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2,3,5-triphenyl tetrazolium chloride as an indicator of the viability and vigour in seeds of *Acacia catechu* Willd.

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ABSTRACT

Seed is the important stage of a plant but biology of the seeds is usually ignored. Seed characteristics depict the sum total effect of various stresses and strains. Viable seed is one which is capable of germinating under the proper circumstances. For the quick test of viability of seeds, tertrazolium (TTZ) is an excellent indicator of reduction in biological materials. On the basis of tetrazolium chloride staining pattern seed vigour classes of *A. catechu* were also made.

Key words : Seed viability, vigour, TTZ.

The term 'viability' has been widely and repeatedly used for seeds beyond several other aspects. Baldwin[5], looking into the possibility of growth of plants, proposed that is is an abstract term referring to the potential capacity of seed to germinate but, Barton[6] used slightly other words. She stated that viability is the condition of seeds in the sence of being capable of growth and survival. Schopmayer[20] stated that viability is the potentiality of seeds to germination. Agrawal[4] defined the term viability as the ability of seeds to live, grow and develop. Recently, Bonner[7] defined seed-viability as the state of being capable of germination and subsequent growth and development of the seedling. Thus, it can 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[11-13-18,21] 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. 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.

TTZ is an indicator or reduction in biological materials. Red colour appears when the Tz is reduced in living tissue whereas non-

living tissue does not bring the reduction of Tz[12].

This method has gained popularity on account of its simplicity and rapidity. Its manifestation can be clearly recorded and interpreted. Several workers have used this method for seed of vegetables, cereals and forest trees[1-3,8,9,17,19].

Smith[21] made a detail study of Tz ia an oxidation-reduction indicator in corn embryos. He concluded that Tz was catalized by diphosphopyriding necleotide linked dehydrogenase (malic and alcohol system) and was mediated by diphorase.

The dehydorgenase enzymes are involved in respiratory activities of biological systems. During respiratory process intermediates are produced which act as subtrats. Tz act as hydrogen acceptor are transferred in several steps. After this, Tz is reduced and converted in to insoluble red colour substance Formazan. Therefore, Tz test indicated respiratory (viable) and non respiratory (non viable) tissues.

Seeds of *Acacia catechu* Willd. has been selected for the present work

Fruit :

Pod, broad, straight, flat, dark brown, shining dehiscent and 3-6 seeded.

Seed :

7.01± 0.31 mm in length and 6.2 ± 0.21 mm in width, broadly ovate or orbicular, dark greenish brown, smooth, shining with a hard tests and 0.0259 g in weight.

Preparation of tertrazolium solution:

Four hundred (4 x 100) seeds from each lot were taken for TTC testing. They were kept between two layers of moist filter papers for 24 hours. Imbibed seeds were bisected longitudinally on a clean sliding cut with a sharp razor. One half of each seed was kept in a petri dish having some water in it as the seed should not dry. All the seed were transferred to 0.1% solution of TTC which was prepared by dissoving 0.500 g 2-3-5, triphenyl tetrazolium chloride in 500 ml of phosphate buffer[10] Seeds were kept in TTC solution for required staining period at 35^{o}C in an oven. Thereafter, the solution was drained off and seeds were washed many times with water. For evaluation of the colour, the seeds were kept on a glass sheet with their cut surfaces upwards. They were kept moist during evaluation.

Preparation of seeds for staining :

Premoistening of seed :

This step is most important for staining of seeds seeds of *Acacia catechu* having hard seed coat so they required scarifications and 10 hours imbibition period at 30^{o}C.

Decoating of seeds :

To allow easier penitration of tetrazolium solution into the seed, the seed coat removal by cutter or forceps. The seed bisected with the help of sharp blade or razor.

Staining methods of seeds :

The bisect seeds were kept in 100 ml glass beaker and each having 20 seeds only.

The solution poured in beakers and covered the mouth of beaker with glass plate for avoiding dust and contamination. All the used glass were sterlized.

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

species	Concentration of solution TTC	Staining period / hr
A. catechu	0.1%	16

The laboratory germination and seed testing rules are as under :

Species	Substrate	Temperature	First count days	Final count days	% laboratory germination
A. catechu	B.P.	27±2·C	6	24	37

Staining patterns of tetrazolium in seed of *A. catechu* are described in table 1, seeds have been classified in different categories on the basis of staining patterns have also been shown 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 germinations was obtained. Comparison of viable categories with actual seed germination was made by 't' test. Various combinations of viability categories after staining when showed no significant differences with germination were considered as most feasible seed categories.

Tetrazolium chloride is reduced to red, water insoluble formazon compound by termination oxidase system in the living tissues of seed. It is perpitated in living tissue where as no reaction takes place in dead tissues. Due to this reaction, red stained areas indicate living tissues and colourless portions of seed are considered as dead.

Result indicates that where embryo along with a part of cotyedons are stained with tetrazolium seeds can be classified as viable. Other categories have been made on the basis of staining pattern.

