Rapid Method for Testing Seed Viability by Using Urine Sugar Analysis Paper[1)]

By KENJI TAKAYANAGI* and KAN-ICHI MURAKAMI**

Researcher* and Chief**, Division of Genetics, Department of Physiology and Genetics, National Institute of Agricultural Sciences (Hiratsuka)

It is desirable to know the seed viability and estimate the percentage of germination of seed stock by easy and quick test before sowing. Many workers have developed various methods for testing the viability of seeds without sprouting; these are tellulate method[1)], tetrazolium method[4)], and X-ray contrast method[6)]. These are comparatively expensive, time consuming and involve elaborate techniques. Steep water turbidity[2)], fluorescent substances[3)] and electrolytes[5)] exuded from seed have also been tried to use as an indicator of seed viability, but these methods require apparatus and techniques which may not be within reach for practical use.

A simple and rapid method of testing viability of rape seed by detecting glucose in the exudates from seed with urine sugar analysis paper was developed[7)] and this method has been employed in cereal crops like rice and malting barley with slight modification.

Principle

The authors while engaged in studies on metabolic changes in the seed with the ageing process, it was observed that the most important biochemical change was the exudation of sugars and other metabolic products in non-viable seeds, far in excess, compared to the viable seeds when they were soaked in water. A simple method of detecting glucose by urine sugar analysis paper was brought to use for seed exudates to estimate the glucose content.

This method of testing seed viability was tried on malting barley and rice seeds, but the exudates reacted to urine sugar analysis paper only after soaking for about 40 hours. This test can be done more quickly with these cereal seeds within six hours of soaking by inserting urine sugar analysis paper into embryo.

This method is based on the fact that in non-viable seed, as the embryo deteriorates more glucose and other metabolic products are exuded as compared to the viable seed. It was evidenced from the experimental results obtained from paper chromatography[8)].

Viability test with exudates from seed

1) Mass test

One gram of rape seed was soaked aseptically in 2 ml of sterile water and kept at 30°C for one to 24 hours. Urine sugar analysis papers, "Tes-Tape" (Eli Lilly Co., U.S.A.) and "Clinistix" (Japan Ames Co., Tokyo), were used for detecting glucose in the exudates. One end of urine sugar analysis paper

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was dipped in the soaked seed water and removed up. Then the color of "Tes-Tape" was changed from yellow to green after a minute, and "Clinistix", from pink to dark blue after three min., showing the reaction to glucose exuded from seeds which have lost their viability. The color of the paper was compared with each standard color chart (Fig. 1).

Experimental results are presented in Table 1. There is a relation between soaking

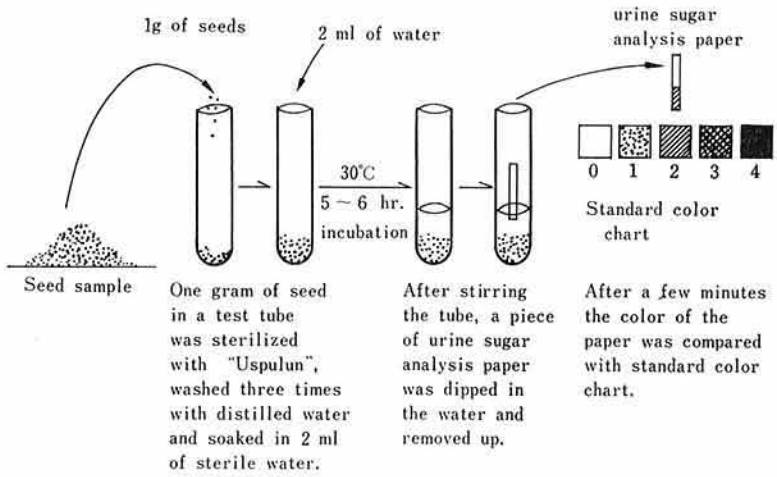

Fig. 1. Procedure of testing seed viability by using a urine sugar analysis paper—mass test.

Table 1. Detection of glucose in the exudate from rape seed by using urine sugar analysis paper. One gram of seed was soaked in 2 ml of water for several hours at 30°C.

