BULL. BOT. SURV. INDIA
Vol. 31, Nos. 1-4 : pp. 117-121, 1989

GERMINATION OF *CASSIA FISTULA* L. SEEDS BY MECHANICAL STRESS

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ABSTRACT

The effect of mechanical stress in presence of different kinds of chemical media on seed germination, percentage of germination and rate of imbibition in seeds of *Cassia fistula* L. have been studied. A unique germination percentage of seedlings free from fungus attack were observed in cracked and punctured seeds. Modifications raised by this stress in seeds enhances quick germination in presence of suitable moisture media. To make comparative study between the rate of imbibition and percentage of germination and to know the susceptibility of seeds towards fungal attack, heating and roasting in addition to cracking and puncturing, were applied and discussed.

INTRODUCTION

Long term dormancy in seeds of economically and ornamentally valued species, *Cassia fistula* L., is due to its arrested regenerative potential of embryonic tissue, caused by calcarious cotyledons with heavily lignified (90%) impermeable seed coat (Basu, D. & Chakraverty, R. K. 1986). The seed coat is impregnented with resting stage of seed borne fungal mycoflora (Randhawa *et al.*, 1986). Such problems specific to these seeds can be eliminated by mechanical stress to the seeds which enhances germination and also produce fungal free populations.

MATERIALS AND METHODS

Cassia fistula L. pods were collected from end of March to 30th April 1988. About 200 seeds were freshly taken out from pods and out of these only 72 healthy seeds were selected and the experiments were conducted in petridishes (Bewley and Black, 1978). These were divided into groups of 12 seeds each and each group was subjected to the different kinds of treatments irrespective of their diameter.

Date of receipt : 29.12.88. Date of acceptance : 26.7.89.

The first set of 12 seeds was cracked and punctured to a depth of 0.5 to 1 cm by a stainless blade and pins, the second set of seeds was kept in hot water at 45°C for 5 minutes, third set of seeds was roasted at 45°C for 5 minutes and remaining set of seeds were kept under normal conditions without any stress and pretreatments.

Treated seeds were surface sterilized by soaking in 0.1% Mercuric chloride solution for 3 minutes. After washing with deionized water, seeds were kept for imbibition in different media (distilled water, 0.5% NaCl, 1% alkaline water, 1% HCl and 3% HCl) for one hour. After soaking, the seeds were arranged (in petridishes) on Whatman No. 1 paper (9 cm size) which served as a single layer moisture resource with respective samples as shown in the Table-1.

Determination of Rate of Imbibition in relation to Water loss and Weight of the Individual seeds (After stresses) :

Twelve seeds were selected for this experiment, which were divided into 4 groups of 3 seeds each and each group was subjected to the different kinds of mechanical stresses.

Table 1 : Percentage of germination in stressed seeds of Cassia fistula L. in different kinds of samples.

Sl. No.	Kinds of samples	No. of Replicates with stressed seeds	No. of seeds germinated	Percentage of germination
1.	Distilled water	1. 1H + 2R 3* + 4N	1H 3*	25% (*)
		2. 1H + 2R 3* + 4N	3*	
2.	0.5% NaCl	1. 1H + 2R 3* + 4N	1H 3*	25% (B), 25% (*)
		2. 1H + 2R 3* + 4N	1H 3*	
3.	1% NaCl	1. 1H + 2R 3* + 4N	1H 3*	25% (B), 25% (*)
		2. 1H + 2R 3* + 4N	1H 3*	
4.	1% Alkaline	1. 1H + 2R 3* + 4N	Nil	12.5% (*)
		2. 1H + 2R 3* + 4N	3*	
5.	1% HCl	1. 1H + 2R 3* + 4N	Nil	Nil
		2. 1H + 2R 3* + 4N	Nil	
6.	3% HCl	1. 1H + 2R 3* + 4N	Nil	Nil
		2. 1H + 2R 3* + 4N	Nil	

Table 2 : Quantitative analysis of Rate of Imbibition in relation to water loss in Cassia fistula L. seeds (after different stresses)

