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The use of indigo carmine for the rapid determination of the germinative capacity of *Cassia fistula* Linn. seeds

Mukta Shrivastava

Department of Botany Govt. M.L.B. Girls College, Bhopal M.P. (India)

ABSTRACT

Viable seed is one which is capable of germinating under the proper circumstances. Seed viability of *Cassia fistula* was assessed with 1% indigo carmine staining which stained the dead tissue. On the basis of staining pattern (embryo + cotyledon) seeds were classified into various staining categories for the detection of viability. An attempt was also made to classify seed vigour on the basis of indigo carmine staining. It was found that indigo carmine test showed viability nearest to the laboratory gemination, easily soluble in water and takes less time to determine the viability.

Key word : Seed viability, seed vigour, Indigo carmine.

Seed characteristics depict the sum total effects of various stresses and strains, which the species has been subjected to during its evolution, in its specific habitat of origin. Seed characters; actually originate due to interactions of various environmental conditions. Viable seed is one which is capable of germinating under the proper circumstances. The term 'viability' has been widely and repeatedly been used for seeds beyond several other aspects. Baldwin[2], looking into the possibility of growth of plants, proposed that it is an abstract term referring to the potential capacity of seed to germinate but, Barton[3] used slightly other words. She stated that viability is the condition of seeds in the sense of being capable of growth and survival. Schopmayer[14] stated that viability is the potentiality of seeds to germination. Agrawal[1] defined the term viability as the ability of seeds to live, grow and develop. Recently, Bonner[4] defined seed-viability as the state of being capable of germination and subsequent growth and development of the seedling. Thus, it can be said that a viable seed is one which is capable of germinating under the proper circumstances.

Actual germination of seeds in soil in the green houses in field or in suitable medium in the laboratory in controlled temperature chambers has been tried by several workers[6,8-12,15] and is the commonest method for determining germination capacity of seeds. But many seed have special requirements for germination and obviously do not germinate or produce seedlings unless these requirements are met

with. This may involve a certain temperature, a particular exposure to light, mechanical or chemical treatment of the seed coats to make them permeable or an after ripening, depending upon the nature of dormance present in the seeds.

Methylene blue was used to predict the viability of seeds by Turesson (1922 - quoted by Barton[3]). Neljubow[13] found that indigo carmine, an aniline dye penetrated merely dead tissues and not the living ones. Interpretation of this test was based principally upon the proportion of the embryo remaining uncoloured. This method has also been included in the official method of seed testing in Poland, Yugoslavia, Romania and Czechoslovakia. Rostovtsev and Lyubich (1978) used indigocarmine to test the viability of tree and shrub seeds.

Seeds of *Cassia fistula* has been selected for the present work.

C. fistula

Fruit :

Very long, pendulous pod, cylindrical dark brown, smooth, hard, indehiscent, numerous septation, each septa having one seed.

Seed :

Embedded in a dark brown sweetish pulp, 13.01± 1.07 mm in length and 6.21 ± 0.25 mm in width, ovate, compressed, light brown, hard smooth shiny with a typical hard leguminous testa and 0.1818 g in weight.

Preparation of indigo carmine solution :

Seed samples (4 x 100) were soaked in water for 16 hours at room temperature after which the seeds were bisected longitudinally and treated with 10 ml of 1:100 solution of indigo carmine for one hour at room temperature. Indigo carmine was dissolved in tap water and no heating of solution being necessary. After treatment the seeds were washed with running water to remove the extra dye and were transferred to glassed class plate for staining studies.

For a parallel germination test, 4x100 seed were taken from each seed lot. Experiments were performed at 27 ± 2ºC in a SEW seed germination incubator. Seeds were kept on a three layered moist sheet of filter paper in germination trays. Diffused light was provided daily for eight hours. Daily observations were made up to 21 days.

Preparation of seeds for staining:

Premoistening of seeds :

This step is most important for staining of seeds.

Seed of *Cassia fistula* has a hard seed coat so they required scarifications and 30 hours imbibition period at 30ºC.

Decoating of seeds :

To allow easier penetration of indigo-carmine solution into the seed, the seed coats were removed by cutter or forceps. The seeds were bisected with the help of Sharp blade or razor.

Staining methods of seeds :

The bisected seeds were kept in 100 ml

glass beaker and each having 20 seeds only. The solution poured in beakers, the mouth of beaker covered with glass plate for avoiding dust and contamination. All the used glass wares were sterilized.

