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THE ANALYSIS OF PROFICIENCY TEST RESULTS ON THE SEED PURITY AND THE OTHER SEED DETERMINATION FOR TEN-YEAR PERIOD

ANALIZA REZULTATA ČISTOĆE SEMENA I DETERMINACIJE DRUGOG SEMENA U PROFICIENCY TESTOVIMA ZA PERIOD OD DESET GODINA

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ABSTRACT

The aim of the present study was to analyse the ten-year results (2001-2010) on the seed purity analyses and the other seed determination by the ISTA proficiency tests in which the Seed Testing Laboratory of the Maize Research Institute, Zemun Polje, participated. The results of the proficiency tests for all ISTA accredited laboratories were statistically evaluated and the A, B, C or BMP (below minimum performance) ratings were given on the basis of standard deviation and the Z scores. Results in proficiency test for the species the Laboratory had been accredited for (purity test and other seed determination) received the A rating. The exception was the species Portulaca grandiflora - the Laboratory had received the C rating for the seed purity analysis and corrective actions had to be implemented. According to the analysis of the ten-year results on the proficiency tests, it is observable that the trend of Laboratory staff proficiency and competence has been increasing, as well as, awareness of a need for a permanent learning and advanced training, supervision over the whole process of testing with the aim to achieve reliable results.

Key word: *Proficiency test, seed purity, other seed determination.*

REZIME

Cilj rada bio je da se analiziraju desetogodišnji rezultati (2001-2010) analiza čistoće semena i udela drugog semena ISTA testovima stručnosti u kojima učestvuje Laboratorija za seme Instituta za kukuruz Zemun Polje. Rezultati testova za sve ISTA akreditovane laboratorije statistički su ocenjivani i A, B, C ili BMP (ispod minimuma) ocene su donošene na osnovu standardnog odstupanja i Z vrednosti. Testovi za vrste za koje je Laboratorija akreditovana ocenjeni su sa A. Izuzetak je vrsta Portulaca grandiflora – Laboratorija je dobila ocenu C za analize čistoće semena i potrebno je izvršiti određene korekcije zbog toga. Prema analizama desetogodišnjih rezultata testova stručnosti, uočava se da je trend stučnosti laboratorijskog osoblja i znanja u porastu, kao I svest o potrebi stalnog učenja I usavršavanja, nadzora nad procesima testiranja sa ciljem postizanja pouzdanih rezultata.

Ključne reči: testovi stručnosti, čistoća semena, udeo drugog semena.

INTRODUCTION

Proficiency tests

Proficiency tests are performed to assess the laboratory staff proficiency and they are organised by the International Seed Testing Association (ISTA) three times per year. Approximately 100 laboratories from different countries participate in the proficiency tests and their results are evaluated. Positive ratings are necessary for the laboratory accreditation prolongation. The goal of proficiency tests is not to identify the best seed testing laboratory in the world, but to identify those laboratories that do not meet the minimum proficiency standard expected from an ISTA accredited laboratory, and to determine if such laboratories are taking corrective action to bring their performance to at least the minimal level. During the process of corrective actions implementation a laboratory can forward a request to the ISTA Secretariat in order to solve the problem that occurred during testing. By its participation in the proficiency tests the laboratory checks the uniformity in the application of recommended test methods, confirms staff proficiency and increases its reference seed collection.

Seed purity analysis and other seed determination

The object of seed purity analysis is to determine the percentage composition by weight of tested sample and by inference the composition of the seed lot; and to determine the identity of the various species of seeds and inert particles constituting the sample. The working sample is separated into the three component parts: pure seed, other seed and inert matter. Pure seed refers to the species stated by the applicant or found to predominate in the test. Other seeds include seed units of any plant species other than that of pure seed. Inert matter includes seed units and all other matter and structures not defined as pure seed or other seed *(ISTA Rules, 2011)*. The object of the seed determination is to estimate the number of seeds of other species stated by the applicant either generally (e.g. all other species) or by reference to one category of seeds (e.g. species scheduled as noxious in a certain country) or specifically (e.g. *Elytrigia repens*). According to the definition, other seeds refer to species other than those under test *(ISTA Rules, 2011)*. The seed determination requires knowledge about various crop and weed seeds (especially quarantine weeds) originating either from our regions or other regions. In alfalfa seed testing, the presence of weed plant species does not only impede harvest, but also contaminate alfalfa seed and obstruct processing *(Dević et al., 2007, Đukanović et al., 2008)*. A referent seed collection facilitates the seed determination. Such a collection, according to the international standard, should be owned by all accredited laboratories and should include most important seeds typical for a given region *(Jensen, 1979)*. However, ISTA now requires from the accredited laboratories to determine seeds not typical for the regions were laboratories are placed and this determination has to be at the level of the species.

