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EVALUATION OF QUALITY PROTEIN MAIZE (*OPAQUE-2*) HYBRIDS AS SUBSTITUTES FOR CONVENTIONAL MAIZE CULTIVARS GROWN IN SRI LANKA

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ABSTRACT

Breeding maize with improved protein quality was started in mid 1960s with the discovery of mutants, such as Opaque-2 that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in the maize endosperm. Research programme was conducted in three seasons, *yala* 2009, *maha* 2009/2010 and *yala* 2010 at Field Crops Research and Development Institute, Mahailluppallama, with the objective of evaluating the suitability quality protein maize single cross hybrids, developed from seven QPM inbred lines (CML161, CML171, CML164, CML168, CML189, CML193 and CML194) received from International Maize and Wheat Improvement Centre (CIMMYT), Mexico. Five promising QPM hybrids, KH74, KH76, KH156, KH157 and KH38 were evaluated along with Sampath, and Pacific 984 in National Coordinated Varietal Test (NCVT) at research stations representing maize growing areas. During *yala* season 2010 five promising QPM hybrids were evaluated along with Sampath, and Pacific 984 in Varietal Adaptability Test (VAT) in farmer fields in maize growing districts. Genotype x environment interaction of multi-locational trials was analysed using Variance Component method. Adaptability (D) and stability parameters (S^2) were also calculated. Results revealed that three QPM hybrids, KH74, KH156 and KH157 showed comparable adaptability with check hybrids over the location for two seasons but the cob appearance of KH156 was poor. VAT trials conducted during *yala* 2010 showed that QPM hybrids KH74, KH76 and KH157 have better adaptability comparable to commercial check hybrid, Pacific984. These hybrids also had a higher tryptophan contents compared to check hybrids. Therefore, KH74, KH76 and KH157 were selected as candidate QPM hybrid for varietal release.

KEYWORDS: CIMMYT, Maize hybrid, QPM hybrid, Quality Protein.

INTRODUCTION

Maize (*Zea mays* L.) occupies the second largest extent of the total cultivated extent of cereals in Sri Lanka, where, about 95 % is rice, 4 % is maize and the rest is occupied by millets. At present the average annual extent under maize cultivation is about 88,857 ha (AgStat, 2012) and national maize requirement is about 176,010 t, which is mainly utilized in animal feed production. In addition, a considerable amount of maize grains are utilized in the formation of a weaning food for lactating mothers and for children. Other forms of uses such as maize flakes, oil and flour are also imported. As the estimated national production is around 239,134 t and at present Sri Lanka is self-sufficient in maize production.

Maize plays an important role in human and animal nutrition mainly in developing countries due to high content of carbohydrates, fats, proteins and some of the important vitamins and minerals. Cereal proteins, however, have poor nutritional value for monogastric animals including humans due to reduced content of essential amino acids such as lysine, tryptophan and threonine. For the human nutrition, lysine is the most important limiting amino acid in the maize endosperm protein followed by tryptophan (Prasanna *et al.*, 2001).

Quality protein maize, or "QPM", contains naturally occurring opaque-2 mutant which alters protein composition of the maize endosperm resulting in increased concentration of lysine and tryptophan (Mertz *et al.*, 1964). The kernel homozygous for the single recessive gene opaque-2 had significantly increased lysine and tryptophan contents in the endosperm. It contains around 4 % lysine 1 % tryptophan which is about 3-4 times higher in comparison to normal corn. Because of the increased concentration of these two essential amino acids, digestibility and nitrogen uptake is high in QPM relative to normal-endosperm maize. The biological value (amount of N that is retained in the body after digestion) of QPM is about 80 %, while it is 40-57 % in normal maize (Bressani, 1995). Introduction of QPM will also improve nutrient security providing better nutrition for the needy sectors of the population. As suggested by (Bressani, 1995), children scuffed from malnutrition in maize-dependent areas benefited a 12 % increased growth rate for weight and a 9 % increased growth rate for height when substituted QPM for conventional maize.

In 1998, improved QPM inbred lines received, from International Maize and Wheat Improvement Centre (CIMMYT), Mexico were used to develop QPM hybrids locally at Field Crops Research and Development Institute (FCRDI). Mahailluppallama. Further, General Combining Ability of parental inbred lines and Specific Combining Ability of single crosses mated in half diallel design were also assessed by Karunaratne and Suriyagoda (2008). Therefore, the present study was conducted in three seasons from *yala* 2009 to *yala* 2010 seasons at different locations in the Dry zone of Sri Lanka with the objective of evaluating QPM hybrid varieties as a possible substitute for the ordinary maize varieties.

