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ASSESSMENT OF ONE MAIZE HYBRID LOT UNIFORMITY BY UPOV MORPHOLOGICAL AND PROTEIN MARKERS

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Abstract

Monitoring genetic uniformity and identity is very important in maize breeding and seed production. Due to practical reasons, molecular and biochemical methods for the determination of genetic purity and uniformity should reliably reflect genetic differences associated with traditional morphological traits, even when they are not agriculturally important. A comparison of genetic purity and uniformity of one maize hybrid lot, based on morphological UPOV markers and the UTLIEF (Ultrathin-layer isoelectric focusing) method is performed in this research. Tested hybrid samples expressed uniform protein markers profiles, but on the other hand, unsatisfactory uniformity of morphological markers in the field, indicating some laches in seed production. Although the UTLIEF method, standardized by ISTA rules, provides enough accurate distinction between F1 seed and self-pollinated maternal seed, a "clean" isozyme or protein profile will not necessarily correlate with morphological homogeneity. It is most likely that the non-uniformity of the tested hybrid lot originates from the non-uniformity of one of the parental lines. Therefore, to establish where the laches occurred, it is necessary to perform post-control tests with reference samples of maize hybrid and parental lines, as well as, the insight into their official descriptions according to the UPOV descriptor. Thus, it is very important to require the deposition of referent samples of hybrids and varieties whose seed production is allowed in Serbia, as well as to have harmonised protocols for conduction of laboratory and field post-control tests.

Key words: genetic seed purity, off-types, post-control tests, UPOV, UTLIEF method

Introduction

Average seed production of agricultural plants in Serbia is conducted at ~ 50,000 ha annually, while maize (*Zea mays* L.) seed production is at ~ 5 – 10,000 ha (Babić et al., 2016). Maize hybrid variety presents the first generation after cross-pollination between parental lines, and it is used in commercial production. The two main characteristics of a hybrid variety are hybrid vigour and high uniformity. The process of hybrid creation, registration, production, processing, packaging and seed quality control, should necessary provide to farmers (end users) seeds of high quality and genetic purity, with appropriate variety identity.

Once chosen, a hybrid combination for seed production needs continuous monitoring to avoid any mistakes or contamination. Female and male components are sown in subsequent rows in the proportion of 4:2 or 6:2 in favour of the female component. The preceding crop should be adequate, out of which small grains are the best, and there should be the isolation of at least 200 m to another maize production, which prevents contamination of female component by out-crossing. During the seed production, field inspector, authorized by the Ministry of agriculture, forestry and water management of the Republic of Serbia are conducted at least six times in certain developmental phases

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when specific traits are expressed. Based on the performed observation, records of seed production quality and seed health certificates, the Ministry of Agriculture issues the certificate of the seed production for every registered seed production plot, necessary for seed packaging and distribution (Official Gazette RS, 2006).

Although seed production is officially monitored, many seed companies additionally perform super-control during seed production, to minimize the occurrence of mistakes that could deteriorate the identity, uniformity or genetic purity of produced seed. Mistakes could happen in case of incorrect labelling of packaged basic seed, or due to sowing of wrong parental component (Semagn et al., 2012). The result of this is a high-quality hybrid seed produced by adequate procedure, but with impaired variety identity. Wrong parental identity could be noticed only by experts such are breeders and seed specialists, especially when dealing with sister lines that are morphologically very similar. Additionally, inadequate hybrid seed purity can occur due to the high percentage of self-pollination (inadequate or untimely detasseling of the female component), or due to genetic non-uniformity of one or both parents.

Genetic maize seed purity is usually estimated by morphological and biochemical markers (Isozymes and storage proteins). Genetic variability among different sources of maize inbred lines with the same identification and origin has been observed since the beginning of the inbred-hybrid system (Semagn et al., 2012). Observation of many morphological traits in the field has been and continues to be, the most widely used approach for varieties description, identification and monitoring purity (Babic et al., 2017). After all, morphologies of varieties will be omnipresent in agriculture, and their comparison encompasses features that will be seen by the farmer. The *Union pour l'Obtention des Protections des Vegetales* (UPOV) prescribes lists of descriptors that are mainly based upon a series of 30 – 50 morphological traits specific for each crop species that are used in the process of PVP (Plant Variety Protection). The application of different markers in control of seed quality, homogeneity and inbred line identity system is widely reported (Yan et al., 2009; Geth et al., 2002; Heckenbergen et al., 2006). The major weaknesses include a small sample size or a limited number of markers, a lack of clear guidelines in data interpretation and no suggested protocols for routine quality control genotyping. Isozymes have been routinely used in checking seed-lot purity in maize for the past 20 years (ISTA Rules, 2013). Recently, the importance of the new generation molecular markers (SNP) in genetic purity control, maintenance of parental line`s genetic identity and minimization of mistakes in different steps of seed production is emphasized (Semagn et al., 2012).