MATERIAL AND METHOD

Sample preparation for the ISTA proficiency tests

Samples prepared for the ISTA proficiency tests are drawn from three seed lots of homogenous morphological traits. The

seed lot homogeneity is determined in the following way: 10 samples are drawn from each seed lot and heterogeneity is determined according to the ISTA Rules *(ISTA Rules, 2011)*. If heterogeneity is determined, seed cannot be used for the proficiency test. The seed amount drawn for samples to be used in proficiency tests depends on the seed size and requires appropriate devices such as a mechanical seed divider with bins of greater capacities. The proper equipment provides seed mixture and division and later combining of sub samples provides a sample of uniform traits. A sample size for the seed purity analysis is prescribed by the ISTA Rules. Samples for proficiency tests that are forwarded to all ISTA accredited laboratories are not purified prior to other seed addition.

Seed purity and other determination

The Seed Testing Laboratory of the Maize Research Institute, Zemun Polje, compulsorily participated in 16 rounds of purity tests and the seed determination and voluntarily participated in three rounds (*Lolium multiflorum, Festuca arundinaceae* and *Portulaca grandiflora).* A voluntary participation in *Portulaca grandiflora* seed testing preceded the laboratory accreditation for flower seed testing, which was performed during the ISTA reaccreditation in 2009.

The following species were tested:

1. 01-2 *Daucus carota;*
2. 01-3 *Brassica napus;*
3. 02-2 *Triticum aestivum;*
4. 03-1 *Trifolium incarnatum;*
5. 03-3 *Lycopersicon esculentum*;
6. 04-1 *Brassica napus;*
7. 05-2 *Secale cereale;*
8. 06-1 *Sorghum bicolour*;
9. 06-2 *Beta vulgaris*;
10. 07-2 *Medicago sativa*;
11. 07-3 *Raphanus sativus*;
12. 08-1 *Lolium multiflorum*;
13. 08-2 *Portulaca grandiflora*;
14. 08-3 *Daucus carota*;
15. 09-1 *Hordeum vulgare*;
16. 09-2 *Linum usitatissimum*;
17. 09-3 *Oryza sativa*;
18. 10-1 *Festuca arundinacea*;
19. 10-2 *Medicago lupulina*.

Results for the species *Sorghum bicolor* and *Lycopersicon esculentum* were not forwarded because the statistical model for data processing in the ISTA Secretariat could not be applied when the seed purity was above 99.5%, as it was a case with these two species. The purity analysis was done according to the ISTA Rules *(ISTA Rules, 2011)*. The other seed determination was done according to Manuals for the Seed Determination *(Heinich, 1955a; Heinich, 1955b; Heinich, 1955c), (Delorit, 1970), (Skender et al., 1998), (Jones et al., 2004),* while Latin names were given according to the ISTA List of Stabilised Plant Names *(ISTA, 2007)*.

