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COMPARISON OF TWO COLD TEST PROCEDURES FOR SEED VIGOUR EVALUATION OF MAIZE INBRED LINES

POREĐENJE DVE PROCEDURE HLADNOG TESTA ZA ISPITIVANJE VIGORA SEMENA SAMOOPLODNIH LINIJA KUKURUZA

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ABSTRACT

Cold test (CT) is a valuable method of assessing maize seed vigour. Although widely used CT is not standardized because of variations in CT procedures. The objective of this study was to evaluate seed vigour of 15 maize inbred lines developed at the Maize Research Institute Zemun Polje, using two different substrates (soil and sand) in a severe CT (7.5 °C for 10 days followed by 7 days at 20/30 °C). Statistical analysis showed that inbred lines differ significantly in seed vigour, and no difference between two applied substrates in CT. Coefficients of variation were lower in CT with sand (6.05 %), compared to the CT with soil (6.74%) due to variation in soil quality and presence of soil-borne pathogens. Both CT procedures were highly correlated with field emergence. Results of this research indicate that CT with sand is appropriate for testing maize seed vigour, with the potential for standardization.

Key words*: vigour, cold test, substrate, maize.*

REZIME

Vigor semena je veoma važan parametar kvaliteta, jer ukazuje na sposobnost semena da klija u suboptimalnim uslovima (niska temperatura, visoka vlažnost zemljišta itd.). Hladni test je važan metod za utvrđivanje vigora semena kukuruza. Iako je dobar pokazatelj nicanja u polju, i koristi se širom sveta, hladni test nije standardizovan zbog razlika u proceduri (temperatura, vrsta i vlažnost supstrata). Cilj ovog rada bio je ispitivanje vigora semena samooplodnih linija kukuruza primenom dva različita supstrata (zemlja i pesak) u hladnom testu. Za ispitivanje je odabrano 15 samooplodnih linija kukuruza stvorenih u Institutu za kukuruz „Zemun Polje“. Primenjeni su izrazito stresni uslovi ispitivanja u hladnom testu (10 dana na 7,5°C, a zatim 7 dana na 20/30°C). Statističkom obradom podataka utvrđeno je da su se samooplodne linije značajno razlikovale po vigoru semena tj. tolerantnosti na niske temperature u periodu klijanja i početnog porasta. Sa druge strane nije utvrđena značajna razlika između dva primenjena supstrata u hladnom testu. Prosečna klijavost 15 samooplodnih linija u hladnom testu sa peskom iznosila je 82,2%, a u hladnom testu sa zemljom 80,1%. Koeficijent varijacije je bio niži u hladnom testu sa peskom (6,05%), nego u hladnom testu sa zemljom (6,74%), što se objašnjava variranjem u kvalitetu zemlje i prisustvu zemljišnih patogena. Obe ispitivane procedure su postigle visoku korelaciju sa nicanjem u polju. Rezultati ovih istraživanja ukazuju na mogućnost korišćenja peska kao supstrata u hladnom testu, čime bi se ispitivanje vigora semena kukuruza znatno olakšalo, a ujedno bi se stekli uslovi za standardizaciju hladnog testa.

Ključne reči*: vigor, hladni test, supstrat, kukuruz.*

INTRODUCTION

Seed vigour is a very important parameter of quality, as it indicates the ability of seeds to germinate in sub-optimal conditions (low temperatures, wet soil, etc.). Moreover, vigour determines seed longevity as well as speed and uniformity of seed germination and emergence. Vigour is a better indicator of seed quality then standard germination test (*Matthews et al., 2012; Tabaković et al., 2013; Vujaković et al., 2015*). Seed vigour testing started in the early 20th century. The first test developed for the evaluation of seed vigour was cold test and now it is applied in seed testing laboratories around the world. Cold test involves exposure of seeds to low temperatures, for specified period of time (10 °C / 7 days) and subsequent transfer to optimal conditions for germination. When soil is used as a substrate in the cold test, soil pathogens are additional stressful factor. Although the cold test proved to be a good indicator of field emergence, it has not been introduced to ISTA (International Seed Testing Association) rules because it was not possible to standardize this test.

