UDC 575.630.15
https://doi.org/10.2298/GENSR1703853B
Original scientific paper

DISRUPTION OF GENETIC IDENTITY FOR GENEBANK MAIZE ACCESSIONS DURING CONSERVATION

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Babić V., V. Andjelković, A. Nikolić, M. Milivojević, J. Srdić, A. Popović, N. Kravić (2017): Disruption of genetic identity for genebank maize accessions during conservation.- Genetika, Vol 49, No.3, 853-864.

Maintenance of the original accessions identity and integrity is one of the priorities among genebank activities. Different factors related to conservation may result in accessions disruption. Regeneration is the most frequent critical point in this process, due to bottlenecks, inbreeding, random genetic drift and unintentional mixing or contamination. On the other hand, genetic drift may occur due to seed viability loss. Therefore, it is very important to establish the balance between the frequency of regeneration and the duration of accession conservation. The aim of the present study was to estimate whether the identity of accessions regenerated after 27 years of medium-term conservation was disrupted. Phenotypic markers were applied on three Maize Research Institute „Zemun Polje" (MRIZP) genebank maize landraces (K2026, K768 and K86), differing in seed viability, kernel type and effective population size. It was estimated that, after the regeneration, there had been no significant changes in the landrace K2026. There were some parameters indicating that genetic drift had occurred in the landrace K768, and that there had been even a certain degree of inbreeding in the landrace K86. According to the results, accession K2026 could still be kept under the same ID number. Due to the genuine identity disruption, assignment of new ID numbers for K768 and K86 should be suggested.

Key words: landraces, phenotypic markers, regeneration, viability, *Zea mays* L.

INTRODUCTION

Principal genebank activities should be aimed towards conservation of genetic identity and integrity of accessions, as well as their viability. Screening for accession viability at adequate intervals is of an exceptional importance. At the same time, the balance between the

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frequency of germination testing and the duration of accession conservation should be established. It is recommended to perform regeneration when seed viability falls below 85% of the initial germination percentage in active collections, and/or when the number of viable seed per accession is < 1500 in active or base collections (TABA *et al.* 2004).

Active collection of MRIZP genebank comprises more than 6000 maize accessions. Out of this number, 2217 accessions belong to landraces collected from the regions of the former Yugoslavia (ANĐEKOVIĆ and IGNJATOVIĆ-MICIĆ, 2012; VANČETOVIĆ *et al.*, 2010). In accordance with standards recommended for plant genebanks (FAO, 2014), activities are directed towards genetic identity and integrity conservation, and seed viability preservation. The application of standards in monitoring of the collection status, regeneration process and accession handling, provide satisfactory level of genetic identity conservation. Unfortunately, disruption of accession identity may occur at any time, even when the influence of anthropogenic factors and errors made during regeneration are minimised. Namely, inadequate labelling, replacement of accessions, etc., contamination with foreign genes (in the process of cleaning and storing of accessions, during sowing, at harvest, by the preceding crop, by foreign pollen) could reflect in accession identity disruption. Other causes for genetic composition disruption, when genetic changes (genetic drift) occur, are as follows: inadequate handling and the landrace/population size, different levels of pollen and ovule production of individual parents, deterioration of certain individuals of accessions during regeneration or absence of reproduction, occurrence of recombination and development of a new genotype, as well as changes in the heterozygosity level. Furthermore, changes can be a result of selection (genetic shift) affected by natural or artificial (anthropogenic) factors.

Additionally, genetic drift could arise due to different loss of individuals viability within the same accession during conservation. In study of germination testing, carried out on 703 accessions (BP, $20 \Leftrightarrow 30^{o}$C, ISTA Rules, 2005), considerable decrease in germination below standards was found among 49 accessions (BABIĆ *et al.*, 2015). TABA and TWUMASI-AFRIYIE (2008) stated that differences in adaptability and problems in seed setting often imposed additional challenges to proper regeneration. However, consistent planning and monitoring the regeneration process reduce the risk of changes that can occur and lead to genetic drift or genetic shift, and thus to disruption of genetic identity and integrity of the accession. Unless due precautions are taken, the regeneration process is potentially prone to a source of a genetic change for accessions in the system, due to bottlenecks, inbreeding, random genetic drift and unintentional mixing or contamination (CROSSA *et al.*, 1994).