Seed germinability	Urine sugar analysis paper	Coloration of the paper				
		Soaking time				
		1	3	5	10	24 hr.
No.	Tes-Tape	0	0.5	1.0	2.0	4.0*
do.	Clinistix	0	0.8	3.0	3.0	3.0
Good	Tes-Tape	0	0	0	0	0.2
do.	Clinistix	0	0	0	0	0.5

* Figures indicate the degree of coloration of the paper, Tes-Tape; Coloration after 1 min., 0: yellow (glucose free), 1.0—4.0: light to dark green (0.05—2% or more glucose in the exudate), Clinistix; Coloration after 3 min., 0: pink (glucose free). 1.0—3.0: light violet to dark blue (0.01—1% or more glucose).

Table 2. Detection of glucose in the exudate from rape seed mixed in various proportions of dead and viable seeds

Mixed proportion		Per cent of viable seed	Soaking time and coloration of the paper							
			3		5		10		24 hr.	
Viable	Dead		T	C	T	C	T	C	T	C
500 mg	0 mg	100%	0	0	0	0	0	0	0.2	0.5
475	25	95	0	0	0	0	0.3	0.8	0.7	2.0
450	50	90	0	0	0	0	0.5	1.8	1.0	3.0
425	75	85	0	0	0	0	1.2	2.0	1.8	3.0
400	100	80	0	0	0.1	0.5	1.5	2.5	2.5	3.0
0	500	0	0.3	0.8	1.0	3.0	3.5	3.0	4.0	3.0

T: Tes-Tape, C: Clinistix. See the note of Table 1 in details.

time and the intensity of color change in the paper, longer the soaking period greater the intensity of color change for the same seed. However, five hours soaking was enough to distinguish the dead from viable seeds.

Samples were prepared by mixing the dead and viable seeds in various proportions to see the reaction to urine sugar analysis paper and their relation to different soaking periods. The results clearly show that even 5% of dead seeds can be detected after 10 hours of soaking (Table 2).

2) Individual seed testing

To test individual seed viability a gadget was used as shown in Fig. 2. A round plastic plate of 9 cm diameter with 50 small depressions on its surface was placed in a petri dish of 12 cm diameter with wet blotters at the bottom. About 0.02 ml of water which is required for soaking a rape seed was dropped

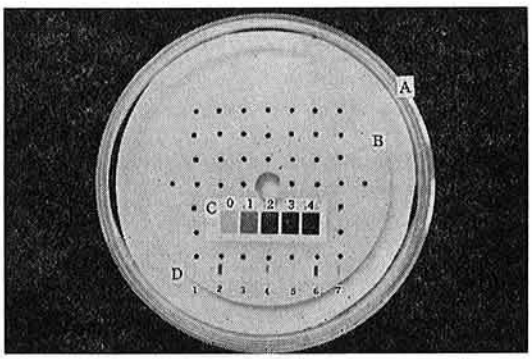

Fig. 2. Seed germinability test for a single seed of *Brassica* sp.
A: Petri dish with wet blotters. B: Germination plate with 50 small depressions. C: Standard color chart of "Tes-Tape", 0 (yellow)—4 (dark green). D: Some pieces of "Tes-Tape" paper tested.
With a simple moist chamber (A) in which a round plastic plate (B) was placed, a seed and a drop of water were put in each hole. After 10 hours of incubation at 30°C, a small piece of "Tes-Tape" was put into water in a hole and removed up. One minute later, the color of the paper (D) was compared with the standard color chart (C); No. 1, 3 and 5 are good germinative seeds. No. 2, 4 and 6 are not good germinative seeds. No. 7 is poor germinative seed.

