

## RING ANALYSES IN THE PROFICIENCY ASSESSMENT OF AUTHORISED SEED SAMPLERS

### KRUŽNE ANALIZE U PROVERI RADA AUTORIZOVANIH UZORKOVAČA SEMENA

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#### ABSTRACT

The objective of this study was to apply the ring analysis in the proficiency assessment of authorised seed samplers from 18 accredited laboratories from the Republic of Serbia and two seed samplers from the Republic of Macedonia. The seed sampling was performed in the Processing plant, while the assessment was performed at the Seed Testing Laboratory of the Maize Research Institute, Zemun Polje. Two maize seed lots, placed in containers and jumbo bags, and one barley seed lot packed in paper bags, were sampled. Drawing of submitted samples and the estimation of the sampling intensity by seed samplers were done during the course of sampling. Seed purity, 1000-seed weight and seed germination were determined in the drawn samples. Results obtained by authorised samplers were within toleration limits.

All samplers from accredited laboratories proved to be well trained, the samples were properly drawn, while insignificant nonconformities were detected in the calculation of sampling intensity.

**Key words:** sampling, seed samplers, ring analyses.

#### REZIME

Cilj ovog rada je primena kružne analize u kontroli ovlašćenih uzorkovača semena iz 18 akreditovanih laboratorija iz Republike Srbije i dva uzorkovača iz Makedonije. Uzorkovanje i ispitivanje obavljeno je na Doradnom centru i u Laboratoriji za ispitivanje semena Instituta za kukuruz „Zemun Polje“. Uzorkovane su dve partije semena kukuruza, smeštene u kontejnerima i džambo vrećama i jedna partija ječma upakovana u papirne vreće. U toku uzorkovanja, praćen je način formiranja prosečnog uzorka i izračunavanja intenziteta uzorkovanja od strane uzorkovača. Na uzetim uzorcima ispitivana je čistoća semena, masa 1000 semena i klijavost semena. Utvrđivanje klijavosti semena kukuruza je izvršeno na standardnom temperaturnom režimu  $20 \leq 30^{\circ}\text{C}$ , naizmenično 8/16 h, a za seme ječma na  $20^{\circ}\text{C}$ . Masa je utvrđena merenjem  $8 \times 100$  semena a čistoća semena procentualnim učešćem nečistoća u uzorku. Ispitivana čistoća semena se kretala od 99,4%-99,9% za prvi uzorak kukuruza, za drugi uzorak kukuruza se kretala od 98,6-99,3% a za uzorak ječma od 99,4-99,7%. Klijavost semena za prvi uzorak kukuruza se kretala od 96-98%, za drugi uzorak od 90-96% a za partiju ječma od 97-99%. Masa se kretala od 351,7 g-357,3 g za prvi uzorak kukuruza, za drugi uzorak kukuruza od 306,3 g-315,0 g a za uzorak ječma od 46,19 g-44,76 g. Analize su rađene prema Pravilnika o kvalitetu semena poljoprivrednog bilja 47/87 i po međunarodnim ISTA pravilima. Eksperimentalni podaci obrađeni su deskriptivnom statistikom (srednja vrednost, standardan devijacija, medijana), koristeći Data Analysis System.

Dobijeni rezultati ovlašćenih uzorkovača su u granicama dozvoljenih odstupanja. Svi uzorkovači iz akreditovanih laboratorija dobro formiraju radni uzorak, dok su manja odstupanja uočena kod izračunavanja intenziteta uzorkovanja.

**Ključne reči:** uzorkovanje, uzorkovači semena, kružne analize.

#### INTRODUCTION

Seed sampling is the first and the most important stage in the process of seed testing. It is also a prerequisite for a reliable estimation of seed lot quality. Sampling can be within the scope of accreditation of seed testing laboratories, but it does not have to be. If sampling is to be accredited, the laboratory has to apply the equal level of auditing to its samplers and analysts. National and ISTA accreditation standards do not specify requirements regarding the auditing and monitoring of the performances of seed samplers, but laboratories have to develop their own solutions and to prove that their procedures and practices are consistent. Results of Kojić *et al.* (2011) indicate that the analyses of results PT tests, 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.

