Kernel modification and tryptophan content in quality protein maize breeding germplasm

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Abstract
Development of quality protein maize (QPM) includes kernel modification and tryptophan content evaluations. As a part of the breeding project at Maize Research Institute analyses of F1 and BC1 kernels for these traits for crosses between QPM x opaque2 lines were performed. F1 kernels had hard endosperm (scores 1 and 2). In BC1 six out of twelve crosses had the kernel modifications 2 and 3. Tryptophan content was high in both F1 (0.069) and BC1 (0.116). All analyzed genotypes showed good kernel modification and high tryptophan content but the influence of parental lines on these traits was perceived. The results will contribute to the choice of genotypes for further selection in breeding QPM.

Key words: kernel modification, opaque2, QPM, tryptophan

Introduction
Maize is one of the most important crops in human and animal nutrition. However, like most other cereals, its nutritional value is poor due to deficiency of two essential amino acids – tryptophan and lysine. It was shown that opaque2 (o2) mutation can nearly double the lysine content of the endosperm compared with the wild type (Mertz et al., 1964). Maize homozygous for the o2 (recessive) mutation has substantially higher lysine and tryptophan content than maize that is either homozygous dominant (O2O2) or heterozygous (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.

Incorporation of opaque2 into high yielding cultivars was not commercially successful, because of its numerous agronomic and processing problems – reduced grain yield, soft endosperm, chalky and dull kernel appearance and susceptibility to ear rots and stored grain pests. These drawbacks have been corrected in genetically improved, hard endosperm quality protein maize – QPM (Vivek et al., 2008). QPM is a genotype in which opaque2 has been incorporated along with associated modifiers. The endosperm hardness modifier genes convert the soft/opaque mutant endosperm to a hard/vitrous endosperm with little loss of protein quality. It has been shown that the increased levels of gamma zein likely contribute to the recovery of a hard endosperm phenotype, as the QPM grains have approximately double amount of gamma zein in the endosperm relative to the o2 mutants (Wallace et al., 1990). The amino acid modifier genes affect the relative levels of lysine and tryptophan content in the grain endosperm and multiple genes have been identified in controlling amino acid content (Wu et al., 2002).

Maize Research Institute maintains a collection of opaque2 inbred lines developed in the seventies (Denić et al., 1979). Recently, these lines have been analyzed for protein and tryptophan content and are used for breeding quality protein maize. Besides good agronomic performances, there are two unique and essential steps in the development of
QPM germplasm. The first is to simultaneously identify segregants in a family or population having the o2 allele in the homozygous recessive (o2o2) condition with a hard endosperm, through kernel modification score. The second step is to identify and confirm the presence of the essential amino acids, i.e. percentage of tryptophan in a sample. The breeding project at MRI involves crosses between QPM and opaque2 lines, with the aim to improve MRI opaque germplasm. Following the selection steps, choice of the material for further selection was based on kernel modification score and tryptophan level in each generation. Herein, we present the results of the analysis of F1 and BC1 generations for kernel modification and tryptophan content, for crosses between QPM and opaque2 lines.

Material and methods
Two QPM lines, CML144 and CML159 (from CIMMYT, Mexico) were crossed with six opaque2 lines, i.e. P801, P802, P803, P806, P809 and P820 (from MRI, Serbia). The F1 plants were backcrossed with the opaque2 parental line for producing BC1 seed. Kernel modification was visually assessed using light table, according to the scoring scale from 1 (completely translucent, with no opaqueness) to 5 (completely opaque). Modification score 2 is given to the kernel which is 25% opaque, while scores 3 and 4 are given to 50% and 75% opaque kernels, respectively.

Tryptophan content was determined according to Nurit et al. (2009). Shortly, the protocol is based on the Hopkins-Cole reaction, in which one molecule glyoxylic acid and two molecules tryptophan form a colored compound with a maximum absorption at 560nm. Briefly, a random sample of 30 seeds was grounded to a fine powder. The grounded samples were defatted and digested with papain. Colorimetric reaction was developed using FeCl3-6H2O dissolved in glacial acetic acid and 30N H2SO4. Absorption was read at 560nm in a spectrophotometer. Tryptophan % was calculated using a standard (calibration) curve, developed with known amounts of tryptophan, ranging from 0 to 30µg/ml.

Results and discussion
Selection of desired level of modification in the kernel is done to pick out kernels with the o2o2 genotypes with hard endosperm. A kernel with o2o2 genotype (soft endosperm) is seen as complete opaqueness, while kernels with O2O2 or O2o2 genotypes (hard endosperm) are translucent. Less opaqueness implies higher/more action of endosperm hardness modifier genes. It is recommended to select only types 2 and 3 in a conventional breeding approach. Type 3 kernels should be selected in early generations as it is a compromise between the guaranteed presence of o2o2 and good modification (Vivek et al., 2008).