Keeping genetic identity and integrity unchanged involves maintaining the joint frequency distribution of all alleles at all loci. However, in cross-pollinated plants, the absence of genetic changes, is generally unattainable. It is known from the theory that the probability of losing an allele from an accession during regeneration depends on its initial frequency and the number of parents used for regeneration. The probability of losing an allele is higher for rare alleles. WEN *et al.* (2011) demonstrated the utility of molecular markers for understanding the extent of changes in the genetic purity of the maize accessions during regeneration adopted by the *ex situ* genebanks, and recommended the best practices for maintaining the original genetic diversity of the genebank accessions.

BOCZKOWSKA and PUCHALSKI (2012), by testing seed samples of the same population conserved under different conditions, concluded that the decrease in seed viability due to ageing resulted in increased homozygosity. Moreover, the analysis showed a clear segregation of the

population with the lowest seed viability in relation to the other, tested samples. Inbreeding coefficient values indicated a small variation of the seed samples related to natural ageing.

The objective of the present study was to estimate whether and to what extent the identity of accessions K2026, K768 and K86 were disrupted, due to the loss of viability bellow the standard for plant genebanks. This was done by the application of phenotypic markers.

MATERIALS AND METHODS

Three maize landraces (K2026, K768 and K86) from MRIZP genebank, stored for 27 years under medium-term conditions (t=4-5°C; RH=45-50%; required seed moisture of 11%) differing in germination rate, were used in this experiment (Tab. 1). Yellow kernel dent population K2026, was collected in 1964 in Croatia (Rab island). White kernel flintlike K86, was collected in Serbia (Aleksinac), in 1963. Semident white kernel population K768 was collected in Bosnia and Herzegovina, in 1964. All three populations were at that time used for human consumption, i.e. for producing white or yellow maize flour. In 2012, routine control germination test for accessions stored in MRIZP gene bank revealed that, in 49 samples, germination rate dropped down below the standard. Populations were multiplied from such seed (Babić et al., 2015). Out of them, populations K2026, K768 and K86 were selected in order to establish whether and to what extent disruption of variety identity occurred, after the regeneration from the seed with reduced viability. The populations were chosen for the following reasons: previous regeneration was performed in the same year (1986), they were stored under the same conditions for 27 years, but the level of seed viability was different. The accessions evaluated varied according to kernel type, as well as in effective population size during the regeneration.

Table 1. Viability testing and the effective population size for evaluated maize landraces

ID	YG	Estimation of accession viability						N_e
		IG (%)	CG1 g/as/ds (%)	CG2 g/as/ds (%)	CG3 g/as/ds (%)	FE1 14/24 (%)	FE2 14/24 (%)	
K2026	1986	98	87/11/2	72/16/12	60/18/22	71/83	54/65	244
K768	1986	93	79/10/11	59/16/25	41/32/26	36/46	36/45	344
K86	1986	99	94/3/3	63/22/15	44/32/24	58/70	31/48	216

YG-year of previous regeneration; IG-initial germination; g/as/ds-germination/abnormal/dead seedlings; CG1-control germination in 2001; CG2-control germination in 2012, CG3-control germination in 2017; FE1-field emergence in 2013 (14/24 - after 14/24 days); FE2- field emergence in 2014 (14/24 - after 14/21 days); N_e-effective population size

In 2013, the landraces were regenerated in the technical isolation using paired crossing mating scheme. Maize was considered as a dioecious plant, i.e. one plant participated just once as a parent (either male or female). Paired cross without reciprocal is recommended for regenerating cross-pollinated species, as it results in the same genetic effect as paired cross with reciprocal or chain cross, but requires only a half the number of labour-intensive crosses (WANG *et al.*, 2004). Each accession was sown manually in 12 rows (six rows as female plants and six rows as male plants), with 15 hills per row. Five seeds per hill were sown and thinned to two plants per hill at the 4^{th}-5^{th}-leaf stage.

The accessions regenerated in 1986 and 2013 were sown in 2014, with the aim to determine morphological differences between plants. Although morphological changes cannot be the only indicator for the existence of genetic changes, the assumption was that we could get preliminary information on linkage between genetic drift and estimated accession germination rates.