Control of the seed production in Maize Research Institute Zemun Polje (MRIZP) is performed every year in laboratory tests of genetic purity by UTLIEF (Ultra-thin layer isoelectric focusing), as well as in field trials (bio-test in winter nursery), all aiming to produce seed with high/required quality (Sečanski et al., 2015). Laboratory genetic purity seed tests are performed by the standard procedure issued by ISTA rules (ISTA Rules, 2013), while the control in field trials is under the supervision of the experts (most often breeders), who identify atypical plants that differ according to one or several morphological traits. The number of atypical plants must be within the limitations required by the Guidelines for seed control production (Official Gazette RS, 2006) and Law on Agricultural Plant Seeds of the Republic of Serbia (Official Gazette RS, 2005).

Mismatches could occur, regardless of the undertaken measures and clearly defined legal rules, and they can cause high financial problems for all participants in the seed production chain. When it happens, it is necessary to define clearly where the failure occurred, especially when interested parties cannot agree.

The results of the uniformity/genetic purity of one lot of maize hybrid unregistered in the National seed variety list, are presented. This study aimed to compare the methodologies for estimation of variety uniformity defined by the UPOV descriptor with a standard laboratory genetic purity control test (UTLIEF method).

Materials and methods

Plant material

For this research, a 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.

Ultrathin-layer isoelectric focusing (UTLIEF) method

For a complete analysis, 400 seeds of F1(e) hybrid were used. Individual dry seeds were ground to fine semolina using an individual seed grinder (Kataskapt-Lederer, Lufa Augustenberg). Upon grinding, approximately 50 mg of every single seed was transferred to individual wells of microtitar plate (96 well, Greiner bio-one, cat. no. 650101). The proteins were extracted in 250 ml of redistilled water (albumins) or 250 ml of 30% 2-chlorethanol (prolamins).

The UTLIEF was conducted according to section 8.9.3 of the International Rules for testing (ISTA Rules, 2013). The polymerization solution for each gel contained 5 ml acrylamide (T=6.8%, C=2.5%), 0.22 ml of pH 2-11 Seed-mix ampholytes (Servalyt, Germany), 0.8 g urea, 30 µl of 20% (w/v) ammonium persulphate and 4 µl N N N' N'- tetramethylethylenediamine. A recommended gel thickness is 0.12mm, so a defined thickness adhesive tape was used as a spacer.

The gel was placed on the pre-cooled (<10°C) cooling plate of the horizontal electrophoresis apparatus (Multiphor II, GE Healthcare, USA). Before placing on the gel electrode wicks were soaked in the appropriate electrode buffers. The anode solution contained 0.332% (w/v) aspartic acid and 0.368% (w/v) glutamic acid, and the cathode solution contained 0.472% (w/v) arginine, 0.364% (w/v) lysine and 12% (v/v) ethylenediamine. Two anodal electrode wicks were placed on either side of the gel and one cathodal electrode wick in between. The application strips were placed about 0.5 cm below the anode buffer wicks. Single isoelectric focussing was run at 2500 V, 15 mA, 40 W for ~1750 volt hours^{-1}.

After 70 min, the gel was removed and fixed in 12% (w/v) trichloroacetic acid for 20 min, then stained by shaking in gel staining solution, which containing 0.015% (w/v) Coomassie R250 and 0.045% (w/v) Coomassie G250, 11% (v/v) acetic acid, 18% (v/v) ethanol and 71% (v/v) water for 60 min and, finally, destained in a solution of 30% (v/v) ethanol, 5% (v/v) acetic acid and 65% (v/v) water for 15 min. After rinsing with water, the gels were air dried at room temperature.