All seed testing laboratories in Serbia are accredited for sampling by the Accreditation Body of Serbia (ATS). Once a year, laboratories conduct internal audits of their accredited samplers using check samples that are sampled and tested within

the same laboratory and that meet requirements of the standard. Results obtained for the observed parameters are compared with the tolerance table and if they are within tolerance limits, the audited sampler performed sampling well and a licence is issued to her/him. Kojić *et al.* (2010) have determined the importance of accreditation of automatic seed samplers, because sampling performed with automatic seed samplers have advantages over manual sampling.

However, checking of the sampling process itself is not possible. Due to major nonconformities occurring in the process of sampling, there is a need to monitor the work of seed samplers, because they work under specific conditions, alone with limited contacts with other colleagues. In recent years, the ISTA has more often organised workshops for seed sampling that have shown the way laboratories could monitor performances of their samplers from problem-solving tests to monitoring trends in order to provide corrective actions before errors become significant and samples compromised and not representative, Don *et al.* (2012).

The ATS evaluation implies a regular check of seed sampling, and the internal audit of seed samplers done by the laboratory. The outdated Regulation on Seed Testing Quality of

Agricultural Crops, issued in 1987, with a poor description of sampling, the keystone for seed testing, is a problem in laboratories accredited by ATS.

## MATERIAL AND METHOD

Two maize seed lots of ZP hybrids, placed in jumbo bags and containers, and one barley seed lot packed in paper bags, were sampled. Sampling was done by a sampling stick/spear for maize seeds and a trier for wheat seeds. The applied sampling intensity was in accordance with the Regulation on Seed Testing Quality of Agricultural Crops. Accredited samplers used their own equipment for sampling. After sampling, samplers drew submitted samples out of composite ones, which were then submitted to the Seed Testing Laboratory of the Maize Research Institute, Zemun Polje. During sampling, the following was monitored: the use of the sampling equipment by samplers, knowledge of sampling intensity, as well as, drawing a submitted sample.

In the laboratory, a soil divider was used to reduce the submitted samples to working samples to the prescribed weight. Seed purity, 1000-seed weight and seed germination were determined in accordance with the *ISTA Rules (2015)*.

Maize seed purity was analysed on a 900-g sample, and then 8 x 100 seeds were counted for the determination of 1000-seed weight. *Tabaković et al. (2016)* pointed out the importance of the production location on the expression of seed mass.

A total of 4 x 100 seeds were separated to test germination, which was performed by applying the between filter paper method. Maize seeds were germinated in a seed germinator at the altering temperature of 20 °C=>30 °C. The first, i.e. final counts were conducted on the fourth, i.e. seventh day, respectively, *ISTA Rules (2015)*. Results of *Pavlov et al. (2015)* indicate that average germination was higher by 2.1 % in large seed size fractions than small seed size fractions.

Barley seed germination was tested by the between filter paper method at the temperature of 20 °C. The first and final counts were done on the fourth and the seventh day, respectively, *ISTA Rules (2015)*.

Obtained results were statistically processed according to the Tattersfield method, *Tattersfield (1979)*, which implies the calculation of a Z score, based on the determination of an "actual" central value (median) that is obtained after discarding outliers.

## RESULTS AND I DISCUSSION

Analysed seed purity ranged from 99.4 to 99.9 % and 98.6 to 99.3 % for the first and the second maize sample, respectively, while it varied from 99.4 to 99.7 % for the barely sample. The corresponding values for seed germination ranged from 96 to 98 %, from 90 to 96 % and from 97 to 99 %, respectively. Seed weight ranged from 351.7 to 357.3 g and 306.3 to 315.0 g for the first and the second maize sample, respectively, while it varied from 46.19 to 44.76 g for the barely sample.