Kinds of treatments	Dry Wt., of Ind. seeds (in mg/seed)			Ind. Wt. of the seeds after 29hr. imb. in different samples			Differences of Wt. (in mg/seed)			Rate of Imb.(in %) (in mg/seed)		
	1	2	3	1% NaCl	0.5% NaCl	1% HCl	1	2	3	1	2	3
1. *(Cracked and Punctured)	248	252	190	400	490	470	(+) 152	(+) 238	(+) 280	(+) 61.3	(+) 94.4	(+) 147
2. H (45°C/5 min.)	170	188	170	152	170	168	(−) 18	(−) 18	(−) 2	(−) 10.6	(−) 2.6	(−) 1.7
3. R (45°C/5 min.)	152	190	172	168	200	195	(+) 16	(+) 10	(+) 23	(+) 10.52	(+) 5.26	(+) 1.33
4. N (normal)	170	190	172	170	202	172	0	12	0	Nil	(+) 6.31	Nil

Abbrev. : Imbn. : —Imbibition
Ind. : —Individual

Note : '*' Cracking and puncturing on each side of the seed at the depth of 1cm.
'H' (45°C/5 min.): —Seeds treated with hot water at 45°C for 5 minutes.
'R' (45°C/5 min.): —Seeds roasted at 45°C for 5 minutes
'N' (Normal): —Seeds without stress.

Table 3 : Percentage of Germination on cracked and punctured seeds

Sl. No.	Kinds of treatments	Kinds of samples	No. of seeds per petridish	No. of seeds germinated	Percentage of germination
1.	Cracked and Punctured	Distilled water	10	8	80
2.	-do-	0.5% NaCl	10	9	90
3.	-do-	1% NaCl	10	9	90

The first three sets were subjected to three types of stresses. The fourth one served as control without any stresses as described above. Dry weight of all seeds was taken before and after the treatments with different stresses. Differences in weight and percentage rate of imbibition were calculated as shown in the Table-2.

Determination of Germination Percentage through Cracked and Punctured seeds :

For this thirty seeds were cracked and punctured. After this kind of stress, these were divided into 3 groups of 10 seeds each, and subjected to pre-treatments such as surface sterilization with 0.1% $HgCl_2$, pre-soaking with 0.5% and 1% NaCl, respectively. Soaking with distilled water served as control. Finally these were allowed to germinate on Whatman No. 1 paper as described above.

RESULTS AND DISCUSSION

Cracked and punctured seeds completed their imbibition in 24 hr. in all media but germinated only in distilled water, 0.5 and 1% NaCl within 16 hr. immediately after imbibition and distorted in 1% and 3% HCl. Hot seeds took long time for imbibition and germination. These seeds were attacked and killed easily by the fungus before and after the initiation of germination. However, it was free from 1% Alkaline medium. Roasted seeds took still longer time than that of cracked and punctured and hot seeds, and were killed by fungal attack before initiation of germination (Plate 1b). No germination was observed in normal seeds as there was no uptake of water within this short period of imbibition. At first the particular species of these fungus starts their life cycle in viable seeds and extending into non-viable seeds.