The trial was set up in three replications, with four rows per accession. Spacing between rows was 0.75 m, with inner-row spacing of 0.40 m (15 hills per row). Sowing was done manually, with four seeds per hill and thinning to two plants per hill was done at the 4^{th}-5^{th}-leaf stage.

Emergence rate (i.e. the number of emerged plants), was observed in the trail (Tab. 1). The first count was done at 2^{nd}-leaf stage (14 days after sowing). The second one was done at 4^{th}-5^{th}-leaf stage, assuming that in this phase maize seedlings are developed into the normal plants (24 days after sowing).

The following morphological traits, according to CIMMYT/IBPGR descriptors for maize (1991), were measured in the sample of 15 plants per replication: plant height (PH), ear height (EH), tassel node height (TNH), first tassel branch height (FTBH), leaf length (LL), leaf width (LW), number of leaves above the ear (NLAE), number of ears per plant (NEP), number of primary tassel branches (NPTB), number of kernel rows (NKR), number of kernels per row (NK), ear length (EL), ear diameter (in the middle) (EDM), cob diameter (CD), kernel length (KL), kernel width (KW), kernel thickness (KT), 1000-kernel weight (KW1000). The yield was measured and expressed as grain yield (g) per plant.

The additional characterisation according to UPOV descriptors for maize (TG/2/7, 2009) was done for the following traits: ear shape (ES), type of grain (TG), colour of tip of grain (CTG), colour of dorsal side of grain (CDG) and anthocyanin coloration of glumes of cob (ACC) (data not presented).

The trend of seed viability was presented for the 30-year period under medium-term storage conditions.

The measures of variation for 16 scale traits observed were calculated using paired ***t*** test, in order to compare performances of the same landrace generated from previous (1986) and new (2013) regeneration.

RESULTS AND DISCUSSION

Regeneration should be performed when seed viability falls below 85% of the initial germination percentage (FAO/IPGRI, 1994). The trend of viability loss for observed maize landraces is presented in Figure 1. The red line indicates the critical germination values of 83, 79 and 84% for the landraces K2026, K768 and K86, respectively. Moment of regeneration is marked by vertical line. After first ten years of storage, viability loss intensifies, which is particularly visible after the intersection with the critical germination value line. This was the case in all three landraces. Therefore, the first viability testing should be done after 10 years of storage, while the next ones should be done in shorter intervals, especially for accessions with increased number of dead seed and/or abnormal seedlings. The landrace K768 lost the highest percentage of viability after a 15-year conservation period (CG1, Tab. 1). The viability loss for landraces K86 and K2026 was a somewhat lower after the same period of conservation and their germination was above the critical value. The significant viability loss was recorded in all three landraces after 26 years of conservation (CG2, Tab. 1): the greatest loss, as well as, the highest

number of dead seeds, were recorded in the landrace K768 (59% and 25%, respectively). The greatest number of abnormal seedlings and a significant drop of germination were observed in the landrace K86. In comparison with the previous test (CG1), the highest viability along with increased number of dead seeds and abnormal seedlings were recorded in K2026 landrace. Optimising the frequency of germination tests, in order to avoid waste of resources, is hampered by the scarce availability of seed longevity data, particularly for material maintained under genebank conditions (TREUREN, 2013). Up to the moment of regeneration, flint population K2026 preserved the highest viability; semiflint K86 expressed slightly lower viability decrease, while the fastest drop in viability was observed in semident population K768. Interestingly, flint K2026 and semiflint K86 populations expressed better field emergence rates (FE1) compared to the rates of the standard germination test on filter paper (CG2) (Table 1). Such regularity was noticed in the large number of populations (i.e. 49 populations), regenerated after the significant drop of germination rate below the standard (BABIĆ *et al.*, 2015). Since the conditions in 2013 were very favourable for field emergence, the large number of seeds from populations K2026 and K86 that produced abnormal seedlings in laboratory conditions, produced normal plants, unlike semident K768 (Tab. 1). The longer conservation period, the greater viability loss; hence, after 30 years of conservation (CG3) under medium-term conditions, the germination recorded on filter paper was 60, 41, 44% for K2026, K768 and K86, respectively. Increase in dead seeds and abnormal seedlings percentage was noticed (CG3, Tab. 1) compared to the previous control germination (CG2). Second evaluation of field emergence (FE2) was estimated in 2014, with less favourable conditions for field emergence. Accordingly, field emergence was lower to the previous year, especially for the populations K2026 and K86. It could be concluded that under the less favourable conditions, abnormal seedlings were not able to develop into the normal plants.

