NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 32, 2015
Print ISSN 1222-4227; Online ISSN 2067-5720

VIABILITY TESTING OF MAIZE LANDRACES ACCESSIONS FROM MRIZP GENE BANK

Vojka Babić[1*], Natalija Kravić[1], Milosav Babić[1], Aleksandar Popović[1], Dragica Ivanović[1]

[1]Maize Research Institute, Zemun Polje, Slobodana Bajica 1, 11185 Belgrade, Serbia
[*]Corresponding author. E-mail: vbabic@mrizp.rs

ABSTRACT

Large number of accessions, usually stored in gene banks, makes the regeneration very expensive, involving at the same time risks to the genetic integrity of accessions. Therefore, monitoring viability of stored seeds is a very important operation in gene banks. In 2013, monitoring for seed viability was conducted on 703 local maize landraces from Maize Research Institute Zemun Polje (MRIZP) gene bank. According to the results of germination test under laboratory conditions on filter paper (BP, 20⇔30°C, ISTA Rules), 49 local landraces were chosen for additional germination testing in field and under laboratory conditions using sand as a growing media (S, 20⇔30°C, ISTA Rules). For testing in sand, extended period of germination monitoring (ISTA Rules, 5.6.4) was applied, while for evaluation of abnormal seedlings less strict criteria than those in ISTA Rules were used. Statistical analysis, showed that the determination of seed viability in the field (24th day) was in the best accordance with the results of germination testing in sand (counting on the 7th day). It was noticed that the extended evaluation in sand did not contribute to more precise results. Also, correlation analysis revealed the existence of a trend, indicating that higher germination rate was associated to higher level of kernel hardness.

Key words: germination, landraces, seed longevity, kernel hardness, *Zea mays* L.

INTRODUCTION

Over six million accessions are stored in the *ex situ* gene bank collections worldwide. As a result of these large collections, most gene banks are facing difficulties in managing their collections to the highest standards (FAO, 1998). Regeneration of gene bank accessions should be done as rarely as possible, because, each time an accession is regenerated, there is a risk that the genetic integrity of accession is compromised by genetic drift, selection, or gene flow (Spooner et al., 2005). A part of the variability loss hypothetically caused by ageing, results in seed death (Revilla, 2009). In general, regeneration of gene bank accessions leads to reduced genetic diversity (Soengas et al., 2009), particularly in out-crossing crops like maize, where it is very difficult to completely maintain the genetic identity. Also, the initial level of maize landrace accessions heterozygosity could have influence on the success of maintaining their genetic integrity during regeneration (Wen, 2011).

Monitoring the viability of stored seed is of the exceptional importance (Murariu, 1996). For that purpose, special procedures of viability testing are recommended (Rao et al., 2006). Seed viability is the measure of how many seeds in a lot are alive and could develop into plants that will reproduce under appropriate field conditions. As viability of seeds stored in the gene bank decreases slowly during storage, the decision on identifying the accessions and their necessary regeneration is based on continuous monitoring of both seed viability and quantity in order to avoid excessive deterioration or reduction in seed quantity.

The accurate assessment of seed viability is important, as it provides a correct decision about regeneration of an accession, which is not only expensive but also risky from the point of loss in genetic diversity. The most accurate and reliable method to test seed viability is the germination test. Viability is monitored by determining the accessions germination ability at predefined time intervals. The interval between two viability checks depends on the expected seed