Statistical analysis of results on seed purity analysis

The ISTA Secretariat statistically analyzed results by Tattersfield's method *(Tattersfield, 1979)* according to which the Z score (normalized score) was calculated. This value is a parameter of the laboratory competency. The individual Z score for pure seeds, other seeds and inert matter is calculated according to the following formula:

$$Z = \frac{Xi - \overline{X}}{SD}$$

where is: Xi – laboratory's mean; $\overline{X}$ - overall mean (ISTA accredited laboratories); *SD* - standard deviation

The in-round rating system for purity is based on the sum of absolute Z scores (sum of individual Z-scores for tree samples). Only pure seeds are taken into consideration. The A, B, C and BMP (below minimum performance) ratings are given as shown in Table 1 *(ISTA, 2010)*:

Table 1. In-round rating system for purity

Score	Sum of absolute Z-scores
A	Sum of absolute Z-scores ≤ 3.5
B	3.5 < Sum of absolute Z-scores ≤ 5.3
C	5.3 < Sum of absolute Z-scores ≤ 7.0
BMP	Sum of absolute Z-scores > 7.0

Rating system for other seed determination

The in-round rating is based on the actual retrieval rate of a distinct species *(ISTA, 2010)*. The percentage of retrieved seeds for each species among all participants is calculated (Table 2). Based on the actual retrieval rate, a factor is assigned to each added species to take the relative difficulty into account.

Table 2. Thresholds defining the factor assigned to each species based on the actual retrieval rate of all seeds of a distinct species

Retrieval rate (%)	Assigned factor
≥ 90	3
≥ 85	2
< 85	1

The factor is multiplied by the total number of seeds added for this species. Then the factor is multiplied by the number of seeds found by the laboratory. The percentage of retrieved and identified seeds determine the in-round rating (Table 3).

Table 3. In-round rating for other seed determination

In-round rating	Percentage of retrieved and identified seeds
A	≥ 90.0
B	≥ 80.0
C	≥ 70.0
BMP	< 70.0

Up to 2003 results of all laboratories participants regardless of their status (accredited or voluntary participant in the proficiency tests) were used for calculation of the overall mean, but since 2003 only results of accredited laboratories have been taken for calculation. Moreover, the A, B, C and BMP ratings for results of seed germination and purity analyses in the proficiency tests were introduced in 2003, while the other seed determination has been evaluated since 2006.

RESULTS AND DISCUSSION

Figure 1 shows the sum of absolute Z scores and laboratory ratings for seed purity analyses of observed plant species during the ten-year period. Based on the sum of absolute Z scores the laboratory received the A rating in 15 out of 17 test rounds, which justified its accreditation. The laboratory received the B rating (sum of absolute Z-scores 3.9) for the species *Tifolium incarnatum*, which was acceptable, while for the species *Portulaca grandiflora* the laboratory received the rating C (sum of absolute Z-scores 5.72).

In table 4 are presented seed purity results for Proficiency test round (PT 08-2) *Portulaca grandiflora*. Detailed analysis of standard deviations and Z-scores for all categories (pure seed,

other seed and inert matter) helps in identification of possible mistakes.

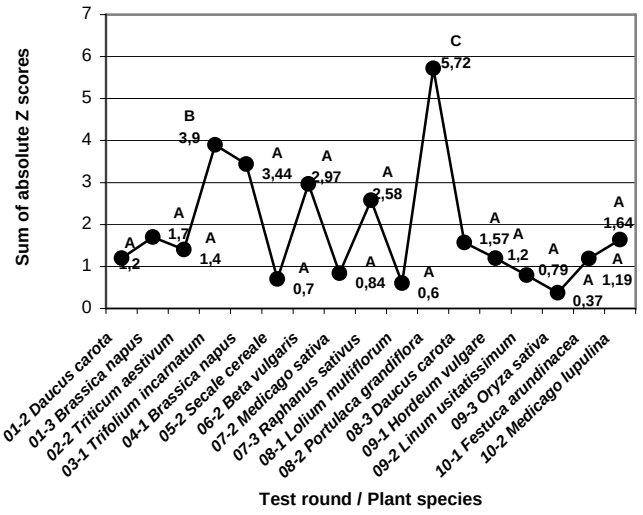

Fig. 1. Sum of absolute Z scores and laboratory ratings for seed purity of tested species