Figure 1. Trend of viability loss for maize landraces accessions during 30 years of conservation

The effective size of population (N_e) under type of crossing applied and sampling female and male gametes, is calculated by $N_e=8N_mN_f/(N_m + N_f)$, where N_m - number of male parents; N_f - number of female parents (HALLAUER *et al.*, 2010). Accordingly, regeneration for the landraces observed was considered successful if minimum 50 well pollinated ears were obtained, reflected in effective population size of 200 individuals. A total of 61, 86 and 54 ears

per landraces K2026, K768 and K86, respectively, were obtained in 2013 regeneration. Therefore, the effective population size for landraces K2026, K768 and K86 was 244, 344 and 216 individuals, respectively (Tab. 1). An equal number of kernels was sampled from each ear and a bulk was made to be used for further evaluation.

In the following 2014, phenotypic characterisation of the accessions regenerated in 1986 and 2013, was performed in order to estimate the level of identity disruption for the landraces K2026, K768 and K86. Significance of differences for 16 phenotypic traits was performed by the paired ***t*** test.

*Table 2. Measures of variation and **t** test for the observed phenotypic traits of accessions of the landrace K2026 from 1986 and 2013*

	Average		St. deviation		CV		Interval		
Trait	1986	2013	1986	2013	1986	2013	1986	2013	***t*** test
PH (cm)	183.8	189.3	14.0	29.1	7.6	15.4	65.0	148.0	-0.162^{ns}
EH (cm)	65.2	70.0	11.7	13.6	17.9	19.5	45.0	55.0	-1.474^{ns}
TNH (cm)	127.7	130.4	10.4	17.2	8.1	13.2	40.0	61.0	-0.728^{ns}
FTBH (cm)	148.5	149.6	11.6	19.0	7.8	12.7	48.0	70.0	-0.279^{ns}
NPTB	21.3	20.7	5.5	5.0	25.8	24.3	21.0	23.0	0.466^{ns}
NLAE	5.2	5.2	0.9	0.5	17.8	10.3	4.0	2.0	0.171^{ns}
LL (cm)	71.4	75.6	8.3	8.0	11.6	10.6	28.0	35.0	-1.983^{ns}
LW (cm)	8.1	8.7	1.0	1.2	12.7	13.8	4.0	5.0	-2.067*
NKR	14.2	13.6	1.6	1.8	11.3	13.0	6.0	8.0	1.373^{ns}
NK	29.6	31.0	5.2	5.4	17.5	17.5	20.0	20.0	-0.974^{ns}
EL (cm)	15.5	16.7	2.7	3.0	17.7	18.1	12.0	12.0	-1.723^{ns}
EDM (cm)	3.7	3.8	0.2	0.3	6.7	9.1	1.0	1.5	-1.035^{ns}
CD (cm)	2.2	2.2	0.3	0.3	11.6	11.7	1.0	1.0	-0.046^{ns}
KL (cm)	1.0	0.9	0.1	0.1	6.7	8.5	0.2	0.3	3.330**
KW (cm)	0.7	0.8	0.1	0.1	10.1	12.4	0.3	0.4	-2.131*
KT (cm)	0.4	0.4	0.1	0.1	15.2	14.1	0.2	0.2	-0.210^{ns}

Plant height (PH), ear height (EH), tassel node height (TNH), first tassel branch height (FTBH), leaf length (LL), leaf width (LW), number of leaves above the ear (NLAE), number of ears per plant (NEP), number of primary tassel branches (NPTB), number of kernel rows (NKR), number of kernels per row (NK), ear length (EL), ear diameter (in the middle) (EDM), cob diameter (CD), kernel length (KL), kernel width (KW), kernel thickness (KT).