Table 1. Results for seed purity, germination and 1000-seed weight and obtained statistical values for 20 laboratories

Lab. no.	Sample	Purity	Total germination	1000-seed weight	Lab. no.	Sample	Purity	Total germination	1000-seed weight
1	1	99.7	96	352.3	11	1	99.8	97	351.7
	2	98.9	94	308.3		2	99.0	93	306.3
	3	99.8	98	45.58		3	99.6	98	45.93
2	1	99.8	97	356.0	12	1	99.8	97	357.3
	2	98.9	93	308.2		2	99.3	94	308.9
	3	99.6	98	45.76		3	99.7	98	45.32
3	1	99.8	98	352.8	13	1	99.8	96	352.2
	2	98.7	93	310.7		2	99.5	92	312.9
	3	99.6	98	45.37		3	99.6	97	45.45
4	1	99.9	97	352.0	14	1	99.9	97	353.0
	2	99.2	91	308.9		2	99.0	93	310.6
	3	99.4	98	45.06		3	99.5	99	45.75
5	1	99.9	97	355.3	15	1	99.8	97	357.1
	2	99.2	94	315.0		2	99.3	91	310.9
	3	99.5	98	45.71		3	99.6	99	45.56
6	1	99.8	96	356.0	16	1	99.8	97	354.9
	2	99.2	90	311.1		2	98.7	96	309.6
	3	99.5	96	45.84		3	99.7	98	44.84
7	1	99.7	96	350.7	17	1	99.8	97	352.3
	2	98.9	93	310.8		2	99.1	91	312.2
	3	99.7	99	46.19		3	99.6	98	45.38
8	1	99.8	98	354.1	18	1	99.6	97	352.3
	2	99.1	92	312.2		2	99.0	92	308.4
	3	99.5	98	45.37		3	99.5	99	45.28
9	1	99.8	96	354.1	19	1	99.7	97	354.8
	2	99.1	93	312.2		2	99.0	92	310.9
	3	99.5	98	45.38		3	99.5	99	46.31
10	1	99.8	96	353.5	20	1	99.4	97	356.4
	2	99.1	93	311.0		2	98.6	93	313.2
	3	99.7	98	45.38		3	99.6	97	44.76
					Overall mean	Uz. 1	99.80	96.86	353.89
						Uz. 2	99.04	92.37	310.99
						Uz.3	99.59	97.85	45.51
					sd	Uz. 1	0.00612	0.4977	1.7597
						Uz. 2	0.1857	1.0160	1.8547
						Uz.3	0.0998	0.5082	0.4163
					Mediana	Uz. 1	99.7-99.9	95.9-97.6	350.4-357.2
						Uz. 2	98.6-99.5	89.7-95.6	306.3-315.4
						Uz.3	99.4-99.8	96.5-98.8	44.7-46.3

According to presented values it is observable that deviations in results for seed purity, germination and 1000-seed weight were greater for the second maize sample due to incorrect calculations of sample intensities and due to seed lot heterogeneity.

The median for pure seed, total germination and 1000-seed weight was determined after outliers had been discarded. The standard deviation and the overall mean were calculated for the laboratories that scored above or equal to the median. In the Student's distribution table, a value *t* was read for the probability level of 99 % and the degree of freedom for the number of laboratories that scored equal to or above the median. This score was deducted and added to the median and limiting values, i.e. a range for discarding outliers were obtained (table1.).

In the first sample for seed purity, 14 out of 20 laboratories obtained values equal to or above the median that ranged from 99.7 to 99.9. The median for seed germination in the first sample ranged from 95.9 to 97.6 and 10 laboratories had values equal to or above the median. Furthermore, 10 laboratories had values for the 1000-seed weight equal to or above the median that ranged from 350.4 to 357.2.

In the second sample for seed purity, nine laboratories had values equal to or above the median, which ranged from 98.6 to 99. The median for total germination in the second sample varied from 89.7 to 95.6 and also nine laboratories had values equal to or above the median. Furthermore, the median for 1000-seed weight ranged from 306.3 to 315.4 and nine laboratories had values equal to or above the median.