Examining uniformity by UPOV descriptor

The field trial was conducted in 2018, on the MRIZP nursery field (44°51' N; 20°18' E; 73 m asl), in two replications. Each replication is sown in four rows. From two inner rows, 40 plants were marked to be analysed by the UPOV descriptor (UPOV, 2009). The uniformity of a variety was assessed by observation of individual plants for all relevant characteristics (Table 1).

According to the method of propagation of variety and type of characteristics expression, in the assessment of uniformity, Off-types and Standard deviations (STDEV) methods can apply (UPOV, 2019a). In this research, for traits metrically measured (MS) STDEV approach is applied, while for the traits visually assessed (VS), the Off-types approach is applied (Table 1). For the assessment of uniformity of inbred lines and single cross hybrids, a population standard of 3% and an acceptance probability of at least 95% should be applied (UPOV, 2009). In the case of a sample size of 80 plants (40 plants in two replicates), five off-types are allowed (UPOV, 2019b). An off-type plant may be different from the variety based on one or several characteristics, but it will only be counted as one off-type plant, irrespective of the number of characteristics for which it has a different expression. The STDEV approach for uniformity assessment means that a candidate variety should not be significantly less uniform than the comparable varieties. The comparison between a candidate variety and comparable varieties is carried out based on STDEV, calculated for metric characteristics of individual plant observations. Comparable varieties are varieties of the same type within the same or a closely related species that have

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)

Tabela 1. Lista ocenjivanih karakteristika po UPOV deskriptoru, rang ocena po deskriptoru (RND), faza razvoja biljke u momentu ocene (DSA) i tip ocene (TO)

No	List of characteristics	DSA	TO	RND
3	Intensity of the leaf green colour	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 the 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 the 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 colouration of silks	Anthesis halfway	VS	1-9
17	Anthocyanin colouration of brace roots	Anthesis to milk devel.	VS	1-9
18	Density of tassel spikelets	Anthesis to watery ripe	VS	3-7
19	Anthocyanin colouration of the sheath	Watery ripe to milk	VS	1-9
20	Anthocyanin colouration of internodes	Watery ripe to milk	VS	1-9
21	Length of the main t. axis above lowest l. b.	Watery ripe to milk	VS/MS	1-9/cm
22	Length of the 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 the ear in the 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 the top of the grain	After harvest	VS	1-9
39	Color of the dorsal side of the grain	After harvest	VS	1-9
41	Anthocyanin colouration 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.

been previously examined and considered to be uniform (UPOV, 2019a). In this study, the threshold value was the value of the single cross comparable hybrid, ZP434, being of the same FAO maturity group as the F1(e), estimated as uniform in MRIZP field trials for seed production control.

Metric characteristics were also visually observed (Table 1). The accordance of the values obtained by MS and VS observations was checked by correlation analysis.

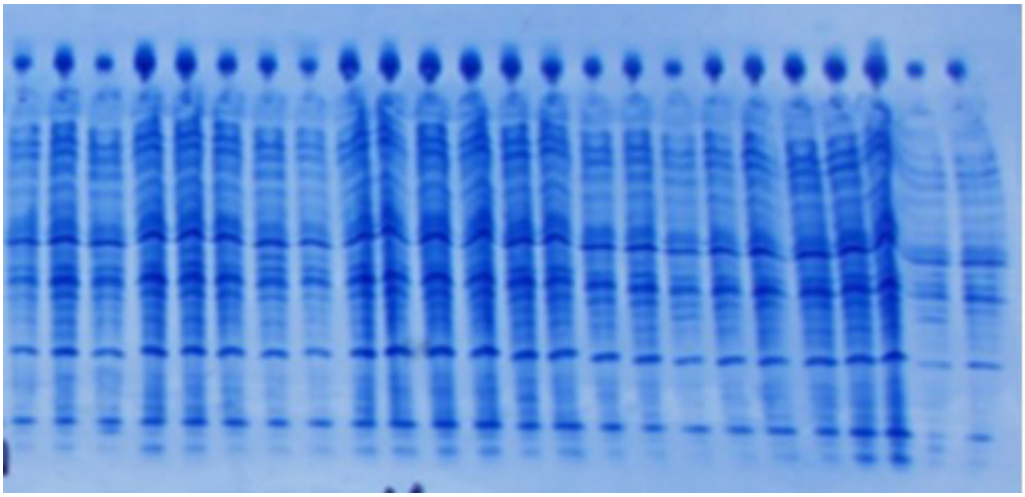
Results

The results of the protein markers analysis showed the uniformity of the tested hybrid F1(e) protein profiles was 395 out of 400 seed samples (Figure 1), which is within the population standard that allows 3% off-types at a probability level ≥ 95%.