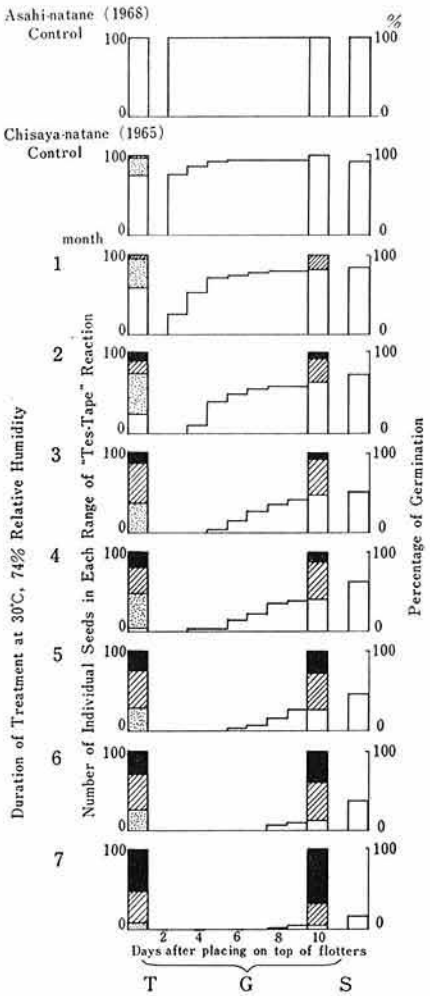

Fig. 3. Relationship between "Tes-Tape" test for a single seed and its germinability with artificially aged seed of rape. Seeds were treated under adverse condition (30°C, 74% R.H.) for various durations (0 to 7 months).
T: Reaction of "Tes-Tape" for individual seed. The coloration of "Tes-Tape" ranged from no change (0)= □, to light green (0.1–0.7)= ▨, green (0.8–1.2)= ▨ and dark green (1.3–4.0)= ■. The length of the column is represented with the number of individuals in each coloration group.
G: Daily germination and development of seedlings for ten days; the number of normal □, abnormal seedlings ▨ and ungerminated seeds ■ were counted at the tenth day.
S: Percentage of germination in soil.

in each small hole and a single seed was placed. After keeping the covered petri dish at 30°C for one to 24 hours the exudate from the individual seed was tested with a small piece of "Tes-Tape" for viability. Ten hours of incubation was enough to distinguish the dead from viable seed.

An experiment was conducted to know viability changes in seeds under artificial ageing condition. Rape variety Chisaya-natane seeds harvested in 1965 and preserved under dry condition with 98% germination were used in this experiment and freshly harvested Asahi-natane seeds were kept as a control. To accelerate seed ageing, seven samples of Chisaya-natane seeds were transferred to the condition of high temperature and high relative humidity (30°C, 74% R.H.) at one month interval from July, 1968 to February 1969.

After seven months of treatment, the seed samples were tested for germination, the percentage of germination ranged from 6% to 97% in various treatments. The seven seed samples along with two controls (seeds of Chisaya-natane and Asahi-natane kept under dry condition) were tested for individual seed viability and also for mass test. One hundred seeds from each of nine samples were tested with "Tes-Tape" for individual seed viability after soaking for 24 hours.

The results of the reaction to the "Tes-Tape" ranged from no change (□) to dark green (■) with two intermediate groups, light green (▩) and green (▨), and the length of the column (T) is represented with percentage of individuals in each group.

These seeds were allowed to germinate on top of blotters under alternative temperature of 20°C and 30°C. Percentage of germination and development of seedlings were recorded for ten days. The number of normal (□), abnormal seedlings (▨) and dead seeds (■) was counted at the tenth day. Another set of nine samples were sown directly in soil and percentage of germination was presented in column S (Fig. 3).

Results of the above experiment show a good correlation between coloration of "Tes-Tape" with germination rate and seedling vigor. The "Tes-Tape" coloration of light green to green shows that in this group the seed may be dead or develop to weak and

Table 3. Comparison of the artificially aged seeds of rape: Germination on top of blotters, in soil and "Tes-Tape" test for mass and individual seed viability

Variety	Duration of the treatment	Germination		"Tes-Tape" reaction		
				Individual test*	Mass test**	
				Percentage of the seeds ranged from	Coloration of "Tes-Tape" after soaking of:	
		on blotter	in soil	0 to 0.7 of coloration	6 hr	24 hr
Asahi-natate	Control	100%	100%	99%	0	0
Chisaya-natane	Control	97	92	96	0.2	0.5
do.	1 month	94	84	95	0.3	0.7
do.	2	90	71	74	0.5	1.0
do.	3	57	50	37	0.8	1.5
do.	4	57	63	47	0.8	2.0
do.	5	17	47	29	1.0	2.0
do.	6	16	39	26	1.0	2.5
do.	7	6	18	8	1.5	3.0

* One hundred seeds were used in each sample. A seed was soaked in 0.2m*l* of water with a gadget shown in Fig. 2, and incubated at 30°C for 24 hours. A small piece of "Tes-Tape" was dipped in the water in which the seed was soaked. The percentage of the seeds which showed no color change (0) to light green (0.7) was shown in the Table as an estimate of seed biability.

