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Original scientific article

NUTRITIONAL ENHANCEMENT OF AN ADAPTED QPM HYBRID DEVELOPED THROUGH MARKER ASSISTED SELECTION

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Kostadinović M., D. Ristić, S. Božinović, V. Galić, J. Vančetović, D. Ignjatović-Micić (2025). *Nutritional enhancement of an adapted QPM hybrid developed through marker assisted selection*- Genetika, Vol 57, No.2, 259-266.

Quality protein maize (QPM) has a high content of the essential amino acids lysine and tryptophan that are deficient in standard maize kernels. Maize Research Institute Zemun Polje has a breeding program aimed to create QPM, using the integrated conventional and molecular breeding approach. The aim of this study was biochemical evaluation of a QPM hybrid, obtained by crossing adapted QPM inbred lines (commercial lines converted to their QPM version). Relevant nutritional parameters, such as protein, starch, lipid and amino acid content were analyzed, and tryptophan to protein ratio (TPR) was determined. The tryptophan content (Trp) was significantly increased ($p<0.05$) in the QPM hybrid in comparison with standard maize (SM) hybrid. Also, the Trp was 0.075%, which corresponds to the QPM threshold value. The tryptophan to protein ratio, as an indicator of improved nutritional protein quality, was significantly higher ($p<0.05$) in the QPM hybrid. The starch and lipid content were both significantly higher ($p<0.01$ and $p<0.05$, respectively) compared to SM. Furthermore, a highly significant correlation ($p<0.01$) was identified between TPR and starch content, as well as a significant correlation ($p<0.05$) between TPR and lipid content, indicating the possibility of simultaneous selection and improvement of these traits in our breeding material. Biochemical evaluation has confirmed the improved nutritional value of this QPM hybrid, thus it was identified as a promising hybrid for use in the feed industry.

Keywords: maize, tryptophan, protein quality, QPM, feed industry

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INTRODUCTION

Traditional breeding relies heavily on phenotype-based selection, made on morphological bases that were highly influenced by the environment (AKHTAR *et al.,* 2023). The backcross procedure method has been extensively used in plant breeding to introgress favorable alleles from a donor plant into a recipient elite genotype (recurrent parent). The goal is to recover a pure line that will contain the novel allele and be as good as the recurrent parent for all other important traits (LÜBBERSTEDT *et al.,* 2023). As the reconstruction of the recurrent parent genotype requires more than six generations, marker assisted selection (MAS) has gained immense importance as it precisely selects desirable plants and eliminates large scale biochemical estimation in the segregating generations (BEGNA, 2020). Considering that the high quality is the main goal of today´s crop breeding, the genetic enhancement of nutritional quality in grains is essential to increase the nutritional value and conduct high quality crop breeding (ZHENG *et al*., 2021).

Quality protein maize (QPM) has a high content of essential amino acids lysine and tryptophan that are deficient in standard maize kernels. Protein composition of maize endosperm changes due to the naturally occurring *opaque2* recessive mutation, resulting in two-three-fold higher concentrations of lysine and tryptophan (VASAL, 2000). Furthermore, the quality of QPM protein is 90% similar to milk due to the nitrogen balance index comparable with milk (JILO, 2022). Although QPM was primarily developed for human consumption, evaluation of the effects of dietary QPM on broilers showed improved livestock performance and decreased use of costly protein ingredients and lysine supplementation (IGNJATOVIĆ-MICIĆ *et al.,* 2025). Therefore, it can be beneficial for use in feeds, especially in developed countries where 78% of total maize production is used for feed (SOFI *et al*., 2009).

Maize Research Institute Zemun Polje has a breeding program aimed to create QPM, using the integrated conventional and molecular breeding approach (KOSTADINOVIC *et al.,* 2016; IGNJATOVIC-MICIC *et al.,* 2020; KOSTADINOVIĆ *et al.,* 2022). The aim of this study was biochemical evaluation of QPM hybrid, obtained by crossing adapted QPM inbred lines (commercial lines converted to their QPM version). As this hybrid is aimed for the feed industry, relevant nutritional parameters, such as protein, starch, lipid and amino acids contents were analyzed, and tryptophan to protein ratio (an indicator of improved nutritional protein quality) was determined.

