# ESTIMATION OF MAIZE HYBRID UNIFORMITY BY PROTEIN AND MORPHOLOGICAL MARKERS

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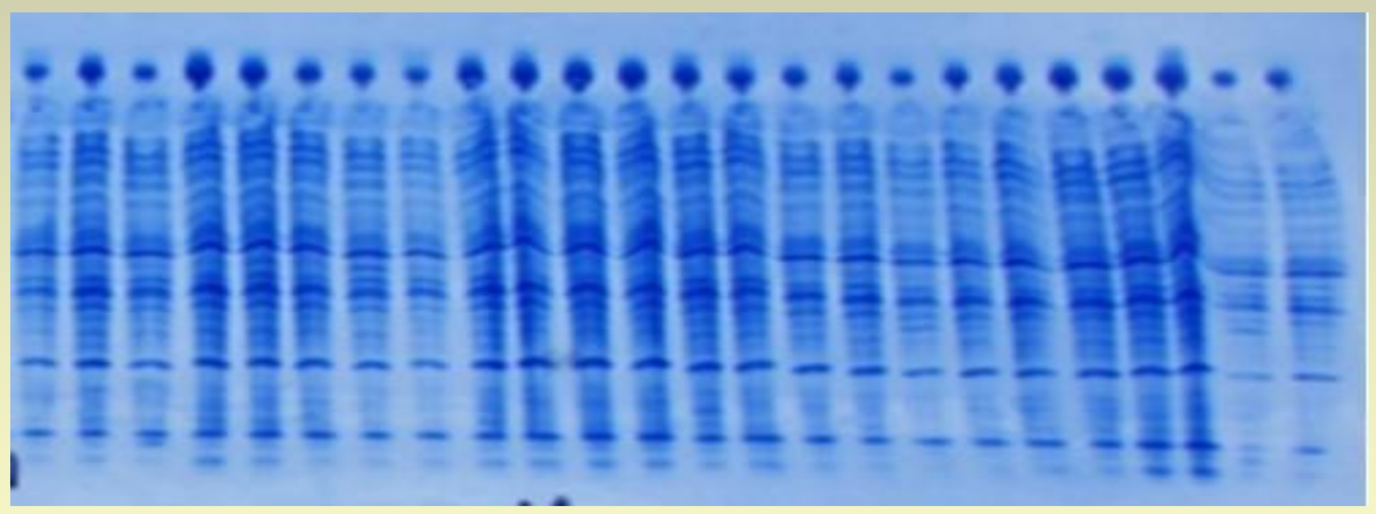
## INTRODUCTION

The process of maize hybrid creation, registration, production, processing, packaging and seed quality control, should necessary provide to farmers (end users) seed of high quality and genetic purity, with appropriate variety identity. Once chosen, hybrid combination for seed production needs continuous monitoring in order to avoid any mistake or contamination. Genetic maize seed purity is usually estimated by morphological and biochemical markers (Isozymes and storage proteins). Due to the practical reasons, molecular and biochemical methods for determination of seed purity and uniformity should reliably reflect genetic differences associated to traditional morphological traits, even when they are not agriculturally important. The aim of this study was to compare the methodologies for estimation of variety uniformity defined by UPOV descriptor, with standard laboratory genetic purity control test (UTLIEF method).



***Picture 1**. The part of the protein profile of the tested maize hybrid*

***Table 1.** List of observed characteristics from UPOV guidelines, Range of Notes by Descriptor (RND), developmental stage for the assessment (DSA) and type of observation (TO)*

