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### STUDY OF EXCISED EMBRYO TEST IS FOUR LEGUMINOUS SEEDS

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#### ABSTRACT

Many factors influence viability of seeds. Some of the important factors are environmental conditions under which the seed has been produced, mechanical injuries during harvesting, processing of seeds and storage environment. Germination test in a laboratory is defined as the emergence and development from the seed embryo, of those essential structures which, for the king of seed being tested, indicate the ability to develop into a normal plant under favourable conditions in soil.

*Key words: Environment, Emergence, Development, Conditions, Indicate.*

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#### I. INTRODUCTION

The excised embryo test is to determine quickly the viability of seed which normally germinate slowly or show dormancy. In this method, the seed coats are removed from the water-soaked seeds and the embryos are excised under sterile conditions and placed on moist filter paper and kept under optimum conditions of light, temperature and moisture. The viable embryos germinate within a very short time.

##### Excised Embryo Test

Germination studies by employing the traditional method involves long durations, sometimes very long as in the case of forest trees. Even the cereals, by normal methods require three to twenty-one days. For testing viability of seeds these methods are time consuming and tardy. Therefore, a quicker method to evaluate viability of seeds became a necessity.

The role and importance of the seed coat in the germinating seeds of common weeds.

Excised embryo test as a speedy method of evaluating seed viability has been recognized by and recorded in the International Rules for seed Testing (ISTA, 1976). The details of this method is given in the report of The Forest Tree Seed Committee - 1974 to 1974.

#### II. MATERIAL & METHODS :-

##### Excised embryo test

Excised embryo test was performed with two replicates of one hundred seeds each drawn at random from seed lots. The excised embryos were obtained from seeds of different ages, namely, fresh, one year old and two years old. The objective of excised embryo test is to determine quickly the viability of seeds which normally germinate slowly or show dormancy. In this method, the seed coats are removed from the seeds soaked at room temperature (20-25° c).

The embryos are examined under sterile condition and placed on moist filter paper and kept under optimum conditions of light, temperature and moisture.

##### Embryo excision :

Excision of seeds was done inside a sterilized glass chamber to avoid any fungal attack. 50% ethanol solution in water was used for sterilization. In most of the species test a was easily removed by thumb and fore finger to push the embryo out of the seed coverings.

**Evaluation :**

The embryos were examined daily and the test was terminated as soon as distinct differentiation between viable and non-viable embryos could be made up to a maximum period of fourteen days.

The following categories of seeds are considered viable.

- Germinating embryos.
- Embryos with one or more cotyledons exhibiting growth or greening.
- Embryos remaining firm slightly enlarged and either white or yellow according to species.

All other types of embryos are considered non-viable. (ISTA, 1976)

- Embryos which rapidly developed severe mould, deteriorated and decayed.
- Degenerated embryos.
- Dead or embryoless seeds detected during preparation.
- Embryos exhibiting extreme brown or black discolouration, off-gray colour or white watery appearance.

Viability and germinability testes were also conducted on fresh, one year old and two year old whole seeds of all the four species.

### III. OBSERVATION AND INTERPRETATION

#### Excised embryo test

The results of germination tests, both of excised embryos as well as whole seeds; fresh seeds and aged seeds, show considerable differences in viability, and percentage germination (table-3). The results, of all the four species, indicate a uniform effect of ageing on seed viability and germination percentage. The seeds of B. monosperma and P. marsupium tend to lose drastically their viability (93 to 50%) and germinability with increasing age. Dalbergia Paniculata however, retains germinability, though somewhat declined (97 to 62%). In Abrus Precatorius the decline is moderate. The number of days taken for germination of seeds as well as of excised embryos, in all the four species, increased considerably with increasing age of the seed source. The germination percentage of excised embryos, of all the four species, was greater than that of whole seeds of all the age categories.

The survival rate of the germinating excised embryos decreases with increasing number of days. However, the results indicate that in all the four species, the survival rate becomes constant with no further death. Amongst the surviving embryos of A. Precatorius in excised embryo test by the fifth day, the radical of 61.4 of germlings reach a length of 7 to 10 mm. Similarly, for B. monosperma, D. paniculata and P. marsupium the figures are 87.0%, 46.98% and 62.9% respectively (Table-2).

The seedling growth in terms of S/R ratio for excised embryo and whole seed in different age groups of the four species shows considerable variation. In A. precatorius and P. marsupium seedling emerging from excised embryos of two year old seeds show a greater value than that of seedlings from whole seeds of the same age group. In B. monosperma, however, the seedlings from two year old whole seeds show a greater S/R ratio than that of the excised embryos from seeds of the same age group, while the value for D. paniculata shows virtually no difference between the two (Table-1).

Excised embryo test is known to be an efficient and accurate method. In the present study where fresh, one year and two year old seeds were tested percentage germination of both the whole seeds and excised embryo decreased with increasing age of the seeds. Between the whole seeds and excised embryos greater percentage of germination was recorded for excised embryos of all the seed lots vis a vis whole seeds. These tests indicate that the ageing of seeds are directly related to the lowering of seed viability. In all the species, except P. marsupium seed viability in one year old seeds was fairly good. In P. marsupium there was drastic reduction in seed viability, in seeds older than one year.