In K2026, the means and measures of variation for the majority of observed traits increased in the accession regenerated in 2013 (Tab. 2). Such results may be partially explained by inability of some individuals generated in 1986 to germinate, as well as, by the weaker status of plants germinated from aged seeds with poor germination energy. However, a part of variability that could not be manifested in the accessions from 1986, due to death of a certain number of individuals, was preserved and expressed in the next generation. The differences in comparison

of new and old accessions of the landrace K2026 were statistically highly significant only for KL ($p<0.01$), and significant for LW and KW ($p<0.05$), respectively. There were no statistically significant differences for the rest of the traits between 1986- and 2013-regenerated accessions. One of the reasons for insignificant changes in this landrace, in spite of a significant germination fall to 72%, is that a considerable number of abnormal seedlings developed into normal plants under favourable field conditions. Thus, the percentage of developed plants at the 5^{th}-6^{th}-leaf stage during regeneration amounted to 83% (Tab. 1). Therefore, it was concluded that identity and integrity of accessions during 2013 regeneration were not disrupted in the landrace K2026. It is almost impossible to completely preserve genetic variability of the initial accession in cross-pollinated plants, such as maize, and regeneration of genebank accessions leads to reduced genetic diversity (SOENGAS *et al.,* 2009). A part of the variability loss hypothetically caused by ageing, results in seed death (REVILLA, 2009).

*Table 3. Measures of variation and **t** test for the observed phenotypic traits of accessions of the landrace K768 from 1986 and 2013*

Trait	Average		St. deviation		CV		Interval		***t*** test
	1986	2013	1986	2013	1986	2013	1986	2013	
PH (cm)	184.5	203.6	17.0	28.5	9.2	14.0	82.0	150.0	-3.149**
EH (cm)	69.2	84.4	13.0	17.1	18.8	20.3	57.0	65.0	-3.878**
TNH (cm)	129.7	143.4	13.7	18.3	10.5	12.8	68.0	80.0	-3.283**
FTBH (cm)	152.9	163.7	14.9	20.4	9.7	12.5	72.0	85.0	-2.347*
NPTB	13.9	17.7	4.1	3.6	29.4	20.4	16.0	15.0	-3.890**
NLAE	5.4	5.5	0.8	0.8	14.2	14.2	3.0	3.0	-0.167ns
LL (cm)	68.0	75.1	5.7	7.7	8.4	10.2	20.0	35.0	-4.031**
LW (cm)	9.1	9.0	1.3	1.4	14.0	16.2	5.0	5.5	0.379ns
NKR	12.2	12.9	1.4	1.9	11.7	14.5	6.0	8.0	-1.930ns
NK	32.8	33.0	7.4	6.4	22.5	19.2	29.0	29.0	-0.150ns
EL (cm)	16.0	16.7	2.5	2.7	15.8	16.4	9.0	10.0	-0.982ns
EDM (cm)	4.4	4.1	0.3	0.5	6.6	12.4	1.4	1.6	3.491**
CD (cm)	2.5	2.3	0.3	0.2	10.9	10.3	1.4	0.8	2.475*
KL (cm)	1.1	1.0	0.1	0.1	6.7	12.7	0.3	0.4	3.021**
KW (cm)	1.0	0.9	0.1	0.2	9.9	22.6	0.5	0.7	3.250**
KT (cm)	0.4	0.4	0.1	0.0	16.1	10.3	0.2	0.2	2.560*

Plant height (PH), ear height (EH), tassel node height (TNH), first tassel branch height (FTBH), leaf length (LL), leaf width (LW), number of leaves above the ear (NLAE), number of ears per plant (NEP), number of primary tassel branches (NPTB), number of kernel rows (NKR), number of kernels per row (NK), ear length (EL), ear diameter (in the middle) (EDM), cob diameter (CD), kernel length (KL), kernel width (KW), kernel thickness (KT).

The magnitude of average values, standard deviation, coefficients of variation, as well as, the interval between the maximum and minimum values for the majority of observed traits

was increased in newly regenerated accessions of the landrace K768. The differences were not statistically significant for NLAE, LW, NKR, NK, EL, while they were statistically significant for the rest of the traits. In Table 1 has been shown a significant germination fall and a great number of dead seeds in accessions from 1986. Furthermore, in this case, abnormal seedlings did not develop into plants in the field (field emergence during regeneration was 46%). Thus, in the moment of regeneration, over 50% of individuals lost a possibility to participate in regeneration. Such results may point out to significant genetic drift that occurred during the regeneration of the landrace K768.

