# Characterization of Quality Protein and Normal Maize Accessions from a Breeding Program: Identification of Variety-Diagnostic Markers

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## INTRODUCTION

Maize is a major cereal crop for both livestock and human globally [1]. Several millions of people particularly in developing countries derive their protein and daily calorie requirements from maize [2]. It accounts for up to 15 to 56 % of total daily calories in diets of people in 25 developing countries [1]. In these countries particularly African and Latin American, animal protein is scarce, expensive and unavailable to vast majority of the society [3]. Normal maize varieties are deficient in two essential amino acids, lysine and tryptophan required for human nutrition [3,4]. Tryptophan and lysine content in normal maize is 2% which is less than half the amount recommended for human nutrition.

## OBJECTIVES

- To determine the amino acid profile of MUDISHI 3.
- To develop variety-diagnostic molecular markers that could be used to specifically track this accession in a maize breeding program

## MATERIALS AND METHODS

### Selection

- The main selection criteria include, spike size, resistance to mildew and maize streak virus, grain yield, nutritional quality (lysine, tryptophan and other amino acid contents), and organoleptic characteristics

### Molecular Analysis

- Total genomic DNA from maize seedlings were extracted using the CTAB protocol.
- A total of 24 ISSR and 46 RAPD primers synthesized by Invitrogen were used for DNA amplification.

### Protein and Amino Acid Analysis

- Analyses were conducted at the University of Missouri Agricultural Experiment Station Chemical Laboratories.

Table 1: List of maize accessions used in this study

| Varieties | Origin | Year | Type |
|---|---|---|---|
| GPS-5 | INEAC-Gandajika | - | NORMAL |
| SALONGO-2** | INERA- Gandajika | 1976 | NORMAL |
| DMR-ESR-W** | IITA-Ibadan | 1994 | NORMAL |
| LOCALE-2 | Farmers- Gandajika | - | NORMAL |
| AK9331-DMRESR-Y | IITA-Ibadan | 1994 | NORMAL |
| MUS-1** | INERA | 1996 | NORMAL |
| LOCALE-1 | Farmers- Gandajika | - | NORMAL |
| DMR-ES-W | IITA –Ibadan | 1994 | QPM |
| QPM-LONGE 5*** | NARI-Uganda | 2008 | QPM |
| ECAQVE-3 | CIMMYT-Kenya | 2008 | QPM |
| ECAQVE-4 | CIMMYT-Kenya | 2008 | QPM |
| ECAQVE-6 | CIMMYT-Kenya | 2008 | QPM |
| QPM-SRSYNTH*** | CIMMYT-Kenya | 2008 | QPM |
| SUSUMA | CIMMYT-Kenya | 2008 | QPM |
| MUDISHI 1 | INERA-DR Congo/LU | 2010 | QPM |
| MUDISHI 3 | INERA-DR Congo/LU | 2012 | QPM |

Figure 1: Field evaluation of a new QPM (MUDISHI 3).

## RESULTS

### Agronomic Characteristics

- MUDISHI 3, a new maize variety with white grain color was released in 2011 and it is adapted to all the maize growing regions in western, central and southern DR-Congo.
- It is highly resistant to downy mildew and to lodging and resistant to maize streak virus.

### Protein Analysis

- The crude protein content were 14 %, 9.9 % and 10 % in MUDISHI 3, Salongo 2 and DMR-ES-W, respectively.
- Overall, the total basic acids, which include lysine, arginine, and histidine constituent 11.4 % of the total amino acids for MUDISHI 3.

### Molecular Analysis

- A variety-diagnostic marker of 480 bp size was identified from amplified products generated by the ISSR HB 15 primer with MUDISHI 3 DNA sample.
- It represents an excellent tool for molecular breeding in the DR-Congo breeding program.

Table 2: Protein and amino acid profile

| Amino acids | MUDISHI 3 W/W | % | Salongo 2 W/W | % | DMR-ES-W QPM W/W | % | LSD |
|---|---|---|---|---|---|---|---|
| Taurine | 0.04 | (0.31) | 0.03 | 0.31 | 0.03 | 0.31 | 0.08 |
| Hydroxyproline | 0.11 | (0.84) | 0.03 | 0.31 | 0.03 | 0.31 | 0.09 |
| Aspartic Acid | 0.92 | (7.05) | 0.60 | 6.26 | 0.59 | 6.03 | 1.09 |
| Threonine | 0.49 | (3.75) | 0.34 | 3.55 | 0.35 | 3.58 | 0.30 |
| Serine | 0.65 | (4.98) | 0.44 | 4.59 | 0.44 | 4.49 | 0.88 |
| Glutamic Acid | 2.24 | (17.2 | 1.89 | 19.71 | 1.93 | 19.71 | 2.04 |
| Proline | 1.14 | (8.74) | 0.86 | 8.97 | 0.90 | 9.19 | 1.1 |
| Lanthionine | 0.00 | (0.00) | 0.00 | 0.00 | 0.00 | 0.00 | --- |
| Glycine | 0.73 | (5.59) | 0.35 | 3.65 | 0.36 | 3.68 | 0.8 |
| Alanine | 0.94 | (7.20) | 0.75 | 7.82 | 0.76 | 7.76 | 1.4 |
| Cysteine | 0.32 | (2.45) | 0.20 | 2.09 | 0.21 | 2.15 | 0.8 |
| Valine | 0.71 | (5.44) | 0.47 | 4.90 | 0.48 | 4.90 | 0.9 |
| Methionine | 0.24 | (1.84) | 0.18 | 1.88 | 0.20 | 2.04 | 0.2 |
| Isoleucine | 0.49 | (3.75) | 0.36 | 3.75 | 0.36 | 3.68 | 0.8 |
| Leucine | 1.47 | (11.3) | 1.31 | 13.66 | 1.33 | 13.59 | 1.6 |
| Tyrosine | 0.33 | (2.53) | 0.26 | 2.71 | 0.26 | 2.66 | 0.3 |
| Phenylalanine | 0.63 | (4.83) | 0.50 | 5.21 | 0.50 | 5.11 | 1.0 |
| Hydroxylysine | 0.03 | (0.23) | 0.02 | 0.21 | 0.02 | 0.20 | 0.03 |
| Ornithine | 0.01 | (0.08) | 0.01 | 0.10 | 0.01 | 0.10 | 0.01 |
| Lysine | 0.47 | (3.60) | 0.28 | 2.92 | 0.28 | 2.86 | 0.6 |
| Histidine | 0.35 | (2.68) | 0.27 | 2.82 | 0.28 | 2.86 | 0.7 |
| Arginine | 0.67 | (5.13) | 0.39 | 4.07 | 0.41 | 4.19 | 1.0 |
| Tryptophan | 0.07 | (0.54) | 0.05 | 0.52 | 0.06 | 0.61 | 0.08 |
| Total | 13.05 | | 9.59 | | 9.79 | | |
| Crude Protein* | 14.20 | | 9.89 | | 10.14 | | |

