Original scientific paper

ROLE OF GENETIC RESOURCES FROM DIFFERENT GEGRAPHIC AND CLIMATIC REGIONS IN SIMULTANEOUS BREEDING FOR HIGH QUALITY PROTEIN MAIZE (HQPM) AND STRESS TOLERANCE

Miloje DENIC[1, 2, 3], Dragana IGNJATOVIC MICIC[1], Goran STANKOVIC[1], Ksenija MARKOVIC[1], Sladjana ZILIC, Vesna LAZIC JANCIC[1], Pedro, CHAUQUE[2], Pedro FATO[2], Constantino SENETE[2], David MARIOTE[2] and Wayne HAAG[3]

[1]Maize Research Institute "Zemun polje", Belgrade, Serbia
[2]Institute for Agricultural Research of Mozambique (IIAM), Maputo, Mozambique
[3]Sasakawa Global 2000, Maputo, Mozambique

Denic M., D. Ignjatovic Micic, G. Stankovic, K. Markovic, S. Zilic, V. Lazic Jancic, P. Chauque, P. Fato, C. Senete, D. Mariote and W. Haag (2012): *Role of genetic resources from different geographic and climatic regions in simultaneous breeding for high quality protein maize (HQPM) and stress tolerance*- Genetika, Vol 44, No. 1, 13 - 23.

Due to the low biological value of proteins of common maize, it was reinitiated breeding for high protein quality maize (HQPM) using three genetic systems, namely: opaque-2 gene, endosperm modifier genes and enhancer genes, which are increasing lysine and tryptophan content in opaque-2 background In order to alleviate effect of abiotic and biotic stress

Corresponding author: Miloje Denic, Maize Research Institute "Zemun polje", Slobodana Bajica 1, 11185 Belgrade, Serbia, Tel.:011/3756704, e-mail: denicm@eunet.rs

factors, the genotypes with tolerance to those factors were included. Genetic resources originating from North, Central and South America, then West, Central and Southern Africa and gene bank of Maize Research Institute "Zemun Polje" were used. Combining breeding approaches in selection of genetic resources, field plot techniques and laboratory analysis, it was created large number of early QPM varieties, inbred lines and hybrids with modified endosperm and high yield potential under poor and good growing conditions. Created lines exhibited high combining ability in conventional and non-conventional hybrids. Yield trials showed that QPM hybrids are competing with commercial hybrids of common maize.

Key words: genetic resources, tryptophan, kernel modification, stress tolerance, yield

INTRODUCTION

Maize is the principal crop in many countries and major staple food in the most African and South American countries. The main problem in human nutrition in developing countries and in livestock feed in developed countries is insufficient production and poor quality of cereal proteins. Maize proteins in livestock feed are very important since they participate up to 80 % in the diet. However, due to the very low content of essential amino acids lysine and tryptophan in grain endosperm, biological value is very low, which is main limiting factor of common maize in feeding of monogastric animals, including human beings. Therefore increased production of high quality protein maize (QPM) (MERTZ *et al*., 1964; CROMWELL *et al*., 1967; MISOVIC *et al.*, 1969; EGGUM *et al*., 1983; BRESSANI, 1975) is essential for the improvement of the nutritional value of the daily diet.

Therefore, it is of special importance breeding for QPM, with high nutritional and biological value of proteins, which are 80 % of milk protein value, as compared to 40 % for common maize. Further, QPM include higher niacin availability due to the higher tryptophan and lower leucine content, higher calcium, carbohydrate and carotene utilization. Besides, QPM can be transformed into edible products without deterioration of its quality and acceptability, and can be used in conventional and new food products. In addition, if high quality protein maize is used as a gradient, it could play an important role in reducing import of the protein supplement in animal feed. With simultaneous approach in breeding for HQPM and drought tolerance, the risk of reduction of maize production under low rain-fall, will be reduced

It should be pointed out that maize production also faces serious constraints caused by agro-ecological conditions and poor socio-economic situation in developing countries. Among the agro-ecological conditions, the main constraints are: lack of rainfall; low soil fertility; poor weed control; diseases and pests (maize streak virus and downy mildew, borers and storage pests); and stem/cob rots, leaf blights, gray leaf spot and rusts. To alleviate many of these constraints, appropriate breeding approaches, field-based methodologies to select for multiple stress tolerance were implemented to develop desirable varieties. . More results on QPM related to history, genetics, breeding, new methods, utility and benefits were reviewed and reported by MERTZ *et al.* (1964), DENIC *et al.* (1979), VILEGAS *et al.* (1992), VASAL (1994), WALLACE *et al.* (2002), VIVEK *et al.* (2008) and MICIC-IGNJATOVIC *et al.* (2008).