MATERIAL AND METHODS

Seven QPM inbred lines, CML161, CML171, CML164, CML168, CML189, CML193 and CML194 were received from CIMMYT, Mexico. These inbred lines (parental inbred lines) were used to develop single cross hybrids, CML161 x CML171 (KH74), CML161 x CML194 (KH76), CML171 x CML193 (KH156), CMLL171 x CML194 (KH157) and CML193 x CML194 (KH 38) in the crossing blocks of the research fields of FCRDI.

Hybrid seed production of the promising crosses

Crossing blocks of parental inbred lines were established considering their days to flowering, since it is important to synchronize the flowering of male and female parents to make relevant crosses. The tassel of female parent was removed before pollinating from the male parent. Ear shoots of female parent were covered by a shoot bag (water proof paper bag or transparent polyethylene bag) before silks are being visible. Viable pollen grains from relevant male parent were collected to the tassel bag (water proof paper bag) which was placed onto tassel on previous day and they were transferred on to the covered silks of female parent. Pollination was done in the morning from 8.00 am to 11.00 am in order to ensure the pollen viability. The pollinated ear shoots were covered using tassel bags for two weeks to avoid contaminations. Aforesaid procedure was used to produce seeds of five selected promising crosses KH74, KH76, KH156, KH157 and KH38.

Evaluation of promising QPM hybrids in National Co-ordinate Varietal Trials (NCVT)

During *yala* 2009, *maha* 2009/10 and *yala* 2010 seasons, five promising QPM hybrids were evaluated using non QPM hybrid Sampath and Pacific 984 (as check hybrid varieties) in 5 NCVT locations at FCRDI, Mahailluppallama (DL1b), Regional Agricultural Research and Development Centre, Aralaganwilla (DL2b), Agriculture Research Station, Madurukatiya (IL1c), a Seed Farm, Okkampitiya (DL1a) and Grain Legumes and Oil Crops Research and Development Center, Angunukolapalasse (DL1a) under supplementary irrigated conditions. The plot size used was 5 m in length, and 1.2 m in width. DOA recommended fertilizer levels (urea-325 kg/ha, TSP - 100kg/ha and MOP - 50 kg/ha) and recommended spacing (60 cm x 30 cm /hill) were adopted. The experimental design was a Randomized Complete Block Design with 3 replicates. Grain yields were obtained at maturity using formula given below.

$$\text{Yield (t/ha)} = \frac{\text{Plot grain yield (kg)} \times 10{,}000 \times (100 - \text{Grain Moisture \%})}{\text{Plot size (m}^2\text{)} \times 1000 \times 85.5\ \%}$$

Ear characters including ear length, ear girth, number of seed rows, seed color, seed texture, cob shape and other agronomic characters including plant height, ear height, stalk lodging days to 50 % tasseling and silking were recorded. Grain yield data were analyzed using the procedure ANOVA in SAS (Statistical Analysis Software) and means were compared using Duncan multiple range test. Genotype x environment interaction was analyzed using Variance Component method Adaptability (D) and stability parameters (S^2) were calculated using the method proposed by Abeysiriwardhana (1991). The variety with significantly high positive mean deviation (D) and non significant stability parameter (S^2) was selected as the most adaptable stable variety.

Evaluation of promising QPM hybrids in varietal adaptability trials (VAT) in farmers field

During *yala* season 2010 five promising QPM hybrids were evaluated along with *Sampath* and Pacific 984 in VAT in farmer fields at Hambantota, Monaragala and Anuradhapura districts under supplementary irrigated conditions. The plot size used was 5 m in length, and 2.4 m in width. The experimental design used was Randomized Complete Block Design with 2 replicates under farmer management system. Grain yields were obtained at maturity and plot yields were calculated same as above.

Grain yield data were analyzed using the procedure ANOVA in SAS (Statistical Analysis Software) and means were compared using Duncan multiple range test. On farm multi-locational yield trials with few varieties with minimum replicates, were evaluated for wide adaptability and stability over diverse environments using the method proposed by Abeysiriwardhana (2001), i.e., the deviations of the plot yield from the maximum plot yield in a given environment are computed and pool analysis of variance is run on these deviations. Variety, with significantly lower mean deviation (D) and non significant stability parameter (S^2) is the most stable and adaptable variety.