Received 05 February 2014; accepted 20 February 2015. First Online: March, 2015. DII 2067-5720 RAR 2015-26

longevity of the crop (Hintum and Visser, 2003). Also, the interval between subsequent checks should be based on experience and may be adjusted depending on the extent of viability loss observed during the first monitouring test (Rao et al., 2006). In previous studies of seed viability, results indicated that seed viability is well maintained for the majority of seed lots during the first 25 years after regeneration, as only 3.3% of the monitoring tests revealed below-threshold germination values. Optimizing the frequency of these germination tests, in order to avoid waste of resources, is hampered by the scarce availability of seed longevity data, particularly for material maintained under gene bank conditions (Treuren, 2013). As recommended in guidelines for standard gene bank germplasm operations (FAO/IPGRI, 1994), the first monitoring of seed viability in the active collection should be conducted after 10 years of storage, and then repeated after every five years. The practice in Maize Research Institute Zemun Polje (MRIZP) gene bank is to regenerate (after 10 years of storage) maize landrace accessions with germination drop below 85% of initial germination, while the landraces stored for 20 years are regenerated automatically, i.e. without germination checking.

In the present study we tried to determine the most suitable germination test of seed viability assessment for long-stored maize landraces with higher decrease in germination since the last test (BP, 20⇔30°C, ISTA Rules). Beside the germination test on filter paper (BP, 20⇔30°C, ISTA Rules), additional viability tests under field conditions and in sand (ISTA Rules, 5.6.4) were conducted.

MATERIAL AND METHODS

For the purposes of planning the regeneration in 2013, viability of the 703 accessions maintained under medium-term storage conditions (t=4-5°C; RH=45-50%), was tested under laboratory conditions (BP, 20⇔30°C, ISTA Rules). Out of this number, 49 accessions, previously regenerated 20-25 years ago, were selected for further viability testing under field conditions, as well as under laboratory conditions in sand (S, 20⇔30°C, ISTA Rules) by application of extended period of evaluation (ISTA Rules, 5.6.4). Criterion for selection of these accessions was germination bellow 30% and/or more than 30% in germination decrease concerning the results of previous germination test (performed in 2001). First count in laboratory was done on seven-day old seedlings (sand 7^{th}) and the final on ten-day old seedlings (sand 10^{th}). At the same time, each seedling with all essential structures, but with slight coleoptile and primary leaf damages, or with less developed root system, was considered as normal.

In the field, selected accessions were sown in two replicates, 3 rows per replicate and 15 hills per row (at the distance of 40 cm) with inter-row distance of 75 cm. As dealing with long-stored seeds with low germination rate according to laboratory testing, only 60% of seed emergence was assumed. Hence, four to seven seeds were sown per hill, expecting two plants per hill, which corresponds to the sowing density in the common process of regeneration for maize accessions. The first count was done at two-leaf stage of maize plant (14 days after sowing). The second one was done at four to five-leaf stage, assuming that maize seedlings reaching this phase will be developed into the normal plants (24 days after sowing), when thinning to a required plant density for regular regeneration is conduced.

Results obtained under laboratory and field conditions were analysed by the analysis of variance (ANOVA) and the regression analysis.

RESULTS AND DISCUSSION

In V6 stage of plant development, nodal root system is the major functioning root system, therefore it is assumed that maize seedlings reaching the four to five-leaf stage will surely develop into normal plants (Ritchie et al., 1997). The second plant counting in the field (24^{th} day), when plants were at that stage, was considered as the most reliable parameter of maize population viability. Correlation coefficients pointed out that the highest similarity was obtained between results in the

field (24th day) and in sand (0.95 and 0.94). Significantly weaker correlations were observed between results obtained in the field (24th day) and on filter paper (0.72).

The lowest correlations were recorded between germination values obtained on filter paper and in sand (0.60 and 0.65) (Figure 1a, b, and c).

