BOOK OF PROCEEDINGS

AgroSym 2023

XIV International Scientific Agriculture Symposium

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TRYPTOPHAN CONTENT INCREASE IN MAIZE INBRED LINES IMPROVED THROUGH MARKER ASSISTED BREEDING

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Abstract

Quality protein maize (QPM) has high content of essential amino acids lysine and tryptophan that are deficient in standard maize. Naturally occurring *opaque2* recessive mutation has been the most widely studied and used as a source for genetic improvement of the nutritional value of maize proteins. Marker assisted conversion of maize inbred lines to QPM adapted to temperate regions is being conducted at Maize Research Institute Zemun Polje (MRIZP). The ultimate goal is selection of the *opaque2* recessive genotypes (foreground selection) with the highest proportion of recurrent parent′s genome (background selection) along with high lysine/tryptophan content without losing good agronomic performances of the original line. After two backcrossing and selection of heterozygous individual plants, *opaque2*-specific molecular markers phi057 and umc1066 successfully identified recessive homozygotes in BC_2F_2 generation. The next step was to confirm expression of the QPM trait through elevated kernel tryptophan content (TC). Laboratory analyses were used to quantify and select for acceptable tryptophan concentrations for QPM - above 0.075%. The results presented in this paper relate to identification of BC_2F_3 progenies with increased TC in the conversion of two MRIZP commercial inbred lines (RP_1 and RP_2). The average TC ranged from 0.070% in RP_1 to 0.074% in RP_2. Out of 60 progenies from these two lines, 27 had TC at or above the QPM treshold. A total of 12 progenies was chosen for the highest tryptophan content. These lines will serve as parental lines for developing QPM hybrids adapted to temperate regions.

Keywords: *maize, marker assisted breeding, tryptophan, quality protein maize.*

Introduction

Maize (*Zea mays* L.) is among the most important cereal crops across the world. However, due to the low level of two essential amino acids – lysine and tryptophan, it does not meet the daily balanced protein requirement. Improvement of the protein quality in maize began in the 1960s with the discovery of mutants that produce enhanced levels of lysine and tryptophan (Mertz et al., 1964). Quality Protein Maize (QPM) was developed using conventional breeding techniques and contains the mutation at *opaque2* (*o2*) loci, which changes the protein composition of the maize endosperm, resulting in 2-3 fold higher concentrations of lysine and tryptophan (Vasal, 2000). Due to its higher biological value (amount of nitrogen that is retained in the body), balanced nitrogen index and leucine-isoleucine ratio, QPM offers significant nutritional benefits, which is well demonstrated both in terms of human food and animal feed (Eshetie, 2017).

Marker assisted selection (MAS) gained considerable importance in complementing conventional breeding as it increases efficiency, reduces time and costs taken to obtain QPM (Babu et al., 2004). The use of molecular markers in QPM breeding programs shortens the selection process during development of improved genotypes, making it more efficient across environments (Tandzi et al., 2017). Molecular markers are used to control all steps for

introgression of a target locus from a donor to a recipient line. Babu et al. (2005) reported conversion of standard maize inbred line to its QPM version through a combination of marker assisted and phenotypic selection. Their rapid line conversion strategy included a two-generation backcross program that employs foreground selection for the *opaque2* gene in two backcross generations, background selection at non-target loci in the BC_2 generation, and phenotypic selection for kernel modification and other desirable agronomic traits in two subsequent selfed generations. As concluded by these authors, this integrated breeding strategy can be applied to reduce genetic drag as well as the time involved in a conventional line conversion program. Gupta et al. (2013) demonstrated the introgression of *o2* allele to the parental lines of a released Indian maize hybrid through marker assisted backcross breeding followed by reconstitution of the original hybrid with 30% enhanced lysine and 41% increase in tryptophan content over the original hybrid.

