	-0.20	0.36	-0.01	-0.18	-0.51	1.33	0.40	0.08
P3	5.38	3.82	**-0.03**	-0.89	0.51	0.44	0.90	-1.50	0.29	-0.17
P4	5.67	4.37	3.53	**-0.04**	-1.36	-0.43	-0.31	0.42	1.21	-0.04
P5	5.58	3.72	4.65	2.87	**-0.31**	-0.85	-0.53	0.69	0.27	0.07
P6	3.12	2.99	4.02	3.14	2.45	**-0.87**	0.21	-0.34	0.93	1.01
P7	4.29	3.70	5.52	4.30	3.81	3.99	**0.16**	0.72	-0.01	0.27
P8	5.22	5.62	3.20	5.10	5.10	3.51	5.61	**0.24**	-1.21	-0.32
P9	4.69	4.90	5.20	6.10	4.89	4.99	5.00	3.96	**0.45**	-1.25
P10	5.70	4.68	4.84	4.95	4.79	5.17	5.47	4.95	4.24	**0.55**

Table 4 Percentages of protein in maize endosperm of F_1-hybrid (below the diagonal) and of tryptophan in protein of maize endosperm (above the diagonal) in the diallel cross.

Parents	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
P1		0.792	0.660	0.847	0.783	0.645	0.786	0.866	0.648	0.756
P2	8.64		0.928	0.835	0.869	0.783	0.724	0.847	0.830	0.743
P3	8.34	6.82		0.786	0.545	0.928	0.781	0.825	0.636	0.882
P4	6.15	8.10	9.18		0.794	0.889	0.738	0.862	0.793	0.868
P5	7.82	7.96	8.49	8.14		0.819	0.901	0.868	0.857	0.783
P6	8.80	7.58	8.39	7.48	8.74		0.883	0.770	0.760	0.905
P7	7.88	7.74	8.24	8.68	8.48	8.58		0.729	0.871	0.948
P8	7.40	6.85	8.12	7.23	9.07	7.71	7.09		0.780	0.890
P9	7.80	7.73	8.51	8.55	8.00	7.83	6.89	7.88		0.976
P10	7.15	6.98	7.12	6.82	8.15	6.51	8.04	7.27	7.74	

to normal maize, but they had similarly overall protein content (Jompuk *et al*., 2006). Moreover, the parental lines had the same tryptophan content as the diallel cross.

CONCLUSION

Inbred lines derived from quality protein maize (QPM) populations were controlled by the opaque-2 and some modifying genes, and their crosses had similar protein content in endosperm as normal maize. Yet, the percentage of tryptophan content in protein had almost two times that of normal maize. Moreover, grain yield of the best diallel cross (P9 x P4) about 6.10 t ha^{-1} was not significantly different from the normal maize (commercial single cross hybrids; 6.61 t ha^{-1}). Inbred P10 had the best combination of GCA effects for grain yield. Inbred lines, P1, P7, P8 and P9 also gave positive GCA effects. Therefore, these inbred lines could be used as tester lines for the quality protein maize program or the positive GCA effect lines could be used as the lines to form new quality protein maize populations. In conclusion QPM hybrids could possibly be grown in Thailand without any difference in grain yield and had greater advantage, in terms of grain nutrition, than the normal maize.

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