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Research Article

Introgression of Opaque-2 Gene into the Genetic Background of Popcorn Using Marker Assisted Selection

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ABSTRACT

Biofortification of a natural snack like popcorn is important for improved nutrition and health of children and adults. This study was aimed at introgressing opaque-2 gene, a high lysine/tryptophan gene into the genetic background of popcorn. Field experiments were carried out at the Teaching and Research Farm (TRF) of the Federal University of Technology, Akure (FUTA) during the dry and wet seasons of 2016 while biochemical and molecular analyses were carried out in the Biochemistry Laboratory, FUTA and Bioscience Laboratory, International Institute of Tropical Agriculture (IITA) respectively. Nine parental genotypes were used which comprised three popcorn maize varieties and six Quality Protein Maize (QPM) varieties. F1 hybrids were developed by crossing the parental genotypes using North Carolina mating design I. The hybrids were then advanced to F2 by self-pollination. Segregated F2 kernels from each genotype were screened on the light box. Kernel modifications 2 (25% opaque) and 3 (50% opaque) were selected. Selected kernels were analysed biochemically for protein and tryptophan contents. Data obtained were subjected to Analysis of Variance (ANOVA) using Minitab software version 17. Significant means were separated using Tukeys HSD ($P \leq 0.05$). The allelic state of opaque-2 gene in the selected F2s was determined using SSR marker phi 112. Protein and tryptophan contents and quality index for unpopped and popped F2 of crosses of QPM and popcorn were significantly ($P \leq 0.05$) higher than popcorn checks. Unpopped protein content, unpopped tryptophan content and popped tryptophan content were highly correlated with one another. Phi 112 SSR marker showed dominant pattern of polymorphism, amplifying DNA fragments between 154bp and 160bp for the F2.

Keywords: Introgression, Opaque-2, QPM, Popcorn, Biochemical properties.

INTRODUCTION

Maize (Zea mays L.) is one of the most important food security crops in developing countries (Avinashe et al., 2012). More than 300 million Africans depend on maize as their main food source (M'mboyi et al., 2010). In Sub–Sahara Africa (SSA), maize is used directly for human food with more than 100kg consumption per capita (Adeyemo et al., 2011). Maize can be classified into specialty types based on kernel morphology, texture, usage, functionality and other characteristics, which include waxy maize, high protein maize, high oil maize, flour maize, sweet-corn and popcorn (Johnson, 2000). Popcorn (Zea mays var. everta) is an extreme form of flint corn (Z. mays var. indurata) that has a very hard corneous endosperm with only a small portion of soft starch and is characterized by its popping ability; the kernel pops upon heating as a result of the unique quality of the endosperm (Acquaah, 2006). Popcorn is a soft and tasty aliment that is appreciated in many countries (Rodovalho et al., 2008). The cultivation, consumption and utilization of popcorn are becoming popular especially in developing countries like Nigeria (Adunola et al., 2017). The advent of cinema, playing ground, arcade, modern schools and corn popping technology (microwave) have been a major driver for the increased consumption of popcorn in Nigeria (Adunola et al., 2018).

Malnutrition is a serious health concern that has been linked to deficiency of diet in protein quantity, protein quality and some other essential nutrients such as vitamins, minerals etc. needed for body growth, development and maintenance (Adunola et al., 2018). Maize, as a source of plant protein, is not just low in protein quantity, but also low in some essential amino acids such as lysine, tryptophan and niacin. The dependence of people on maize as a major food source predisposes them to the risk of malnutrition because these amino acids cannot be synthesized in human body. Hence, boosting the protein quality of maize, particularly in terms of lysine and tryptophan contents will avail people living in the developing countries of quality diet on regular basis. This will also help to reduce nutrition related diseases, deaths and significantly improve nutritional status of individuals who depend primarily on maize for sustenance (Ado, 1999).