MATERIALS AND METHODS

Part of the work presented in this paper is from two maize breeding programs under tropical and subtropical conditions and related to entries with opaque - 2 background with endosperm modifier genes (QPM) and tolerance to principal diseases and drought. Other part of genetic resources is from temperate and continental climatic conditions and related to two subgroups of entries: a) germplasm with opaque – 2 background but without endosperm modifier genes, and b) germplasm of common maize (non-opaque -2 background) with tolerance to principal diseases and drought. All genetic resources included in this work were at advanced stage, meaning that at the initial stage of breeding wide range of other genetic resources were used.

In order to fulfill requirements in breeding for HQPM and tolerance to biotic (diseases) and abiotic (drought) stress factors, various genetic resources of different geographic origin and type of maize were used (Tab.1). In total, there were 55 entries, where 25 entries were common maize and 30 entries were with homozygous recessive opaque-2 allele. In the case of origin, 27 entries were from tropical to subtropical climate and 28 entries were from temperate to continental climate. Relatively large number of selected entries was necessary because of the action of the three additional genetic systems in breeding for HQPM. Therefore, besides good yield potential, each selected entry has to have at least three good breeding target traits.

Tab. 1. Groups and number of genetic resources

Origin and type	Number	Origin and type	Number	Total
Tropical - Subtropical	27	Temperate - Continental	28	55
Common Maize	15	Common Maize	10	25
Opaque - 2	12	Opaque – 2	18	30

Some principal data on subgroups of the group of genetic resources originating from tropical to subtropical conditions are given in Table 2. All entries of common maize group, besides good agronomic traits and drought tolerance, they have strong tolerance to principal diseases and hard endosperm. In the group of entries with opaque-2 background all of them are with all three genetic systems for HQPM and disease resistance.

Tab.2 Genetic resources orginated from tropical and subtropical region

Genetic resources	Orgin	Composition	Main characteristics
Common maize: 10 Mantuba lines	Mozambic lines extracted by SEMOC[1] originating from Nigeria, IITA[2] chatrel pool	Selected from pool early-late white/yellow crosses as EWF-DMR-SR ETO population	Early (E), White (W), Flint (F), resistent to maize Streak virus (SR), down meldew (DMR), ETO type
5 SEMOC lines	Mozambican Maize program: 3 lines extracted from CIMMYT[3] Mexico population 2 lines derived from other source of IITA maize program	2lines ETO type and 1 line Tuxpenho type 1 line ETO type and 1 line Tuxpenho type	ETO type is flint and Tuxpenho is dent type of grain.this is also type of heterotic pattern grouping All lines are with strong DMR
Opeque-2 maize			
5 lines from pool 15 QPM-LSMQ,	CIMMYT Mexico QPM pool	QPM lines extracted by SEM –OC Mozambique	Medium early, white, flint QPM lines with strong SR,
CML 144, 159, 161, 165	CIMMYT, mexico QPM lines	Exctracted from CIMMYT germplasm	ETO type 2 white/yellow, 2 A and B heterotic group
1entry 5 (E 5 lines)	QPM line from Ghana	Exctracted from CIMMYT, Mexico Population 62 QPM by Ghana maize program	Medium late. Flint QPM line ETO type, good combainer with Tuxpenho type
Sussuma flint	Re-selected by IIAM from Sussuma dent, which was Selected Gnana QPM OPV Obatanpa	Obatanpa (good nursering mother was obtained by converting CIMMYT Pop 63 in Ghana	Medium late, flint QPM population with strong maize streak virus resistance
Matuba Pro	Synthetic from IIAM4 by crossing SML/LSMQ/MTL/LSMQ lines	Composed of Matuba, SEMOCN amd SEMOC QPM lines	Early white flint, resistence to MS virus and downy mildew