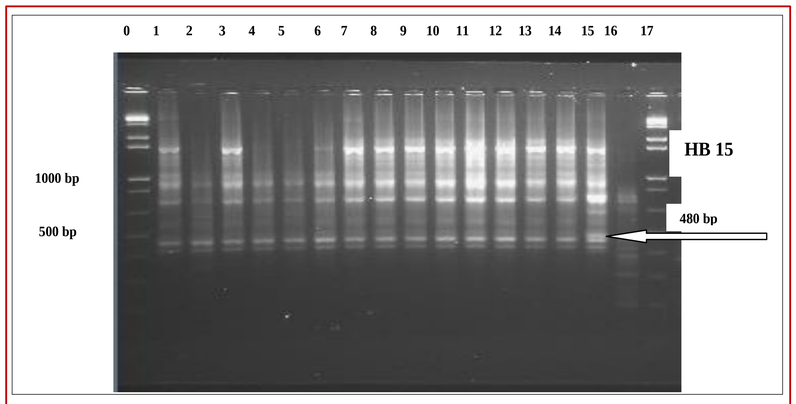


Figure 2: ISSR amplification of maize accessions DNA with primer HB 15.

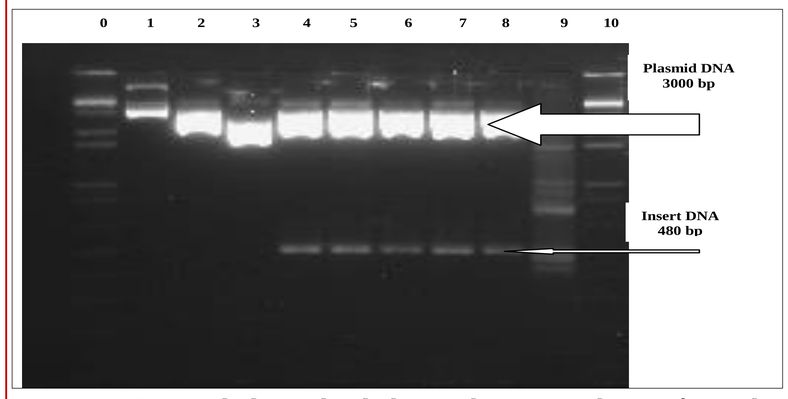


Figure 3: Digested plasmid gel electrophoresis with transformed bacteria clones.

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GTGGTGGTGG  CTCTGGGGGA  AACCGgGCTG  GATTATCACT  ATCAGCCAGA  AACAAAAGAT  CAGCAGCAGC
GCTCGTTCCT  GGAACATATC  CGTACCGGTA  TTGCGCTGAA  CAAACCGATC  ATTGTTCACA  CCCGTGATGC
CCGCGAAGAT  ACCCTGACGA  TTCTGCGTGA  AGAGCAGGTT  GAACGTTGCG  GCGGCGTGCT  GCACTGCTTC
ACTGAGGATC  AGCCCACCGC  AGCAAAACTG  CTGGATATGG  GCTTTTACAT  CTCTTTTTCC  GGCATCGTCA
CATTCCGCAA  TGCCGAGCAG  TTACGTGAAG  CCGCACGCTA  TGTGCCGCTG  GATCGGATGC  TGGTGGAAAC
GGATTCGCCT  TATCTGGCAC  CGGTGCCTTT  CCGTGGTAAA  GAGAATCAGC  CCGCTTATAC  GCGCGATGTT
GCCGAATATC TGGCTATCCT GAAAGGGGTG GATTTAGAAA CGCTGGCAGC CACCACCAC
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Figure 4: Consensus sequence of variety-diagnostic ISSR marker of 480 bp from MUDISHI 3 generated by ISSR primer HB 15.

## CONCLUSIONS

- Protein analysis data revealed that MUDISHI 3 is a QPM variety that is distinct from its original population (DMR-ES-W).
- One primer revealed a diagnostic marker for QPM MUDIHI 3.
- Primers flanking the diagnostic marker sequence were developed.
- Further analysis of several other ISSR and RAPD primers is required to achieve the main goal of developing variety-specific markers in the targeted breeding program.

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