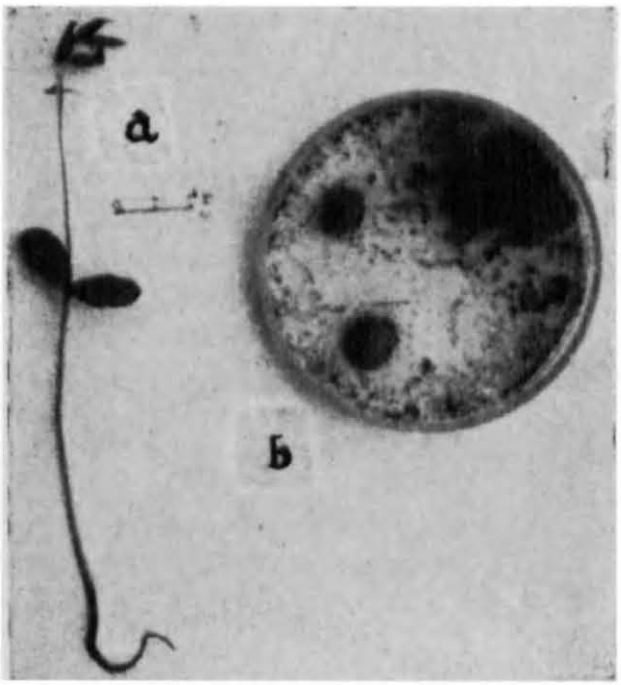

Plate 1 : (a) Seedling of cracked and punctured (1% NaCl), showing its fungus free tolerance (6 days after transplantation). (b) Consuming of Roasted seed by the growth of fungus. (deposited in the seedling Herbarium of P. P. & B. Section, C.B.L., BSI.)

Seedlings obtained from cracked and punctured seeds both in distilled water as well as in 0.5 and 1% NaCl produced no differences and abnormalities although establishment and tolerance capacity was somewhat poor in the seedlings from distilled water when compared to seedlings from 0.5 and 1% NaCl (Plate 1a-b). Hence it may be concluded that 0.5 and 1% NaCl acts as a suitable

co-factor to enhance quick germination and also facilitates fungal free populations from cracked and punctured seeds. Rate of imbibition was high in cracked and punctured seeds instead of their weight and water loss enhanced by the stress. It also helps the seeds to face surrounding with high water potential during imbibition and the water uptake takes place continuously since the protrution of primary radicle. Reversably the stress of hot water increased the water potential of seeds at the time of treatment. So those seeds regulate disorbption up to 28 hr. to neutralize the increased water potential with its surrounding of low water potential. Again imbibition starts after 42 hr. disorbption. Generally roasted seeds and normal seeds shows low rate of imbibition or totally nil due to its 90% impermeable seed coat as it didn't absorb water