The concentration of solutions and time period required for staining are given below[7].

species	Concentration of solution TTC	Staining period / hr
C. fistula	0.1%	1

The laboratory germination and seed testing rules are as under :

Species	Substrate	Temperature	First count days	Final count days	% laboratory germination
C. fistula	sand	28±2·C	3	20	26

Staining patterns of indigo carmine in seeds of *C. fistula* are described in Table - 1. seeds have been classified in different categories on the basis of staining. Staining patterns have also been shown in figure 1. Comparisons of staining classes of indigo carmine along with actual germination of seeds of *C. fistula* is depicted in figure 1. Percentage value of viability categories from 1 down 2, 3 and so on were totalled till a nearest value to the laboratory germination was obtained.

Comparison of viable categories with actual seed germination was made by 't' test. Various combinations of viability categories after staining when showd no significant differences with germination were considered as most feasible seed categories.

Indigo carmine stained non living tissues as blue whereas the living tissue remained unstained. In case of Indigo carmine staining viability and seed categories have been determined. As such, viable seeds are those which are capable of producing normal seedlings in a germination test under favourable conditions. Well developed viable seed possess the ability to repair small, superficial necrosis to an extent within the decisive tissue. Thus, when such necrotic areas are present indecisive tissue blue coloured areas were found in indigo carmine staining.

The result indicates that in seeds of *C. fistula* indigo carmine tests seemed to be reliable and showed viability nearest to the laboratory germination. On the basis of indigo carmine staining pattern seed vigour classes were also made. These classes have been made taking six vigour categories. It was observed that indigo carmine test is better, since it is economic, easily soluble in water, remains unaffected by medium (pH), no carcinogenic effect and takes less time to determine the viability.

Table - 1

Indigo carmine staining viability and vigour classes in seed of Table - 1

Tetrazolium staining, viability and vigour classes in seeds of *C. fistula*

Category	Description	Viability percentage (Average of 3 replicates)	Viable/non viable	Vigour class
1	Embryo and cotyledons both are stained	13	V	Very FAST
2-4	Opposite to recicle minor portion of cotyledons stained with light blue colour.	5	V	Fast
5	One third portion of cotyledons stained with light blue colur.	8	V	Slow
6	Peripheral portion, some patched on central portion and minute portion of embryo stained.	1	V	sluggish growth
7	Embryo and less than half portion of cotyledon is unstained.	3	NV	No growth
8	Embryo proper stained with light blue colour and cotyledons stained dark blue colour.	34	NV	No growth
9	Embryo and cotyledons stained with large number of dark blue colour dots.	9	NV	No growth
10	Embryo & cotyledons both stained with dark blue colour.	27	NV	No growth