The results of the ***t*** test performed for the landrace K86 indicated statistically significant differences for the traits EH, TNH, FTBH, LL, NK, EL, while differences for the rest of the traits were not significant (Tab. 4). Although average values of a newly regenerated accession were higher for almost all traits, standard deviation, CV and the min-max interval were lower for almost all observed traits. Such a result may indicate the increase in inbreeding and homozygosity in a landrace, suggesting that in this case accession identity was disrupted during the regeneration. WEN *et al*, (2011) stated that the initial level of maize landrace accessions heterozygosity could have influence on the success of maintaining their genetic integrity during regeneration, as well.

*Table 4. Measures of variation and **t** test for the observed phenotypic traits of accessions of the landrace K86 from 1986 and 2013*

Trait	Average		St. deviation		CV		Interval		***t*** test
	1986	2013	1986	2013	1986	2013	1986	2013	
PH (cm)	175.3	185.8	24.0	22.0	13.7	11.8	95.0	89.0	-1.758^{ns}
EH (cm)	59.3	70.7	13.0	9.3	21.9	13.2	55.0	37.0	-3.880**
TNH (cm)	119.3	132.9	20.9	15.0	17.5	11.3	80.0	61.0	2.890**
FTBH (cm)	140.0	152.0	21.8	18.9	15.6	12.4	82.0	79.0	2.286*
NPTB	14.8	15.7	4.4	6.0	29.6	38.1	19.0	24.0	0.640^{ns}
NLAE	5.3	5.6	0.9	0.9	16.5	16.1	3.0	3.0	-1.316^{ns}
LL (cm)	70.3	73.9	5.5	5.1	7.8	6.9	20.0	20.0	2.604*
LW (cm)	8.4	8.4	1.3	1.1	15.8	13.6	5.5	5.0	0.000^{ns}
NKR	13.7	14.1	1.6	2.0	11.9	14.4	4.0	8.0	0.840^{ns}
NK	24.6	31.6	9.6	5.7	39.0	17.9	33.0	26.0	-3.442**
EL (cm)	13.0	15.7	3.0	2.1	23.1	13.2	11.0	10.0	-4.003**
EDM (cm)	4.2	4.2	0.3	0.3	7.8	6.5	1.2	0.9	0.606^{ns}
CD (cm)	2.5	2.5	0.2	0.2	8.6	7.7	0.8	0.8	1.235^{ns}
KL (cm)	1.0	1.0	0.1	0.1	9.1	5.4	0.4	0.2	-1.005^{ns}
KW (cm)	0.8	0.8	0.1	0.1	10.9	11.1	0.3	0.3	-1.005^{ns}
KT (cm)	0.4	0.4	0.0	0.1	13.1	15.6	0.1	0.2	-0.476^{ns}

Plant height (PH), ear height (EH), tassel node height (TNH), first tassel branch height (FTBH), leaf length (LL), leaf width (LW), number of leaves above the ear (NLAE), number of ears per plant (NEP), number of primary tassel branches (NPTB), number of kernel rows (NKR), number of kernels per row (NK), ear length (EL), ear diameter (in the middle) (EDM), cob diameter (CD), kernel length (KL), kernel width (KW), kernel thickness (KT)

According to obtained results, average values and measures of variation increased in newly regenerated accessions of the landraces K2026 and K768, but not of the landrace K86.

Why did this happen? The increase in average values may be explained mainly by ability of fresh seeds, being with good germination and energy, to produce more vigorous plants. Moreover, a part of the increase in measures of variation may be explained by the fact that a certain number of individuals from accessions regenerated in 1986 died, while a part of variability was preserved through recombination in crossing during regeneration. If so, why it did not happen in the landrace K86? It is obvious that the effective population size had its role in preservation of the accession integrity. The effective population size was 244, 344 and 216 for landraces K2026, K768 and K86, respectively. Although control germination was lower and the number of dead seeds was higher in the landrace K768 (59/16/25) than in the landrace K86 (63/22/15), its effective population size was far more higher (344 vs. 216) (Tab. 1). Extent of genetic drift increases with reduced effective population size (SPOONER *et al.*, 2005). The effective population size affected the degree of variability preservation within the accessions of the landrace K768 and, also, prevented inbreeding, which is probably the case in landrace K86 during regeneration.