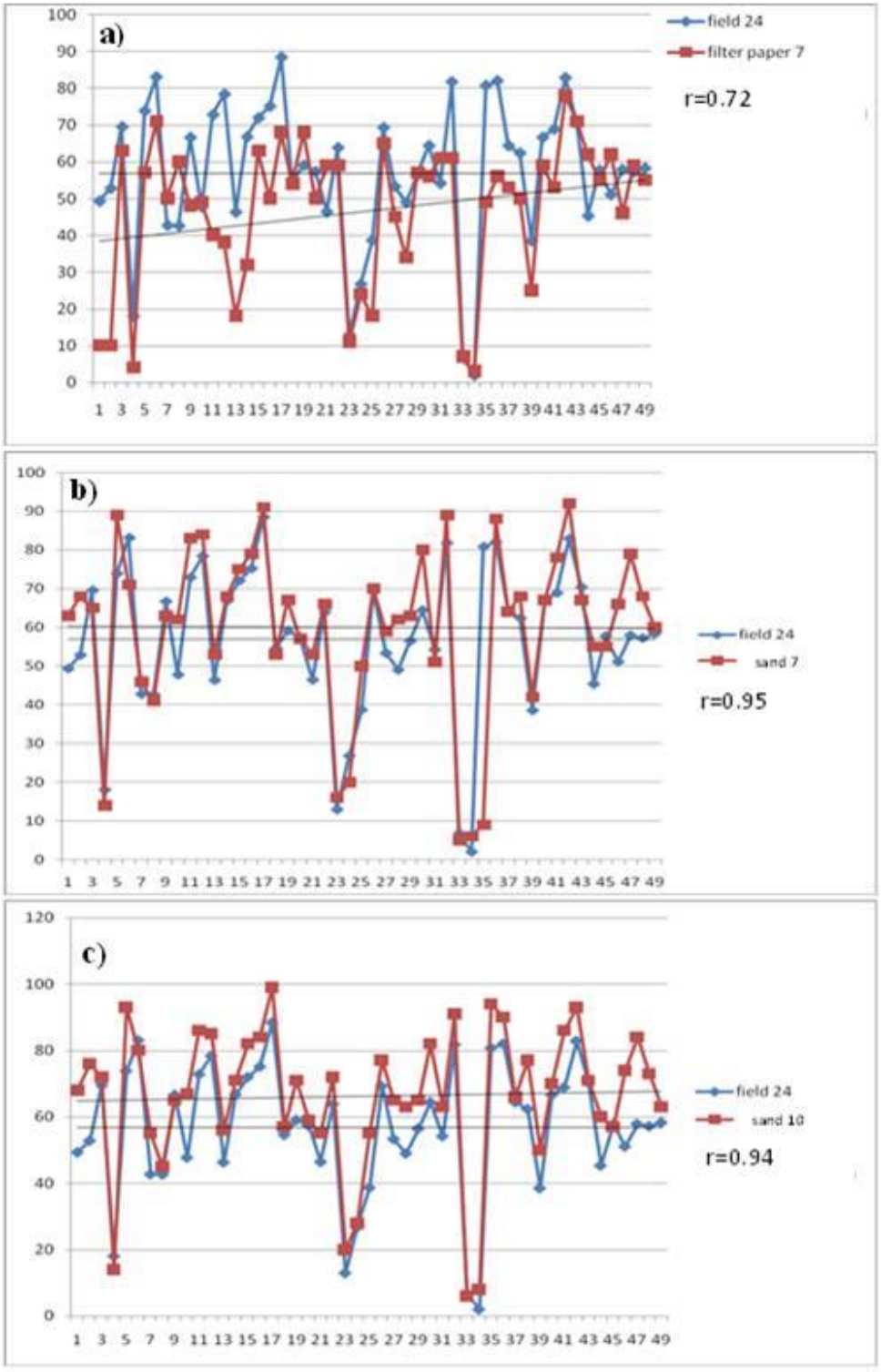

Figure 1. The comparison of values obtained on emergence in the field (24th day) and laboratory germination: a) field 24th: filter paper 7th; b) field 24th: sand 7th; c) field 24th: sand 10th