| No | List of characteristics | DSA | TO | RND |
|---|---|---|---|---|
| 3 | Intensity of leaf green color | Inflorescence visible | VS | 1-3 |
| 4 | Undulation of leaf blade margin | Inflorescence visible | VS | 1-3 |
| 5 | Angle between blade and stem | Anthesis | VS | 1-9 |
| 6 | Curvature of leaf blade | Anthesis | VS | 1-9 |
| 7 | Degree of stem zig-zag | Anthesis | VS | 1-3 |
| 9 | Anth.col. at base of tassel glume | Anthesis | VS | 1-9 |
| 10 | Anth.col. of tassel glume exclude base | Anthesis | VS | 1-9 |
| 11 | Anth.col. of tassel anthers | Anthesis | VS | 1-9 |
| 12 | Angle between main axis and lateral t. br. | Anthesis | VS | 1-9 |
| 13 | Curvature of lateral t. branches | Anthesis | VS | 1-9 |
| 14 | Number of primary tassel branches | Anthesis to milk devel. | VS/MS | 1-9/no. |
| 16 | Anthocyanin coloration of silks | Anthesis hafway | VS | 1-9 |
| 17 | Anthocyanin coloration of brace roots | Anthesis to milk devel. | VS | 1-9 |
| 18 | Density of tassel spikelets | Anthesis to watery ripe | VS | 3-7 |
| 19 | Anthocyanin coloration of sheath | Watery ripe to milk | VS | 1-9 |
| 20 | Anthocyanin coloration of internodes | Watery ripe to milk | VS | 1-9 |
| 21 | Length of main t. axis above lowest l. b. | Watery ripe to milk | VS/MS | 1-9/cm |
| 22 | Length of main t. axis above upper l. b. | Watery ripe to milk | VS/MS | 1-9/cm |
| 23 | Length of lateral branch | Watery ripe to milk | VS | 1-9 |
| 24 | Height of plant | Milk to dough develop. | VS/MS | 1-9/cm |
| 25 | Ratio Height of ear/ Height of plant | Milk to dough develop. | VS/MS | 1-9/cm |
| 26 | Width of blade | Milk to dough develop. | VS/MS | 1-9/cm |
| 27 | Length of ear peduncle | Milk to dough develop. | VS | 1-9 |
| 28 | Length of ear | After harvest | VS/MS | 1-9/cm |
| 29 | Diameter of ear in middle | After harvest | VS/MS | 1-9/cm |
| 30 | Shape of ear | After harvest | VS | 1-3 |
| 31 | Number of rows of grain | After harvest | VS/MS | 1-9/no |
| 36 | Type of grain | After harvest | VS | 1-9 |
| 38 | Color of top of grain | After harvest | VS | 1-9 |
| 39 | Color of dorsal side of grain | After harvest | VS | 1-9 |
| 41 | Anthocyanin coloration of glumes of cob | After harvest | VS | 1-9 |

VS – Visual assessment of a number of individual plants or parts of plants; MS – Metric measurement of a number of individual plants or parts of plants; No – order of characteristics in UPOV descriptor.

***Table 2.** Uniformity assessment of the examined maize hybrid based on the STDEV for metric traits*

| No | Plant trait | Corr. VS/MS | F1(e) mean | STDEV | | CV (%) | |
|---|---|---|---|---|---|---|---|
| | | | | F1(e) | ZP434 | F1(e) | ZP434 |
| 14 | Number of primary tassel branches | 0.69** | 6.6 | 1.69 | 0.49 | 25.70 | 7.07 |
| 21 | Length of tassel above lowest l. b. | 0.34** | 182.2 | 18.25 | 13.70 | 10.02 | 7.44 |
| 22 | Length of tassel above highest l. b. | 0.13ns | 190.6 | 18.46 | 16.46 | 9.69 | 7.50 |
| 24.2 | Height of plant | 0.70** | 214.4 | 19.93 | 13.63 | 9.30 | 6.10 |
| 25 | Height of ear | 0.62** | 63.6 | 11.18 | 10.49 | 17.57 | 14.91 |
| 26 | Width of blade | 0.50** | 7.7 | 0.84 | 0.58 | 10.91 | 6.78 |
| 28 | Length of ear | 0.50** | 17.8 | 3.89 | 1.20 | 21.81 | 5.88 |
| 29 | Diameter of ear in middle | 0.80** | 3.9 | 0.27 | 0.10 | 6.88 | 2.32 |
| 31 | Number of rows of grain | 1.00** | 13.7 | 1.42 | 0.80 | 10.37 | 6.48 |

No – number of characteristic in UPOV descriptor; F1(e) – examined hybrid; Corr – Pearson's correlation coefficient; ** – significant at P≤0,01; ns – non-significant; VS – visual assessment of a number of individual plants or parts of plants; MS – metric measurement of a number of individual plants or parts of plants;