** One gram of seed in each sample was soaked in 2m*l* of wate.ı and incubated at 30°C for 6 hours and 24 hours. Degree coloration of "Tes-Tape" was shown in the Table (mean of three replicates).

abnormal seedlings. Under soil condition the seed samples of Chisaya-natane showed percentage of germination from 18% to 92% in various treatments; however, it was observed that the seedlings are weak corresponding to reaction of "Tes-Tape" to the light green to green range.

One gram of seed from each of the nine samples was tested with "Tes-Tape" for mass test. The degree of coloration ranged from 0 to 1.5 after six hours of soaking and 0 to 3.0 after 24 hours in various treatments showing clear relationship between percentage of germination and the intensity of coloration. Comparative account of results obtained under normal germination test in the laboratory, in soil, and individual and mass seed viability tests with "Tes-Tape" are summarized in the Table 3.

Viablility test with glucose in the embryo

In cereal crops like rice and malting barley, we could not succeed in rapid germinability test with exudates from seed, because the exuded glucose was not enough to react with "Tes-Tape" within a few hours of incubation. However, after 40 hours soaking exuded glucose was enough to react with "Tes-Tape" (Table 4). We are able to conduct viability test more rapidly in these cereal seeds by inserting "Tes-Tape" into the embryo after a few hours of soaking the seed. Experimental results show that only three to six hours incubation was enough to distinguish the dead from viable seed (Table 5). Dormant seed similar to viable seed did not show any change in the coloration of "Tes-Tape". Procedure for application of this method is given in Fig. 4.

Chromatographic analysis of rice seed embryo from viable and non-viable seeds showed an increase of mono-saccharide content in the dead embryo as compared with viable seed, further confirming the above results.

Further problems

The techniques described here are simple

Table 4. Exudation of glucose from the seed of malting barley. One gram of seed was soaked in 2m*l* of water at 30°C for 17-40 hours.

Variety	Percentage of germination	Soaking time and coloration of the paper					
		17		24		40 hours	
		T	C	T	C	T	C
Tochigi Golden Melon	100%	0.5	0.9	0.5	0.8	0.5	0.9
do.	0	0.5	0.8	0.5	0.7	1.3	3.0
New Golden	99	0.3	0.3	0.3	0.5	0.3	0.7
do.	0	0.5	0.9	0.7	1.5	1.3	3.0

T: Tes-Tape, C: Clinistix.

Table 5. Relationship between germinability and glucose in the embryo of malting barley. After soaking in water, a small piece of "Tes-Tape" was inserted into the embryo.

Variety	Harvested year	Per cent of germ.	Soaking time and coloration of "Tes-Tape"			
			3	4	6	24 hr.
Tochigi Golden Melon	1963	0%	3~4	—	3~4	4*
New Golden	1963	0	2~4	—	2~4	4
Tochigi Golden Melon	1967	100	0~0.5	—	0~0.5	0.5
New Golden	1967	99	0~0.5	—	0~0.5	0.5
U.S.—6**	1967	38	0~0.5	—	0.5	0.5

* Figres indicate the range of coloration of "Tes-Tape" which was observed in each sample.
** Dormant seed.