Relationships between results obtained under field (24th day) and laboratory conditions on filter paper (7th day) and in sand (7th and 10th day) were determined by the linear regression. The smallest standard error of regression was determined for sand (7th

day), with the highest coefficient of determination (R^2), meaning that this method is in the highest accordance to field testing (Figure 2a, b, c). Comparing the germination on filter paper (7th day) with that in the field (24th day), it was noticed that results mainly differ for populations with a large number of estimated abnormal seedlings on filter paper. With respect to expected germination ability, it was concluded that a large number of abnormal seedlings developed into normal plants under field conditions. Although correct in this case, one has to be cautious in generalising the conclusion that a large number of abnormal seedlings will develop into normal plants. First of all, it has to be emphasised that agro-ecological conditions during the period of emergence in 2013 were optimal for maize. It could be supposed that the obtained results would be much different even if only temperature conditions were not optimal (with the assumption that the soil moisture content could be controlled by irrigation). During germination testing of maize landrace accessions stored in gene banks for a longer period, certain specificities should be taken into account, e.g. ISTA Rules have been made for commercial hybrid maize seed, genetically uniform, with seedlings having hybrid or increased vigour. Maize landraces, as open-pollinated varieties, are composed of genetically different individuals. Hence, seedlings of certain individuals within the accession can differ in size of the entire seedling, root system, as well as in size and form of the primary leaf and vigour itself. Also, negative effects of sib pollination can be expressed in some individuals.

Seed respiration, as the main producer of reactive oxygen species (ROS), could increase during storage, due to exogenous and endogenous factors such as temperature, air humidity, etc. (Dragičević et al., 2010). Moreover, a long period of storage (20-25 years) definitely affects germination viability, especially in accessions with lower moisture content. As the moisture content of investigated accessions ranged from 10.8% to 14.7%, seeds were not pre-treated as recommended in cases of seeds with moisture content below 8% (Rao et al., 2006). Period of germination testing in sand was extended, due to a long storage of accessions. Instead of standard counting on the 4th day, the first counting was performed on the 7th day, while the second one was performed on the 10th day. The results obtained in the field (24th day) were in higher accordance to the first counting in sand (7th day) (R^2=0.896; $\sigma_{Y24Xsend7}$=5.67).

