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Decrease in Lysine and Tryptophan Content in S_2 Inbred Lines from a Quality Protein Maize (QPM) Variety in a Breeding Program

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Abstract

Several countries in Africa, Latin America along with China have incorporated QPM in their Agricultural development plan. A new quality protein maize variety (QPM) was developed by breeders and farmers using the participatory breeding approach in the DR-Congo. It is adapted to all the maize growing regions in the country. Inbred lines from this new variety were produced for further development of maize synthetic populations. The main objective of the present study is to determine the level of amino acid changes in early generations of inbred lines. The results of the study revealed a significant decrease of 33% and 38% of tryptophan in S_1 and S_2 inbred lines compared to the original parental MUDISHI 3 population, respectively. There was a decrease of 15% of lysine in S_2 inbred lines compared to the parental MUDISHI 3. Actually, S_2 inbred lines of MUDISHI 3 contain similar level of lysine compared to the genetically improved normal maize (Salongo 2) that is currently released. The development of composite lines is recommended over synthetic populations to maintain the high levels of lysine and tryptophan along with other desirable agronomic characteristics since they involve the intercrossing of open pollinated varieties.

Keywords

Quality Protein Maize, Lysine, Tryptophan, Amino Acid Profile, Inbred, Maize Synthetic Population, MUDISHI 3, DR-Congo

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1. Introduction

Several countries in Africa, Latin America along with China have incorporated Quality Protein Maize (QPM) in their Agricultural development plan [1]. The majority of these national programs limit their breeding effort to evaluate and selecte from international germplasms QPM varieties already released. Because of variations in climate and regional needs, QPM adapted varieties designed for local applications need to be developed. Conversion of non-QPM varieties to QPM has been used by several programs based on the backcross method. However, development of synthetic maize varieties combining different agronomic characteristics from several parents requires the development of inbred lines through self-pollination.

Inbred line development requires several generations of plant selfing (up to S6) until homozygous lines are obtained. Synthetic maize populations are usually developed by intercrossing inbred lines [2] [3]. They can be used by either farmers for commercial production or breeders as source populations from which to select new lines.

A new QPM maize variety adapted to DR-Congo maize growing areas has been developed [4]. But this variety showed high level of susceptibility to insects in storage after harvest. A breeding program aiming at developing synthetic variety combining several qualities of new QPM and insect resistance from local varieties has been initiated. Several inbred lines have been produced and characterized using molecular techniques [4]. Studies have shown that postharvest insect-pest resistance is quantitatively inherited [5] [6]. Transmission of these genes in advanced maizeinbred lines has been confirmed [7]. The genetic control of lysine and tryptophan content in Quality Protein Maize Varieties is also qualitative [8] [9]. But the transmission of these genes in inbred lines has not been investigated.

The main objective of the present study is to determine the level of amino acid changes in early generations of inbred development.

2. Materials and Methods

2.1. Development of MUDISHI 3

MUDISHI 3 is an open-pollinated quality protein variety developed by the National Institute for Agronomic Study and Research, (INERA—DR-Congo) and Laurentian University, Sudbury, Ontario, (Canada). It was developed by breeders and farmers using the participatory breeding approach. The original variety used to develop MUDISHI 3 was DMR-ESR-W-QPM obtained from the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria.

The variety is the results of open pollinations of the parental line with several QPM and normal maize varieties that were grown in the same location for few seasons. The QPM accessions include QPM Longe 5, ECAVE-3, ECAVE-4, ECAVE-6, QPM-SR-Synth, and Susuma and the normal maize involved are DMR-ESR-W, AK9331-DMR-ESR-Y, Salongo 2, MUS 1, GPS 5, and Locale 1. The open pollinated plants were grown and progenies were selected in isolation for different agronomic characteristics for several cycles in different environments. The main selection criteria include, spike size, resistance to mildew and maize streak virus, grain yield and nutritional quality (lysine, tryptophan and other amino acid contents), and organoleptic characteristics. Plant selection and variety evaluation were performed using participatory variety selection (PVS) approach with local farmers led by breeders.