The search for means to boost protein quality of maize met with an important discovery in 1963 when three scientists (Edwin T. Mertz, Oliver E. Nelson, Jr., and Lynn S. Bates) from Purdue University, Illinois, USA found a maize natural mutant with increased levels of lysine and tryptophan, twice as much as the normal maize. A single gene mutation called opaque-2 gene was found to be responsible for this superior protein quality. However, other natural maize mutants conferring higher lysine and tryptophan were identified in the 1960s and 1970s, viz., floury-2 (fl2), opaque-7 (o7), opaque-6 (o6), and floury-3 (fl3) (Vivek et al., 2008). Among these natural mutants, major breakthrough has been the isolation of the opaque-2 mutant (o2), with superior nutritional properties, albeit undesirable agronomic traits (Mertz et al., 1964). Identification of the nutritional benefit of recessive opaque-2 mutant present on chromosome 7, followed by accumulation of endosperm-modifiers, led to development of Quality Protein Maize (QPM) at the International Maize and Wheat Improvement Center (CIMMYT), Mexico (Vasal et al., 1980). Afterwards, efficacy testing in humans and animals was enthusiastically undertaken (Bressani, 1992; Knabe et al., 1992). Subsequent conventional breeding efforts generated numerous cultivars with improved agronomic characteristics, and these were referred to as QPM (Krivanek et al., 2007; Vivek et al., 2008). Maize homozygous for the o2 (recessive) mutation was shown to have substantially higher lysine and tryptophan content than maize that was either heterozygous (O2o2) or homozygous dominant (O2O2) for the opaque-2 locus (Crow and Kermicle, 2002). Bressani (1992) showed that increased concentrations of these two amino acids in the grain endosperm can double the biological value of maize protein. However, the amount of protein in such maize remains at about 10%, the same as that of common (or normal endosperm) maize. Maize homozygous for the o2 mutant has a quality value equivalent to 90% that of milk (Vivek et al., 2008).

The use of Marker-assisted selection (MAS) helps in phenotype screening and selection with high reliability. Breeding for QPM genotypes becomes straightforward with the help of o2 locus-specific simple sequence repeats (SSRs) (Gupta et al., 2013). phi57, phi112 and umc1066 are the three SSRs (www.agron.missouri.edu) located within the o2 locus, which are useful for the QPM breeders to discard non-QPM genotypes at seedling stage and to select heterozygous and homozygous genotypes for o2 during marker-assisted breeding (Babu et al., 2005, Gupta et al., 2009; Kostadinovic et al., 2015). Significant successes have been recorded with the use of MAS in QPM breeding in terms of selecting for o2 allele genotypes alongside their modifier genes as well as full recovery of recurrent parent. Babu et al. (2005) used MAS for development of QPM parental lines of Vivek-9 hybrid and could develop QPM hybrid in less than half the time required through conventional breeding. Danson et al. (2006) explored various markers to introgress o2 gene into herbicide tolerant elite maize inbred lines. They found that using marker for QPM and endosperm modification can greatly enhance the selection efficiency for isolating fully modified kernels in QPM background (Sofi et al., 2009). In view of the foregoing, the objective of this study is the introgression of opaque-2 gene into the genetic background of popcorn using marker assisted selection.

MATERIALS AND METHODS

Experimental site

The research experiment was carried out on the Teaching and Research Farm (TRF) of the Federal University of Technology, Akure (FUTA) during the 2016 dry and wet seasons. FUTA's TRF is located at an altitude of 332 m above sea level, 7016'N, 5012'E in rainforest south-western region of Nigeria. Biochemical analysis and molecular analysis were carried out in the Biochemistry Laboratory, FUTA and Bioscience Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan respectively.

Description of parent genotypes

Nine parental genotypes were employed in this study which comprised different varieties of popcorn maize and QPM. The three popcorn maize varieties used as female parents were PopAkr, PopLag and PopIb while the remaining six QPMs as male parents were 2009TZEORSTRQPM, Pop66-SR/ACR-91-SUWAN-1-SR-C1, ART/98/SW1-OBQPM, ART/98/SW6, ILE-1-OB

and ART/98/SW1-Y. The variety, source, type and description of the genotypes are presented in Table 1.

Table 1: Description of parental genotypes.