The standard deviation value in the first sample amounted to 0.34, which resulted in a low individual Z score of 0.10. The individual Z scores close to zero indicate that the laboratory results are close to the mean of all laboratories. The laboratory mean for the second sample for the pure seed analysis was lower than the mean of all other participants, hence the individual Z score was negative. The laboratory mean for the third sample for the pure seed expressed in percentages was lower than the mean of all other laboratories by 1%, while the corresponding percentage for inert matter was higher by 1%. This leads to a conclusion that the laboratory increased inert matter at the account of pure seeds by 1% in relation to other laboratories and thereby it deviated from the mean. Standard deviation for the third sample amounted to 0.21, while the individual Z score was -4.92. The sum of absolute Z scores was 5.72 and the laboratory received the C rating. The highest value of standard deviation (0.77) for the ten-year period of the ISTA proficiency samples was determined in the species *Brassica napus* in the third sample for the pure seed (these results are not presented in this study). Due to the deviation in the third sample and the C rating, the laboratory applied corrective actions in order to determine the cause of the occurred deviation. These actions included re-testing of the same sample and separation of pure seeds from inert matter and advanced training of the staff. Implemented corrective actions were checked and positively evaluated by ISTA auditors during the audit performed in 2009.

Table 5 shows that a low value of standard deviation (0.19) for pure seeds in the third sample resulted in the increased individual Z score (2.07). A low value of standard deviation indicates that the majority of laboratories had results close to the mean. The laboratory received the B rating for the species *Trifolium incarnatum* with the sum of absolute Z score of 3.90, which was acceptable. *Figure 2* presents the averages of individual Z scores for pure seeds, other seeds and inter matter. According to instructions for the proficiency tests *(ISTA, 2010),* results should be within the range of ± 1, which was obtained by the laboratory, except for inert matter in the species *Beta vulgaris, Lolium multiflorum* and *Portulaca grandiflora* with the values of 2.31, 1.71 and 2.63, respectively. The value for pure seeds (-1.84) in the species *Portulaca grandiflora* also deviated. *Figure 2* shows that the laboratory tended to give lower values for pure seeds and higher values for inert matter. Eleven out of 17 carried out analyses had values for pure seeds below zero, while six analyses had corresponding values above zero. Thirteen, i.e. four out of 17 performed analyses had values of inert mater above, i.e. below zero, respectively.

Figure 3 presents the laboratory ratings and percentage of retrieved and identified seeds. According to this Figure the percentage of retrieved and identified other seeds was above 90% for all species, hence the laboratory received the A rating for the determination of other seeds except in the species *Portulaca grandiflora.* The B rating in the species *Portulaca grandiflora* was received since the percentage of retrieved and identified seeds was 87%. This rating is acceptable and it was received due to the incorrect determination of two added seeds.

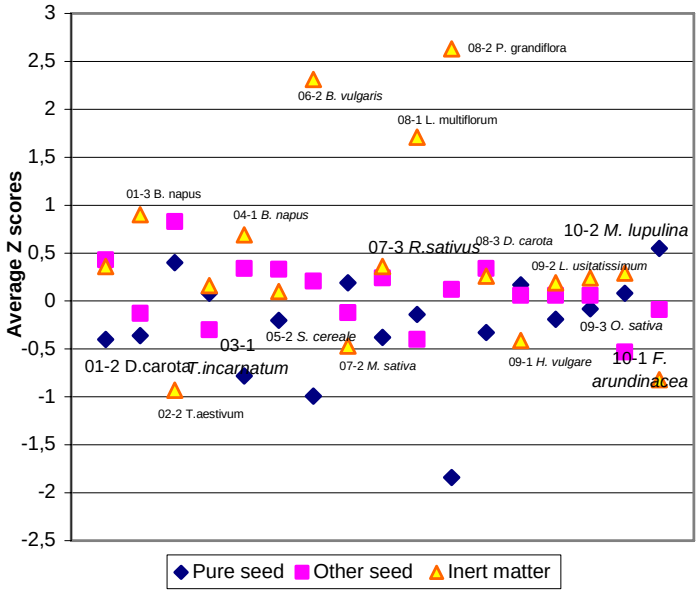

Fig. 2. The average of individual Z scores for pure seed, other seed and inert matter over tested species