2.2. Inbred Development Method

Inbred lines have been developed by inbreeding selected heterozygous plants from MUDISHI 3 QPM. Selected plants (S_0) in MUDISHI 3 population were hand-pollinated and pedigree selection was practiced. This latter method consists essentially of selfing the individual plants selected. Best ears were planted using ear-to-row method at 0.75 m × 0.25 m with one plant per hill in a separate block of selfing. Fertilizers were applied based on local recommended rate (64-46-0). Prior to the initiation of flowering, plants to be hand pollinated were checked daily for signs of ear shoot emergence and pollen shedding. Before the silks emerge, the ear shoot on the plant to be pollinated was covered with a semitransparent shoot bag anchored securely against the stalk to prevent dislodging by wind or rain. While doing that, care was taken to leave enough space between the shoot tip and the shoot bag to allow silks to develop properly.

About 24 hours prior to pollination, the tassel already shedding pollen was covered with a tassel bag (polli-

nating bag) secured with a paper clip. On the day of pollination, the covered tassel was lightly shacked inside the tassel bag to dislodge pollen and the tassel bag containing pollen was then unstapled and carefully removed and emptied over the exposed silks. Finally, the tassel bag was placed over the pollinated shoot and stapled and remained in place until harvest to avoid undesirable probable pollen.

The selection of desirable genotypes was done during growth stage and at harvest based on different agro-morphological criteria. Vigorous plants resistant to maize downy mildew, streak virus and stem borers were selected. Short genotypes with low position of ears on the plants were preferred. Lines with cobs with abnormal shape and with few kernels were discarded. Inbred lines with few kernels per cob and cob with abnormal shape, non-straight kernel per row, chalky kernels, and high level of husk exposed tips were not selected. Likewise, rotten ears were also discarded. Selected ears were planted the following season using the same scheme described above up to S_2 stage.

2.3. Protein and Amino Acid Analysis

Amino acid analyses were conducted at the University of Missouri (USA) Agricultural Experiment Station Chemical Laboratories (ESCL). Total amino acid profiles were determined for MUDISHI 3, S1, and S2 inbred lines. One locally released and genetically improved normal maize variety (Salongo 2) was also analyzed as reference. All the analyses were conducted in triplicates. The grain amino acid concentration was evaluated using AOC standard method (Method 982.30 E (a, b, c), AOAC [10]. Crude protein was determined by combination analysis (Method 990.03, AOAC [10] using the formula crude protein = N × 6.25. ANOVA (two-way) was used to identify significant variation for each amino acid and crude protein. The least significant differences were determined to compare means.

3. Results and Discussion

MUDISHI 3 was developed in the DR-Congo maize breeding program using a QPM variety (DMR-ESR-W-QPM) from Ibadan, Nigeria. Although this variety is recommended for low to middle altitudes in the DR-Congo, it can perform well in other regions under optimal growing conditions. A complete agronomic and morphometric profile of this variety is described in **Table 1**. Additional information has been reported in Mbuya *et al*., [1] and Nkongolo *et al*., [4]. This variety is relatively short (150 cm on average), has a relatively short reproductive cycle (100 days) and can reach 3 T to 4 T under mineral fertilization. It is very resistant to lodging, down mildew, and maize mosaic virus, but highly susceptible to maize weevil (*Sitophilus zeamais*) and larger borer (*Prostephanus truncates*). **Figure 1** shows the locations in the DR-Congo where this line has been field-evaluated. **Figure 2** shows MUDISHI 3 in a field trial and **Figure 3** illustrates cob characteristics.