Different modifications of CT methods prescribed in handbooks for vigour testing (*Hampton and TeKrony, 1995; AOSA, 2002*) were used in seed testing laboratories. Variations were in temperatures and duration of stressful period, as well as type and moisture content of substrates (*Nijënstein, 1995*). Laboratories in the warmer parts of the world used 7 days cooling and sand as a substrate, while laboratories in colder regions preferred soil and more stressful conditions (cooling for at least 10 days).

Seed vigour evaluation by cold test conducted in 20 seed laboratories in four different climate zones (*Nijenstein and Kruse, 2000*) have shown that it is possible to achieve uniformity and repeatability when using a standardized protocol, but due to different preferred CT methods standardization worldwide is very difficult.

Seed testing laboratory in the Maize Research Institute Zemun Polje has accredited CT method with 7 days cooling period at 10 °C, followed by 7 days at 20/30 °C. In previous research *Milivojević (2005)* high seed vigour of ZP maize hybrids were detected in CT method described above, very close to standard germination percentage. Similar reactions of maize hybrids were observed by other authors (*Đukanović, 1999; Opra, 2002; Radić, 2003; Noli, 2008*). Therefore, more stressful conditions in CT are necessary in order to achieve better classification of maize genotypes and seed lots.

The objective of this study was to evaluate seed vigour of maize inbred lines using two different substrates (soil and sand) in a severe CT (7.5 °C for 10 days followed by 7 days at 20/30 °C), to compare those two CT procedures regarding precision and effectiveness in prediction of field emergence.

MATERIAL AND METHOD

For this study 15 maize inbred lines, components of commercial ZP hybrids were selected. Inbred lines belong to different maturity groups (FAO 300 to FAO 700), and possess different kernel types. Seed was obtained by self-pollination in 2011 at the nursery field in Zemun Polje. The harvest and husking was performed manually. Obtained seed was sieved through 5 mm sieve, and dried until moisture between 11 and 13 % was reached. Laboratory testing was carried out in 2015. Until testing seed was kept in laboratory storage room where storage conditions were 18 °C and 60 % relative humidity.

***Cold test* (CT)**

The cold test method in rolled paper towels described in Handbook for Vigour Testing *(Hampton and TeKrony, 1995)* was applied. Two different substrates sand and soil were used. In CT with sand untreated seed was tested, while for CT with soil seed was treated with Maxim XL 035FS. Testing conditions during stressful period were severe (7.5 ° C for 10 days). After that period samples were transferred to optimal temperature for germination 20/30 °C, with day/night period lasting 8 h / 16 h. Evaluation of normal seedlings was conducted according to ISTA Rules (2015) after 4 (first evaluation) and 7 days (final evaluation) of germination in optimal conditions.

***Field emergence* (FE)**

Field emergence was performed in 2015 by early sowing of 2x100 treated seeds per inbred line. Preparation of the soil was carried out by conventional methods. Sowing was performed manually, at the beginning of April – 04. 02. 2015. at location Umka (brown forest soil). Number of emerged seedlings was recorded frequently until complete emergence was reached. During field experiment, soil min and max temperatures at the depth of 5 cm were registered.

Statistical analysis

Results of CT were processed by the two-way analysis of variance (ANOVA), and subsequently the significance of differences between mean values was tested by LSD test using MSTAT program. Correlations and regressions between two CT methods and FE were calculated by Excel.

RESULTS AND DISCUSSION

Analysis of variance showed no significant differences between the two applied substrates in CT (Table 1). Inbred lines were significantly different in terms of seed vigour i.e., tolerance to low temperatures during germination and initial growth. Same level of significance was recorded in the first and final evaluations, except for interaction between the applied factors (substrate x genotype). The average germination of 15 inbred lines after 4 days in optimal conditions in CT with sand was 77.3 % (Table 2), while slightly lower percentage was in CT with soil (76.5 %). The average final germination in CT with sand and CT with soil was 82.2 % and 80.1 %, respectively. LSD test for interaction (substrate x genotype) showed significant differences between two substrates in CT only in two inbred lines (ZPL 10 and ZPL 15 - first evaluation), while for final germination, significant difference between CT with sand and CT with soil was observed only in inbred line ZPL 15.