The „General Introduction to the Examination of Distinctness, Uniformity and Stability" (TG/1/3) explains how the variation in the expression of relevant characteristics within varieties is used as the basis for the assessment of uniformity and provides an overview of the two main approaches to the assessment of uniformity: namely Off-types and Standard deviations (UPOV, 2019a).

In the process of plant variety protection (PVP), UPOV guidelines TGP/10/2 (i.e. Examining uniformity) for the uniformity assessment, explains that the STDEV approach means that a candidate variety should not be significantly less uniform than the comparable varieties (UPOV, 2019a). Average values of the measured traits, STDEV, and coefficient of variation (CV) are given for examined and comparable hybrids (Table 2). It could be noticed that for all examined traits, the STDEV of the F1(e) hybrid is higher than the ZP434. It is also noticed that CVs of the comparable hybrid does not exceed 15%, while the values of the examined hybrid for a number of primary tassel branches, the height of ear and length of ear, are higher, which pointed out to the high deviation from the mean values.

Visual assessment of uniformity encompassed the counting of off-types for each trait while combining two replicates. In the estimation of individual plants, variation of expression for almost all traits is noticeable. The undulation of leaf blade margin and degree of stem zig-zag was discarded from the analysis because of their high dependence on the subjectivity of the examiner. Despite the environmental/genotypic influence, the degree of trait expression that could be considered as off-type is marked red bold (Table 3). According to recommended UPOV standards, in the case of a sample size of 80 plants, five



Picture 1. The part of the protein profile of the tested maize hybrid
Slika 1. Deo protenskih profila ispitivanog hibrida kukurza

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

Tabela 2. Ocena uniformnosti ispitivanog hibrida kukuruza na osnovu standardne devijacije (STDEV) metrički merenih karakteristika

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 the ear in the 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 characteristics 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;

off-type plants are allowed (UPOV, 2019b). The highest number of off-types is noticed for: the intensity of leaf green colour (13), the angle between the main axis and lateral tassel branches (12), the curvature of lateral tassel branches (13), anthocyanin colouration of internodes (22), the shape of the ear (15) and type of grain (14). An unacceptable number of off-type plants is also noticed for the following traits: anthocyanin colouration of tassel glumes excluding base (7), anthocyanin colouration of silk (8), the density of tassel spikelets (8), anthocyanin colouration of glumes of cob (7) (Table 3).

If the modal value (the note with the highest frequency) is taken for the real value of the expression of the specific trait for the given hybrid, only seven out of 80 individually estimated plants (VS) didn't express any type of non-uniformity. Thereafter, 73 plants could be considered as off-types in accordance with at least one observed trait. Even if traits with noticed high variability were to be disregarded (with the assumption that their variations derived more from environmental than genotypic influence and/or highly depend on the subjectivity of the examiner), then the number of off-type plants surpasses the threshold value for the UPOV standard (i.e. allowed five in the sample of 80 plants).

Metrically measured traits (scale level of measurement) were also visually assessed (ordinal level of measurement) (Table 1). Statistically significant correlations between results obtained by two measuring scales for the same evaluated trait (Table 2) indicated the high consistency of these two types of assessments.

Discussion

Monitoring genetic homogeneity and identity is very important in the breeding process and during maize seed production. UTLIEF method applied in this research is known as a fast, cheap and reliable technique for seed protein separation (Milivojević et al., 2017). This method has a satisfactory level of precision because single cross hybrids are created from the crossing of unrelated inbreds that usually exhibit one to four polymorphic isozyme loci. In process of purity control, this enables distinguishing between maternal and true F1 seeds (Smith and Register, 1998). Also, pollination by unrelated lines can be detected.