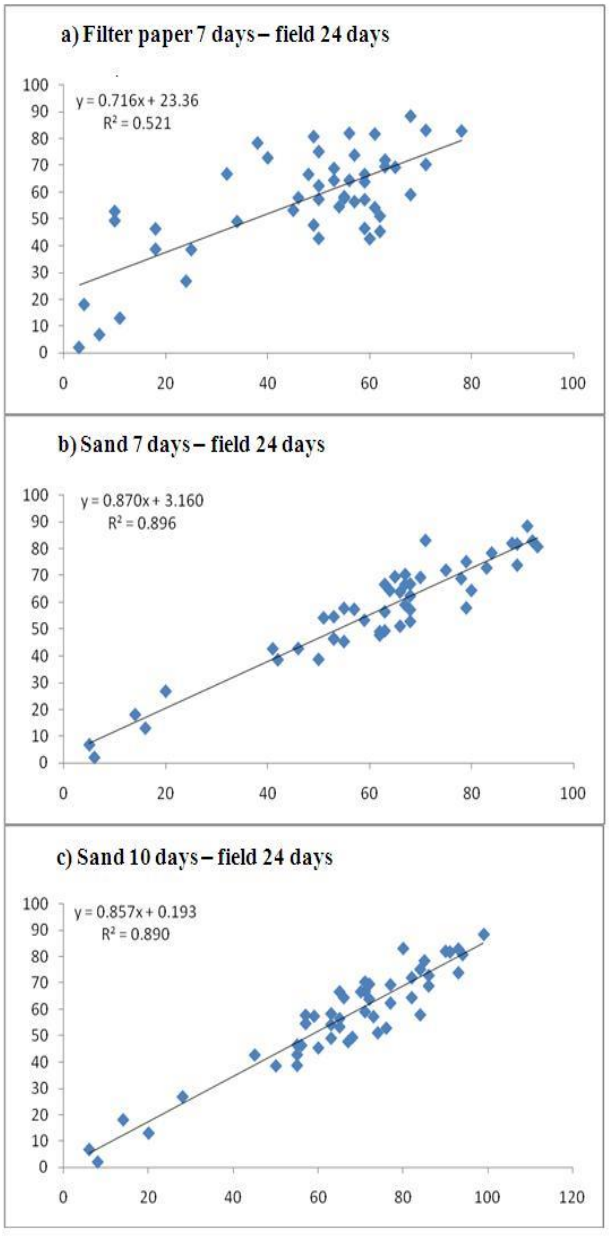

Figure 2. Linear regression: a) filter paper 7th- field 24th; b) sand 7th- field 24th; c) sand 10th- field 24th

The analysis of variance (ANOVA) showed that the differences between treatments (five different evaluations of germination: filter paper – 7th day; sand – 7th day; sand – 10th day; field conditions – 14th day; field conditions – 24th day) were

statistically highly significant (P>0.01) (Table 1). According to least significant differences (LSD) for treatments, the evaluation of germination under field conditions (24th day) was significantly different (P>0.01) from evaluations on filter paper (7th day), in sand (10th day) and in the field (14th day). The differences determined between germination (%) estimated in the field on the 24th day and in sand on the 7th day, were not statistically significant (P>0.01) (Table 2). If counting in the field after 24 days is considered as the most reliable estimation of maize landraces viability, the results of the ANOVA confirmed that testing of seed germination in sand on the 7th day, by application of modified ISTA Rules for abnormal seedlings evaluation, gives the most useful information for maize landraces viability estimation.

Table 1. Analysis of variance of different germination testing methods for maize landraces

Source	Degrees of freedom	Sum of squares	Mean square	F value
Testing methods	4	6836.148	1709.037	61.842*
Populations	48	37513.781	781.537	28.280**
Error	192	5306.048	27.636	
Total	244	49665.976		

Coefficient of variation: 10.94%.

Table 2. Mean differences and least significant differences among germination testing methods

Methods	Filter paper 7 days	Sand 7 days	Sand 10 days	Field 14 days	Field 24 days
Sand 7 days	9.545**				
Sand 10 days	12.620**	3.075^{ns}			
Field 14 days	0.940^{ns}	10.485**	13.560**		
Field 24 days	6.356**	3.189^{ns}	6.264**	7.296**	
Mean	42.528	52.073	55.148	41.588	48.884

$LSD_{0.01}$=4.823; $LSD_{0.05}$=4.266; ** P>0.01; ^{ns} non significant.

Substantial differences may sometimes exist in longevity among accessions in the same species and even among genotypes within the same accession (Engels and Visser, 2003). An assumption of the Ellis-Roberts viability equation, that all seed lots of a species deteriorate at the same rate in the same storage environment, was not valid for hybrid corn (*Zea mays* L.) seed (Tang et al., 2000). In study on the regularity of the behaviour in different samples of maize seeds kept in the mid-term storing conditions, Muminovic (1998) concluded that, besides the initial germination and moisture content in the grain, several other factors affected the loss of seed viability. Therefore, mathematical models proposed by Ellis and Roberts can be used only for a rough estimate of the predictions of seed viability in maize maintained in a gene bank. According to kernel type, certain regularities were observed from results obtained on germination of all maize landraces (703) tested on filter paper. Sixty-nine landraces (≈10%) maintained high vigour (germination >95%). Out of this number, 63% were flints, 28% dents and 9% were intermediate types, respectively. On the other hand, 25% of tested landraces (143 accessions) expressed germination below 72%. Within this set of landraces, 54% were dents, 12% intermediate types and 34% were flints. A considerable decline in germination was recorded for 49 accessions, which is 7% of the total number of tested landraces, with dents (65%) prevailing. Landraces, with high germination rate and with germination decline below the standards, were arranged according to grain

hardness. It was observed that the increase in hardness was associated with the increase of germination, with positive (r=0.37) and statistically significant (P>0.01) correlation between kernel hardness and germination rate, i.e. the higher dentiness the lower germination rate relation (r=-0.37) (Figure 3). Such results pointed out the fact that flint landraces maintain vigour longer than dent landraces.