Marker assisted conversion of commercial maize inbred lines to QPM adapted to temperate regions is being conducted at Maize Research Institute Zemun Polje (MRIZP) (Kostadinovic et al., 2014, 2016). Molecular markers are being used to identify the *opaque2* recessive genotypes (foreground selection) with the highest proportion of recurrent parent′s genome (background selection) along with high lysine/tryptophan content without losing good agronomic performances of the original line. After two backcrossing and selection of heterozygous individual plants, *o2*-specific molecular markers successfully identified recessive homozygotes in BC_2F_2 generation. The next step was to confirm expression of the QPM trait through elevated kernel tryptophan content. The results presented in this paper relate to identification of BC_2F_3 progenies with increased tryptophan content in the conversion of two MRIZP commercial maize inbred lines.

Material and methods

Plant material

Two MRIZP commercial inbred lines, adapted to the local environmental conditions in Serbia, were chosen for marker assisted conversion to high tryptophan maize. These lines, used as the recurrent parents (RP_1 and RP_2), are components of the leading MRIZP hybrids. One commercial MRIZP inbred line ZPL5, which was converted to its QPM counterpart (Kostadinovic et al., 2016), was used as *o2* donor. Throughout the conversion process, F_1, BC_1, BC_2, BC_2F_2 and BC_2F_3 generation were developed (Figure 1). Molecular markers were employed both in foreground (BC_1, BC_2, BC_2F_2) and background (BC_2) selection to ultimately identify recessive homozygotes with the highest recovery of recurrent parent's genome (RPG). Their progenies (BC_2F_3) were subjected to biochemical analysis to confirm expression of the QPM trait through elevated kernel tryptophan content.

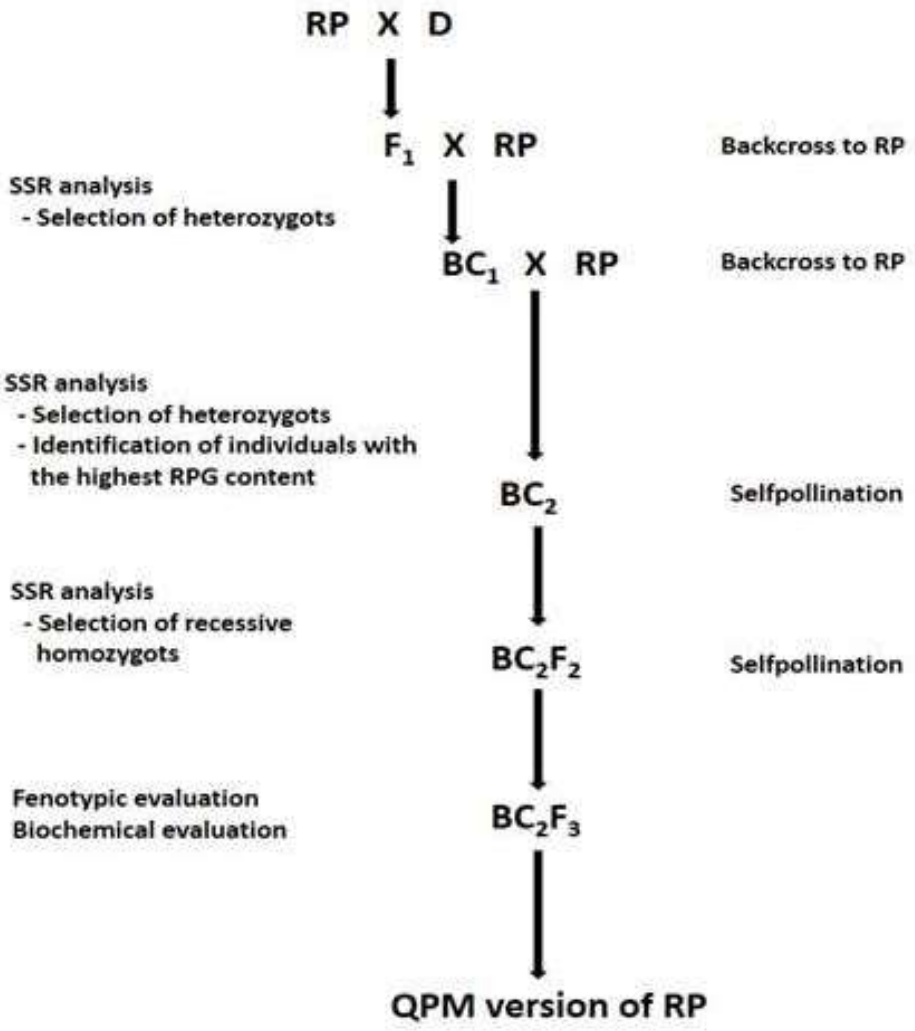

Figure 1. Schematic presentation of marker assisted conversion of a standard maize inbred line to its QPM version (RP-recurrent parent, D-donor line, RPG-recurrent parent's genome, QPM-quality protein maize)