It is often questioned whether molecular and biochemical methods in the determination

Table 3. Uniformity assessment of hybrid on the basis of Off-types combining two replicates
Tabela 3. Procena uniformnosti hibrida na osnovu Off-types metode koja kombinuje dva ponavljanja

No	List of characteristics	Note (numb. of plants with such a note)	M	OT
3	Intensity of the leaf green colour	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 the 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 the main axis and la. t.b.	**1 (12);** 3 (39); 5 (30);	3	**12**
13	Curvature of lateral t. branches	**1 (13);** 3 (50); 5 (17);	3	**13**
16	Anthocyanin colouration of silks	**1 (4);** 3 (43); 5 (29); **7 (4);**	3	**8**
17	Anthocyanin colouration 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 colouration of sheat	1 (80);	1	0
20	Anthocyanin colouration 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	Colour of the top of the grain	**3 (1);** 4 (79);	4	1
39	Colour of the dorsal side of the grain	5 (80);	5	0
41	Anth. col. of glumes of cob	1 (73); **3 (3); 5 (4);**	1	**7**

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

of seed purity could present reliably enough genetic differences associated with traditional morphological traits. Due to practical reasons, the molecular approach must be able to identify seed lot that differs according to morphological and technological characteristics important to farmers and the processing industry, even when those differences are not of agronomic importance. As an example, farmers will not be satisfied with the hybrid that had expressed high genetic purity according to isoenzyme profiles but varies in kernel type or plant height. Although isozymes or zein seed storage proteins method is an internationally accepted method for genetic seed purity testing for species such as maize and sunflower (ISTA Rules, 2015), Smith and Register (1998) pointed out that it has some imperfections: „because isozymes and genes affecting morphological traits are most usually coded by different and unlinked loci, a 'clean' isozyme profile will not necessarily correlate with morphological homogeneity". Dou et al. (2012) stated that UTLIEF profiles of salt-soluble proteins of single seeds are highly efficient and reproducible for the genetic purity testing of commercial maize hybrids, but it proves difficult to find diversities when there are close genetic relationships.

The results of this research showed that the maize hybrid sample, which has expressed uniform protein markers profiles, didn't show a satisfactory level of uniformity of morpholog-

ical characteristics in the field. Although morphological markers are considered by many authors as unreliable indicators of genetic relationships, the choice of quality descriptors (UPOV, 2009), as well as, adequate biometric methods, can result in higher quality information (Babić et al., 2017). The standard deviations approach (UPOV, 2019a), suitable for the 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. In addition, CVs for some traits exceeded 15%, which is considered a great variation in maize trials (Gupta, 2015).

Within visual assessment of the off-types, special attention should be paid to traits that have exhibited more than two, out of five levels of trait expression, such as anthocyanin colouration of silk, anthocyanin colouration of brace roots and type of grain. Although part of the variation could be attributed to the environmental effect and subjectivity of the examiner, such high variation undoubtedly points out the genetic non-uniformity of the hybrid. It could be the consequence of the parental non-uniformity and/or out-cross pollination during hybrid seed production. Some traits could be omitted from evaluation because they are considered unreliable indicators of uniformity due to: a) a high influence of environmental factors (i.e. intensity of leaf green colour); b) altering by ageing (i.e. anthocyanin colouration of internodes); c) a high effect of pollinator – xenia effect (i.e. type of grain) (Vančetović et al., 2009); d) high dependence on the subjectivity of examiner (i.e. undulation of leaf blade margin, degree of stem zig-zag) (Babić et al., 2007). Despite this, recorded non-uniformities observed herein exceeded the limitation of five allowed off-type plants.

The result of examined hybrid uniformity according to the UPOV descriptor pointed to some mismatch in seed production. When maize hybrid is registered on the National plant variety list, according to the Law on Agricultural Plant Seeds of the Republic of Serbia (Official Gazette RS, 2005), and Law on Recognition of Varieties of Agricultural Plants (Official Gazette RS, 2010), hybrid's owner is obliged to provide referent sample which is kept for the needs of official post-control tests (Đoković et al., 2018). For now, referent samples of parental components are not required, but in the process of registration, their characterization by UPOV descriptor is being performed. Seed production of hybrids unregistered in the Republic of Serbia is allowed only in cases when there is a contract between producer and foreign client, with an obligation for export of the total production. In such a case, the competent Ministry does not possess referent samples for hybrid and parental lines, as well as descriptions according to the UPOV descriptor (Official Gazette RS, 2005).