Low temperatures and long stressful period in CT contributed to great differences among genotypes. Inbred line ZPL 4 achieved the highest average results in CT with both substrates (98/98 % - first/ final evaluation), followed by ZPL12 (92.5/97 %), ZPL 1 and ZPL 2 (94/94 %). Inbred lines ZPL 3 and ZPL 6 have shown sensitivity to low temperatures. Percentage of normal seedlings on the first evaluation in both lines was below 10 %, while final germination was 27 % (ZPL 3) and 17 % (ZPL 6). Evaluation after 4 days in optimal conditions of CT gave more clearly separation of genotypes than the final evaluation, indicating that the period of testing in the CT can be shortened. However, coefficients of variation (Table 2) were higher in the first than in the final evaluations for both substrates. Coefficients of variation were lower in the CT with sand (6.64 % first, 6.05 % final evaluation) than in the CT with soil (6.92 % first, 6.74 % final evaluation), which is explained by variation in soil quality and presence of soil pathogens. This is consistent with the results of other authors (*Loeffler et al., 1985; TeKrony and Woltz, 1997*).

Table 1. Effect of substrate on cold test results of first and final evaluation

Source of variation	Degree of freedom	First evaluation		Final evaluation	
		Mean squares	F value	Mean squares	F value
Replication	1	84.017	3.1955^{ns}	70.417	2.7080^{ns}
Substrate (S)	1	10.417	0.3962^{ns}	66.150	2.5439^{ns}
Genotype (G)	14	3442.031	130.9129**	2670.531	102.7014**
SxG	14	77.202	2.9363**	48.579	1.8682^{ns}
Error	29	26.293		26.003	

*ns, ** – non significant, significant at 0.01 probability level, respectively*

Table 2. Average germination on first and final evaluation in cold test (CT) with sand and soil of 15 maize inbred lines, coefficients of variation, correlation with field emergence (FE)

Inbred line	First evaluation			Final evaluation		
	CT sand	CT soil	Average	CT sand	CT soil	Average
ZPL 1	93^{abc}	95^{ab}	94.00^{AB}	93^{ab}	95^{ab}	94.00^{AB}
ZPL 2	95^{ab}	93^{abc}	94.00^{AB}	95^{ab}	93^{ab}	94.00^{AB}
ZPL 3	7^{h}	3^{h}	5.00^{H}	27^{e}	18^{ef}	22.50^{E}
ZPL 4	98^{a}	98^{a}	98.00^{A}	98^{a}	98^{a}	98.00^{A}
ZPL 5	83^{cdef}	88^{abcde}	85.50^{CDE}	85^{bc}	93^{ab}	89.00^{BC}
ZPL 6	5^{h}	11^{h}	8.00^{H}	17^{ef}	14^{f}	15.50^{E}
ZPL 7	87^{bcde}	80^{def}	83.50^{DEF}	94^{ab}	89^{ab}	91.50^{AB}
ZPL 8	73^{fg}	73^{fg}	73.00^{G}	77^{cd}	75^{cd}	76.00^{D}
ZPL 9	78^{ef}	87^{bcde}	82.50^{EF}	89^{ab}	89^{ab}	89.00^{BC}
ZPL 10	83^{cdef}	95^{ab}	89.00^{BCDE}	88^{ab}	95^{ab}	91.50^{AB}
ZPL 11	87^{bcde}	92^{abc}	89.50^{BCDE}	90^{ab}	94^{ab}	92.00^{AB}
ZPL 12	92^{abc}	93^{abc}	92.50^{ABC}	97^{a}	97^{a}	97.00^{A}
ZPL 13	96^{ab}	87^{bcde}	91.50^{ABC}	97^{a}	88^{ab}	92.50^{AB}
ZPL 14	94^{ab}	87^{bcde}	90.50^{ABCD}	94^{ab}	91^{ab}	92.50^{AB}
ZPL 15	89^{abcd}	65^{g}	77.00^{FG}	92^{ab}	72^{d}	82.00^{CD}
Average	77.3	76.5		82.2	80.1	
Coefficient of variation	6.64%	6.92%		6.05%	6.74%	
Correlation with FE	0.765**	0.816**		0.768**	0.797**	

a, b - different lowercase indicate significant difference (LSD test, interactions, 0.05)