Biochemical analyses

Two sub-samples per genotype, consisting of 30 kernels each, were dried in a controlled oven at 65°C/16-18 hours to constant dry weight, milled using a Perten 120 lab mill (Perten, Sweden) to obtain fine powder (<500 μm). Flour was defatted by hexane treatment in Soxhlet extractor. Tryptophan content was determined by the colorimetric method given in Nurit et al. (2009). Shortly, the color was developed in the reaction of flour hydrolysate, obtained by overnight digestion with papain solution at 65°C, with a reagent containing glyoxylic acid and ferric chloride dissolved in sulfuric acid. After incubation at 65°C/30 min, absorbance was read at 560 nm. Tryptophan content was calculated using a standard calibration curve, developed with known amounts of tryptophan, ranging from zero to 30 μg/μl. Analyses were carried out in duplicate for each sample and the results were presented as mean ± SD.

Results and discussion

The biggest challenge in QPM breeding is to improve the protein quality while simultaneously achieving grain yield at the level of standard maize hybrids. The development of QPM genotypes involves three distinct genetic systems: *opaque2* genetic system, endosperm-modifier genetic system and amino acid-modifier system (Maqbool et al., 2021).
Opaque2 gene, recessive mutant alleles, comprising the first genetic system for QPM development. This naturally occurring mutation was widely studied as a source for genetic improvement of the nutritional value of maize proteins. Simple sequence repeats phi057 and umc1066, located within *opaque2* gene, were effectively used to distinguish between recessive and dominant alleles (Kostadinović et al., 2014; Kostadinović et al., 2022). Consequently, these molecular markers are thus being used to increase the value of conventional breeding through indirect selection of plants which possess the desirable gene in segregating populations (Afolayan et al., 2019). After two backcrossing and selection of heterozygous individual plants, these *o2*-specific molecular markers successfully identified recessive homozygotes in BC_2F_2 generation. Homozygous recessive plants were selfpollinated to produce BC_2F_3 kernels for endosperm modifications score and tryptophan content analyses.
Alleles of endosperm hardness modifier genes constitute the second genetic system for QPM breeding. Kernels that have very soft endosperm and no modifiers also have undesirable characteristics such as susceptibility to ear rots and pests, and kernel cracking. Therefore, it is important to accomplish sufficient degree of endosperm modifications to consequently achieve higher grain yields (Eshetie, 2017). Prior to biochemical analyses, kernel endosperm modifications were visually assessed using light table, according to the scoring scale from 1 (completely translucent, with no opaqueness) to 5 (completely opaque), as presented in Vivek et al. (2008). This visual phenotypic selection served as an appropriate tool to separate *o2o2* genotypes with modified endosperm (Twumasi-Afriyie et al., 2016). Over 95% kernels showed good (types 1 and 2) and medium (type 3) endosperm modifications, which corresponds to standard maize kernels (Vivek et al., 2008). Only hard endosperm kernels (≤50% opaque) were selected for tryptophan content determination. Derivations with soft kernels, as well as those with the insufficient number of kernels, were discarded out of further biochemical analyses.
Amino acid modifier genes, the third genetic system, affects the lysine and tryptophan contents of maize grain. Lysine and tryptophan concentration in maize kernels are highly correlated (3:1 ratio of lysine to tryptophan). Therefore, in a QPM breeding program only one of these amino acids is typically monitored, tryptophan being the more commonly measured of the two (Nurit et al., 2009). When interpreting laboratory results for making selections, the tryptophan content (TC) has to be above the acceptable limit (QPM threshold) - above 0.075% (Vivek et al., 2008). Out of 60 progenies from two lines analyzed here, 27 had TC value at or above the QPM treshold (Figure 2). The average TC ranged from 0.070% in RP_1 to 0.074% in RP_2. Somewhat higher TC values were found in RP_2 (0.070-0.077%) compared to RP_1 progenies (0.068-0.077%).