Although monitoring of traits by UPOV descriptor during official control of seed production is recommended by the Guidelines for seed control production in the Republic of Serbia (Official Gazette RS, 2006), in practice it is difficult to perform. Control is most often based on the estimation of so-called out-cross plants, relying on the estimator's familiarity with the material whose uniformity and purity are assessed. According to our results and some previous experience (Babić et al., 2007; 2012), it is found that visual assessment by UPOV descriptor is suitable for the control of uniformity in the process of commercial seed production. However, the number of traits to be evaluated should be reduced. Visual assessment of the group of plants with the counting of the number of off-type plants is very fast and cheap. Based on the reliability of the visual assessment, we consider that the following trait could be recommended as sufficient for the successful assessment: angle between leaf blade and stem, the curvature of the leaf blade, anthocyanin colouration at the base of tassel glume, anthocyanin colouration of tassel glume excluding base, anthocyanin colouration of anthers, anthocyanin colouration of silk, anthocyanin colouration of brace roots, type of grain, anthocyanin colouration of glumes of cob. Moreover, correlations between obtained

estimates for the same trait, based on two types of measurements (VS/MS) were significant, pointing out the fact that metric traits could be visually assessed reliably enough, especially plant and ear height, a number of primary tassel branches, ear diameter and kernel row number, so they could also be recommended for uniformity assessment in maize seed production. It would be of special usefulness to include the assessment of these traits in seed production control of parental lines.

Evaluating the purity of hybrid seed lots produced from segregating parental lines by isozyme loci, are of limited use (Gowda et al., 2017). In case when segregation for isozyme alleles in lines is unknown, this lack of information will result in an incorrect interpretation of hybrid seed lots purity. On the other hand, for correct estimation of non-uniformity cause in the field post-control tests, it is necessary to include besides examined hybrid lot, the referent samples of maize hybrid and its parental components provided by the authorized institution. In this study, it could only be speculated that F1(e) non-uniformity originates from the non-uniformity of one parent, most likely the female component. As this hybrid is not registered in the Republic of Serbia, the authorized institution (i.e. Ministry of Agriculture) possesses neither the referent seed samples nor the official descriptions by UPOV descriptor. For relevant field inspection during the seed production, and post-control test, it would be necessary to obtain the samples and descriptions, provided by a 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 a 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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Procena uniformnosti jednog lota hibrida kukuruza primenom UPOV morfoloških I proteinskih markera

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Sažetak

Procena uniformnosti i identiteta sorte je veoma važna u oplemenjivanju i semenarstvu kukuruza. Iz praktičnih razloga, molekularne i biohemijske metode za utvrđivanje genetičke čistoće i uniformnosti semena bi trebalo pouzdano da odslikavaju genetičke razlike vezane za tradicionalne morfološke osobine čak i kada one nisu od agronomskog značaja. U datom radu je prikazano poređenje rezultata procene genetičke čistoće i uniformnosti jednog lota hibrida kukuruza, koji nije registrovan u Srbiji, na osnovu morfoloških (UPOV) i proteinskih (*UTLIEF - Ultrathin-layer isoelectric focusing*) markera. Testirani uzorak hibrida je ispoljio uniformne profile proteinskih markera, ali je sa druge strane ispoljio nedovoljnu uniformnost morfoloških karakteristika u polju, što je ukazalo na postojanje propusta u semenskoj proizvodnji. Mada je UTLIEF metod, standardizovan od strane ISTA-e, pouzdan za razlikovanje F1 semena hibrida od samooplođenog semena majčinske komponenete, „čisti" izozimski, odnosno proteinski profili ne moraju nužno da budu u korelaciji sa morfološkom uniformnošću. Najverovatnije je da pribeležena neuniformnost ispitivanog uzorka hibrida potiče od neuniformnosti jedne od roditeljskih linija. Stoga, da bi se pouzdano ustanovilo gde se dogodio propust u semenskoj proizvodnji, potrebno je uraditi postkontrolni test koji uključuje referentne uzorke kako hibrida kukuruza, tako i njegovih roditeljskih komponenata, kao i uvid u zvanične izveštaje opisa po UPOV deskriptoru. Zato bi bilo veoma važno zahtevati deponovanje referentnih uzoraka hibrida čija se proizvodnja odobrava u Republici Srbiji, kao i postojanje usklađenih protokola za izvođenje laboratorijskih i poljskih postkontrolnih testova.

Ključne reči: genetička čistoća, off-types, postkontrolni test, UPOV, UTLIEF metod

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