In accessions regenerated in 2013, grain yield per plant increased in all landraces, especially in K86 (110g p^{-1}-2014; 65g p^{-1}-1986). The 1000-kernel weight of the previous and subsequent regeneration did not express significant differences. A significant difference in the number of barren plants was recorded. The percentage of barrenness (10, 23 and 35%) obtained in K2026, K768 and K86 accessions from 1986, respectively, was significantly higher than in accessions of these landraces (4, 19 and 20%, respectively), regenerated in 2013. The increased barrenness and lower yield per plant of accessions from 1986 can be explained, to a great extent, by a poor seeds status resulting in weaker plants. The differences in plant early growth and vigour were observed, more pronounced in landraces K86 and K768 (Photo 1a, 1b).

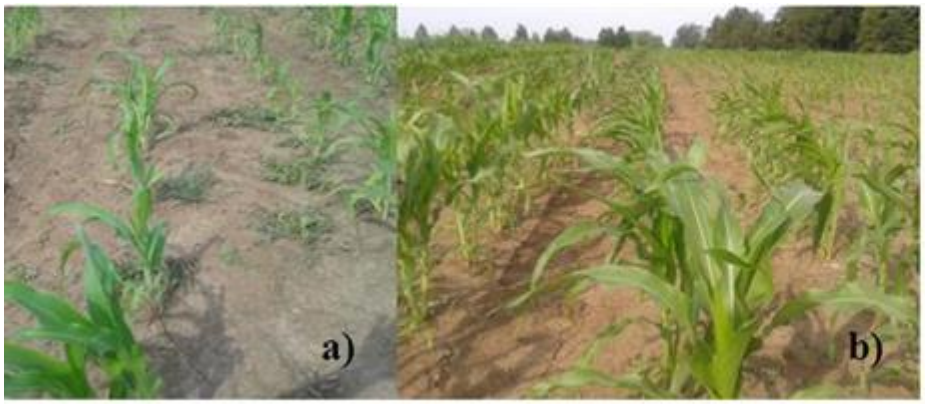

Photo 1. Plants appearance of the landrace K768: a) accession regenerated in 1986; b) accession regenerated in 2013, (Photo by Vojka Babić, June 13, 2014)

Based on the phenotypic analysis, it can be concluded that in spite of the viability loss bellow standards, the identity of the landrace K2026 was well preserved, and that it can be

conserved under the same ID accession number. However, the results point out that genetic identity of landraces K86 and K768 was seriously disrupted, indicated the necessity for new ID accession numbers assignment. In order to conserve original genetic diversity, the application of molecular markers is necessary for understanding the extent of changes in the genetic purity of the maize accessions during regeneration (WEN *et al*. 2011). Hence, more accurate assessment of the degree and nature of changes requires molecular-based characterisation of the accessions, contributing to adequate decision about ID accession numbers labelling.

ACKNOWLEDGEMENTS

Part of this work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia through the Project TR 31028. Authors would like to thank the Ministry for the support.

Received, January 22th, 2017
Accepted August 18th, 2017

REFERENCES

ANDJELKOVIĆ, V. and D. IGNJATOVIĆ MICIĆ (2012): Maize genetic resources – Science and benefits, edt by Serbian Genetic Society; Maize Research Institute "Zemun Polje", Belgrade, Serbia: 53-92.

BABIĆ, V., N. KRAVIĆ, M. BABIĆ, A. POPOVIĆ, D. IVANOVIĆ (2015): Viability testing of maize landraces accessions from MRIZP Gene bank. Romanian Agric. Res., *32*: 85-91.

BOCZKOWSKA, M.K. and J. PUCHALSKI (2012): SSR studies of genetic changes in relation to long-term storage and field regeneration of rye (Secale cereale) seeds. Seed Science and Technology, *40* (1): 63-72.

CIMMYT/IBPGR (1991): Descriptors for Maize. International Maize and Wheat Improvement Center, Mexico City/International Board for Plant genetic Resources, Rome.

CROSSA, J., S. TABA, S.A. EBERHART, P. BRETTING, R. VENCOVSKY (1994): Practical considerations for maintaining germplasm in maize. TAG *89*: 89–95.

FAO (2014): Genebank Standards for Plant Genetic Resources for Food and Agriculture. FAO, Rome, Italy.