S/N	Variety	Source	Parent	Type
1	2009TZEORSTRQPM	IITA	Male	OPV
2	Pop66-SR/ACR-91-SUWAN-1-SR-C1	IITA	Male	OPV
3	ART/98/SW1-OBQPM	IAR&T	Male	OPV
4	ART/98/SW6	IAR&T	Male	OPV
5	ILE-1-OB	IAR&T	Male	OPV
6	ART/98/SW1-Y	IAR&T	Male	OPV
7	PopAkr	Akure market	Female	OPV
8	PopLag	Lagos market	Female	OPV
9	PopIb	Ibadan market	Female	OPV

OPV=Open Pollinated Variety; IITA=Inter Institute of Tropical Agriculture, Ibadan; IAR&T=Institute for Agricultural Research and Training, Moor Plantation, Ibadan.

Plant materials

Five rows of each female parent were planted while three rows of each male parent were planted. Each row contained ten plant stands. At anthesis, brown shoot/tassel bags were used to collect pollen from each male parent. The pollens from respective male parents were bulked and used to pollinate good female parents. Pollination was done using North Carolina mating design I. At harvest, dried clean cobs devoid of rot and of good husk cover were selected. The F1 genotypes obtained were advanced to F2. The F1 genotypes were sown in five pots each at three seeds per pot and were thinned to two plants per pot after plant establishment. At flowering, four to five good plants in each genotype were self-pollinated to generate the F2 progeny.

Experimental design

This experiment was laid out in a Completely Randomized Design (CRD) with three replications. It was used to analyze the crude protein and tryptophan contents and quality index for unpopped kernels of the selected F2 genotypes.

F2 selection using the light box

From the materials obtained from F1s advancement (i.e., selfed F1 to produce F2), selection for endosperm modification was made. F2 materials were screened on the light box using (Vivek, 2008) descriptors. For analysis of endosperm modification, the F2 kernels were rated on a scale of 1-5, with 5 indicating 100% opaque; 4 indicating 75% opaque; 3 indicating 50% opaque; 2 indicating 25% opaque; and 1 indicating 100% normal (vitreous). Endosperm modification scores were derived based on analysis of 100 randomly chosen kernels from the ears of F2. Modification Type 2 and 3 segregating for endosperm modifiers were selected. Laboratory analysis was done for the parents and selected F2 generation for their protein and tryptophan content. The derived value was used to determine the lysine and protein quality index.

Biochemical analysis

Tryptophan content was determined using the colorimetric method of Eric et al. (2009). 10 g of sample were milled, using a cyclotec mill (manufactured by Tecator, Hoeganaes). The milled sample was then defatted with n-Hexane in a Soxhlet-type continuous extractor for 6 h. After n-Hexane evaporation, 80 mg of milled sample was digested using 3 mL of 4 mg/mL papain. A blank with only papain solution was included as a control. The tubes were incubated at 650°C for 16 hours (shaken at least twice in the first hour of incubation), allowed to cool to room temperature, and centrifuged at 3600 g for 10 minutes, ensuring a very clear supernatant.

One milliliter of the hydrolysate (supernatant) was carefully transferred to a clean tube, and 3 ml of reagent D (glyoxylic acid and ferric chloride dissolved in sulfuric acid) was added. Samples were thoroughly stirred and then incubated for 30 min at 650°C. The samples were allowed to cool to room temperature before reading their optical density (OD) at 560 nm in a Beckman DU-6 UV-visible spectrophotometer.

Crude protein of the samples was estimated by using Kjeldahl (Villegas et al., 1984). A sample of 0.5 g and a blank was estimated in the digestion tube. For digestion at high temperature, 10 ml of concentrated sulfuric acid and 1.1 g digestion mixture were added in the tube. Then the digestion tubes were set in digestion chamber fixing at 4200°C for 45 minutes ensuring water supply, easier gas outlets etc. After digestion the tubes were allowed to cool and 5 ml of sodium thio-sulphate ($Na_2S_2O_3$, 33%) and 30 ml sodium hydroxide (NaOH) solution was added in each tube. Then the distilled extraction was collected with 25 ml of Boric acid (4%) and titrated with standard hydrochloric acid (0.2 N). The nitrogen values obtained was converted into percentage of crude protein by multiplying with a factor of 6.25 assuming that protein contains 16% nitrogen.