[1] Sementes de Mocambique; [2] International Institute for Tropical Agriculture;
[3] Centro International de Melhoramento de Milho Y Trigo; [4] Instituto de Investigacio Agraria de Mozambique

Group of genetic resources of common maize lines from temperate to continental climate (Tab. 3) are subject of introgression of recessive opaque – 2 gene and conversion to HQPM. They are commercial lines tolerant to drought and with good combining ability for yield potential. Opaque – 2 lines of soft endosperm are with homozygous recessive opaque – 2 allele and with enhancer genes for high lysine and tryptophan content. However, they are missing endosperm modifier genes which are involved in control of grain texture.

Regarding breeding procedures, *i.e.* incorporation of opaque – 2 recessive allele, endosperm modifier genes, enhancer genes tryptophan/lysine content and genes for other target traits, the modified method of recycling of non-QPM with QPM donors was used, followed by recurrent full-sib selection for OPVs or by inbreeding selection for lines (VIVEK *et al*., 2008). Testing of disease resistance was done under strong infestation using modified spreader rows technique (DENIC, 1996), originally developed by CARDWELL *et al*., 1994. In the case of protein quality, which was estimated with tryptophan content (highly correlated with lysine content), the method with salicylic acid in colorimetric reagent was used (NURIT *et al.*, 2009).

Tab. 3 Genetic resources orginated from temperate to continental region

Genetic resources	origin	composition	Main characteristics
Common maize			
7 CMS elite lines	MRI-ZP lines, Serbia	Not aviable	Lancaster and BSSS heterotic pattern yellow dents and flints
3 lines	USA Corn Belt	B73,Mo17, Oh43	Lancaster and BSSS heterotic pattern yellow dents and flints
Opaque-2 lines (soft endosperm)			
14 lines	MRI-ZP lines, Serbia	Converted elite common maize lines	Lancaster and BSSS heterotic pattern yellow dents and flints
4 lines	USA Corn Belt	B73,Mo17, Oh43. W64A	Lancaster and BSSS heterotic pattern yellow dents and flints

Maize Research Institute Zemun polje, Belgrade, Serbia

RESULTS AND DISCUSSION

Because of the large number of data, only results from examples of individual breeding operations are presented. Data presented in Fig. 1 show tryptophan content in: a) female parents opaque – 2 lines with soft endosperm (without endosperm modifier genes) from temperate-continental regions, b) male parents CIMMYT QPM lines with hard endosperm from tropical-subtropical regions, c) their F_1 crosses, and d) line of common maize (B 73) from temperate-continental region. These data show that all parents and their F_1 crosses demonstrated higher tryptophan content in comparison with B 73 line of common maize. Opaque-2 lines exhibited also higher tryptophan content than QPM lines. Most of opaque – 2 lines (8 out of 12) showed higher tryptophan content than their F_1 crosses. Among the F_1 crosses, 7 of them with female parent CML 144 showed higher tryptophan content than those of female parent CML 159. Part of the data related to tryptophan content in the whole grain of BC_1 progenies (after back-crossing F_1 with opaque-2 lines) were published by IGNJATOVIC-MICIC *et al.* (2010).

Regarding disease resistance remarkable results were obtained with resistance to downy mildew (DMR) in tropical to subtropical germplasm. Data presented in Fig. 2. show percent of plants with downy mildew (DM) symptoms of synthetics (submitted to four cycles of selection for DMR), and DMR of check, which is commercial variety not selected to selection for DMR. These data show that, under strong pressure of infestation, check demonstrated 95 – 98 % of plants with DM symptoms, whereas in the case of synthetics 15 % - 30 % of plants with DM symptoms were registered. Results on resistance to stalk and ear rot (*Fusarium graminearum*), which is serious disease in temperate – continental climate, were published by IGNJATOVIC-MICIC *et al.*, (2010).