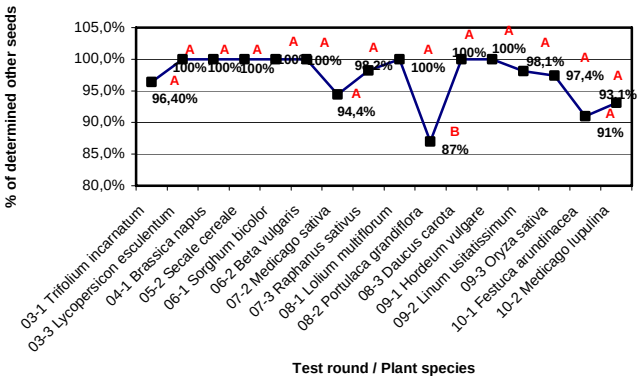

Fig. 3. Laboratory rating and percentage of retrieved and identified other seeds of tested species

In *Table 6* are shown results of the other seed determination for the species *Portulaca grandiflora.* It is observable from the column of assigned factors that majority of added seeds were difficult to determine (factor 1). Instead of *Sysimbrium* sp. in the first sample the laboratory recorded *Erysimum cheiranthoides* (*Figure 4*), while in the third sample the species *Spermacoce alata* was recorded instead of the species *Campanula carpatica (Figure 5),* which resulted in a lower percentage of determined

seeds. The laboratory registered an exact number of added seeds, but the determination was not correct. Seeds of *Amaranthus albus, Rumex acetosella* and *Capsella bursa-pastoris* determined by the laboratory were not difficult to determine, and they had a factor 3. Furthermore, *Figure 3* shows that the lower percentage of retrieved and identified seeds was in the family *Fabaceae*. By studying results for these species it was observed that the laboratory was not able to determine species more difficult to be determined, i.e. species with the factor 1, what reduced the percentage of retrieved and identified seeds, but it did not affected laboratory rating. The laboratory was not able to determine *Helianthemum nummularium* seeds in the second sample of PT round 10-2 *Medicago lupulina* (*Figure 6*), nor it was able to determine *Eleusine indica* seeds *(Figure 7)* in the third sample, hence the percentage of retrieved and identified seeds amounted to 93.1%. The laboratory did not determined seeds of *Chloris gayana (Figure 8)* in the *Medicago sativa* second sample (PT round 07-2), nor seeds of *Polygonum argyrocoleon* in the third sample. These seeds had factor 1, hence the percent of retrieved and identified seeds was 94.4%. Analysis of proficiency test results by ISTA proficiency committee demonstrate that non accredited laboratories have significantly lower percentage of retrieved and identified seeds compared to accredited labs (*Muller, 2010*). For example in PT round 09-1 *Hordeum vulgare* distribution of A, B, C, and BMP ratings was 84%, 10%, 3%, 3% respectively for accredited laboratories, while voluntary laboratories had following distribution 45%, 23%, 8%, 26%.

Table 4. Seed purity results for ISTA PT round 08-2 Portulaca grandiflora

Name	Pure seed				Other seed				Inert matter			
	Lot 1 L1	Lot 2 L2	Lot 3 L3		Lot 1 L1	Lot 2 L2	Lot 3 L3		Lot 1 L1	Lot 2 L2	Lot 3 L3	
lab. mean (%)	98.54	98.98	98.15		0.32	0.67	0.61		1.14	0.35	1.25	
overall mean (%)	98.51	99.15	99.18		0.33	0.62	0.58		1.11	0.24	0.25	
SD	0.34	0.24	0.21		0.14	0.17	0.15		0.30	0.16	0.14	
Z Score	0.10	-0.70	-4.92	Ø-1.84	-0.10	0.28	0.18	Ø 0.12	0.10	0.69	7.11	Ø 2.63

Sum of absolute Z-scores for pure seed ↔ 5.72

Laboratory in-round rating for this test round **C**

Table5. Seed purity results for ISTA PT round 03-1 Trifolium incarnatum

	Pure seed				Other seed				Inert matter			
	Lot 1 L1	Lot 2 L2	Lot 3 L3		Lot 1 L1	Lot 2 L2	Lot 3 L3		Lot 1 L1	Lot 2 L2	Lot 3 L3	
lab. mean (%)	98.09	97.87	99.49		0.18	0.17	0.12		1.72	1.96	0.38	
overall mean (%)	96.63	97.92	99.10		0.23	0.15	0.19		1.11	1.89	0.69	
SD	0.31	0.54	0.19		0.10	0.08	0.09		0.31	0.60	0.19	
Z Score	-1.73	-0.10	2.07	Ø0.08	-0.45	0.28	-074	Ø -0.30	1.97	0.12	-1.62	Ø0.16

