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APPLICATION OF THE TETRAZOLIUM TOPOGRAPHICAL TEST IN THE SUNFLOWER SEED VIABILITY DETERMINATION

ZOIA CSERESNYÉS
(Romania)

The tetrazolium test (2, 3, 5-triphenyltetrazolium TTC chloride) started by Lakon (1942) and continuously improved by Lakon and Bulat (1957), Lindenbein and Bulat (1960) and Kietreiber (1960) has a wide application in the seed testing practices under the following circumstances: to obtain in a shorter time data concerning the germination capacity of the seed (Germ, 1957) for the acquirement of data refering to the germination of the seed in dormancy (Lindenbein and Bulat — 1960) as well as for the check-up of the germination when the test conditions for germination cannot be achieved. In all the other cases as a rule, the tetrazolium test should not replace the germination as it expresses the viable seed percentage and not the germinating seed in the given conditions (Germ 1957).

When determining the sunflower seed quality, the TTC-test is required to all the above-mentioned situations, especially as there is no specific method to determine the real germination of the dormant seed.

Although this is a widespread method in a number of countries such as the Soviet Union (Gost 12039-66), Hungary (MSZ 6354-68) a diversity of the recommended methods can be observed, including a certain inadvertences. The diversity refers mainly to a couple of test's aspects, namely: the embryos preparation and treatment as well as: the basical criteria of their classification into viable and non-viable embryos (Manjok — 1968), Blagodir — 1970, Gost 12039-66, MSZ 6354-68).

The aim of this paper was to establish the simplest and the fastest method for the embryos' preparation and treatment, to offer precise, unitarian and clear criteria for the embryos classification (into viable and non-viable) and finally, to draw comparative study of the TTC-obtained results with the results obtained through the germination test.

MATERIALS AND METHODS

The study was carried out with samples representing different germination levels, from the very high ones (96—100%) to very low levels (40—50%). The samples were from different phases, covering all kinds of Romanian varieties, lines and hybrids. Special attention was given to a lot of seed samples in dormancy. The seed preparation method was established with the help of a number of preliminary essays of various practices known in the literature (Blagodîr, 1970; MSZ 6354—68). The seeds were placed in a beaker of water for 16 hours at room temperature and, after this period, are freed from their coatings. The achenes are sectioned (0.5—1 mm width) opposite to the radicle in order to ease this operation; then, easily pushing the seed, it emerges from the coatings. The seeds thus prepared are treated with an aqueous 1% solution of tetrazolium chloride with a pH-value between 6.5—7.0. The treatment was effected with 2—4 replicates of 100 seed each, taken in darkness, at the temperature of 30°C. When the treatment time elapsed, i.e. when we obtained a bright red colour, the solution was decanted and the seed were washed and wet-evaluated. The seed examination can be postponed for several days, provided they are stored at lower temperatures (3—4°C).

In order to find out the compatibility of the results obtained through the TTC-test, the garmination of the same seed samples was determined in parallel with the TTC-test. The germination test was effected according to the provisions of STAS 1634—73 and the International Rules for SEED TESTING, in rolled industrial filter paper.

RESULTS

The main result of the above-mentioned experiments consists in the making up of the classification schedules for the stained embryos — dividing them into "viables" and "non-viables". In this respect, we considered, first of all, the criteria established by Lakon (1942) concerning the size and topography of necroses; amputations of the embryos followed, analogous to the necroses to be studied as Razumnaia did in 1967 with the pea-seed. The seed with different amputations were tested in order to establish their capacity to develop normal plants under laboratory and field conditions. The number of normal seedlings was determined and their growth pattern under laboratory conditions, as well as their penetration power through sand.

The results obtained (table 1) helped us to find out that the radicle necroses (amputations) influence more the seedling's subsequent growth than the necroses (amputations) suffered by the cotyledons. It is therefore recommended to rate as "non-viable" the embryos where amputation (necrosis) exceeds half from the radicle's length because these embryos will give seedlings with a hypocotyl between 1.6—3.1 cm long (as compared to 7.8 cm at the check plant) with a poor penetration power (60%).

In a similar way were estimated the necroses on the cotyledons, rating as "non-viable" the embryos where the necrosis holds more than

half of the respective embryos area — as the embryos with 2/3rd of the cotyledons amputated revealed a poor penetration power (88%) and, especially, a severely reduced weight of their seedlings (28 g/100 seedlings as compared to 60 g/100 seedlings at the check sample) — see table 1.