Laboratory analysis of amino acids

Tryptophan was determined using colorimetric method (Opienska-Blauth *et al.*, 1963) modified by Hernandez and Bates (Hernandez and Bates, 1969). Seven QPM parental lines, nine promising QPM hybrids were evaluated against two non QPM hybrids, Pacific 984, the commercial check hybrid and Sampath, the local hybrid. Reagent A was prepared by dissolving 270 mg $FeCl_3.6H_2O$ was in 0.5 ml distilled water and diluted to 1 l with glacial acetic acid. Reagent C was prepared by mixing volume-to-volume of reagent A and 30N sulfuric acid (Reagent B). Papain solution 4 mg/ml of 0.1N sodium acetate buffer (pH 7.0) was prepared just before use. Standard tryptophan solutions for making the standard curve were prepared using L-tryptophan.

About 85 mg finely ground defatted maize endosperm samples were weighted into 13 x 100 mm screw capped test tubes and 3 ml papain solution was added. Tubes were shook carefully to make sure the samples were thoroughly wet. Blanks were prepared using papain solution. Samples were incubated for 16 hours at 63±2 °C. Tubes were then centrifuged at 2,500 rpm for 15 minutes. One ml hydrolysate was added to test tubes containing 4 ml of reagent C and after shaking they were incubated for 15 minutes at 63±2 °C. Absorbance was read at 560 nm using UV-visible spectrophotometer. Protein contents of the seeds were determined by Micro-kjeldahl method (Miller and Houghton, 1945). Moisture contents of the endosperms were determined using oven drying method and crude fat contents were determined by Soxhlet method (AOAC, 2005).

RESULTS AND DISCUSSION

Evaluation of promising QPM hybrids in National Co-ordinate Varietal Trials (NCVT)

Promising QPM hybrids evaluated in NCVT during *yala* season 2009 at FCRDI, Mahailluppallama and GLORDC, Agunukolapalasse showed significantly lower mean grain yields compared to that of commercial check hybrid Pacific 984 (Table 2). However, all promising QPM hybrids showed comparable yields with the check hybrid Pacific 984 at RARDC Aralaganwilla and at Seed Farm Okkampitiya (Table 1). In the NCVT of promising QPM hybrids in *maha* 2009/10 at FCRDI, Mahailluppallama and GLORDC, Angunukolapalasse, KH157 produced comparable yield with that of Pacific 984. All promising QPM hybrids showed comparable yields with the check hybrids at RARDC, Aralaganwilla. However, all the tested hybrids recorded very poor grain yield at ARS, I ladurukatiya due to bird damage.

During *yala* season 2010 all the QPM hybrids, except KH38 showed comparable yield with that of Pacific 984 at FCRDI, Mahailluppallama and GLORDC, Angunakolapalasse. QPM hybrids evaluated at ARS, Aralaganwilla showed that all the hybrids produce comparable yield with Pacific 984. Considering the overall results, KH156 and KH157 showed comparable yield with Pacific 984 at most of the locations. Analysis of Genotype x Environment interaction showed that Pacific 984 was the most adaptable hybrid with significantly high positive mean deviation (Table 2).

Among the promising QPM hybrids, KH157 showed positive mean deviation value with non significant stability parameter in *yala* season 2009. KH156 and KH157 showed positive mean deviation with non significant stability parameter during *maha* season 2009/10 and KH74, KH76 and KH156 showed positive mean deviation with non significant stability parameter during *yala* season 2010.

Characters other than grain yield of promising hybrids evaluated at FCRDI, Mahailluppallama

Important agronomic characters for selecting promising hybrids other than the yield were recorded in each season (Table 3). All the QPM hybrids and check hybrids showed almost similar days to 50 % tasselling, silking and days to maturity. However, each variety can be distinguished based on seed colour and cob shapes.