Sum of absolute Z-scores for pure seed ↔3.90

*Laboratory in-round rating for this test round **B***

Table 6. Results for other seed determination of ISTA PT round 08-2 Portulaca grandiflora

Species name	Seeds added	Mean rate %	Factor	Seeds found	Seeds added x factor	Seeds found x factor	Miss-pelled	Correct species	Corect genus	Different species
Lot 1										
Nicotiana tabacum	3	82.2	1	3	3	3		X		
Papaver rhoeas	3	83.3	1	3	3	3		X		
Petunia x hybrida	2	65.9	1	2	2	2	X	X		
Sysimbrium sp.	2	27.3	1	0	2	0				
Lot 2										
Ageratum houstonianum	2	67.6	1	2	2	2		X		
Amaranthus albus	2	97.2	3	2	6	6				X
Rumex acetosella	2	92.6	3	2	6	6		X		
Lot 3										
Campanula carpatica	4	54.0	1	0	4	0				
Capsella bursa pastoris	4	90.3	3	4	12	12		X		
Cuscuta epythymum	3	84.1	1	3	3	3			X	
Matricaria recutita	3	82.6	1	3	3	3				X
SUM	30			24	46	40				
Percentage						87%				
In round rating						B				

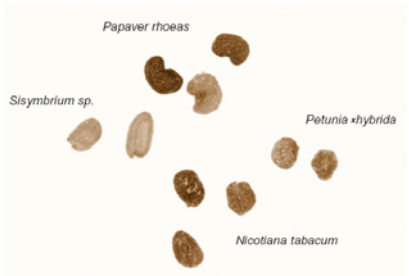

Fig. 4. Other seed in lot 1 of ISTA PT round 08-2 Portulaca grandiflora

Fig. 5. Other seed in lot 3 of ISTA PT round 08-2 Portulaca grandiflora

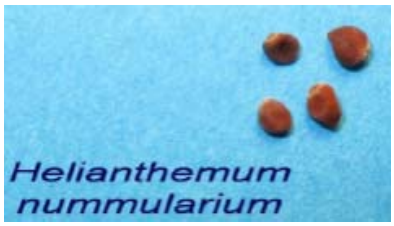

Fig. 6. Seed in lot 2 of ISTA PTound 10-2 Medicago lupulina

Fig. 7. Seed in lot 3 of ISTA PT round 10-2 Medicago lupulina

Fig. 8. Seed in lot 2 of ISTA PT round 07-2 Medicago sativa

Fig. 9. Seed in lot 3 of ISTA PT round 09-3 Oryza sativa

Figure 9 presents undetermined seeds of ISTA PT round 09-3 *Oryza sativa*. When the analyses were completed all seeds were included into the seed reference collection.

CONCLUSION

During the ten year testing the laboratory received the A rating for the seed purity analyses of all tested plant species, except for the species *Trifolium incarnatum* and *Portulaca grandiflora* for which it received B and C ratings. The B rating was acceptable. After receiving rating C, laboratory implemented corrective actions that were positively estimated by ISTA auditors in 2009. The laboratory tended to increase an inter matter weight at the account of pure seeds, but this tendency did not affect the total laboratory rating. However, the staff was informed about it. The laboratory received the A rating for the other seed determination except for the species *Portulaca grandiflora* for which the laboratory received the B rating that was acceptable. In the last proficiency test rounds other seeds untypical for our region were added. By conducting proficiency tests staff gained experience, the laboratory enlarged its seed reference collection, while difficult other seeds were used in staff training for other seed determination.

By the analyses of results achieved in testing, the laboratory improves the proficiency and competency of its staff and emphasises the necessity of permanent learning, specialisation and supervision of the whole testing process with the aim to obtain reliable results.

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