%Nitrogen=(Milliequivalent of nitrogen(0.014) Ч titrant value(ml) Ч strength of HCL Ч 100)/(Sample weight (g))

%Crude protein=%Nitrogen Ч 6.25

Quality index (QI) is the tryptophan-to-protein ratio in the sample, expressed as a percentage (Vivek et al., 2008).

QI=(Tryptophan content in sample Ч 100)/(Protein content in sample)

MAS for accessing allelic state of opaque-2 gene

Dominant SSR marker phi112 was used in order to access the allelic state of the opaque-2 locus of the maize samples. The DNA was isolated from the grain using the Genomic DNA Purification Kit (Thermoscientific) in accordance with the manufacturer's protocol. Approximately 100mg of fresh young, 2-weeks old per genotype leaf sample was grounded with liquid nitrogen using mortar and pestle. The samples were incubated at 65°C in water bath for 30 minutes with continuous gentle rocking (the rpm set to 20 to 30; high ratio will result in degraded DNA). The tubes were inverted once in every 10 minutes to properly homogenize the tissue with extraction buffer. The tubes were removed from the water bath and allowed to cool for 5-10 mins in a fume hood. Samples were gently mixed and centrifuged at 10000 rpm for 10 mins. About 500 µl aqueous phase were transferred into new tubes and 500-600 µl chloroform: isoamylalcohol (24:1) was added into the side of the tubes. The content was mixed very gently with continuous rocking of the tube for 5-10 min (or about 50 times). The samples were centrifuged at 10000 rpm for 10 min. The upper aqueous part was later transferred to fresh strip tubes and the chloroform: isoamylalcohol wash was repeated. About 600 µl of the upper aqueous portion was later transferred into fresh strip tubes. 600 µl of 100% cold (stored at -20°C) isopropanol (2-propanol) was added and mixed very gently for about 5 min (or gently invert for about 50 times) to precipitate the nucleic acid. The samples were centrifuged at 10000 rpm for 10 min to form a pellet at the bottom of the tube. Supernatant was discarded. 400 µl of 70% ethanol was added; the tubes were gently flapped to let the pellet float for ease in washing. Samples were again centrifuged for 15 min and ethanol discarded by decantation. The pellet was washed with 70% ethanol once again. The samples were centrifuged for 15 min and ethanol discarded by decantation again. The pellet was allowed to air dry until ethanol evaporated completely (until smell for ethanol disappears) for 30-60 minutes. 97µl of sterile distilled water was added and 3µl of RNAse A to degrade RNA.

Polymerase Chain Reaction (PCR) was performed on the extracted DNA of the samples which were subjected to PCR cocktail mix and condition for the PCR (Table 2) and temperature profile (Table 3). The PCR products were loaded on 1.5% agarose gel. The ladder used is 50bplus Generuler from Thermo Scientific.

Table 2: PCR Cocktail mix.

PCR Cocktail mix	Quantity
10× PCR buffer	2.5
25 mM $MgCl_2$	1
5 pMol forward primer	1
5 pMol reverse primer	1
DMSO	1
2.5 Mm DNTPs	2
Taq 5 µ/µl	0.2
50 ng/µl DNA	3
H_2O	13.3
Reaction mixture	25 µL

Table 3: PCR Temperature Profile.

	Initial denaturation	Denaturation	Primer Annealing temperature	Extension	Final extension	Hold temperature
9 Cycle						
Temperature	94°C	94°C	65°C	72°C		
Duration	5 min	15 sec	20 sec	30 sec		
35 Cycles						
Temperature		94°C	55°C	72°C	72°C	10°C
Duration		15 sec	20 sec	30 sec	7 min	∞

Popping method

Popped kernel (%) was determined by taking a sample of 100 kernels and placed into brown envelope. Microwave popping was performed using a 900 W Defy DMO 351 microwave oven with power of 230 V. The samples were popped for two minutes. Number of popped kernels was counted.

