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TESTING OF MAIZE INBREED LINES SEED GERMINATION IN THE SOIL

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Abstract

The aim of this paper is to present soil as a growing medium used in germination tests of seeds of some insecticide-treated maize inbred lines that have been carried out at the seed testing laboratory of the Maize Research Institute, Zemun Polje in Belgrade. Although soil is not recommended as a primary growing medium for seed germination because it is not included into standard methods, it is the most natural and the best growing medium for seed germination testing under laboratory conditions. Soil is recommended when seedlings show phytotoxic symptoms or when results of the standard germination test are uncertain. The standard method B(etween) P(aper) with 4x100 seeds and filter paper + soil method with 4x50 seeds were applied in the study in 2015 and 2016. Seeds were treated with the fungicide Maxim Xl and the insecticide Sonido. Soil used in the study was degraded chernozem taken from a maize field and prepared for the medium according to the standard procedure. Seeds were germinated in the germination room at the temperature of 20<=>30^{o}C and 16:8 of light : dark photoperiod. The first count and total germination were recorded on the 4th and the 7th day, respectively, in both methods. Obtained results indicate that total germination for all maize inbred lines was greater in both years of investigation when filter paper + soil method was applied.

Key words: *Seed germination, Maize inbreds, Soil*

Introduction

Seed germination is the most important parameter of seed quality and its laboratory testing provides information on the maximum potential of germination under optimum conditions. The maximum seed germination potential is often directly opposed to seed germination under environmental conditions, due to which there are differences between laboratory and field germination.

Beside standard methods in seed germination testing, non-standard methods that provide natural conditions for seed germination, are often applied, and they usually give better results of testing. Non-standard methods have to be verified to be applicable. A cold test is a non-standard method applied in seed vigour testing, where soil, sand or their mixture are used as a substrate. Nijënstein and Kruse (2000) established that the least variation of the results was obtained when sand was used as a substrate in the cold test.

The seed germination testing in the soil as a substrate is non-standard method and it is not recommended as a primary method for germination tests, but as an alternative to organic substrates, when results of standard germination tests are doubtful and when a germination expresses phytotoxic symptoms. When the soil is used as a substrate in the seed germination tests, it is necessary that soil meets criteria given in the paragraph 5.4.2 of the ISTA Rules, ISTA Rules (2017), according to which there should be a sufficient amount of air and moisture to provide the growth of the root system; pH has to be within the range of 6.0-7.5

and the substrate has to be microbiologically pure and non-toxic. Each new soil supply for seed germination tests is subject to quality control of these parameters, with the recommendation that the germination substrate is used only once. The two-year research of seed germination in soil in the Missouri laboratory showed that the soil was the best substrate for the growth of all kinds of seeds, Fuhr (1933). Comparing germination substrates and their effects on germination, the seedling development was the most advanced only in the soil, ISTA Handbook of Seedling Evaluation (2013). Although the soil is the most natural substrate for seed germination, it is difficult to standardise this method to be applicable in the laboratory, because each laboratory uses soil from its surroundings, and due to it test results are inconsistent. More consistent results may be gained by using the same soil in the inter-laboratory test comparisons. Radić et al. (2004) established that the seed testing methods should be developed and that their standardisation should be performed in the future, so that they would meet current quality standards and market requirements. A greater application of soil as a seed germination substrate has begun by insecticide treatments of seeds due to phytotoxicity and uncertainty of the results obtained by the standard method. It has been observed in laboratories that seed germination in the soil had yielded the best results of total germination, especially in seeds of inbred lines treated with insecticides, which otherwise had slower and lower germination than hybrids.

Material and methods

During 2015 and 2016, 10 seed lots of inbred lines, produced in various locations and different years, were tested at the Seed Testing Laboratory of the Maize Research Institute, Zemun Polje. The seed was processed and treated with fungicide Maxim XL 035FS and insecticide Sonido. According to Ferguson (1993), seed lots with similar values of seed germination, may differ in physiological maturity, and therefore in seed vigour. The following two test methods were applied: BP (between filter paper method) with 4x100 seeds (standard method) and the filter paper + soil method with 4x100 seeds (non-standard method). The soil was degraded chernozem taken from the maize field, sieved to obtain a smaller and more uniform fraction. In 2015, 20 bags with soil were taken from the field (pH=7.5, moisture=17.9%, and moisture capacity=48%). Seeds of the species *Festuca rubra*, susceptible to toxicity, were germinated to establish non-toxicity of the collected soil. The soil was sieved through the 5-mm sieve and 67.6% soil passed through the sieve. In 2016, 60 bags with soil were taken from the field. Seeds of the species *Festuca rubra* were germinated to establish non-toxicity of the collected soil, and at that time it was established that the soil did not contain toxic matters and that it was microbiologically pure (pH=7.5, moisture=14.4%, and moisture capacity=46%). Furthermore, the soil was sieved through the 5-mm sieve and 93.24% soil passed through the sieve with this mesh size, ISTA Handbook of Seedling Evaluation (2013). When the standard method was applied, the seed was placed on water saturated filter paper, which was covered by another filter paper, then it was rolled and placed into the germination cabinet at the alternating temperatures 20<=>30^{o}C and the light regime 16 h : 8 h (light : dark). When the non-standard method was applied, the seed was placed on previously water saturated filter paper, to which soil was added, then it was rolled and placed into the germination cabinet under the same test conditions (alternating temperatures 20<=>30^{o}C and the light regime 16 h : 8 h (light : dark)). The first count, in both methods, was made on fourth day, while the total germination was determined on the seventh day, ISTA Rules (2017). The obtained values were expressed as the percentage of normal seedlings.

Gained results were statistically processed by the computer program MSTAT. The two factorial analysis of variance and the least significant difference (LSD) test were applied, Hadživuković (1991).