FIG.1. Tryptophan content in parents and their F1 of QPM x o2 lines crosses
Females: CML 144 & CML 159

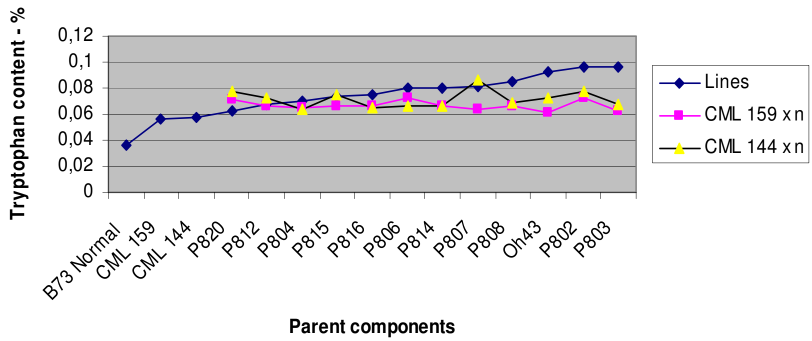
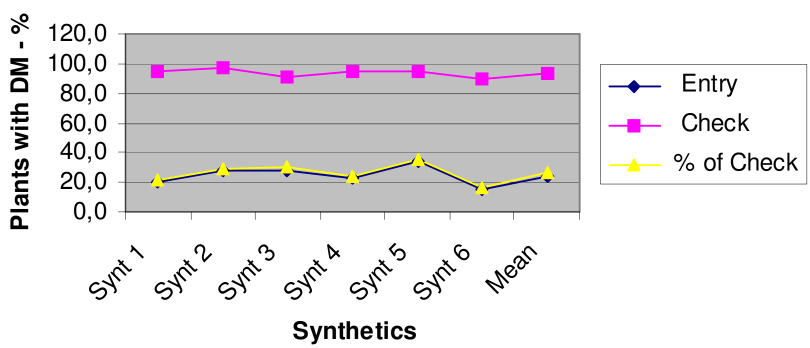
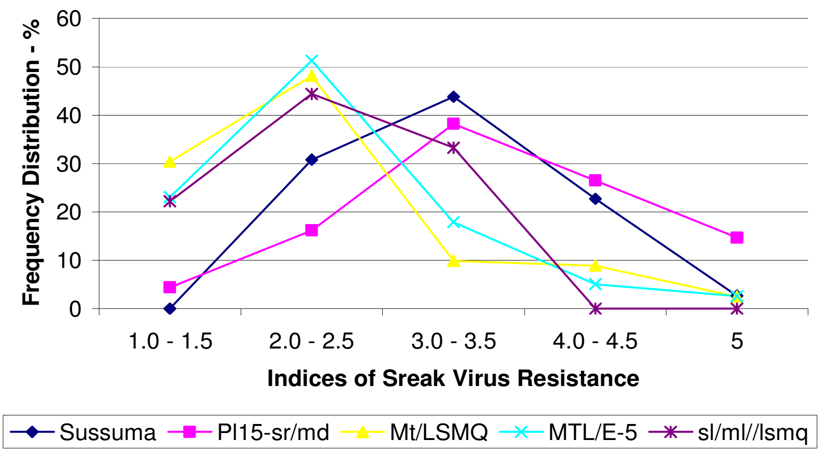


Fig. 2. Downy mildew resistance of QPM synthetics



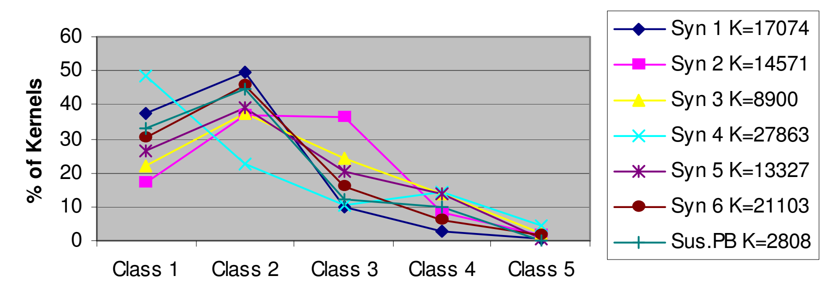
Another very important trait in tropical-subtropical regions is resistance to maize streak virus (SR). Data presented in Fig. 3. show frequency distribution of lines with streak virus symptoms within each class of resistance (indices) of five entries. Presented data illustrate higher frequency distribution of lines with low plant disease severity, *i.e.* strong and good resistance (indices 1.0-1.5 and 2.0-2.5, respectively) in entries MTL/LSMQ, MTL/E5 and sl/ml//lsmq than entries Sussuma lines and Pl15-sr/md lines, were the highest frequency distribution of lines was with intermediate resistance (indices 3.0-3.5).