Table 1

The influence of the sunflower embryos amputation upon their capacity to produce normal seedlings

The amputated side of the embryo	Germination			Penetration	
	normal seedling %	length of:		germ. %	100 germs weight (g)
		radicle	hypocotyl		
		cm			
Check (non-amputated)	100	20.9	7.8	100	60
1/3 radicle	98	17.6	6.0	98	58
1/2 radicle	98	16.1	5.4	98	58
2/3 radicle	85	14.9	3.1	90	48
1/1 radicle	85	12.1	1.6	60	44
1/3 cotyledon	100	20.1	8.6	95	50
1/2 cotyledon	100	18.3	8.2	90	39
1/3 cotyledon	100	17.7	6.5	88	28
1 cotiledon	100	17.1	4.9	98	40
1/3 cotyledon length	100	13.3	7.6	95	45
1/3 radicle + 1/3 cotyledon	94	15.6	4.3	98	44
1/3 radicle + 1/2 cotyledon	98	15.4	4.1	98	33
1/2 radicle + 1/2 cotyledon	92	14.6	2.3	85	30

Summing up the above-mentioned conclusions, the embryos were classified as "viable" and "non-viable" according to the embryo's structure and extension of the observed necrosis. The actual classification is the following (figure 1).

Viable embryos :

1.V. = Completely stained embryos.
2.V. = Embryos showing the cotyledon fully stained but with up to half of the radicle's length non-stained, starting from the tip.
3.V. = Embryos with fully stained radicle but with unstained spots on the cotyledons over less than 50% of the cotyledons' area located either opposite to the radicle, sidewise, or just on a single cotyledon.
4.V. = Embryos with combined flaws from sections 2.V. and 3.V.

Non-viable embryos :

1.N. = Completely unstained embryos with small red spots, rotten, shrivelled seed.
2.N. = Embryos with completely stained cotyledons but with a less than 50% of the stained radicle's length, or, with unstained spots at the base of the radicle.

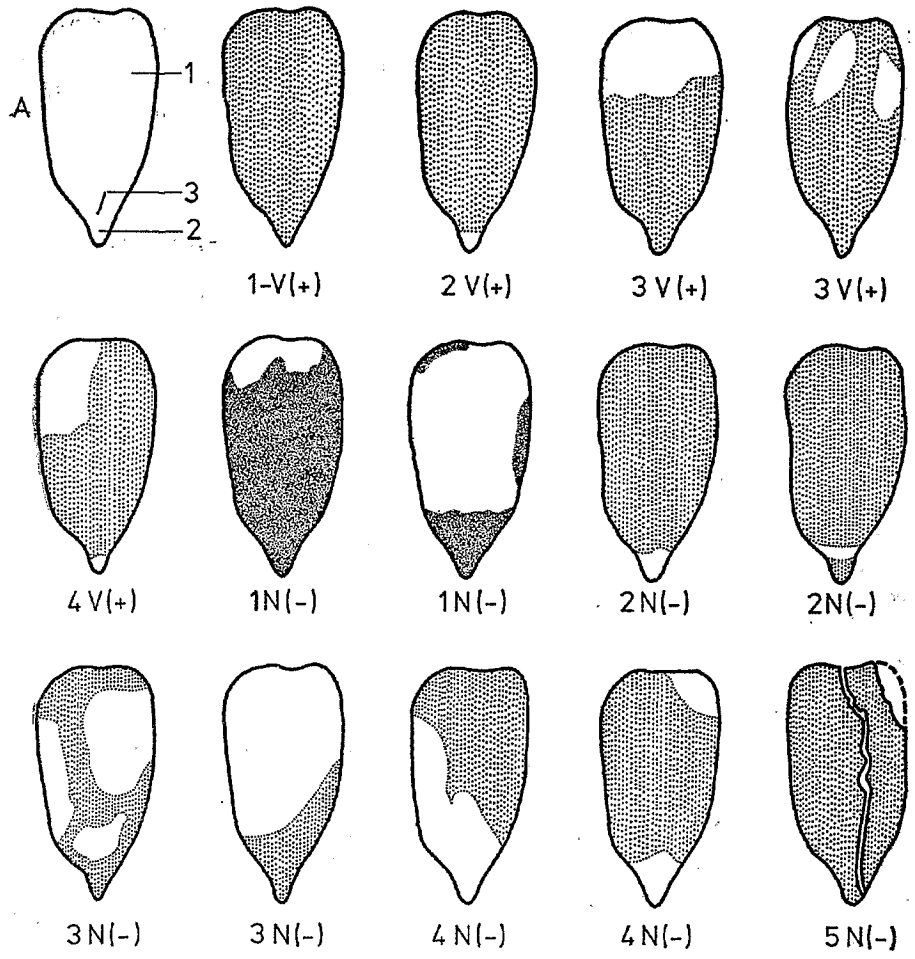

Fig. 1 — The classification of the viable and non-viable embryos.

3.N. = Embryos with fully stained radicle but with unstained spots on the cotyledons over more than 50% of their area or located on the half adjoining the radicle.