Kernel modification scores for F1 and BC1 generations of QPM x opaque2 crosses are presented in Table 1. As expected, all kernels had hard endosperm (scores 1 and 2), due to the heterozygosity in F1 and effects of the QPM parent alleles. However, BC1 included also type 3 kernels. In six out of twelve presented crosses the kernels were scored 2 and 3, what is in accordance with recommendation for selection in early generations. One cross comprised kernel types 1, 2 and 3, and all the other crosses only types 1 and 2. Although kernel types 1 and 2 are not the best choice for early selection, they were chosen for the next generation due to their tryptophan content. The results also indicate the potential influence of opaque2 parent on kernel modification, since with both CML144 and CML159 the same opaque2 lines, i.e. P801, P802 and P809 comprised kernel modification type 3.
Both lysine and tryptophan concentrations are increased in QPM, but only tryptophan is analyzed on routine basis. This is because lysine and tryptophan are highly correlated and, normally, the value of lysine is four times that of tryptophan. Due to the well-established relationship between these amino acids in the protein of opaque2 maize endosperm (Hernandez and Bates, 1969; Villegas et al., 1992), tryptophan can be used as a single parameter for evaluating the nutritional quality. When interpreting the results of laboratory analysis for making selections, tryptophan has to be above the acceptable limits (Vivek et al, 2008). These limits are 0.07 in endosperm and 0.7 in whole grain. As the protocol for tryptophan content determination is time consuming and relatively complex, tryptophan can be measured either in endosperm or whole grain. Decision depends on the breeder’s choice. The crosses of QPM x opaque2 lines, as well as tryptophan content determination in F1 kernels, were done in Mozambique (IIAM - Instituto de Investigacao Agraria de Mocambique), where tryptophan is routinely measured in the endosperm and that is why our data for F1 kernels are for tryptophan content in the endosperm. However, breeders at MRI share opinion that, from the agronomic point of view, utilization of whole grain is more important than of endosperm alone and it was decided that from BC1 onwards tryptophan will be determined in whole grains. Tryptophan analysis of opaque2 inbred lines (data not published) revealed that tryptophan content over the acceptable limit in the endosperm was also over the acceptable limit in whole grain.

Tryptophan content in F1 kernels (endosperm) of the analyzed crosses is given in Graphic 1. Average content for all analyzed genotypes was 0.069, which can be considered the limit set for QPM germplasm. Our data (unpublished) on normal maize inbred lines showed that tryptophan content in the endosperm ranged from 0.05 to 0.059. Considering different QPM parental lines, it is clear that genotypes with CML144 parent had higher tryptophan content (0.072 on average) compared with CML159 genotypes (0.067 on average), CML144 x P806 and CML159 x P806 being the only exceptions. The later cross had higher tryptophan content.

The results of tryptophan determination in maize BC1 kernels (whole grain) are given in Graphic 2. In all analyzed genotypes tryptophan content was significantly higher than the limit set for QPM germplasm, i.e. the average content was 0.116. The same as in F1 kernels, the crosses with CML144 as a parental line had higher tryptophan content. On average, tryptophan content in CML144 x opaque2 genotypes was 0.124 and in CML159 x

<table>
<thead>
<tr>
<th>Cross</th>
<th>F1</th>
<th>BC1*</th>
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<tbody>
<tr>
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<td>CML159 x P809</td>
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<td>CML159 x P820</td>
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*recurrent parents are opaque2 lines

Table 1 Kernel modification scores for F1 and BC1 generations of QPM x opaque2 crosses
opaque2 lines 0.108. Only crosses with P806 parental line had the same tryptophan content. These results are in agreement with Okello et al. (2006), where agronomical, biochemical and phytopathological characteristics of the QPM inbred lines (including CML144 and CML159) indicated differences in the expression of various traits between them.

![Graphic 1 Tryptophan content (%) in F1 maize kernels (endosperm).](image1)

![Graphic 2 Tryptophan content (%) in BC1 maize kernels (whole grain).](image2)

**Conclusion**

Although it can be concluded that all analyzed genotypes showed good kernel modification and high tryptophan content, the results indicated the influence of parental lines in F1 and BC1 populations of QPM x opaque2 crosses. The degree of modification depended on the opaque2 parent and tryptophan content on the QPM parent. Most crosses with CML144 were superior in tryptophan content compared with crosses that had CML159 as a parental line, both in F1 and BC1. These results will contribute to the choice of genotypes, together with phenotypic and disease resistance evaluation, for further selection in breeding high quality protein maize.
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References