Data analysis

Analysis of Variance for this study was carried out using Minitab software version 17. Significant means were separated using Tukey's Honest Significant Difference (Tukey's HSD).

RESULTS

Biochemical analysis for protein and tryptophan contents (%) and quality index (QI) of Un-popped and Popped F2 generation

Biochemical analysis of un-popped and popped F2 generation kernels for crude protein and tryptophan contents (%) and quality index (QI) is presented in Table 4. There were significant differences ($p \leq 0.05$) for crude protein and tryptophan contents (%) and quality index (QI) among the un-popped F2 generation kernels. Crude protein for all the un-popped F2 was above the QPM threshold (8%) except cross of P6 Ч P1 (7.88%). Tryptophan content for all the un-popped F2 were below the QPM threshold (0.075) except P5 Ч P1 (0.075). Quality index for all the un-popped F2s was below the QPM threshold (0.8) except crosses of P4 Ч P1 (0.81), P7 Ч P1 (0.84), P7 Ч P3 (0.85) and P5 Ч P1 (0.91). Similarly, there were significant differences ($p \leq 0.05$) for crude protein, tryptophan and lysine contents (%) and quality index (QI) among the popped F2 generation. Crude protein for all the popped F2s were below the QPM threshold (8%). Also, tryptophan content and quality index for all the popped F2s were below the QPM threshold. The two checks had the highest popped kernels (%) that differed significantly from the F1. Cross of P7 Ч P3 (65%) had the highest popped kernels (%) among the F1. It was observed that no grain popped in cross of P4 Ч P3.

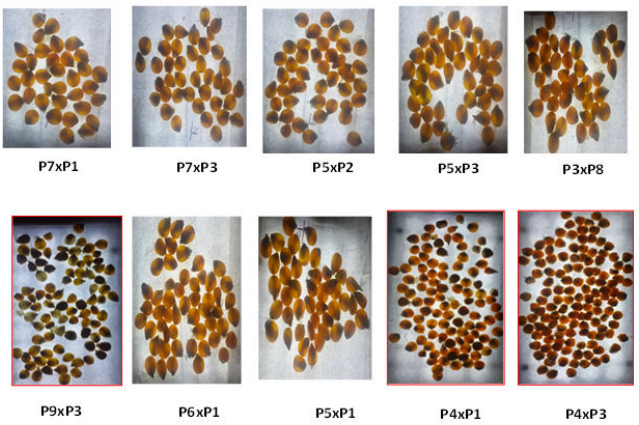

Plate 1: Photograph of the abaxial side of the 10 F2 generations kernels on the light box, Pearson's correlation coefficient (r) among various biochemical components of F2 generation (Popcorn x QPM).

Table 4: Biochemical analysis for protein, tryptophan (%) and quality index (QI) of Un-popped and Popped F2 generation.

	Unpopped			Popped			100-grain weight (g)
F2s	Crude protein (%)	Tryptophan (%)	Quality Index	Crude protein (%)	Tryptophan (%)	Quality Index	
P7 × P1	8.31ab	0.070a	0.84ab	7.66ab	0.057ab	0.75abc	21.57b
P7 × P3	8.08abc	0.068ab	0.85ab	7.44bc	0.056bc	0.75ab	21.26b
P5 × P2	8.12abc	0.061cd	0.75bcde	7.32cd	0.052cd	0.71bcde	21.67b
P5 × P3	8.04abc	0.059cde	0.74cde	720cde	0.050de	0.69cdef	25.31a
P3 × P8	8.01abc	0.057cde	0.72cdef	7.08def	0.047ef	0.67ef	18.53c
P9 × P3	8.29ab	0.063bc	0.76bcd	6.46g	0.043fg	0.67ef	21.23b
P6 × P1	7.88bc	0.055def	0.70def	6.85f	0.046ef	0.68def	22.18b
P5 × P1	8.15abc	0.075a	0.91a	7.73a	0.061a	0.79a	16.01d
P4 × P1	8.40a	0.068ab	0.81bc	7.80a	0.057ab	0.73bcd	15.67d
P4 × P3	8.10abc	0.053ef	0.66efg	6.41g	0.041g	0.65f	9.86e

Check 1	7.81c	0.048fg	0.62fg	7.21cde	0.039gh	0.55g	15.43d
Check 2	7.75c	0.044g	0.57g	7.06ef	0.035h	0.50g	16.00d
QPM threshold	≥ 8	≥ 0.075	≥ 0.8				

Mean values in the same column followed by different alphabets are significantly different from one another (p ≤ 0.05).