The overall amino acid composition of the maize varieties and the levels of statistical significance obtained from the analysis of variance are shown in **Table 2**. There was a significant decrease of 33% and 38% of tryptophan in S_1 and S_2 inbred lines compared to the original parental MUDISHI 3 population, respectively. Lysine content was 3.5 g and 3.6 g of lysine/100 g for MUDISHI 3 and the S_1 Inbred lines, respectively. There was a decrease of 15% of lysine in S_2 inbred lines compared to the parental MUDISHI 3. Actually, S_2 inbred lines of MUDISHI 3 contains similar level of lysine compared to the genetically improved normal maize (Salongo 2) that is currently released.

The other potentially limiting amino acids are threonine, isoleucine and methionine. Threonine and isoleucine levels were relatively similar in MUDISHI 3, S_1 and S_2 inbred lines and Salongo 2. A small but significant decrease of 9% of methionine in S_2 inbred lines compared to MUDISHI 3 was observed. The levels of leucine and glutamic acid, were higher in S_2 inbred lines compared to S_1 inbred lines and MUDISHI 3 parental population.

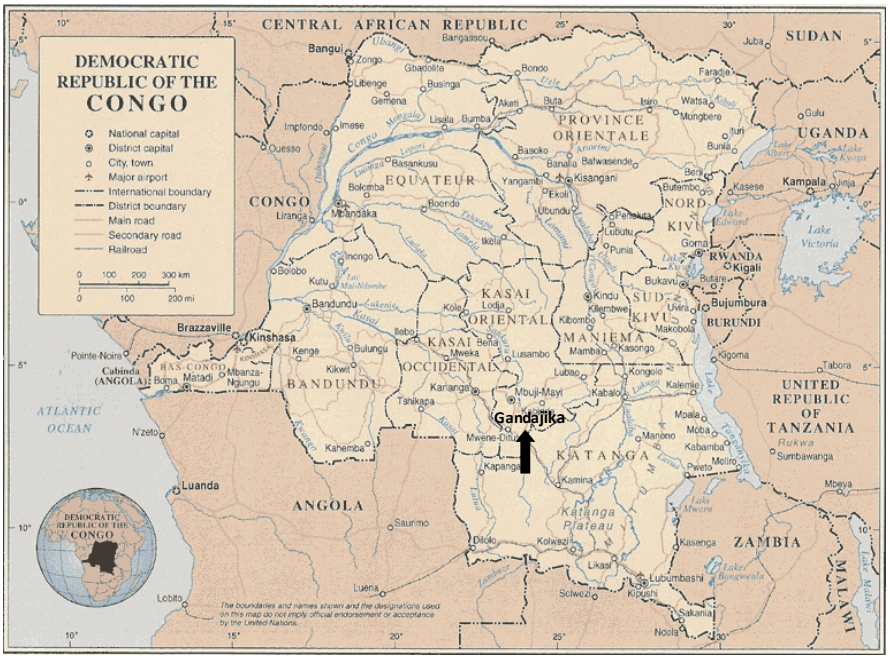
Overall, the total basic acids, which include lysine, arginine, and histidine constituent 11.5%, 11.4%, and 13.3% of the total amino acids for MUDISHI 3, S_1, and S_2 inbred lines, respectively. This value was lower (9.8%) in normal maize Salongo 2. In general, the total basic acids are considerably lower than the acidic amino acids (aspartic acid and glutamic acid), which represent around 24.5%, 24.3%, and 26.1% of the total amino acid residue for both MUDISHI 3, S_1 and S_2 inbred lines, respectively. For Salongo 2, the acidic amino acid level was 26.0%.

Negative correlation between protein content and grain yield in maize is well established. But knowledge on relationships between agronomic traits and lysine and tryptophan content is limited. In the present study, inbred lines from a QPM variety were selected for resistance to maize downy mildew, streak virus and stem borers, and

Table 1. Morpho-agronomic profile of MUDISHI 3.