Evaluation of promising QPM hybrids in Varietal Adaptability Trials in farmers fields (VAT)

Average grain yield and adaptability parameter (D) of the VAT conducted at farmer fields in Hambantota was given in Table 4. Average grain yield of all the hybrids tested in Hambantota was lower than that of Hambantota. Analysis of Gene x Environment interaction showed that there was no significant interaction. Hence all varieties were stable at test locations. Based on the adaptability parameter D (where the variety with significantly low D value is the most adaptable variety), all the other hybrids, except KH 156 and KH 38 showed comparable lower mean deviation with check hybrid Pacific 984. Farmers liked QPM hybrids equally as check hybrid Pacific 984. They have observed vigorous growth and there was no lodging of plants of all varieties tested.

Table 1. Mean grain yield (t/ha) of promising QPM hybrids evaluated in NCVT.

*Entry**	*Yala 2009*				*Maha 2009/2010*				*Yala 2010*		
	MI	*Angunakola palessa*	*Aralagan wila*	*Okkam pitiya*	*MI*	*Angunakola palessa*	*Aralagan wila*	*Okkam pitiya*	*MI*	*Angunakola palessa*	*Aralagan wila*
KH74	5.12b	7.30b	3.88ab	5.22ab	3.90b	3.72c	2.73b	0.95b	4.88ab	4.33ab	3.53ab
KH76	5.22b	7.08bc	3.92ab	ne	4.37b	4.17c	3.38b	1.47ab	4.77ab	4.75ab	2.70ab
KH156	4.49b	5.75d	4.01ab	6.44a	4.44b	5.50b	4.39a	1.49ab	4.84ab	4.33ab	3.55ab
KH157	6.34ab	6.92bc	4.66a	5.84ab	4.89a	5.67ab	3.87ab	1.70a	4.89ab	4.21ab	2.23b
KH38	4.61b	6.12cd	3.84ab	6.05ab	3.77b	4.17c	4.29a	1.79a	3.76b	3.78b	4.88a
Sampath	4.57b	8.79a	2.97b	4.26b	4.57b	6.06a	4.42a	1.99a	4.47ab	4.59ab	3.16ab
Pacific 984	7.97a	9.32a	4.55a	7.09a	7.22a	5.94ab	3.44b	1.90a	5.77a	5.43a	4.11ab
CV (%)	18.1	8.2	16.6	18	26	5.6	21	20	20	16	37

Note: Mean values with same letter were not significantly different at $p<0.05$. ne=not evaluated.

KH74=*CML161 x CML171, KH76=ML161 x CML194, KH156=CML171 x CML193, KH157=CMLL171 x CML1 and KH38=CML193 x CML194.

Table 2. **Mean grain yield and deviation over environments (t/ha) and adaptability parameter of QPM maize hybrids tested in NCVT during *yala* 2009, *maha* 2009/10 and *yala* 2010 seasons.**

Entry	*Yala 2009*			*Maha 2009/10*			*Yala 2010*		
	*Grain yield (t/ha)**	*Mean deviation (D^*)*	*Stability (S^2)*	*Grain yield (t/ha)**	*Mean deviation (D^*)*	*Stability (S^2)*	*Grain yield (t/ha)**	*Mean deviation (D)**	*Stability (S^2)*
KH74	5.38b	-0.29b	0.22	2.83c	-0.98c	0.21	4.25ab	0.01ab	0.07
KH76	5.43b	-0.20b	0.06	3.35bc	-0.46bc	0.25	4.07ab	0.16ab	0.84
KH156	5.23b	-0.45b	3.02^s	3.96ab	0.15ab	0.6	4.24ab	0.00ab	0.06
KH157	5.90b	0.22b	0.96	4.03ab	0.23ab	0.23	3.78b	-0.46b	1.43
KH38	5.16b	-0.51b	1.36	3.50bc	-0.30bc	1.6	4.14ab	0.09ab	5.32^s
Sampath	5.20b	-0.48b	5.45^s	4.26ab	0.46ab	0.76	4.07ab	0.16ab	0.15
Pac. 984	7.23a	1.56a	1.99	4.63a	0.83a	4.47^s	5.10a	0.87a	0.1
CV%	15.5			21.5			23.7		

Note: *Mean values of each column with the same letter are not significantly different at $p < 0.05$. S- Stability parameter significant at $p < 0.05$.

Table 3. Variation in plant height (cm), ear height (cm), days to 50% tasseling, 50% silking and harvest, number of seed rows, cob length(cm), seed colour within . planting row and mean 1000 seed weight (g) of promising hybrids evaluated at FCRDI, Mahaillupallama during *yala* 2009 and *maha* 2009/10.