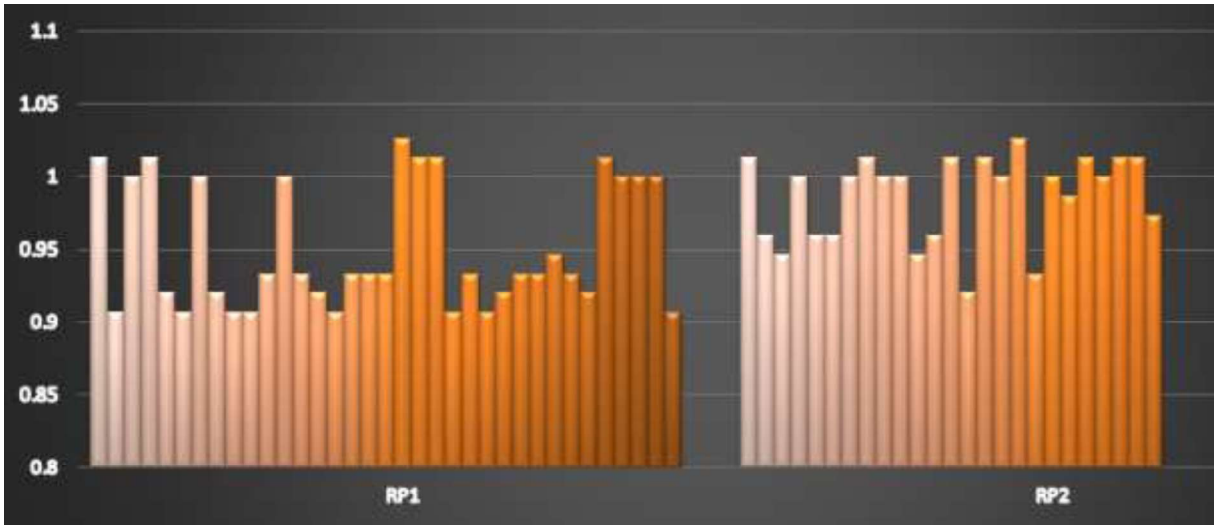

Figure 2. Tryptophan content of the two recurrent parents′ (RP_1 and RP_2) derivations given as % of the QPM treshold (given as 1).

Similar results were found in Jompuk et al., (2011) where TC ranged from 0.070 to 0.084% in QPM inbred lines obtained by the backcross method using marker-assisted selection of the *opaque2* gene. In Babu et al. (2005) biochemical analysis showed somewhat higher TC (0.078 to 0.094%), which was in accordance with our previous work (Kostadinović et al., 2022). In another study, the introgression of *o2* allele was made into the β-carotene-rich inbred lines (Chandran et al., 2019). Besides higher β-carotene content, these improved lines were having higher TC (0.073% to 0.081%) along with better agronomic performance.
A total of 12 progenies was chosen for the highest tryptophan content. These lines will serve as parental lines for developing QPM hybrids adapted to temperate regions. Developed QPM hybrids will be tested for their field performance and relevant biochemical components. A promising hybrid for use in broiler feeds will be identified, with the aim to substitute costly synthetic lysine in their diets.

Conclusions

Co-dominant nature of the polymorphism exibited by the *opaque2*-specific molecular markers phi057 and umc1066 enabled successfull selection of heterozygotes and recessive homozygotes throughout this MAS process. Almost half of the progenies from selected recessive homozygotes with the highest recovery of recurrent parent's genome had tryptophan content at or above the QPM treshold. Among them, progenies with the highest tryptophan content were chosen to represent improved lines and to serve as parental lines for developing QPM hybrids adapted to temperate regions.

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