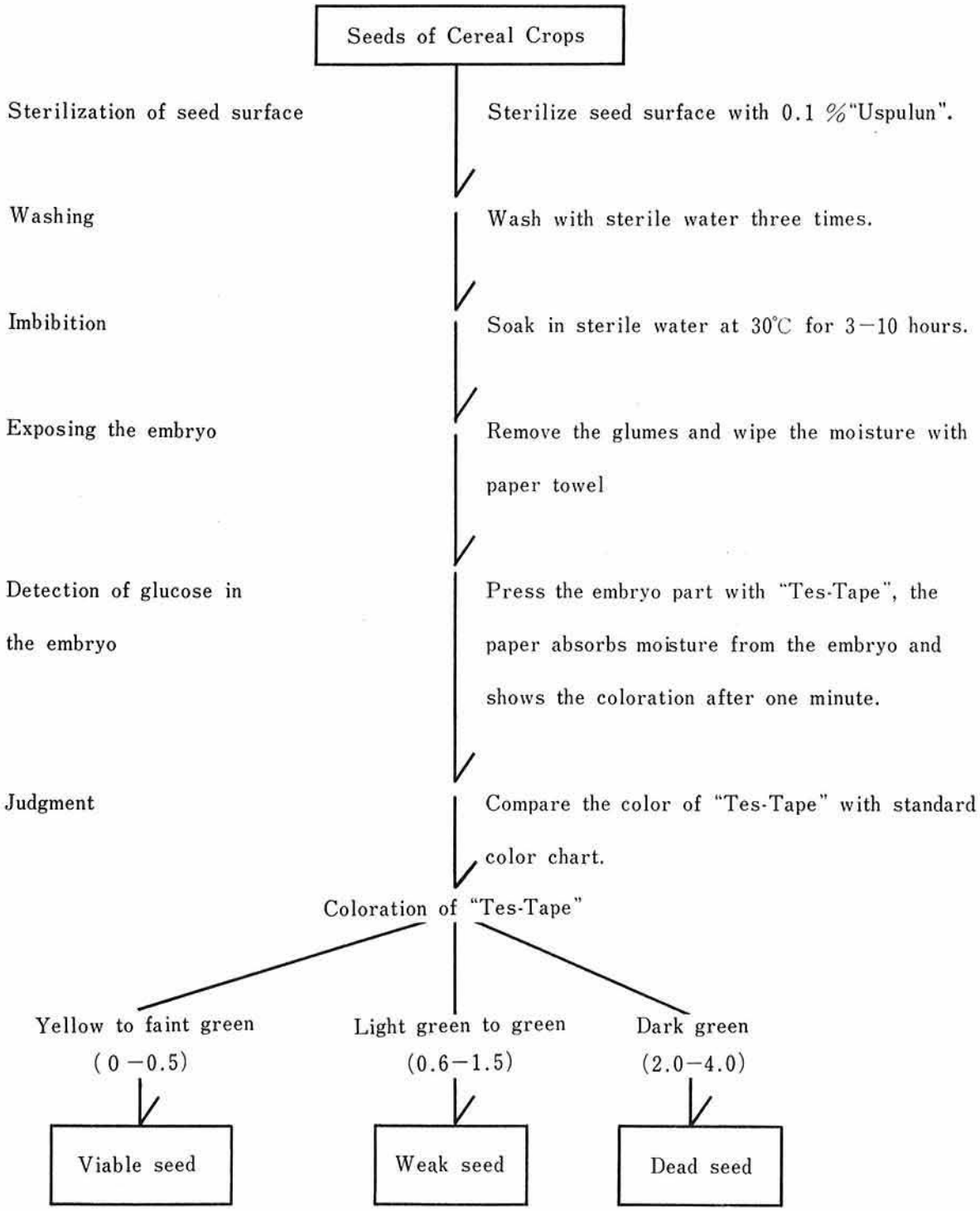

Fig. 4. Procedure of testing for viability of cereal seed by using urine sugar analysis paper, "Tes-Tape".

and convenient, and can be put to practical use for farmers, seedsmen and researchers. This test can estimate only glucose content in the exudates from seed or in the embryo and as such it is useful for the varieties of seed that exude more of glucose. As an example we are not successful in soybean seed, because exuded substances may be other than glucose. Similar simple test can be developed for detecting other metabolic products which may be helpful to test seed viability of many plant genera.

In the experiment conducted with "Tes-Tape" paper, the changes in coloration showed from yellow to light green, green and dark green. Viable seed did not show any change in color, dark green showed all are dead. Intermediate coloration from light green to green showed a proportion of seeds either dead or germinate and gave rise to weak or abnormal seedlings.

This test is used to distinguish the dead from viable seed and it may be also useful to test vigor of seed and seedlings. However, further works on these lines are necessary to standardize the techniques.

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