Where; P1=PopAkr; P2=PopLag; P3=PopIb; P4=ART/98/SW1-OB; P5=ART/98/SW1-Y; P6=Pop66-SR/ACR91SUWAN-1-SR; P7=ILE-1-OB; P8=ART/98/SW6; P9=2009TZEORSTRQPM.

Table 5: Pearson's correlation coefficient (r) among 100-grain weight, popping percentage and various biochemical components of F2 generations (Popcorn Ч QPM).

	Un-popped crude protein	Popped crude protein	Un-popped tryptophan	Popped tryptophan	100-grain weight
Popped crude protein	0.284				
Un-popped tryptophan	0.657**	0.540**			
Popped tryptophan	0.604**	0.737**	0.925**		
100-grain weight	0.064	0.151	0.272	0.299	
Popped kernels (%)	-0.314	0.309	-0.205	-0.173	0.101

** (P ≤ 0.01)

Table 5 presents Pearson correlation coefficient (r) among various biochemical components of the F2 generations. Un-popped crude protein content was highly significantly and positively correlated with un-popped tryptophan and popped tryptophan contents, with correlation coefficient (r) (0.657) and (0.604) respectively. Popped crude protein content was highly and positively correlated with un-popped tryptophan and popped tryptophan contents, with coefficient (r) (0.540) and (0.737) respectively. Un-popped tryptophan content was highly significantly and positively correlated with popped tryptophan content, with coefficient (r) = 0.925.

Screening of F2 for Phi 112 polymorphism in the opaque-2 locus

The SSR locus analyzed phi 112 polymorphism across the screened materials (Figure 2). Phi 112 SSR marker shows a dominant pattern of polymorphism, amplifying DNA fragments between 154 bp and 160 bp for the ten F2.

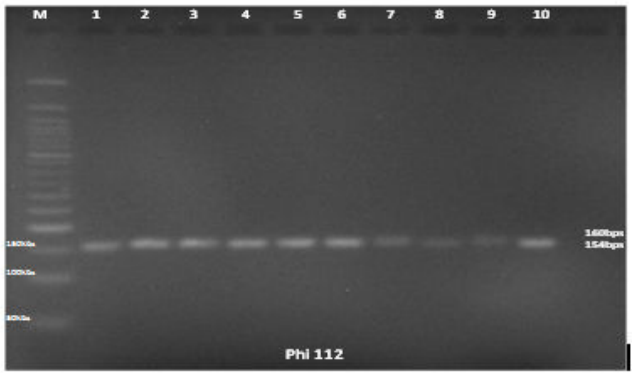

Figure 2: SSR polymorphism observed for F2 generations of QPM x Popcorn using phi 112 marker. Where, M=50bp ladder; 1=P7 × P1; 2=P7 × P3; 3=P5 × P2; 4=P5 × P3; 5=P3 × P8; 6=P9 × P3; 7=P6 × P1; 8=P5 × P1; 9=P4 × P1; 10=P4 × P3.

DISCUSSION

F2 generations of the selfing progenies differentiate for endosperm modification (Figure 1). In each cross, it was observed through light box screening that 5 classes of kernel modification consisted of 0%, less than 25%, 25 -50%, 50-75% and more than 75% opaque (Singh and Ram, 2012). Though the degree of endosperm modification was not quantified in this study, visual observation of the F2s showed that some kernels were opaque while others were translucent. This suggests the presence of the opaque-2 in some of the F2. Lopes and Larkins (1995) revealed the existence of two additive modifier genes that significantly affect the endosperm modification in their population. Crosses of P4 Ч P1 and P4 Ч P3 have the same QPM donor parent (ART/98/SW6) which may suggest the reason why they have more opaque kernel modifications than other genotypes. Scott (2009) reported that kernels of the inbred lines used in his study were more vitreous than a typical o2o2 grain sample. This was similar to the result obtained from this study as seven of the ten F2 were translucent. This could be due to selection for modifier genes most likely donated by the QPM lines used (Scott, 2009). This could also be due to the presence of two to three major loci influencing endosperm modification from the opaque to vitreous phenotype, with several minor factors possibly fine-tuning the process. This enhances