Laboratory germination 26%

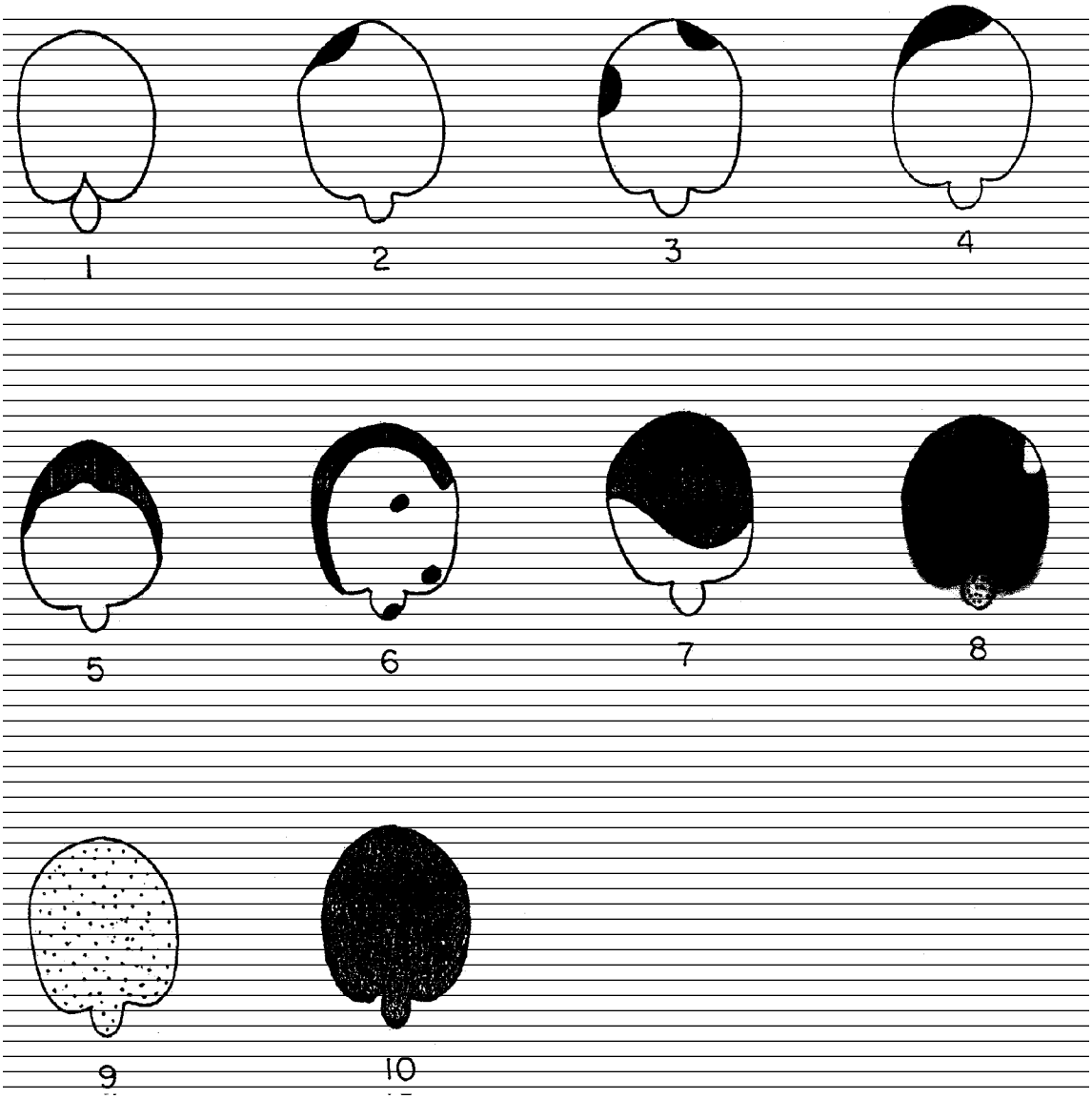

Figure 1. Seed categories in *C. fistula* having received various topographical Patterns of staining by indigo carmine.

References :

1. Agrawal, R.L. (1980). *"Seed Technology"* p.p. 685 Oxford and IBH Publishing Co., New Delhi.
2. Baldwin, Henry Ives (1942) "Forest tree seed of the north temperate regions with speical reference to North America". p.p. 240, Chronica Botanica Co., Waltham Mass, U.S.A.
3. Barton, L.V. (1961). "Seed preservation and logevity", p.p. 216, Leonard Hill., London
4. Bonner, F.T. (1984). Glossary of seed germination terms for seed workers. Gen. Tech. Rep., 50+49 : 1-4.
5. Grabe, D.F. (1970) *"Tetrazolium Testing Hand book for Agricultural seed"* contribution no. 29 to the Hand Book on Seed Testing, p.p. 1-62. Association of official Seed Analysts.
6. Hill, Joseph A. (1976). *Tree plant notes, 27(2):* 2-3.
7. ISTA (1983). Biochemical test for viability (The Topographical tetrazolium test. p-46-52, 20th ISTA Congress, Ottawa, June 19-25.
8. Johnston, M.E.H. (1975). Seed gernination. In "Advance in Research and Technology of seeds", (ed. W. T. Brandnock) Part 1, pp. 8-33, PUDOC -Centre for Agricultural Publishin and documentation, Wageningen.
9. Johnston. M.E.H. (1977) Seed gernination. In "Advance in Research and Technology of seeds"', part 3 (ed. J.R. Thompson) pp. 7-53, PUDOC -Centre for Agricultural Publishing and documentation, Wageningen.
10. Johnston, M.E.H. (1977). Seed gernination. In "Advance in Research and Technology of seeds" part 4 (ed. J.R. Thompson) pp. 43-83, PUDOC-Centre for Agricultural Publishin and documentation, Wageningen.
11. Justice, O.L. (1972). Essentials of seed testing In : "Seed Biology" (ed. T.T. Kozliwski), vol. 3 p.p. 301-370.
12. Mackay, D.B. (1972). "The measurement of viability' In: viability of seeds". (ed. Robets E.H.)p.p. 172-208, Chapman and Hall, London.
13. *Neljubow, D. (1925). *Ann. Ess. Semences heningrad, 4(7):* 31-35.
14. Schopmeyer, C.S. (1974). (ed.) "Seeds of woody plants in the united states". Forest service USDA Hand book No. 450, 883 pp.
15. Simak, M. (1970). New use of X-ray method for the analysis of forest seed. IUFRO Int. Symp. on Sexual Reproduction of forest trees. Varparanta, Finland pp. 11.
16. Smith, F.G. (1952). *Plant Physio1, 27:* 445-456.
17. Yadav, V.K., P.K. Khare and G.P. Mishra (1986). *Trop. Flor, 2:* 213 - 216.