MATERIALS AND METHODS

Plant material

Two commercial maize inbred lines, components of a leading MRIZP hybrid, were converted to their QPM versions through marker assisted backcross breeding (KOSTADINOVIĆ *et al*., 2022). The resulting QPM hybrid was subjected to biochemical evaluation and the results were compared with its standard maize (SM) counterpart.

Biochemical analyses

Each genotype was represented by 60 kernels, divided into two samples consisting of 30 kernels each. Samples were dried in a controlled oven (COLO LabExperts, Slovenia) at 65°C/16-

18 hours, milled (Perten Instruments, Sweden) and flour was defatted by hexane treatment in Soxhlet extractor (INKOLAB, Croatia). Analyses were performed in three replicates.

Tryptophan content (Trp) 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 of proteins 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 on spectrophotometer (Shimadzu UV-1601, Japan) 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. Protein content (PC) was determined by the standard Kjedahl method using FOSS Kjeltec^{TM} 8400 (FOSS, Sweden), based on nitrogen determination as explained in VIVEK *et al.* (2008). The protein was estimated from the nitrogen value as: PC (%) protein = N (%) nitrogen * 6.25 (conversion factor for maize). Tryptophan to protein ratio (TPR) in the sample was calculated as: TPR = 100 * (tryptophan content in the sample / protein content in the sample).

Lipids, starch and amino acids (lysine, threonine, methionine and cysteine) were analyzed commercially by the accredited reference SP Laboratory, Bečej, Serbia (http://www.splaboratorija.rs). Lipid content (LC) was determined by a Weibull-Stoldt standard gravimetric method, starch content (SC) by a standard polarimetric method (ISO 6493:2000), and amino acid content by a modified standard method (ISO13903:2005), using ion exchange chromatography with electrochemical detector.

Statistical analyses

The t-test (Microsoft Excel) was used to determine the significance of differences between the results obtained with two hybrids. Pearson's correlation coefficients (Microsoft Excel) were calculated between the estimated traits.

RESULTS AND DISCUSSIONS

The resulting QPM hybrid with improved nutritional value can be considered as a successful outcome of the conversion process. The expression of the QPM trait is confirmed through elevated kernel tryptophan content. The minimum criteria for QPM include Trp of 0.075% in whole grain samples (TWUMASI-AFRIYIE *et al.,* 2016). Biochemical analysis (Table 1) revealed that Trp was above the QPM threshold and higher in QPM compared to the SM hybrid.

The percentage of differences in the measured traits between standard maize and quality protein maize, as well as statistical significance of the differences, is illustrated in Figure 1. All amino acids were elevated in the QPM hybrid. Tryptophan content was significantly increased ($p<0.05$) in QPM hybrid in comparison with SM hybrid, which could affect the secretion of hormones, development of immune organs, and meat production and quality (FOUAD *et al.,* 2021). Also, Trp was 0.075%, which corresponds to the QPM threshold value. Lysine was significantly higher ($p<0.01$) in QPM hybrid (an increase of 147.37%). This improvement could lead to broiler meat yield increase, as lysine is crucial to protein synthesis for the growth of tissues, and the main essential amino acid required for muscle building (TESSERAUD *et al.,* 2001).

Table 1. Biochemical analysis of standard maize (SM) hybrid and its quality protein maize (QPM) version

Trait	SM				QPM			
	Average	SD	Max	Min	Average	SD	Max	Min
Macronutrients (%)								
Proteins	12.86	0.07	12.94	12.78	11.7	0.02	11.72	11.68
Lipids	4.76	0.01	4.77	4.75	4.84	0.01	4.85	4.83
Starch	62.24	0.13	62.35	62.13	63.95	0.12	64.06	63.84
Essential amino acids (%)								
Tryptophan	0.065	0.01	0.067	0.063	0.075	0.02	0.077	0.074
Lysine	0.19	0.01	0.2	0.18	0.47	0.02	0.49	0.45
Threonine	0.28	0.02	0.3	0.26	0.37	0.01	0.38	0.36
Methionine	0.2	0.01	0.21	0.19	0.22	0.01	0.23	0.21
Cystine	0.03	0.01	0.03	0.03	0.05	0.01	0.05	0.05