Entry	*Plant height*	*Ear height*	*50% tasseling*	*50% silking*	*Harvest*	*No of seed rows*	*grain weight*	*cob length*	*Seed color*
KH74	175-195	78-90	47-52	47 -55	105-110	12-14	258	16.0-19.0	Orange - yellow
KH76	155-160	75-85	48-57	49-58	105-110	10-12	267	17.3-19.0	Dark orange
KH156	175-183	75-87	56-59	58-60	105-110	14-16	318	15.3-18.0	yellowish orange
KH157	170-180	84-95	56-58	60-61	105-110	12-16	271	15.6-18.5	Orange
KH38	170-190	70-83	58-60	60-62	105-110	10-16	270	16.3-20.8	yellow
Sampath	140-170	92-95	50-55	56-59	105-110	14-16	290	15.6-18.5	yellow- orange
Pacific 984	170-200	73-78	59-58	58-60	105-110	14-16	264	16.0-21.6	yellow - orange

Table 4. Mean grain yields (t/ha) of QPM hybrids tested during *yala 2010* in farmers field.

	Entry	*Average grain yield (t/ha)* Hambantota*	*Average grain yield (t/ha)* Anuradhapura*	*Mean grain yield (t/ha)**	*Mean deviation (D)*
CML161xCML171	KH74	4.71 b	5.51 ab	5.11 ab	3.12 ab
CML161xCML194	KH76	4.27 b	6.38 ab	5.33 ab	2.90 ab
CML171xCML193	KH156	2.19 c	4.86 b	3.53 b	4.71a
CML171xCML194	KH157	3.17 bc	5.41 ab	4.29 ab	3.94 ab
CML193xCML194	KH38	2.30 c	5.65 ab	3.98 b	4.26 a
Sampath		2.91 bc	5.88 ab	4.40 ab	3.84 ab
Pacific 984		5.69 a	7.4 a	6.59 a	1.65 b[B]
CV%		15%	14.5%	29.8%	

Note: Values in each column with the same letter are not significantly different at $P<0.05$.

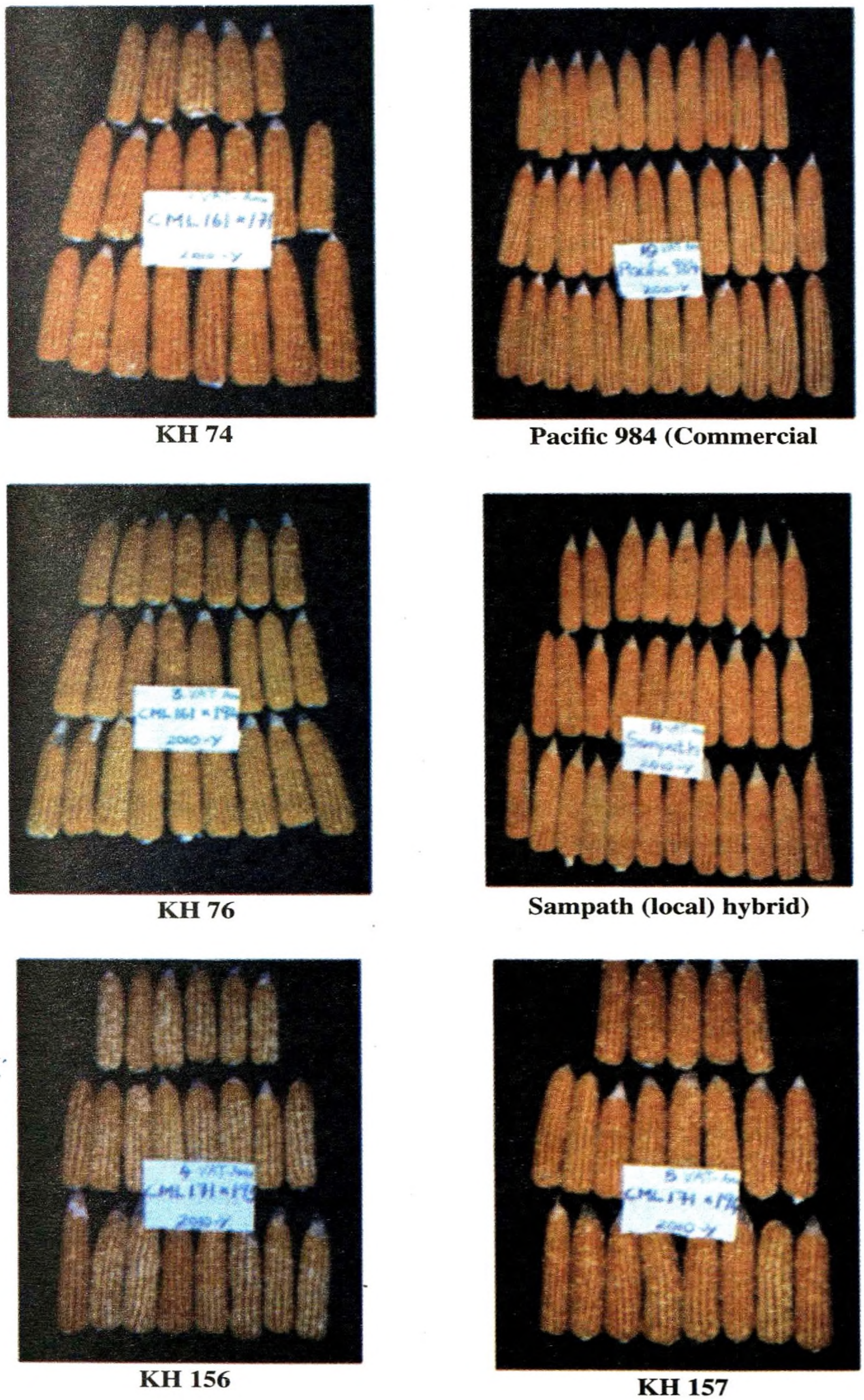
Table 6. Mean moisture, fat, protein and tryptophan (as a % of protein) percentages of maize endosperms.