Items	Characteristics
Species	*Zea mays*
Family	Graminaea
Variety name	MUDISHI 3
Variety type	Quality Protein Maize
Parental line	DMR-ESR-W QPM
Year of release	2012
Institution	INERA/Gandajika, DR-Congo (in collaboration with Laurentian University-Canada)
Recommended region	Low to middle altitudes in the DR-Congo (Kasaï, Nord Katanga, Bas Congo, Bandundu)
Rainfalls	400 - 800 mm
Soil type	Sandy-clay
Recommended spacing	75 cm × 50 cm
Seed quantity per Ha	25 kg
Days to 50% male flowering	51
Days to 50% female flowering	54
Plant height	154 cm
Reproductive cycle	100 days
Cob length	16.6 cm
Cob form	Cylindrico-conical
Number of row per cob	14 - 16
Number of grain per cob	495
Spikes position at maturity	Vertical
Grain form	Flint-dent corn
Grain color	White
Weight of 1000 grains	250 grams
Ginning percentage	84%
Female flower color	Purple
Male flower color	Purple
Rachis color	White
Stem color	Dark green
Leaf color	Dark green
Yield at research station (with fertilizers)	3 - 4 tonnes/hectare
Yield in farmer field (without fertilizers)	0.8 - 1.0 tonnes/hectare
Lodging resistance	Very high
Down mildew resistance	Very high
Maize streake virus resistance	High
Weevil and larger borer resistance	Very low (highly susceptible)

(a)



(b)

Figure 1. (a) A section of an African map showing DR-Congo; (b) DR-Congo map showing the location of MUDISHI 3 development (arrow). The multinational testings were conducted in Kasai Oriental, Kasai Occidental, Katanga, Bandundu, Bas Congo, Maniema, Sud Kivu and North Kivu provinces.

Figure 2. Illustration of MUDISHI 3 Cobs.

Figure 3. MUDISHI 3 field trial in Gandajika (Kasai Oriental-DR-Congo).

Table 2. Total protein and essential amino acid content in quality protein maize (QPM) and normal maize varieties from DR-Congo breeding program.

	QPM	**QPM****	**QPM****	**Normal**	
	MUDISHI 3	MUDISHI 3	MUDISHI 3	SALONGO 2	
Essential AA w/w (%)*		INBRED-S1	INBRED-S2		**LSD**
Taurine	0.11 (1.2)	0.04 (0.31)	0.03 (0.30)	0.03 (0.3)	0.05
Hydroxyproline	0.02 (0.3)	0.11 (0.84	0.03 (0.30)	0.03 (0.3)	0.05
Aspartic Acid	0.56 (6.3)	0.92 (7.05)	0.65 (6.50)	0.60 (6.3)	0.60
Threonine	0.31 (3.5)	0.49 (3.75)	0.35 (3.5)	0.34 (3.6)	0.30
Serine	0.41 (4.6)	0.65 (4.98)	0.45 (4.48)	0.44 (4.6)	0.45
Glutamic Acid	1.63 (18.2)	2.24 (17.2)	1.96 (19.56)	1.89 (19.7)	1.00
Proline	0.82 (9.4)	1.14 (8.74)	0.90 (9.00)	0.86 (9.0)	0.70
Lanthionine	0.00 (0.0)	0.00 (0.00)	0.00 (0.00)	0.00 (0.0)	-
Glycine	0.38 (4.3)	0.73 (5.59)	0.40 (3.99)	0.35 (3.7)	0.45
Alanine	0.65 (7.3)	0.94 (7.20)	0.77 (7.68)	0.75 (7.8)	0.70
Cysteine	0.22 (2.5)	0.32 (2.45)	0.22 (2.20)	0.20 (2.1)	0.40
Valine	0.44 (5.0)	0.71 (5.44)	0.49 (4.89)	0.47 (4.9)	0.45
Methionine	0.20 (2.3)	0.24 (1.84)	0.21 (2.10)	0.18 (1.9)	0.10
Isoleucine	0.31 (3.5)	0.49 (3.75)	0.37 (3.69)	0.36 (3.8)	0.40
Leucine	1.05 (11.9)	1.47 (11.3)	1.30 (13.0)	1.31 (13.7)	0.80
Tyrosine	0.22 (2.5)	0.33 (2.53)	0.27 (2.69)	0.26 (2.7)	0.15
Phenylalanine	0.43 (4.9)	0.63 (4.83)	0.51 (5.09)	0.50 (5.2)	0.60
Hydroxylysine	0.02 (0.2)	0.03 (0.23)	0.02 (0.20)	0.02 (0.2)	0.17
Ornithine	0.01 (0.1)	0.01 (0.08)	0.01 (0.10)	0.01 (0.1)	0.00
Lysine	**0.29 (3.5)**	**0.47 (3.6)**	**0.30 (3.0)**	**0.28 (2.9)**	0.30
Histidine	0.29 (3.3)	0.35 (2.68)	0.29 (0.29)	0.27 (2.8)	0.35
Arginine	0.42 (4.7)	0.67 (5.13)	0.44 (4.4)	0.39 (4.1)	0.60
Tryptophan	**0.07 (0.8)**	**0.07 (0.54)**	**0.05 (0.50)**	**0.05 (0.5)**	0.05
Total	8.86	13.05	10.02	9.59	-
Crude Protein	9.55	14.20	10.56	9.89	-