Results and Discussion

The analysis of variance shows statistically significant differences in total germination over tested seed lots, as well as over the applied germination methods. The seed germination method, year of seed germination testing and the analysed lots of inbred lines affected seed germination. Interactions among observed factors were also very significant (Table 1).
The year of investigation did not affect tested seed germination. There were no statistically significant differences between replications and between the interactions of the factor B.

Table 1. Statistical significance of effects of observed factors on total germination of seeds

Factor	Degree of freedom (DF)	Mean squares (MS)	F value
Replication	1	2.450	0.261^{ns}
Method (A)	1	4681.800	500.8646^{**}
Year (B)	1	31.250	3.3432^{ns}
AB	9	352.800	37.7430^{**}
Lot (C)	9	356.856	38.1768^{**}
AC	9	145.439	15.5592^{**}
BC	9	182.944	19.5716^{**}
ABC	9	76.439	8.1775^{**}
Error	39	9.347	

ns – not significant; ** - significant at the level of 0.01%; df – degree of freedom

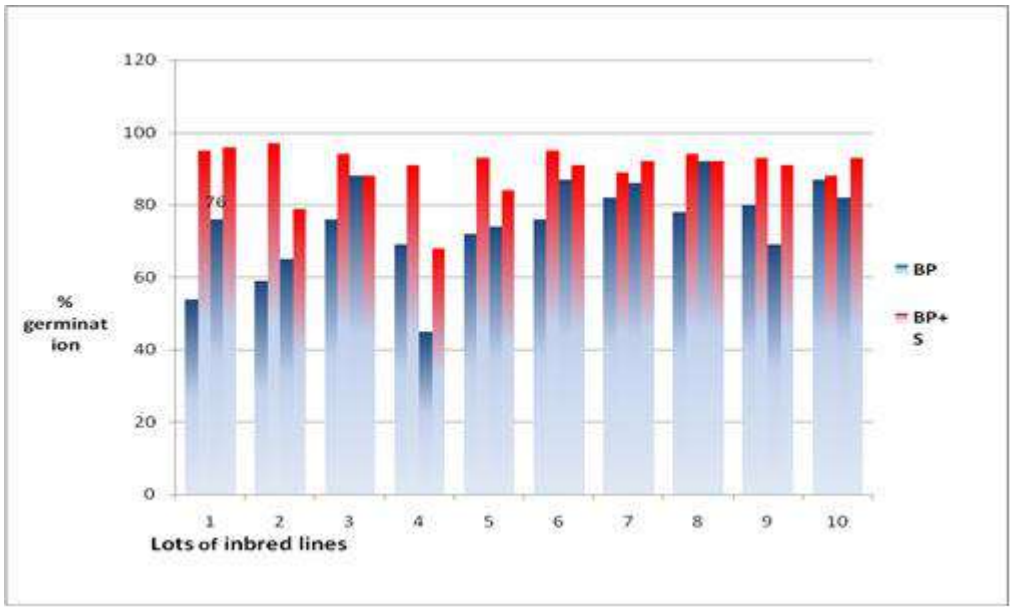
The highest seed germination percentage in both years was recorded when the filter paper + soil method was applied. Lower values were obtained by the standard seed germination method.
In 2015, average total germination over methods was higher by 19% when the filter paper + soil method was applied. Moreover, in 2016, total germination was greater by 11% in the variant when the filter paper + soil method was applied The lowest total seed germination was recorded in the lots 1, 2 and 4 (80.00%, 74.88 and 67.75%, respectively), while the highest value was established in the lots 6, 8 and 10 (87.00%, 88.75% and 87.38%, respectively).
The analysis of results by the LSD test for the interaction between two methods and lots shows that the lowest value of total germination was in the lot 4 (F), which is a statistically significant difference, while differences obtained in lots 8, 10, 6 and 7 (A) were not statistically significant.
The gained results on seed germination (Table 2) indicate that the filter paper + soil method is suitable for testing seeds treated with insecticides. The comparison of results obtained by the standard and non-standard method shows that seed germination was significantly lower when the standard method was applied including all 10 lots and both years of investigation.

Table 2. Average seed germination (%) in 2015 and 2016 over methods and lots

2015	Method	Tested lots										Average over methods
		1	2	3	4	5	6	7	8	9	10	
	BP	54	59	76	69	72	76	82	78	80	87	73,1 D
	FP+S	95	97	94	91	93	95	89	94	93	88	92,60 C
2016	BP	76	65	88	45	74	87	86	92	69	82	76,05 A
	FP+S	96	79	88	68	84	91	92	92	91	93	87,15 B
Average over lots		80.00 D	74.88 E	86.13 AB	67.75 F	80.38 CD	87.00 A	86.88 A	88.75 A	83.13 BC	87.38 A	

FP+S-filter paper + soil, BP – between filter papers

Figure 1. Total seed germination (%) over methods



BP – between filter papers, FP+S-filter paper + soil

Conclusion

Based on achieved results it can be concluded that soil as a substrate for seed germination tests is suitable for seeds treated with insecticides. It was also confirmed that soil as a substrate in seed germination tests is more natural environment for seed, hence the results of germination tests were higher than the results gained by the standard method. Despite the defined properties that the soil has to have in order to be used as a substrate for seed germination tests, it is difficult to standardised soil as a method due to its inconsistency in chemical, microbiological and physical traits. The soil method should always be an alternative to the standard seed germination method when there is a doubt in test results. Results of germination tests obtained by the filter paper + soil method were higher in both years than those obtained by the standard BP method. The Seed Testing Laboratory has been using soil as the substrate for seed germination tests. Although this method is very demanding, as it is

necessary to collect soil, sieve and check it, certainty of results is the reason why we have decided to use this method.

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