

Macro-micro morphological and dormancy breaking studies in *Cassia fistula* L.

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ABSTRACT

An investigation was carried out to study the morphological characteristics of fruit and seeds of *Cassia fistula*. This study characterized and compared various fruit and seed morphological traits, *i.e.*, pod length (cm), pod thickness (cm), pod weight (g), locule distance in pods (mm), no. of seeds/pod, wt. of pod residue/pod, wt. of pulp/pod, seed weight/pod (g), single seed weight (g), 100 seed weight (g), seed length (mm) and seed width (mm). As *Cassia fistula* has the hard seed coat, presowing treatments were studied to enhance the seed germination. The experiment was laid out in Completely Randomized Design (CRD) with three replications. There were nine treatments, *viz.*, T1 - Conc. Sulphuric acid (5 min.) + 12 hours water soaking, T2 - Conc. Sulphuric acid (5 min.) + 24 hours water soaking, T3 - Conc. Sulphuric acid (10 min.) + 12 hours water soaking, T4 - Conc. Sulphuric acid (10 min.) + 24 hours water soaking, T5 - Hot water + 12 hours water soaking, T6 - Hot water + 24 hours water soaking, T7 - Water soaking (12 hours), T8 - Water soaking (24 hours), T9 - Control (Without treatment). The observations were recorded for the seedling characters like days for germination (Days), germination percentage (%), no. of leaves, root length (cm), shoot length (cm), vigour index. The pre-sowing treatment of seeds in concentrated sulphuric acid (5 or 10 min) followed by water soaking (24 hrs) was the most effective for inducing better seed germination in *Cassia fistula*.

KEYWORDS

Morphology, fruit and seed, dormancy, pre-sowing treatment, hard seed coat, Sulphuric acid, germination

INTRODUCTION

Cassia fistula Linn., is an ornamental and medicinally used deciduous tree commonly called “Golden shower tree” and Indian Laburnum”. It is of the large genus *Cassia* and it belongs to the subfamily Caesalpinaceae and the family Fabaceae (Sartorelli *et al.*, 2003). Its origin is in the Indian subcontinent and is distributed in various tropical regions including Asia, South America, Australia and Africa (Orwa *et al.*, 2009). It is widely used for landscaping not only because of its golden yellow flowers which last for several months, but it also has landscaping properties like drought tolerance and low maintenance requirement (Okusanya *et al.*, 2015). It flowers nearly all year round (August - June) and produces enormous amounts of seeds that do not germinate readily in nature due to impermeable seed coat. The present study was aimed to evaluate the “Macro-micro morphological and dormancy studies in *Cassia fistula*”.

MATERIALS AND METHODS

FRUIT COLLECTION

An extensive survey at SRM Institute of Science and Technology, Kattankulathur was made and mature, phenotypically superior or healthy *Cassia fistula* trees of reasonably good form and average growth were marked for seed collection. The times of seed development and maturation were ascertained through consultation of literature and by frequent field visits. A total of 100 brown to black mature fruits pods were collected directly from superior trees (Plate 1).

MORPHOMETRIC PARAMETERS OF FRUIT/POD

The fruits/pods were studied for the pod length, pod girth, pod weight, no of seeds/pod, locule distance and residual pod mass. After recovery of seeds, residual pod mass (Pericarp) was weighed in gram (g). Locule distance inside the fruit was measured under stereo zoom microscope in millimeter (mm).

MORPHOMETRIC PARAMETERS OF SEED

Extraction of seeds was done manually immediately after the collection of pods. The pods were dried under sunlight (35–38°C) for 2 days, and seeds were extracted by splitting the pods. Extracted seeds were dried in shade, cleaned and packed in polyethylene bags for the detailed study of seed characteristics. The shape, color, and size of the seeds were observed and length, width, thickness of the seeds were measured in millimeter (mm) under stereo zoom microscope. No of seeds per pod, Single seed weight (g), 100 seed weight (g) were also measured. To follow a general pattern of seed weight distribution, 100 randomly selected seeds were weighed individually. Seeds were imbibed for a 24 hours and then longitudinally sectioned using a cutting blade to check if the embryo was fully developed at seed maturity. Presence (or not) of mucilage, seed coat structures, and embryo development of *C. fistula* seeds were also evaluated under a stereozoom microscope.

PRESOWING SEED TREATMENT

Uniform sized seeds of *Cassia fistula* were used in the experiment to study the presowing dormancy breaking treatment. The experiment was laid out in Completely Randomized Design (CRD) with three replications. There were nine treatments, viz., T₁- Conc. Sulphuric acid (5 min.) + 12 hours water soaking, T₂ - Conc. Sulphuric acid (5 min.) + 24 hours water soaking, T₃ - Conc. Sulphuric acid (10 min.) + 12 hours water soaking, T₄ - Conc. Sulphuric acid (10 min.) + 24 hours water soaking, T₅ - Hot water + 12 hours water soaking, T₆ - Hot water + 24 hours water soaking, T₇ - Water soaking (12 hours), T₈ - Water soaking (24 hours), T₉ - Control (Without treatment). The observations were recorded for the seedling characters like days for germination (Days), germination percentage (%), no. of leaves, root length (cm), shoot length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g), dry root weight (g) and vigour index. The data obtained from the experiments were analysed by the 'F' test for significance following the methods described by Panse & Sukhatme (1985).