Result of present study showed that when the seed were completely stained with Tetrazolium (small protion of cotyledon remained unstained) they remain viable. Decisive tissues are redicle, plumule, their juncture and cotyledonary portion surrounding redicle and plumule when they remain stained, seeds can be classified as viable.

In addition to the embryo proper, about half of cotyledonary portion must also be stained to make the seeds detectable as viable.

Table-1. Tetrazolium staining, viabilty and vigour classes in seeds of *A. catechu.*

Category	Description	Viability percentage (Average of 3 replicates)	Viable/non viable	Vigour class
1	Embryo and cotyledons both are completely stained	21	V	Very FAST
2	Embryo and cotyledons both are completely stained but minor area of cotyledon is unstained.	2	V	Fast
3	Some parts opposite to embryo is unstained rest parts of seed stained	4	V	Fast
4	Some parts opposite to embryo is unstained rest parts of seed stained	7	V	slow
5	Cotyledons are completely stained except some portion of embryo.	1	V	sluggish growth
6	Peripheral portion of cotyledons is unstained except embryo and some portion of cotyledons	3	V	sluggish growth
7	Tip of redicle and minor unstaied areas on cotyledon	1	NV	No growth
8	One third portion of cotyledon and embryo are unstained	1	NV	No growth
9	More than half portion of cotyledons and embryo unstained	14	NV	No growth
10	Embryo and small portion of cotyledon unstained	6	NV	No growth
11	Embryo and more than half portion of cotyledons unstained.	13	NV	No growth
12	Embryo and cotyledon both stained light pink colour doughts.	7	NV	No growth
13	Embryo and cotyledons both are unstined.	20	NV	No growth

Laboratory germination 37%

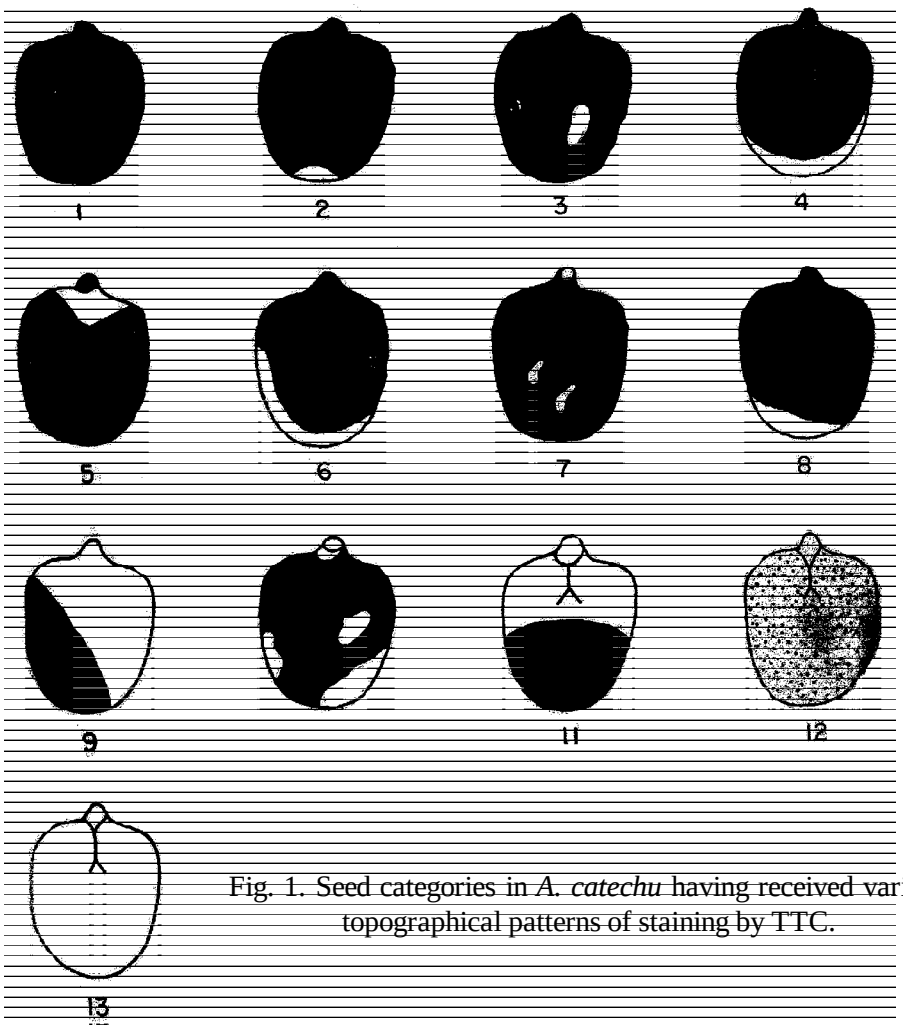

Fig. 1. Seed categories in *A. catechu* having received various topographical patterns of staining by TTC.

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