the possibility of selecting lines with reasonable levels of endosperm modification, while retaining the nutritional value of o2 gene (Prasanna et al., 2001). Glover and Mertz (1987) also indicated that the modified endosperm texture is polygenically controlled with additive type of genetic variation playing an important role, although in some genotypes a few major genes may contribute significantly to kernel modification. The genetic background of the genotype and its kernel texture could also influence kernel modification and frequency of various modification classes. Phenotypic variation ranging from completely unmodified to completely modified kernels was observed in single ears of F2 progenies segregating for kernel modification (Lopes and Larkins, 1991).

This study shows that protein and tryptophan contents and quality index for the unpopped F2 genotypes were higher than those of both popcorn checks. This finding agrees with Vasal (1994) and Prasanna et al. (2001) that QPM genotypes have almost double amounts of tryptophan content compared to normal maize but were similar in overall protein content. The percentage increase in tryptophan content ranged from about 20% - 40% more than that for both checks in this study. This result was close to that reported in Paes and Bicudo (1995) and Bressani (1995). The authors indicated that QPM protein contains, in general, 55% more tryptophan, 30% more lysine and 38% less leucine than that of normal maize. Mertz et al. (1964) first reported that the lysine content in o2 was 3.3 to 4.0 g per 100 g of endosperm protein, which was more than twice that of normal maize endosperm (1.3 g lysine/100 g endosperm protein). This could be as a result of the reduction in zein protein fraction by the presence of o2 gene which resultantly increases lysine and tryptophan rich non-zein protein fraction. Due to the well established relationship between lysine and tryptophan in the maize protein (4:1 ratio), tryptophan can be used as a single parameter for evaluating the nutritional quality of the protein (Villegas et al., 1992; Vivek et al., 2008). When interpreting the results of laboratory analysis for making selections, tryptophan content and quality index have to be above the acceptable limits (Vivek et al., 2008). All the unpopped F2 genotypes were above the QPM threshold for crude protein (%) except cross of P6xP1 and both checks. This is a confirmation that the presence of opaque-2 gene not only improves protein quality but might also increase protein quantity. As reported by Singh and Ram (2012), there was an improvement (1.02 - 1.5%) in protein content of converted lines. Quality index for both unpopped checks were significantly lower than the unpopped F2 genotypes. Quality index above the QPM threshold was observed in four of the unpopped F2 generations. Differences in the protein and tryptophan contents in the F2 could have been as a result of variation in intensity of heterogeneousity of F1. Motukuri, (2012) screened the F1 (BML 6 Ч CML 181) populations using SSR marker umc1066 and found that they all exhibited heterogeneity for gene of interest but varying band intensity where band with higher intensity was used for scoring and that of lower intensity was rejected.

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement (Singh and Narayanan, 1993) on character(s) of interest. This study examined the correlation among 100-grain weight, popping percentage and unpopped crude protein, popped crude protein, unpopped tryptophan and popped tryptophan contents. Significant positive correlations were observed between unpopped crude protein, unpopped tryptophan and popped tryptophan contents. Also, significant and positive correlations exist between popped crude protein and unpopped tryptophan and popped tryptophan contents. Unpopped tryptophan and popped tryptophan content were found to be significantly correlated. However, positive but non-significant correlation was observed between 100-grain weight and all biochemical characteristics considered while negative relationship was observed between popped kernel percentage and unpopped crude protein, unpopped tryptophan and popped tryptophan contents. Zaidi et al. (2008); Reddy et al. (2013); Kostadinovic et al. (2016) in their studies reported positive but non-significant correlations between grain yield and biochemical traits. This result shows the strong relationship that exists between protein and tryptophan contents in popcorn. Therefore, these biochemical components should be accurately determined during QPM conversion program. The result also shows that the pattern of inheritance for quantitative traits is different from qualitative traits. Hence, the selection for good yield and quality traits should occur simultaneously during QPM breeding for popcorn.