4.N. = Embryos with combined flaws from sections 2.N. and 3.N.

5.N. = Broken embryos.

In order to ascertain the compatibility between the results obtained with this kind of viability test and the results obtained by the germination test, a comparison was made between the two sets of experiments using the ISTA tolerance margins (M i l l e s, 1963).

Studying the data listed in table 2, it results that out of 278 analysis performed, 86% (239 analyses) were compatible with a probability P = 2.5% and 94% (261 analyses) for the probability P = 0.1%.

Table 2

The germination and viability analysis compatibility to the TTC-test at sunflower seed

	Total	per germination classes					seed in dormancy
		96—100 %	91—95 %	81—90 %	61—80 %	below 60%	
Number of analysed samples	278	118	76	41	28	15	61
Average germination, %	—	97.8	93.5	86.4	71.4	38.7	97.3
TTC-viability, average %	—	98.1	94.7	88.6	72.5	41.6	97.8
Compatible analysis percentage:							
— P = 2.5%	86	96	86	78	71	60	95
— P = 0.1%	94	97	91	93	86	73	97
$\overline{d}$		0.3	1.2	2.2	1.1	2.9	0.5
Difference significance		—	***	*	—	—	—
LSD 5%		0.36	0.66	1.90	5.12	3.33	1.20
LSD 1%			0.87	2.54			
LSD 0.1%			1.13				

Table 3

The comparison between field emergence, germination, viability and percentage of viable embryos (class 1.V.) at sunflower seeds (1972)

Variety (hybrid)	Germ. %	TTC viability		Field emerg. %	differences:		
		Total %	1.V. %		%	%	%
					columns:		
1	2	3	4	5	2 less 5	3 less 5	4 less 5
Record	98	99	90	91	7	8	—1
Record	95	93	82	85	10	8	—3
Orizont	97	98	92	90	7	8	2
VNIIMK 8931	98	100	94	94	4	6	0
LC 2	89	89	67	70	19	19	—3
AD 66	89	89	74	73	16	16	1
$\overline{d}$					10.5	10.8	0.5
HS 52	98	100	98	88	10	12	10
HS 53	96	98	93	79	17	19	14
HS 90	97	100	98	84	13	16	14
HS 301	98	95	80	60	18	15	0
HS 29	96	98	96	84	12	14	12
$\overline{d}$					11.6	12.6	8.3

From the same table we find out that the percentage of compatible analysis is very high for the upper values of the germination (96% at the germination class between 96—100%), gradually decreasing towards the lower germination classes (60% at the samples with the germination under 60%). The percentage of compatible analysis was 97% at 61 tests carried on dormancy seed samples, with an average germination of

Table 4

The comparison between field emergence, germination, viability and percentage of viable embryos (class 1.V.) at sunflower seeds (1973)

Variety (hybrid)	Germ. %	TTC viability		Field emerg. %	differences:		
		Total %	I.V. %		%	%	%
					columns:		
1	2	3	4	5	2 less 5	3 less 5	4 less 5
Record	99	99	97	96	3	3	1
Record	99	99	90	94	5	5	—4
Record	97	97	92	92	5	5	0
Record	97	95	90	92	5	3	—2
Record	97	93	87	87	10	6	0
Record	96	96	90	91	5	5	—1
Record	96	93	82	88	8	5	—6
Record	95	96	90	89	6	7	1
Record	94	98	91	85	9	13	6
Record	92	96	83	81	11	15	2
Record	90	94	88	83	7	11	5
VNIIMK 8931	94	95	87	83	11	13	4
VNIIMK 8931	89	92	81	78	11	14	3
VNIIMK 8931	82	86	65	70	12	16	—5
VNIIMK 8931	74	79	60	65	9	14	—5
VNIIMK 8931	67	67	55	57	10	10	—2
LC 3	81	75	69	69	12	6	0
$\bar{d}$					8.1	8.3	0
HS 52	95	97	89	80	15	17	9
HS 52	95	96	74	69	26	27	5
HS 52	92	95	89	84	8	11	5
$\bar{d}$					16	18	6.3
LC 2	92	96	81	81	11	15	0
LC 2	91	98	88	79	12	19	9
LC 2	90	98	89	84	6	14	5
LC 2	89	98	83	82	7	16	1
LC 2	87	85	61	51	36	34	10
$\bar{d}$					14	20	5

95%, thus presenting a considerable interest for the application of the TTC-test on these seeds.

It was also calculated the variation coefficient s% for the differences between the two analyses methods (Ceapoiu, 1968). Based on this coefficient, we estimate that, the differences at the germination class between 81—100% show little variation (s% = 1.43—4.86) while the differences at the other germination ranges show a medium variation (s% = 11.3—12.63).