Fig. 3. Frequency Distribution (%) of Lines Related to Streak Resistance of QPM Varieties



One of the essential issues in breeding for HQPM is incorporation and accumulation of endosperm modifier genes in genotypes with homozygous recessive opaque-2 allele and soft endosperm. It has been found that QPM grains with hard endosperm have approximately double amount of gamma zein in the endosperm relative to the *o2* mutants with soft endosperm (WALLACE *et al.*, 1990). Data on frequency and degree of kernel modification, obtained with over 100,000 kernels from OPV Sussuma and six QPM synthetics (after four cycles of selection for this trait), are given in Fig. 4. Presented data show that entries Syn 1, Syn 2 and OPV Sussuma have higher frequency distribution of kernel modification in class 1 and class 2 (100 % and 75 % of kernel modification, respectively).

Fig.4. Frequency Distribution of Kernel Modification of INIA QPM Synthetics (K=number of kernels)



In the case of yield potential some of these advanced entries were tested in conventional inbred line hybrid combinations or in top-cross combinations (lines crossed with OPV, or *vice versa)*. Grain yield of crosses between CIMMYT female parent lines and IIAM male parent lines are given in Tab.4. Data presented in this table show that hybrids WH054450 and VH054418, both IIAM male parent, gave yields over 7 tones/ha, some what higher than CIMMYT hybrids (underlined male CYMMYT parent line) CZH03033 and CZH03009.

Tab.4. Grain yield of crosses between CIMMYT lines and IIAM lines

Hybrid	IIAM male parent	Yield t/ha	Location
VH054450	Synthetic 6QS6-1-7	7.20	Angonia
VH054418	Synthetic 3QFS	7.15	Angonia
CZH03033	[CML389/CML176]S6 - CML line	6.91	Angonia
VH054479	Synthetic 6QS6-1-31	6.37	Angonia
CZH03009	CML 142 - CIMMYT QPM line	6.30	Mean
VH054470	Composite 1/1Q	6.20	Angonia
VH054452	Composite 1/1Q	6.05	Angonia
VH054518	Synthetic 6QS6-1-22	6.00	Sussundenga
VH054473	Synthetic 6QFS9/04	5.93	Angonia

Tab. 5. Summary of grain yield of groups of QPM reciprocal crosses between S3/S4 lines and Sussuma

Locations	Umbeluzi					Sussundenga				
Groups of Entries	Trial mean	Best entry	Best check	B.E[1]./T.M[2].	B.E./B.C[3].	Trial mean	Best entry	Best check	B.E./T.M.	B.E./B.C.
	Grain yield in tones per hectare									
[sml/lsmq]S_4 x Sussuma	5,33	8,49	4,98	1,59	1,70	3,40	6,55	3,52	1,93	1,86
[sml/lsmq//mtl/lsmq]S_3 x Sussuma	5,19	7,16	6,51	1,38	1,10	3,01	5,68	4,40	1,89	1,29
Sussuma S_1 x Pop 62 SR Q	4,30	6,41	3,00	1,49	2,14	4,59	7,82	5,74	1,70	1,36
Sussuma S_1 x [CML 144/CML 159]F1	3,69	5,64	2,38	1,53	2,37	3,82	7,94	3,50	2,08	2,29
[2mtl+2e5+1lsmq]S_4 x Sussuma	4,07	5,60	4,43	1,38	1,20	2,41	4,59	2,69	1,90	1,70
[mtl/e5//sml/e5]S_3 x Sussuma	4,30	5,60	2,20	1,30	2,55	2,60	4,78	2,83	1,84	1,69
CNSLQEGL-S_4 x Sussuma	3,40	3,95	3,13	1,16	1,26	2,91	5,65	4,36	1,94	1,30
CIMMYT Zimbabwe Hybrid	7,17	8,45	6,16	1,18	1,37					

[1]Best entry; [2]Trial mean; [3]Best check

Very good yields were also achieved with top crosses in both testing locations (Tab. 5.). At Umbeluzi Research Station, under intermediate fertilizer inputs, top-cross [sml/lsmq]S_4 x Sussuma achieved grain yield of 8.49 t/ha, which is equal to the yield of CIMMYT Zimbabwe hybrid. Two top-crosses Sussuma S_1 x [CML144/CML159]F_1 and Sussuma S_1 x Pop 62 SR Q, at Sussundenga Research Station, gave yield of 7.94 t/ha and 7.82 t/ha, respectively. These data clearly show that the best trial entries over-yielded the best trial checks.