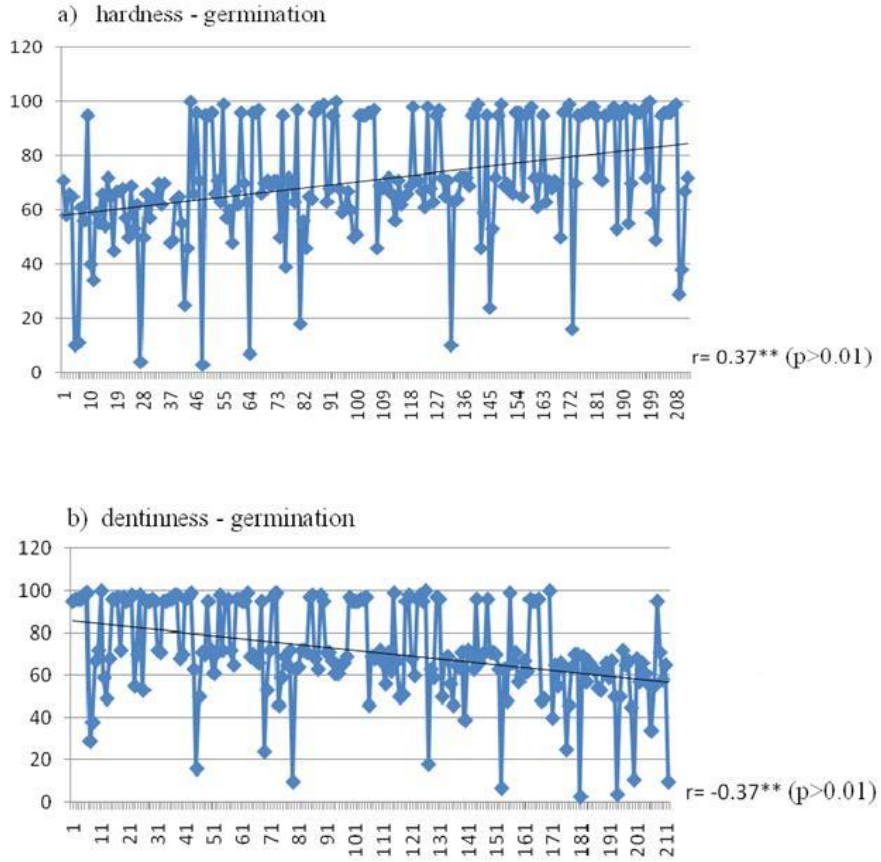

Figure 3: Relationship between kernel type and germination rate:
a) hardness - germination; b) dentinness – germination

Maintaining the integrity and variability of conserved material is a gene bank priority. In relation to open-pollinated maize varieties, mainly very heterogeneous in genetic constitution, the question of the loss in variability within the landrace, as a consequence of long period of storage, arises. To be exact, what are the differences among individuals within population concerning longevity? In order to minimize the loss of accession integrity and variability, the balance between the frequency in regeneration and duration of seed storage is of great importance.

CONCLUSIONS

Application of regression analysis and the ANOVA, showed that the results of seed viability determination in the field (24th day) was in the best accordance with the results of germination testing in sand (7th day). The extended evaluation in sand (10th day) did not contribute to more precise results.

The evaluation of abnormal seedlings in maize landrace accessions, stored in gene banks for a long time, might be done by application of less strict criteria than those in ISTA Rules. However, one has to be careful. Under unfavourable growth conditions (low temperatures, inadequate soil moisture content), there is less possibility for abnormal seedlings to develop into normal plants.

Within the same species, there are differences in longevity. Hence, viability of certain kernel types (in this case of dents) should be tested more frequently. Finally, the higher variability within the same landrace, the higher is the probability of some individuals to be different in longevity, as

well as higher probability of genetic drift as a result of prolonged storage.