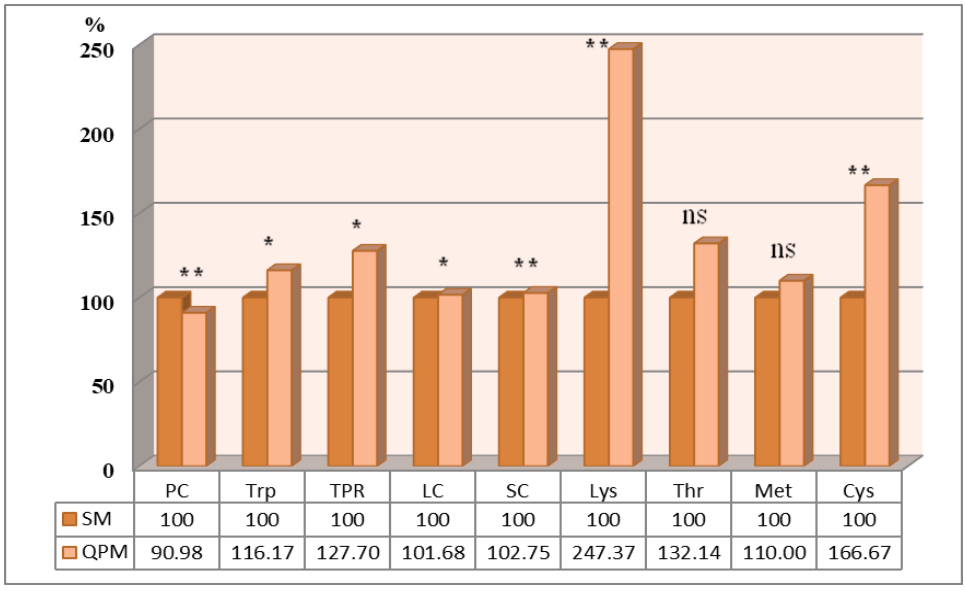

Figure 1. Difference (%) in protein (PC), tryptophan to protein ratio (TPR), lipid (LC) and starch content (SC), as well as essential aminoacids (tryptophan-Trp, lysine-Lys, threonine-Thr, methionine-Met and cysteine-Cys) content between standard maize (SM) and quality protein maize (QPM). Values for SM are given as 100%.

Methionine, precursor for cysteine, a dietary sulfur source, and an integral portion of body protein (OJANO-DIRAIN and WALDROUP, 2002), was increased for 10%, while threonine, important for the synthesis of gut mucosal proteins, as well as acting as a nutrient immune modulator that affects the intestinal barrier function of broilers (JI *et al.,* 2019), was increased for 32.14%. Cysteine was significantly higher ($p<0.01$) in QPM hybrid (an increase of 66.67%).

Protein content was lower by 9.02%. However, its value of 11.7% is still higher than 10% in the ZPQPM13 hybrid in our previous research (IGNJATOVIC-MICIC *et al.,* 2020). Nonetheless, the selection for QPM relates to the improvement of protein quality whose major indicator is tryptophan to protein ratio. As TPR was significantly higher ($p<0.05$) in the QPM hybrid, it can be concluded that the nutritional quality of the protein was improved. Starch and lipid contents were both significantly higher ($p<0.01$ and $p<0.05$, respectively) compared to SM. Also, LC was on a par and SC was higher (2.3%) in comparison to the aforementioned previous research.

Pearson's correlation coefficients and levels of significance are given in Table 2. Considering the PC decrease, resulting negative correlations with all other traits were expected. On the other hand, highly significant positive correlations ($p<0.01$) were found between LC and Lys and Thr, as well as between SC and Lys, Thr and Cys. Furthermore, highly significant correlation ($p<0.01$) was identified between TPR and SC, as well as significant correlation ($p<0.05$) between TPR and LC, indicating the possibility of simultaneous selection and improvement of these traits in our breeding material.

Table 2. Statistical significance of the correlations between biochemical traits of two hybrids.