Our results confirm those obtained by Razumnaia (barley seed) and Kalosnia (1964) and Blagodîr (1970), with sunflower seed, experiments pointing out that, higher differences between the two methods are obtained with seed having a lower germination power (using a number of 8 to 12 samples).

The classification system presented (figure 1) tells us the degeneration degree of the embryos and thus we can predict their behaviour under less favourable field conditions. The same goal was pursued also by Moore (1961), who used a 1 to 5 notation system for the embryos, and by Lindenbein (1965) who pointed out that the weakening of the embryo's vegetative power was due to certain necrosis on its structures. In order to study the possibility of expressing the sunflower seed vigour with the help of the TTC-test, the percentage was compared of the field-germination seed, under less favourable temperature conditions, with the percentage of viable seed from the first class-1. V. (completely stained embryos). Tables 3—6 list the results obtained in the years 1972—1974; here we find the smallest differences (0.5% average) exist between the field germination and the percentage of viable seed — 1. V. This small difference between the two mentioned indices underlines the importance of the TTC-test and the viable embryos' classification in order to appreciate the seed behaviour under less favourable conditions.

Table 5

The comparison between field emergence, germination, viability and percentage of viable embryos (class 1.V.) at sunflower seeds (1974)

Variety (hybrid)	Germ. %	TTC viability		Field emerg. %	differences:		
		Total %	1.V. %		%	%	%
					columns:		
1	2	3	4	5	2 less 5	3 less 5	4 less 5
Record	95	98	93	90	5	8	3
Record	93	93	86	85	8	8	1
Record	90	93	88	84	6	9	4
Record	89	92	84	87	2	5	—3
Record	86	92	86	86	0	6	0
Record	86	88	75	78	8	10	—3
Record	86	90	79	76	10	14	3
LC 1	98	96	92	93	5	3	—1
LC 1	76	82	69	62	14	20	7
LC 3	88	93	83	83	5	10	0
LC 3	89	89	74	79	10	10	—5
$\bar{d}$					6.6	8.4	0.5
HS 52	99	98	96	96	3	2	0
HS 52	97	99	96	89	8	10	7
HS 52	92	93	87	77	15	16	10
HS 52	93	97	93	87	6	10	6
HS 52	93	97	96	86	7	11	10
$\bar{d}$					7.8	9.3	6.6

The samples from the HS 52 hybrid make an exception to the rule as, in spite of their good germination and a high percentage of viable seed belonging to the 1.V.-class — the HS 52 seed show a lower field germination percentage experiencing a greater difference as compared

Table 6

The comparison between field emergence, germination, viability and percentage of viable embryos (class 1.V.) at sunflower seeds (1972—1974)

Year	Average temperature °C		Variety (hybrid)	No. of samples	Germination (less) field emergence			TTC viability (less) field emergence			Viability class 1.V. (less) field emergence		
					d		$\bar{d}$	d		$\bar{d}$	d		$\bar{d}$
	air	soil			min.	max.		min.	max.		min.	max.	
1972	2	3.2	Record lines	6	4	19	10.5	6	19	10.8	—3	2	0.5
			Hybrids	5	10	18	11.6	12	19	12.6	0	14	8.3
1973	10	9.9	Record, VNIIMK 8931	17	3	12	8.1	3	16	8.3	—6	6	0
			HS 52	3	8	26	16.0	11	27	18.0	5	9	6.3
			LC 2	5	6	36	14.0	14	34	20.0	0	10	5.0
1974	9.6	9.6	Record lines	11	0	14	6.6	3	20	8.4	5	7	0.5
			HS 52	5	3	15	7.8	2	16	9.3	0	10	6.6

to the „Record" variety : 8.3% in 1972, 6.3% in 1973 and 6.6% in 1974. Their behaviour is due perhaps to a slower growth of the hybrid seed germs at low temperatures, this also resulting from another experiment where low temperatures were inflicted at different germination phases. The respective results showed that the hypocotyl length at the HS 52 germs decreased 36% as compared to the check sample, due to the low temperature, while in the „Record" variety, the reduction was only 22% (under the same conditions).

CONCLUSIONS AND RECOMMENDATIONS

1. The results therein presented prove that the methods developed for the TTC viability testing allow us to obtain compatible results to those obtained by germination test.

2. The TTC-test yields results superior to those obtained by germination from +0.6 to +2.15%.

3. The TTC testing, according to the method therein described can be applied in situations such as when we want to obtain very quickly information concerning the seed germination, if we want to check-out the potential germination capacity, when several germination tests lead to diverging results and, finally, if we want to obtain certain data regarding the germination of seed in dormancy.

4. The tetrazolium test can be further used when we want to evaluate the emergence in the field of different seed samples under less favourable conditions (i.e. the soil temperature under +10°C at the sowing depth).

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