For maize landraces accessions regenerated more than 10 years ago, viability testing should be conducted within shorter intervals (not longer than five years), in order to avoid not only the deterioration of accessions, but also a change in the genetic structure from the original, collected accession.

Acknowledgement

This work was supported by Ministry of Education, Science and Technological Development, Republic of Serbia, through the project TR31028 "Exploitation of maize diversity to improve grain quality and drought tolerance".

REFERENCES

Dragičević, V., Sredojević, S., Spasić, M., 2010. *Introduction of the interdependence between the glutathione half-cell reduction potential and thermodynamic parameters during accelerated aging of maize seeds*. J. Serb. Chem. Soc., 75, 3: 323-331.

Engels, J.M.M. and Visser, L. (eds), 2003. *A guide to effective management of germplasm collections*. IPGRI Handbooks for Genebank, No. 6, IPGRI, Rome, Italy.

FAO, 1998. *The state of the World's Plant Genetic Resources for Food and Agriculture*. FAO, Rome, Italy. (ISBN: 92-5-104200-4)

FAO/IPGRI, 1994. *Genebank Standards.* FAO and IPGRI, Rome, Italy. (ISBN: 92-9043-236-5)

Hintum, T. and Visser, B., 2003. *Case study on the collection management strategies of the Centre for Genetic Resources, the Netherland*. In: A guide to effective management of germplasm collections, (editors, Engels, J.M.M. and Visser, L.), IPGRI, Rome, Italy; CGN, Wageningen, The Netherlands.

ISTA, 2005. *International Rules for Seed Testing. Edition 2005.* International Seed Testing Association, Bassersdorf, Switzerland.

Muminovic, J., 1998. *Prediction of the relative storability of maize (Zea mays L.) seed in a genebank.* Master Thesis, University of Novi Sad, Faculty of agriculture, Serbia.

Murariu, D., 1996. *Prediction of life duration of maize (Zea mays L.) seed preserved in gene banks by basic viability equations.* Romanian Agricultural Research, 5-6: 69-76.

Rao, K., Hanson, J., Dulloo, M.E., Ghosh, K., Nowell, D., Larinde, M., 2006. *Manual of seed handling in genebanks*. Handbooks for Genebanks, No. 8, Bioversity International, Rome, Italy.

Revilla, P., Butrón, A., Rodríguez, V.M., Malvar, R.A., Ordás, A., 2009. *Identification of genes related to germination in aged maize seed by screening natural variability.* Journal of Experimental Botany, 60, 14: 4151-4157.

Ritchie, S.W., Hanway, J.J., Benson, G.O., 1997. *How a Corn Plant Develops*. Iowa State University of Science and Technology, Ames, Iowa, USA.

Soengas, P., Cartea, E., Lema, M., Velasco, P., 2009. *Effect of regeneration procedures on the genetic integrity of Brassica oleracea accessions*. Mol. Breeding, 23: 389-395.

Spooner, D., van Treuren, R., de Vicente, M.C., 2005. *Molecular markers for genebank management*. IPGRI Technical Bulletin, No. 10, International Plant Genetic Resources Institute, Rome, Italy.

Tang, S., Tekrony, D.M., Egli, D.B., Cornelius, P.L., 2000. *An alternative model to predict corn seed deterioration during storage*. Crop Science, 40: 463-470.

Treuren, R., Groot, E., Hintum, Th., 2013. *Preservation of seed viability during 25 years of storage under standard genebank conditions*. Genetic Resources and Crop Evolution, 60, 4: 1407-1421.

Wen, W., Taba, S., Shah, T., Chaves Tovar, V.H., Yan, J., 2011. *Detection of genetic integryty of conserved maize (Zea mays L.) germplasm in genebanks using SNP markers.* Genet. Resour. Crop Evol., 58: 189-207.