SEED SOWING AND WEED CONTROL

The pre-treated seeds were sown in polybags filled with sand: soil: FYM in the ratio of (1:2:1). One seed was sown in each polythene bag; the sowing depth was 2 cm. There was no serious weed problem as the experiment lasted, however, the few weeds that germinated with the seeds were removed by hand pulling.

RESULTS AND DISCUSSION

MORPHOMETRIC PARAMETERS OF FRUIT/POD

Fruit of *Cassia fistula* is an indehiscent pod, cylindrical, pendulous, terete, smooth, green when young and black after ripening, containing 25-100 **seeds** embedded in a dark black coloured sweetish pulp and separated by transverse partitions (Table 1 & Fig. 1, 2, 3 & 4). The morphology description of fruit/pod structure of *Cassia fistula* agrees with the work of Sanyal, 2015 and another study on species of Cassia genus (Sahai *et al*, 1997).

Morphometric characters of fruit



Fig. 1 Fruit of *Cassia fistula*



Fig. 2 Microscopic view of locules with seed and pulp



Fig. 3 Microscopic view of locules with pulp



Fig. 4 Pulp

MORPHOMETRIC PARAMETERS OF SEED

The seeds are ellipsoid, 8-9 mm long, myxospermous (Rodrigues-Junior *et al.*, 2020), glossy, light brown in colour (Fig. 5 & 6). Mucilage in myxospermous seeds expands upon hydration and flake off upon drying (Fig.8). The whole seed coat had several cracks but only in the mucilaginous layer. Under moist conditions, sticky mucilage expands and increase the seed volume (Table & Fig. 9). The presence of hard, water impermeable seed coat is the reason for physical dormancy which control of seed germination in this genus. This coat-imposing dormancy is related only to the water impermeability of the testa, and the embryo is ready to start germination when the water enters through the water gaps located on the seed coat (Rodrigues-Junior *et al.*, 200; Lewis, 2005 and Baskin *et al.*, 2000). Myxospermous nature of *Cassia fistula* showing the release of mucilage in the myxospermous seeds of *C. fistula* was reported by Rodrigues-Junior *et al.*, 2020 and also stated that Myxospermous seeds came out of dormancy faster than non-myxospermous. Thick endosperm surrounding the fully developed embryo was observed during the longitudinal sections of the seeds (Fig. 7). Seeds had a hilar region composed of a narrow lens, hilum, and a tiny micropyle. The micrometric description of the seeds are in line with the Orwa *et al.*, (2009) and Bosch (2007).



Fig. 5 Dry seeds of *Cassia fistula*



Fig. 6 Microscopic view of Seeds



Fig. 7 Microscopic view of dry papery layer on seed



Fig. 8 Microscopic view of Myxospermous nature of *Cassia fistula*



Fig. 9 Microscopic view of Embryo development in imbibed seeds

PRESOWING SEED TREATMENT

Pre-sowing treatment breaks the dormancy and enhances rapid and uniform germination of seeds. There are several methods of pretreating seeds, but knowledge of a few simple techniques is sufficient to get reasonable germination of almost all species. To determine the optimum pretreatment methods for inducing better seed germination, the present study was conducted and the results were discussed below.

Statistically significant differences were observed among the various dormancy breaking seed treatments for the germination percentage (Fig.10). Soaking the seeds in Conc. Sulphuric acid (10 min.) followed by 12 hours of water soaking (T_3) resulted in higher seed germination percentage (83.33 per cent) and was significantly superior to all other treatments. The second highest germination percentage was observed in Conc. Sulphuric acid (10 min.) + 24 hours water soaking (T_4) (79.66 per cent). The untreated seeds does not germinate (T_0).



Fig. 10 Stages of seedling growth

This is in agreement with the results of Chauhan and Johnson (2009), which reported that nonscarified seeds of the leguminous trees did not reveal any imbibition or germination as a result of the hard seed coat. Mechanical abrasion or chemical degradation of the seed coverings and submersion of the seed in hot water are the most common practices to induce seeds with physical dormancy to imbibe water (Geneve, 2003). Myxospermous seeds come out of dormancy faster than non-myxospermous ones (Rodrigues-Junior *et al.*, 2020). The unabrated seeds of *C. fistula* didn't germinate for quite long time (Soliman & Abbas, 2012; Babaloba *et al.*, 2014).