HALLAUER, A.R., M.J. CARENA, F.J.B. MIRANDA (2010): Germplasm *in* Jaime Prohens and Ferando Nuez (eds), Quantitative genetics in Maize Breeding. Springer: 531-576

ISTA (2005): International Rules for Seed Testing. Edition 2005. International Seed Testing Association, Bassersdorf, Switzerland.

REVILLA, P., A. BUTRÓN, V.M. RODRÍGUEZ, R.A. MALVAR, A. ORDÁS (2009): Identification of genes related to germination in aged maize seed by screening natural variability. J. Exp. Bot., *60*, 14: 4151-4157.

SOENGAS, P., E. CARTEA, M. LEMA, P. VELASCO (2009): Effect of regeneration procedures on the genetic integrity of Brassica oleracea accessions. Mol. Breeding, *23*: 389-395.

SPOONER, D., R. VAN TREUREN, M.C. DE VICENTE (2005): Molecular markers for genebank management. USDA/ CGN/ IPGRI: 58-61.

TABA, S., S. TWUMASI-AFRIYIE (2008): Regeneration guidelines: maize. In: Dullo ME, Thormann I, Jorge MA, Hanson J (eds). Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme, Rome: 10.

TABA, S., S.A. EBERHART, L.M. POLLAK (2004): Germplasm resources. *In*: Smith C.W., J. Betrán, E.C.A. Runge (eds) Corn: origin, history, technology and production. Wiley, Hoboken.

TREUREN, R., E. GROOT, T.H. HINTUM (2013): Preservation of seed viability during 25 years of storage under standard genebank conditions. Gen. Res. Crop Evol., *60* (4): 1407-1421.

UPOV TG/2/7 (2009): Guidelines for the conduct of tests for distinctness, uniformity and stability. International Union for the Protection of New Varieties of Plants, Geneva.

VANČETOVIĆ, J., S. MLADENOVIĆ DRINIĆ, M. BABIĆ, D. IGNJATOVIĆ MICIĆ, V. ANDJELKOVIĆ (2010): Maize genebank collections as potentially valuable breeding material. Genetka, *42* (1): 9-12.

WANG, J, J. CROSSA, M. GINKEL, S. TABA (2004): Statistical genetics and simulation models in genetic resource conservation and regeneration. Crop Sci., *44*: 2246–2253

WEN, W., S. TABA, T. SHAH, V.H. CHAVES TOVAR, J. YAN (2011): Detection of genetic integryty of conserved maize (Zea mays L.) germplasm in genebanks using SNP markers. Genet. Resour. Crop Evol., *58*: 189-207.

PROCENA NARUŠAVANJA GENETIČKOG INTEGRITETA UZORAKA KUKURUZA IZ BANKE GENA TOKOM PROCESA ČUVANJA

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Izvod

Jedan od prioriteta aktivnosti banaka gena je očuvanje identiteta i integriteta originalnog uzorka. Brojni su uzroci usled kojih može doći do njihovog narušavanja. Najčešća kritična tačka u ovom procesu je regeneracija, usled mogućeg uskog grla, ukrštanja u srodstvu, genetičkog drifta i slučajnog mešanja i kontaminacije. Sa druge strane, u toku čuvanja dolazi do gubljenja životne sposobnosti semena, pa do genetičkog drifta može doći i usled izumiranja jednog broja jedinki unutar uzorka. Stoga je veoma važno naći balans između učestalosti regeneracije i dužine čuvanja uzoraka. Cilj datih istraživanja je bio da se na osnovu fenotipskih markera proceni da li je došlo do narušavanja identiteta uzorka kod tri populacije kukuruza (K2026, K768 and K86) iz bake gena Instituta za kukuruz „Zemun Polje“ (MRIZP) različitog stepena vijabilnosti, koje su regenerisane nakon 27 godina čuvanja u srednjeročnim uslovima. Procenjeno je da kod populacije K2026 nije došlo do značajnijih promena nakon regeneracije. Za populaciju K768 postoje pokazatelji da je došlo do genetičkog drifta, a kod populacije K86 i do odredjenog stepena inbridinga. Dodatna karakterizacija populacija K768 i K86 molekularnim markerima, doprineće preciznijoj proceni stepena narušavanja genetičkog identiteta i integriteta uzoraka.

Primljeno 22.I.2017.

Odobreno 18. VIII. 2017.