CONCLUSION

Data obtained demonstrated that, using genetic resources from different geographic and climatic regions, in combination with appropriate breeding approaches, field plot techniques and chemical methods, play one important role in creating risk avoiding HQPM varieties, lines and hybrids. Combining all these four components, large number of early, white and flint synthetics, composites, inbred lines and hybrids were created with resistance or tolerance to downy mildew (DMR), maize streak virus (SR) and drought (DT). Created genotypes exhibited very good kernel modification and yield potential under low and normal inputs. In the case of synthetics and composites, besides tolerance to multiple stress factors, they were competing in yield with local QPM and normal maize checks. In the case of created inbred lines high combining ability was exhibited both in non-conventional and conventional maize hybrids. Trial data revealed that in the most cases the best entries were over-yielding the best checks.

Received December 12th, 2011
Accepted February 23rd, 2012

REFERENCES

BRESSANI, R. (1992): Nutritional Value of high-lysine maize in humans. In E.T. Mertz (ed.).Quality Protein Maize. American Association of Cereal Chemists, St. Paul, MN.Cardwell, K.F., C. Bock, O.F. Akinnuoye, D.Onukwu, V. Adenie, and A.O. Adetoro (1994): Improving screening methods for resistance to downy mildew of maize in Nigeria. Plant Management Research Monograph, Number 3. International Institute of Tropical Agriculture.

CROW, J.F., and J. KERMICLE (2002): Oliver Nelson and quality protein maize. Genetics *160*:819.

DENIĆ, M., V.HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, D.JELENIĆ, J.DUMANOVIĆ, I.BOŽOVIĆ (1979): Comparison of some chemical methods for screening maize genotypes with improved protein quality. Genetika *11*(1): 75-83.

DENIC, M.(1996): Simultaneous selection for earliness and resistance to downy mildew and streak virus in maize. Fifth Regional Maize Conference for Eastern and Suthern Africa, June 3 – 7, 1996, Arusha, Tanzania.

DENIC, M., P. CHAUQUE, P. FATO, C. SENETE, D. MARIOTE and W. HAAG (2008): Approaches in Breeding for High Quality Protein Maize. Genetika , Vol. *40*, No. 3, 237- 247.

EGGUM, B.O., J. DUMANOVIC, D. MISEVIC and M. DENIC (1983): Grain yield and nutritive value of high oil, opaque-2 and waxy maize hybrids. J. Cereal Sci. *1*, 139-145.

IGNJATOVIC-MICIC, D., K. MARKOVIC, D. RISTIC, S. MLADENOVIC-DRINIC, S. STANKOVIC, V.LAZIC-JANCIC and M. DENIC (2009): Variability analysis of normal and *opaque-2* maize inbred lines. Genetika. Vol. *41*, No. 1, 81-93.

IGNJATOVIC-MICIC, D., S. STANKOVIC, G. STANKOVIC and M. DENIC (2010): Comparative Resistance of quality protein maize F2 and BC1 populations to *F. graminearum.* Proceedings of 45th Croation and 5th International Symposium on Agriculture. 15-19 February, Opatija, Croacia. Pp 432-436.

MERTZ, E.T., L.S.BATES, O.E. NELSON (1964): Mutant that changes protein composition and increases lysine content of maize endosperm. Science *145*: 279-280.

MICIC-IGNJATOVIC, D., G. STANKOVIC, K. MARKOVIC, LAZIC-JANCIC and M. DENIC (2008): Quality Protein Maize – QPM. Genetika Vol. *40*, No. 3, 205-214.

MICIC-IGNJATOVIC, D., G. STANKOVIC, S. MLADENOVIC-DRINIC K., LAZIC-JANCIC and M. DENIC (2010): Kernel modification and tryptophan content in QPM segregating populations. Genetika Vol. *42*, No.2, 267-278.