The median in the third sample ranged from 99.4 to 99.8 for pure seed, and 12 laboratories had values equal to or above the median. The corresponding values for total germination amounted to 96.5-98.8 and nine laboratories had values equal to or above the median, while the median for 1000-seed weight ranged from 44.7 to 46.3 and also nine laboratories had values equal to or above the median.

According to obtained values of standard deviation, it can be concluded that variations among samplers in results for pure seed, total germination, and 1000-seed weight were small.

Table Samplers were given ratings A, B, C, BMP on the basis of the Z value that is a sum of absolute values (Table 2).

According to the total Z score that is obtained by summing individual Z scores for the pure seed analysis of all three samples and for normal seedlings in germination tests the laboratories receive their ratings:

- Z score up to 3.5 ↔ A rating
- Z score ranging from 3.5 to 5.3 ↔ B rating
- Z score ranging from 5.3 to 7.0 ↔ C rating
- Z score over 7.0 ↔ BMP (below the minimum standard)

All samplers were given acceptable ratings, except the sampler number 20, who was rated BMP for seed purity test. All samplers used the sampling equipment well and drew submitted samples well. Smaller nonconformities occurred in calculations of the sampling intensity and samplers were informed about. The first seed lot was placed into eight containers of 1,587 kg, making the total size of the lot 12,700 kg. The range of primary

Table 2. Rating for purity, germination and 1000-seed weight for 20 laboratories

Lab. no.	Sample	Marks for			Lab. no.	Sample	Marks for		
		Purity	Germination	1000-seed weight			Purity	Germination	1000-seed weight
1	1	B	B	A	11	1	A	A	B
	2					2			
	3					3			
2	1	A	A	A	12	1	A	A	B
	2					2			
	3					3			
3	1	A	A	A	13	1	A	A	A
	2					2			
	3					3			
4	1	B	A	A	14	1	A	A	A
	2					2			
	3					3			
5	1	A	A	A	15	1	A	A	A
	2					2			
	3					3			
6	1	A	B	A	16	1	A	B	B
	2					2			
	3					3			
7	1	A	A	B	17	1	A	A	A
	2					2			
	3					3			
8	1	A	A	A	18	1	A	A	A
	2					2			
	3					3			
9	1	A	A	A	19	1	A	A	A
	2					2			
	3					3			
10	1	A	A	B	20	1	BMP	A	B
	2					2			
	3					3			

samples stated by samplers was 10-32, while the required intensity was a minimum of 26 primary samples. The second seed lot was placed into 13 jumbo bags of 1,001 kg, making the total size of the lot 14,320 kg. The range of primary samples stated by samplers was 6-30, while the required intensity was a minimum of 29 primary samples. The third seed lot was placed into 400 bags of 20 kg each, making the total size of the lot 8,000 kg. The range of primary samples stated by samplers was 10-80, while the required intensity was a minimum of 80 primary samples. Samplers were more precise in the determination of sampling intensity for the third lot that had been already formed in the paper bags unlike maize lots placed into containers and jumbo bags

## CONCLUSION

Based on obtained results the following can be concluded:

- The greatest variations in obtained results were detected in the second sample, which can be explained by incorrect calculations of the sampling intensity and seed lot heterogeneity.
- All authorised samplers from the laboratories accredited by the ATS sample well and use the sampling equipment well.
- All samplers were given acceptable marks for seed purity, 1000-seed weight and seed germination, except the sampler number 20, who was rated BMP for seed purity test.
- Smaller nonconformities occurred in calculations of the sampling intensity and samplers were informed about it. It was noticed that samplers more precisely determined the sampling intensities in already formed lots, while these calculations of sampling intensities of lots in bulks showed minor nonconformities.
- Upon completion of sampling and the analysis of results it was concluded that ring analyses are necessary in the process of sampling and that they should be applied in a single ITC circle, because in this way it is easier to observe problems that samplers have in the course of sampling and it is also easier to control the sampling process itself.

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