	TPR	Trp	Lys	Thr	Met	Cys
PC	-0.976^{**}	-0.939^{**}	-0.986^{**}	-0.911^{**}	-0.563^{ns}	-0.925^{**}
LC	0.740^{*}	0.634^{ns}	0.865^{**}	0.968^{**}	0.723^{ns}	0.833^{*}
SC	0.975^{**}	0.947^{**}	0.984^{**}	0.913^{**}	0.537^{ns}	0.910^{**}

TPR – tryptophan to protein ratio, Trp - tryptophan, Lys - lysine, Thr - threonine, Met - methionine, Cys - cysteine, PC - protein content, LC - lipid content, SC - starch content, ns - not significant, * and ** - significant at 0.05 and 0.01 level, respectively.

Essential amino acids, such as lysine, tryptophan, methionine and threonine are critical for optimal broiler performance. Therefore, the use of QPM in broiler feeds could decrease the need for costly protein meals and synthetic lysine supplementation (MUSHIPE *et al*., 2017). Moreover, it has been shown that QPM can increase the growth performance and feed efficiency, as well as carcass yield of broiler chicken (IGNJATOVIĆ-MICIĆ *et al.,* 2025). Also, the increase of lipids and starch, the main sources of energy in broiler diets, supports the advantage of QPM use in the diets. As biochemical analysis of QPM hybrid in this research confirmed high lysine and tryptophan contents, as well as other limiting amino acids, it can be concluded that a promising hybrid was identified for use in the feed industry.

CONCLUSIONS

Biochemical evaluation of the QPM hybrid presented in this paper revealed an increase of tryptophan to protein ratio, lipids and starch, indicating the nutritional enhancement. Besides

being the main energy source, QPM hybrid could serve as a source of protein and lysine, decreasing the percentage of protein component (e.g. soybean meal) in diets and the need for synthetic lysine supplementation. An elevated level of other limiting amino acids implies that the QPM hybrid is advantageous for use in the feed industry. The possibility of simultaneous selection and improvement of significantly correlated traits in our breeding material (TPR and SC, as well as TPR and LC) was established.

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POBOLJŠANJE NUTRITIVNE VREDNOSTI ADAPTIRANOG QPM HIBRIDA DOBIJENOG SELEKCIJOM POMOĆU MOLEKULARNIH MARKERA

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Izvod

Kukuruz poboljšanog kvaliteta proteina (eng. *Quality Protein Maize - QPM*) ima visok sadržaj esencijalnih aminokiselina lizina i triptofana koje su deficitarne kod kukuruza standardnog kvaliteta zrna (SM). Institut za kukuruz Zemun Polje ima program stvaranja QPM kombinovanim pristupom konvencionalnog i oplemenjivanja uz pomoć molekularnoih markera. Cilj ovog rada bila je biohemijska evaluacija QPM hibrida dobijenog ukrštanjem adaptiranih QPM inbred linija (QPM verzije komercijalnih linija). Analizirani su relevantni nutritivni parametri kao što su sadržaj proteina, skroba, masti i aminokiselina, a određen je i odnos triptofana i proteina (TPR). Sadržaj triptofana (TC) je statistički značajno ($p<0.05$) bio povećan kod QPM hibrida u odnosu na SM. Takođe, TC je bio 0.075%, što odgovara pragu za QPM. TPR, indikator poboljšanog nutritivnog kvaliteta proteina, bio je statistički značajno ($p<0.05$) povećan u poređenju sa SM. Sadržaji skroba i masti bili su statistički značajno ($p<0.01$ i $p<0.05$, redom) povećani u odnosu na SM. Takođe, visoko značajne korelacije ($p<0.01$) nađene su između TPR i sadržaja skroba, kao i značajne korelacije ($p<0.05$) između TPR i sadržaja masti, što ukazuje na mogućnost istovremene selekcije i poboljšanja ovih parametara. S obzirom na to da je biohemijska evaluacija potvrdila poboljšanu hranljivu vrednost QPM hibrida, može se smatrati pogodnim hibridom za industriju hrane za životinje.

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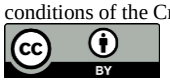

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