Entry	*Moisture (%)*	*Fat (%)*	*Protein (%)*	*Tryptophan (per % protein)*
ML161	13.69	4.27	8.28	0.92 de
CML164	13.81	4.74	8.00	0.98 cde
CML168	12.66	4.97	8.35	1.07 abcd
CML171	12.73	5.11	8.34	1.09 abc
CML189	13.21	4.58	8.32	0.96 cde
CML193	13.22	4.06	9.53	0.84 ef
CML194	13.82	3.42	9.68	0.84 ef
Sampath (check 1)	11.40	5.16	8.63	0.65 g
Pacific 984 (check 2)	12.44	3.38	7.49	0.78 f
CML16 X CML171	11.89	4.51	7.52	1.15 ab
CML161x CML 193	12.00	6.09	9.15	0.94 de
CML161x CML 194	11.81	4.70	8.20	1.01 bcd
CML168x CML 189	11.50	5.37	7.20	1.15 ab
CML168x CML 194	10.68	4.42	7.45	1.14 ab
CML171x CML 193	11.47	5.02	7.37	1.16 a
CML171x CML 194	11.60	5.86	9.27	0.92 de
CMl193x CML 194	11.76	5.42	8.18	1.01 abcd
CMl189x CML 194	10.69	6.44	7.70	1.10 abc

Note: Values in each column with same letter are not significantly different at $p<0.05$.

Laboratory analysis of amino acids

Moisture content ranged between 10.7-13.8 %. No significantly difference was observed in protein or fat contents of the seeds. Tryptophan contents of inbred lines tested ranged between 1.09-0.84 %, while it was 1.16-0.92 % in QPM hybrids. Two non QPM hybrids had significantly lower tryptophan contents than most of the QPM inbred lines and QPM hybrids. KH156, KH74, KH76 and KH 157 hybrids showed tryptophan contents above 1%



KH 74 | **Pacific 984 (Commercial**

KH 76 | **Sampath (local) hybrid)**

KH 156 | **KH 157**

Figure 1. General appearance of ears of promising QPM hybrids and check hybrids.

CONCLUSIONS

Results of NCVT showed inconsistent results of QPM hybrids over three consecutive seasons. QPM hybrids, KH74, KH76, KH 156 and KH157 showed comparable adaptability with check hybrids over the location for at least two seasons. QPM hybrids, KH74, KH76 and KH157 showed comparable adaptability with commercial check hybrid Pacific 984 in VAT. These hybrids also had a higher tryptophan contents compared to check hybrids. Since the cob appearance of KH156 was not attractive, KH74, KH76 and KH157 were selected as candidate QPM hybrids that can be consumed as substitute for ordinary maize varieties.

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