**Table-1 : Various growth parameters of the seedlings developed from variously stored seeds (s) and excised embryos (E) of four selected forest tree species.**

S = Shoot, R = Root, E = Excised embryo, S = whole seed

Species	GROWTH PARAMETERS OF SEEDLINGS								
	Seed age		Shoot length (cm)	Root Length (cm)	Seedling Length (cm)	Fresh weight (mg)	Dry weight (mg)	S/R length ratio	Weigh ratio
<u>Abrus precatorius</u>	Fresh	E	8.27	6.19	14.7.0246	0.6874	0.3675	1.3366	0.5356
		S	5.38	2.64	7.02	0.3979	0.1998	2.037	0.5021
	One year	E	6.00	3.83	9.83	0.4786	0.2692	1.566	0.562
		S	3.27	1.54	4.81	0.2967	0.1274	2.123	0.4293
	Two year	E	3.19	1.08	8.27	0.3298	0.1582	2.953	0.4796
		S	1.08	0.76	4.01	0.1579	0.0624	1.421	0.3951
<u>Butea monosperma</u>	Fresh	E	12.20	8.81	21.01	0.7856	0.5231	1.3847	0.6658
		S	9.71	7.00	16.71	0.5693	0.3264	1.3871	0.5733
	One year	E	10.12	6.29	16.41	0.5963	0.3251	1.6089	0.5445
		S	8.52	3.32	11.84	0.3281	0.2864	5.1325	0.8729
	Two year	E	6.29	2.88	9.17	0.2743	0.1982	2.1840	0.7225
		S	3.18	1.11	4.19	0.1827	0.0728	3.1485	0.3984
Species	GROWTH PARAMETERS OF SEEDLINGS								
	Seed age		Shoot length (cm)	Root Length (cm)	Seedling Length (cm)	Fresh weight (mg)	Dry weight (mg)	S/R length ratio	Weigh ratio
<u>Dalbergia peniculata</u>	Fresh	E	9.12	5.87	14.99	0.6979	0.3735	1.5536	0.5351
		S	6.48	3.43	9.91	0.4281	0.220	1.8892	0.5152
	One year	E	7.58	5.22	12.8	0.4732	0.2131	1.4521	0.4503
		S	5.29	3.01	8.31	0.2851	0.1220	1.75165	0.4279
	Two year	E	4.50	1.72	6.22	0.2732	0.1118	2.6162	0.4062
		S	3.38	1.28	3.38	0.2251	0.08261	2.64062	0.3669
<u>Pterocarpus marsupium</u>	Fresh	E	8.59	6.27	14.86	0.6914	0.3723	1.3700	0.5384
		S	5.88	2.90	8.78	0.4008	0.2018	2.0275	0.5034
	One year	E	6.39	4.00	1.039	0.4812	0.2700	1.5975	0.5610
		S	3.77	1.82	5.59	0.3002	0.1292	2.0714	0.4303
	Two year	E	3.28	1.14	4.42	0.3310	0.1598	2.8771	0.4827
		S	1.33	0.93	2.26	0.1582	0.06	1.43010	4.03919

**Table-2 : Various categories of embryos and their fastness of germination as observed by radical length in excised embryo test in different forest tree species.**

Species	Days	Dead embryos	Firm embryos (No radical growth)			Germinating embryos			
			A	B	C	Length of radical (mm)			
						1-2	3-5	6-10	7-10
<u>Abrus precatorius</u>	1	32	68	-	-	-	-	-	-
	2	36	12	-	-	2	4	13	33
	3	39	4	-	-	4	2	18	33
	4	42	-	-	-	10	5	9	34
	5	42	-	-	-	9	4	10	35

Butea monosperma	1	-	100	-	-	-	-	-	-
	2	15	15	-	-	13	38	19	-
	3	23	2	-	-	7	22	32	14
	4	23	2	-	-	2	5	12	56
	5	23	-	-	-	2	3	5	67

Species	Days	Dead embryos	Firm embryos (No radical growth)			Germinating embryos Length of radical (mm)			
			A	B	C	1-2	3-5	6-10	7-10
<u>Dalbergia peniculata</u>	1	4	96	-	-	-	-	-	-
	2	7	23	2	1	25	32	10	-
	3	8	12	3	5	20	21	19	12
	4	9	8	4	4	12	18	21	23
	5	11	2	1	3	10	10	24	39
Pterocarpus marsupium	1	34	66	-	-	-	-	-	-
	2	35	10	-	-	4	9	32	10
	3	38	2	-	-	6	3	16	35
	4	40	-	-	-	2	2	3	53
	5	40	-	-	-	-	1	2	57

Table-3 : Different aged seeds and excised embryos and their beginning and completion of germination observed in excised embryo test in different forest tree species.

Name of the Species	Age of Seed	Germination Time in Days				Germination %	
		Seed		Excised embryos		Seed	Excised embryos
		Beginning	Completion	Beginning	Completion		
Abrus precatorius	Fresh	3	9	3	8	36	43
	One Year	3	10	3	9	23	35
	Two Year	4	12	4	11	14	20
Butea monosperma	Fresh	2	20	1	19	75	93
	One Year	3	22	2	20	54	75
	Two Year	5	25	4	22	4	12
Dalbergia paniculata	Fresh	7	34	6	33	85	97
	One Year	8	42	7	35	67	82
	Two Year	10	47	7	38	52	62
Pterocarpus marsupium	Fresh	11	29	8	24	43	50
	One Year	13	32	10	26	5	18
	Two Year	16	39	11	28	2	14

#### IV. CONCLUSION

By this method, the seeds are soaked for some time and the embryos are then excised from the seeds and placed on moist filter paper or blotter disc in Petri dishes. The tests are conducted under normal light at a constant temperature of 25°C. The condition of the embryos is examined daily. Depending upon the species and lot differences, the tests can be terminated after only a few days, up to a maximum of 14 days, or as soon as distinct differentiation into viable and non-viable embryos can be made.

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