The use of phi 112 in this study was employed to assist in foreground selection, amplifying for dominant O2 allele. This helps to determine the presence or absence of the gene thereby ascertaining the precision of the gene's presence. The 10 F2 screened in this study using the marker were amplified at DNA fragment 154 bp - 160 bp, meaning that none exhibited null allele for the genotype o2o2. This result is similar to the results obtained by Kostadinovic et al. (2015), Danson et al. (2006) and Babu et al. (2005), where band sizes of 144bp and 170bp, 136bp and 160bp, and 150bp respectively were amplified for non-QPM lines analyzed. Hence, it can be concluded that opaque-2 gene was not present in recessive form (o2o2) in all the F2 genotypes. This does not correlate with results from phenotypic observation of kernel modification and

biochemical analysis. This disharmony in results could be as a result of the following: firstly, codominance in the gene action between the two allele types (O2 and o2). Acquah (2006) states that codominance occurs when both alleles of a heterozygote are expressed to equal degrees such that the two alleles code for two equally functional and detectable gene products. phi 112 does not discriminate between O2O2 and O2o2 genotypes, thus, kernel modification and improved protein and lysine contents among the F2 which were amplified (as O2O2 or/and O2o2) can be attributed to the presence of o2 allele in heterozygous form; secondly, tryptophan content which was not above QPM threshold for all the F2s except cross of P5XP1 (F2) is an indication that the opaque-2 gene was probably not present in recessive form. The significant increase in protein and lysine contents compared to the checks (PopAkr and PopLag) could have been due to the presence of other high lysine/tryptophan mutants (floury-2 (fl2), opaque-7 (o7), opaque-6 (o6), and floury-3 (fl3)). Studies by Schmidt et al.(1987); Coleman et al. (1997); Miclaus et al. (2011); Ignjatovic-Micic et al. (2014) have reported that different modes of action through the presence of some other high lysine/tryptophan natural mutants result in high tryptophan/lysine genotypes. Ignjatovic-Micic et al. (2014) in their study have also indicated that some of the high tryptophan accessions did not have the recessive opaque2 mutation, thus indicating the presence of some other mutation, such as floury 2 or opaque 7; and lastly, difference in kernel characteristics of the popcorn and QPM (dent corn) could have influenced selection on the light box due to their vitreous and opaque endosperm composition which may cause confusion. This kernel modification could be easily mistaken for endosperm modifier for opaque-2 gene. Varying degrees of kernel modification in the F2 could be as a result of the presence of an o2 allele pleiotropic effect which has an influence on zein synthesis (Ignjatovic-Micic et al., 2014). Therefore in QPM popcorn breeding, light box selection should be made only from cobs that are well modified. Also, it is important to note that O2o2 or O2O2 genotypes may show a small but insignificant degree of softness (Vivek et al., 2008) which could be misleading when selecting endosperm modifiers of the o2 locus in the F2 genotypes. Hence, laboratory biochemical analysis and MAS should corroborate light box screening. The former should not be made to replace the latter.

CONCLUSION

The importance of protein in human diet cannot be overemphasized. Lysine and tryptophan are essential amino acids which are required for food catabolism, body growth and tissue repair, but cannot be synthesized by the human body. Findings from this study show that more research works are required to understand the dynamics of QPM breeding for popcorn. Selection for biochemical analysis should be from large population of well modified endosperm of crosses of QPM and popcorn. Also, unpopped genotypes with high tryptophan content should be selected. This is because unpopped tryptophan content is highly correlated with popped tryptophan content. More so, selection for qualitative and quantitative traits should occur simultaneously during QPM breeding for popcorn program. Molecular screening for the allelic state of opaque-2 status among the crosses of QPM and popcorn should be carried out using the widely accepted SSR markers (Phi 112, Phi 057 and umc 1066).

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