MISOVIC, M., V. ANASTASIJEVIC, N. SHEVKOVIC, I. RAJIC, M. DJOKIC, M. PETROVIC (1969): Examination of the nutritional value of corn with improved protein composition (opaque-2) on growth and feed utilization of early weaned piglets. Journal of scientific agricultural research XXII, Sv. 79, 71-82.

NURIT, E., A.TIESSEN, K.PIXLEY, N.PALACIOS-ROJAS (2009). Reliable and Inexpensive Colorimetric Method for Determining Protein-Bound Tryptophan in Maize Kernels. J.Agric. Food Chem. *57*, 7233–7238.

VASAL, S. K. (1994): In Specialty Corns (ed. Hallauer, A. R.), CRS Press. Boca Raton, Fl., pp. 80-121.

VILLEGAS, E., S.K.VASAL, M.BJARNASON (1992). Quality protein maize – what is it and how was it developed? Pages 24-48 in: E.T.Mertz. eds. Quality Protein Maize. American Association of Cereal Chemists. Minnesota, USA.

VIVEK, B.S., A.F.KRIVANEK, N.PALACIOS-ROJAS, S.TWUMASI-AFRIYIE, A.O. DIALLO (2008): Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. exico, D.F.: CIMMYT.

WALLACE, J.C., M.L.LOPES, E.PAIVA, B.A. LARKINS (1990): New methods for extraction and quantification of zeins reveal a high content of γ-zein in modified *opaque*-2 maize. Plant Physiol *92*: 192-196.

WU, R.L., X.Y.LOU, C.X MA, X.L WANG, B.A LARKINS, G.CASELLA (2002: An improved genetic model generates high-resolution mapping of QTL for protein quality in maize endosperm. Proc Natl. Acad. Sci. USA *99*: 11281-11286.

ZNAČAJ GENETIČKIH RESURSA IZ RAZLIČITIH GEOGRAFSKIH I KLIMATSKIH REGIONA U SIMULTANOM OPLEMENJIVANJU KUKURUZA NA VISOK KVALITET PROTEINA (VKP) I TOLERANTNOST NA STRES

Miloje DENIC[1, 2, 3], Dragana IGNJATOVIC MICIC[1], Goran STANKOVIC[1], Ksenija MARKOVIC[1], Sladjana ZILIC[1], Vesna LAZIC JANCIC[1], Pedro CHAUQUE[2], Pedro FATO[2], Constantino SENETE[2], David MARIOTE[2] i Wayne HAAG[3]

[1]Institut za kukuruz "Zemun polje", Begrad, Srbija
[2]Institut za poljoprivredna istrazivanja Mozambika, Maputo, Mozambik
[3]SG 2000, Maputo, Mozambik

I z v o d

Zbog niske bioloske vrednosti proteina zrna standardnog tipa kukuruza se pristupilo stvaranju sorata i linija kukuruza visokog kvaliteta proteina (VKP) koriscenjem tri geneticka sistema: opaque-2 gena, gena modifikatora endosperma i gena enhansera koji povecavaju sadrzaj lizina i triptofana u opaque-2 osnovi. Da bi se istovremeno ublazilo i delovanje abiotskih i biotskih faktora stresa ukljuceni su i genotipovi sa tolerantnoscu na ove faktore. Korisceni su geneticki resursi poreklom iz Severne, Centralne i Juzne Amerike, zatim Zapadne, Centralne i Juzne Afrike i resursi iz banke gena Instituta za kukuruz "Zemun polje". Kombinovanjem selekcionih pristupa u izboru genetickih resursa, tehnika u poljskim i laboratorijskim uslovima stvoren je veliki broj ranih sorata, inbred linija i hibrida VKP, modifikikovanog endosperma i visokog potencijala rodnosti pod losim i normalnim uslovima gajenja. Takodje, stvorene inbred linije su ispoljile visoku kombinacionu sposobnost u nekonvencionalnim i konvencionalnim hibridima. Poljski ogledi su pokazali da VKP hibridi konkurisu po prinosu najboljim komercijalnim hibridima standardnog tipa kukuruza.

Primljeno 12. XII. 2011.
Odobreno 23. II. 2012.