*The values are expressed in w/w = grams per 100 grams of sample. The number is parentheses represent the percent (%) of individual amino acid in the crude protein. AA = Amino Acid. **, Inbred S1 and S2 represent first and second generation of inbred lines derived from MUDISHI 3, respectively.

other agronomic characteristics. Results showed a significant decrease of tryptophan and lysine in selected S_2 inbred lines compared to the original MUDISHI 3. This is consistent with previous studies indicating that whole-grain protein content and quality are generally negatively correlated with other agronomic traits [11] [12].

Reduced lysine and tryptophan in S_2 inbred lines is likely the result of targeted traits that were not associated with opaque-2 modifiers genes. In fact, most of the agronomic traits used for selection of inbred in the present study are controlled by several genes located on different chromosomes. These characters included grain yield, resistance to downy mildew, and maize mosaic virus. Sixteen quantitative trait loci (QTLs) have been mapped for tropical grain yield on seven chromosomes (1 to 7) [13]. Studies have shown that resistance to downy mildew resistance in maize is also polygenic. Three QTLs have been detected that affected significantly resistance to this disease. Two of these mapped closely on chromosome 1 and the third one is located on chromosome 9 [14]. On the other hand, resistance to maize mosaic is controlled by a major dominant gene located on chromosome 3 [15]. Mapping for opaque-2 modifiers influencing the tryptophan and lysine content in quality protein maize revealed five significant QTLs on chromosomes 5, 7, and 9 [16].

Conversion of normal maize to QPM through back crossing and recurrent selection has been successful because the agronomic characteristics of recipient parent are maintained and the level of lysine and tryptophan is monitored over generations. Breeders at CIMMYT have developed a large number of elite QPM varieties for distribution [17] [18]. QPM breeding protocols have been geared towards increasing/maximizing the frequency of modifiers at each step. However when developing inbred, segregation of genes controlling different desired traits make it difficult to maintain both the protein quality and the agronomic traits in late generations. Inbred combination and backcrossing might be required to develop synthetic populations that carry modifiers and genes for high yield and disease resistance.

To improve the odds of combining protein quality and agronomic performance, development of composites might be more suitable than synthetics. For this purpose, open pollinated varieties (OPVs) are intercrossed instead of inbred [3]. These populations are easy to maintain and to produce in large quantities [19].

4. Conclusion

Several S_1 and S_2 inbred lines derived from the newly released QPM variety (MUDISHI 3) were developed in the DR-Congo maize breeding program. Protein and amino acid analysis revealed a significant decrease of lysine and tryptophan in S_2 inbred lines compared to the parental variety (MUDISHI 3). Based on this result, the development of composite lines is recommended over synthetic populations to maintain the high levels of lysine and tryptophan along with other desirable agronomic characteristics.

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