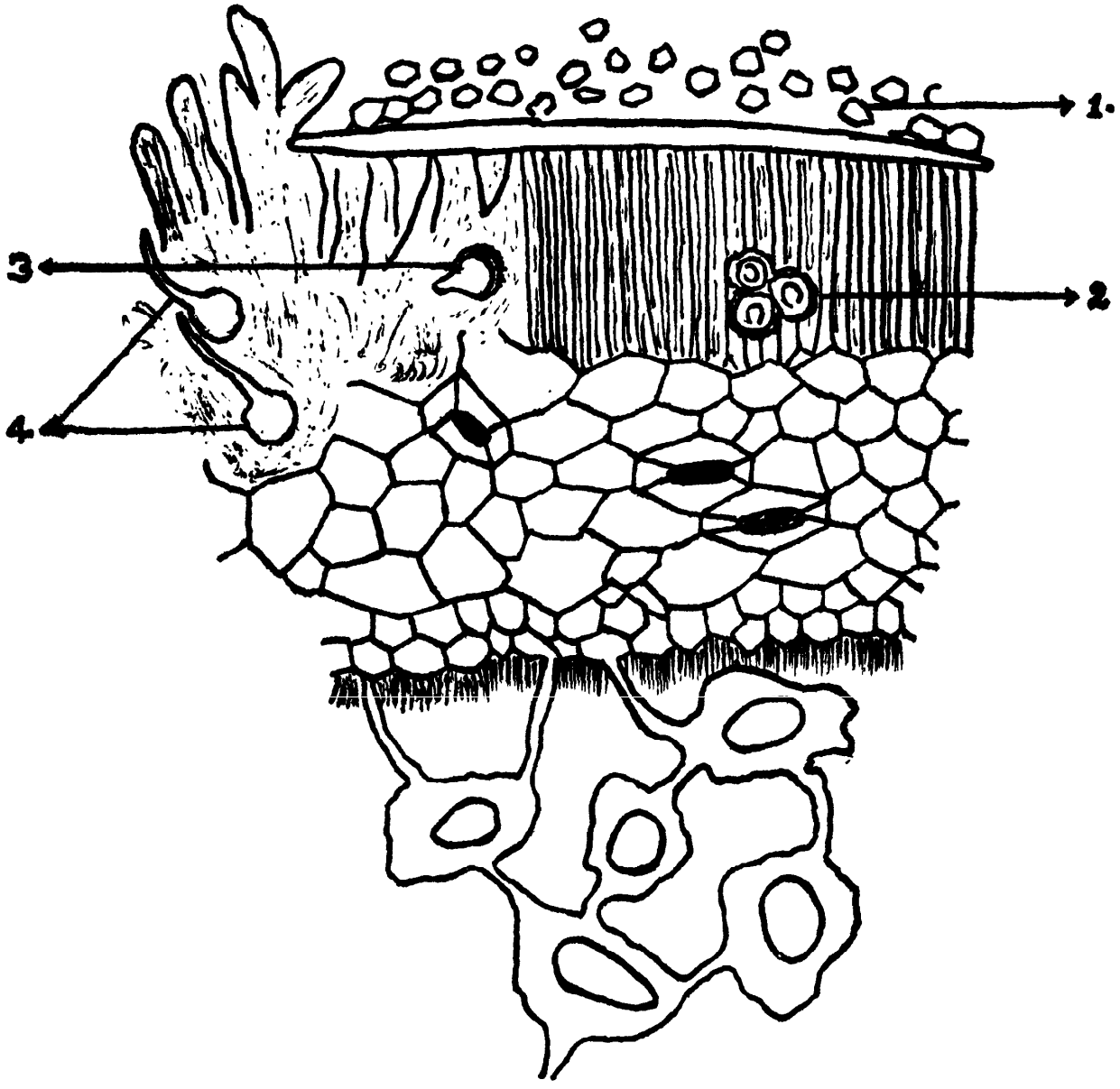

Fig. 1. C. S. of *Cassia fistula* L. seed (treated with Hot, after 29 hr. imbibition.) A Portion enlarged under 15x×45x. 1. Brokened epidermal cells. 2. Resting stage fungus spores located between the cells of tight fibrous hypodermal layer. 3 & 4. Begining and Later stage of germinating spores in the matrix of gelatinous substances released by the brokened hypodermal and cortical cells of seed coat.

within a short period of imbibition. Rarely some normal seeds may also absorb water within a short period of imbibition in 0.5 and 1% NaCl due to the presence of a small water-percentage and a large absorptive capacity in their embryonic tissues.

80 to 90% germination was observed in all media within 45 hr. (on seeds those were applied with the stress of cracking and puncturing only). Eight seeds among 10 were in distilled water and nine among 10 seeds each both in 0.5 and 1% NaCl moisture media as shown in the Table-3. The remaining 10-20% nongerminated seeds may be due to their health and imbalanced water content of embryonic tissue, and also due to the error of stress.

Breaking of dormancy in *Cassia fistula* seeds by concentrated sulphuric acid (H_2SO_4) and the concerned abnormalities of seedlings related to the particular species of fungal attack had been described by Randhawa *et al.* 1986. Scope of this present paper is getting seedlings without any abnormalities in the growth and development of scuttellum and also to produce seedling free from fungal attack. The significance of fungal attack fully depends upon the period of imbibition and the release off gelatinous substances in seeds. There was no fungal attack on cracked and punctured seeds because of their quickest periodicity of imbibition, and germination. Whereas in the hot and roasted seeds, the resting stage of fungus spores (which are from the seed borne fungal mycoflora) attained full growth before the initiation of radicle because of their delayed imbibition and germination. Normal seeds never killed by these fungi even though it was located in between the hot and roasted seeds which are fully occupied by the densed fungal mycelium, why because in the normal seeds gelatinous substances (chemincal nature unknown) not released (by the seed coat) within short period of imbibition. However, it may be concluded that the suitable environment, *i.e.*, the gelatinous substances released by the long time imbibing seeds facilitates to fungus spore germination (Fig. 1).

ACKNOWLEDGEMENTS

Thanks are due the Director, Botanical Survey of India for facilities, Dr. A. K. Sarkar, Central Botanical Laboratory for encouragements and I am also grateful to Dr. S. L. Gupta for critically going through the manuscript.

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