***Table 3.** Uniformity assessment of hybrid on the basis of Off-types combining two replicates*

| No | List of characteristics | Note (number of plants with such a note) | M | OT |
|---|---|---|---|---|
| 3 | Intensity of leaf green color | 2 (67); 1 (13) | 2 | 13 |
| 4 | Undulation of leaf blade margin | 2 (46); 1 (34) | 2 | 0* |
| 5 | Angle between leaf blade and stem | 1 (41); 3 (36); 5(3) | 1 | 3 |
| 6 | Curvature of leaf blade | 1 (32); 3 (44); 5 (4) | 3 | 4 |
| 7 | Degree of stem zig-zag | 1 (59); 2 (21); | 1 | 0* |
| 9 | Anth.col. at base of tassel glume | 1 (79); 3 (1); | 1 | 1 |
| 10 | Anth.col. of tassel glume exclude. base | 1 (5); 3 (73); 5 (2) | 3 | 7 |
| 11 | Anth.col. of tassel anthers | 3 (15); 5 (60); 7 (5) | 5 | 5 |
| 12 | Angle between main axis and lat. t. br. | 1 (12); 3 (39); 5 (30); | 3 | 12 |
| 13 | Curvature of lateral t. branches | 1 (13); 3 (50); 5 (17); | 3 | 13 |
| 16 | Anthocyanin coloration of silks | 1 (4); 3 (43); 5 (29); 7 (4); | 3 | 8 |
| 17 | Anthocyanin coloration of brace roots | 1 (1); 3 (4); 5 (29); 7 (46); | 7 | 5 |
| 18 | Density of tassel spikeltes | 3 (72); 5 (8); | 3 | 8 |
| 19 | Anthocyanin coloration of sheat | 1 (80); | 1 | 0 |
| 20 | Anthocyanin coloration of internodes | 1 (22); 3 (26); 5 (32); | 5 | 22 |
| 23 | Length of lateral branch | 3 (15); 5 (65); | 5 | 15 |
| 27 | Length of ear peduncle | 1 (2); 3 (48); 5 (30); | 3 | 2 |
| 30 | Shape of ear | 1 (9); 2 (66); 3 (5); | 2 | 14 |
| 36 | Type of grain | 1 (1); 2 (6); 3 (18); 4 (34); 5 (21); | 4 | 25 |
| 38 | Color of top of grain | 3 (1); 4 (79); | 4 | 1 |
| 39 | Color of dorsal side of grain | 5 (80); | 5 | 0 |
| 41 | Anth. col. of glumes of cob | 1 (73); 3 (3); 5 (4); | 1 | 7 |

*No – number of characteristic in UPOV descriptor; M – modal value of the note; OT – number of off-type plants for each characteristic; * – discarded from the analysis*

Color of silks. Photos by Momčilo Babić, in „***Description of Morphological Variation of Maize (Zea mays L.)***"

## MATERIAL AND METHODS

For this research, sample of one maize hybrid lot F1(e) is chosen. It was produced in Serbia for the foreign seed company requirements, but it was not registered on the National plant variety list.
Genetic maize seed purity is estimated by:
- morphological markers according UPOV descriptors (Table 1)
- biochemical markers by Ultrathin-layer isoelectric focusing (UTLIEF) method (ISTA Rules, 2015)

## RESULTS AND DISCUSSION

Tested hybrid samples expressed uniform protein markers profiles (Picture 1), but on the other hand, unsatisfactory uniformity of morphological markers in the field, indicating some laches in seed production.
Standard deviations approach, suitable for determination of off-types using measurement of single plants (MS), showed that examined hybrid varies much more than the comparable hybrid ZP434. Values of STDEV were above those for the hybrid ZP434 (Table 2).
Within visual assessment of the off-types 73 plants (out of 80 individually estimated plants) could be considered as off-types in accordance with at least one observed trait (Table 3).
According to our results and some previous experience it is found that visual assessment by UPOV descriptor is suitable for the control of uniformity in the process of commercial seed production. However, number of traits to be evaluated should be reduced. We consider that following traits could be recommended as sufficient for the successful assessment: angle between leaf blade and stem, curvature of leaf blade, anthocyanin coloration at base of tassel glume, anthocyanin coloration of tassel glume excluding base, anthocyanin coloration of anthers, anthocyanin coloration of silk, anthocyanin coloration of brace roots, type of grain, anthocyanin coloration of glumes of cob. It would be of special usefulness to include the assessment of these traits in seed production control of parental lines.

## CONCLUSION

Evaluating purity of hybrid seed lots produced from segregating parental lines by isozyme loci, are with limited use. In case when segregation for isozyme alleles in lines is unknown, this lack of information will result in incorrect interpretation of hybrid seed lots purity.
For correct estimation of non-uniformity cause in the field post-control tests, it is necessary to include beside examined hybrid lot, the referent samples of maize hybrid and its parental components provided by the authorized institution.
As evaluated hybrid is not registered in the Republic of Serbia, authorized institution (i.e. Ministry of Agriculture) possess neither the referent seed samples, nor the official descriptions by UPOV descriptor. For relevant post-control test, it would be necessary to obtain the samples and descriptions, provided by foreign authority where the hybrid is registered. Otherwise, results of uniformity estimation for this hybrid based only upon the seed lot sample provided by the seed testing laboratory, could not be the valid argument in case of the litigation between the interested parties, and determination of the errors and omissions in seed production.

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