A,B - different capitalization indicate significant difference (LSD test, genotype, 0.05)

***, significant at 0.01 probability level*

Cold test proved to be a good indicator of field emergence (*Woltz and TeKrony, 2001; Noli et al., 2008*), when stressful conditions prevail during germination and emergence. During this field trial, minimum soil temperatures did not exceed 10 °C for 19 days after sowing. In this study, significant correlation between laboratory testing and field trials were achieved (Table 2). Slightly higher correlation was in case when CT with soil was used (r = 0.816** first evaluation, r = 797** final evaluation). Highly significant correlation between CT with sand and FE (r = 0.765** first evaluation, r = 0.768** final evaluation) indicate that soil pathogens did not affect germination of treated seed. A more detailed analysis of the relation between field emergence and two CT procedures are shown in Figure 1. Results of the regression analysis indicate that behaviour of inbred lines during field emergence largely corresponds to the laboratory results in CT with sand (R^2 = 0.665) and CT with soil (R^2 = 0.635).

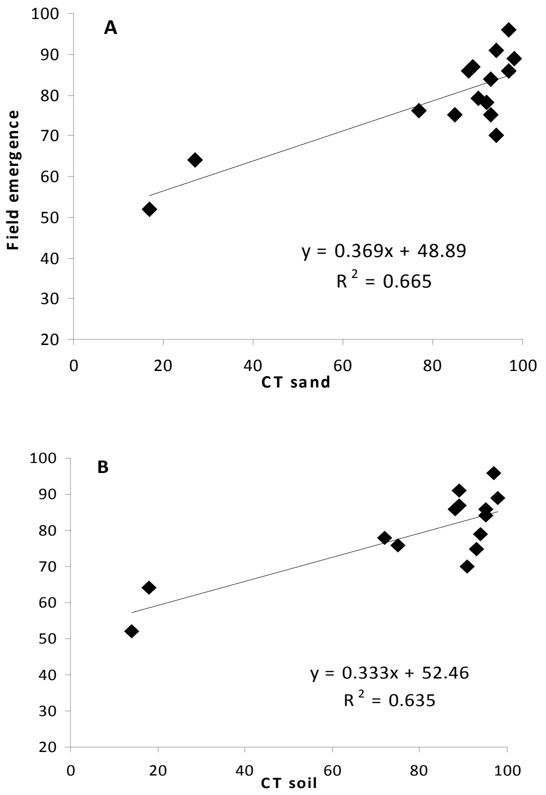

Fig. 1. Relationship between field emergence and final germination in CT with sand (A), and CT with soil (B) for 15 maize inbred lines

In second half of 20th century harmful effect of soil pathogens on maize seedlings was very pronounced (*Woltz et al., 1998*). Nowadays, seed treatment is very efficient, so detrimental effect of soil pathogens is minimized. That is way the use of soil in CT loses its significance. Therefore, hard work on soil purchase (digging, sieving, transport) and storage (maintenance of soil moisture), as well as variation of CT results between seed laboratories due to soil quality could be avoided by the application of sand.

CONCLUSION

This comparative study of two CT procedures confirmed that substrate didn't have a significant impact on CT results. Both CT procedures clearly classified inbred lines according to seed vigour, and achieved a significant correlation with field emergence, which was also confirmed by the regression analysis. CT with soil had a slightly higher correlation coefficient with field emergence, while CT with sand had a slightly lower CV value. This leads to the conclusion that sand is appropriate substrate for the cold test. This CT procedure would be considerably easier for testing maize seed vigour, with the potential for standardization.

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