The earliest germination (13.63 days) was observed in the seeds soaked in Conc. Sulphuric acid (10 min.) + 24 hours water soaking (T₄), followed by soaking in Conc. Sulphuric acid (10 min.) + 12 hours water soaking (T₃) which took 14.36 days.

Analysis of variance in the present study revealed significant difference in shoot length, root length, stem girth, number of leaves, vigour index, fresh biomass and dry biomass over time (P=0.05). The seed treatment with Conc. Sulphuric acid (10 min.) + 12 hours water soaking resulted better seedling characteristics *viz.*, stem length (35.100 cm), root length (35.633 cm), no of leaves (9.667), stem girth (2.200 cm), fresh shoot weight (13.133 g), fresh root weight (6.800 g), dry shoot weight (5.423 g), dry root weight (5.423 g), and vigour index (5894.39).

The efficiency of the sulphuric acid to raise the integumentary inhibition in forest, agro-forestry and pastoral species had been demonstrated by several researchers (Saied *et al.*, 2008; Tanaka-Oda *et al.*, 2009; Zare *et al.*, 2011). However, a prolonged treatment of seeds in the acid can damage the embryo and reduce germinal performances (Kestring *et al.*, 2009). The optimal duration of soaking is proportional to the coat rigidity. Indeed, in this study, the seeds of *Cassia fistula* seems very sensitive to the increase of the duration of soaking in acid. The soaking time in sulphuric acid depends on the thickness and on the solidity of the coat. Our results are in agreement with this conclusion, revealing that the use of sulphuric acid allowed increasing germination percentage and seedling growth on *Cassia fistula*. The best method for breaking dormancy of *Canna indica* L. which resulted in an increased germination percentage to 95% and gave highly quality of golden shower seedlings is acid scarification for 4 hour (Fallah *et al.*, 2014). Concentrated sulphuric acid has been used for many years for softening of hard seed coats. (Hopkins, 1923).

It was observed that hot water treatment imposed for 12 and 24 hours in *Cassia fistula* also recorded the maximum seedling growth parameters. The highest number of leaves per seedling (5.33) was observed in the treatment *viz.*, Conc. Sulphuric acid (10 min.) + 12 hours water soaking (T₃) followed by Conc. Sulphuric acid (10 min.) + 24 hours water soaking (T₄). The treatment Conc. Sulphuric acid (10 min.) + 12 hours water soaking (T₃) recorded the highest shoot length (10.467 cm) as well as root length (14.00 cm). Conc. Sulphuric acid (10 min.) + 24 hours water soaking (T₄) recorded second higher shoot length (9.644 cm) and root length (15.96 cm). The vigour index was maximum for (T₄) hot - Conc. Sulphuric acid (10 min.) + 24 hours water soaking (2040.35), which

was significantly superior over other treatments (Fig.11). This was followed by Conc. Sulphuric acid (10 min.) + 12 hours water soaking (T₃), which recorded 2038.90.



Fig. 11. Seedling growth at 300 days after sowing

Soaking of seeds in hot water could help in enhancing the seed germination by softening the hard seed coat and facilitating leaching out of the germination inhibitors. According to Alamgir & Hossain (2005), hot water treatment can conquer physical dormancy in leguminous species.

The results are similar to the observation made in *Leucaena glauca*, where the boiling water significantly reduced the percentage of abnormal seedlings and dead seeds (Venkatraman, 1948). Sarker *et al.* (2000) also reported that steeping *Sesbania rostrata* seeds in boiling water for one minute showed the highest percentage of germination (62.63 %). Singh *et al.*, (1984) found that water soaking enhanced the seed germination in *Tephrosia purpurea* and *Abrus precatorius*. Sneh and Verma (1993) concluded that a seeds of *Grewia optiva* when treated with hot water for 24 hours resulted into higher germination (49.66%).

Studies by Ibrahim and Otegbeye (2004) showed that seed immersion in cold water improved the germination of some tropical trees. Owonubi *et al.* (2005) observed that soaking *Azadirachta indica* seeds in cold water for 1, 12 and 24 hours increased seed germination, implying that the seed coats of different species are differently permeable to water and gas (Owonubi *et al.*, 2005).

It is concluded from this study, that pre-sowing treatment of seeds in concentrated sulphuric acid (5 or 10 min) followed by water soaking (12 and 24 hrs) was the most effective for inducing better seed germination in *Cassia fistula*.

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Parameters	Average value (M±SE)
Pod length (cm)	82.33±1.00
Pod thickness (cm)	1.78±0.04
Pod weight (g)	152.00±4.05
Locule distance in pods (mm)	5.39±0.10
No. of seeds/pod	99.1±3.66
Wt. of pod residue/pod	53.3±2.37
Wt. of pulp/pod	80.95±2.72
Seed weight/pod (g)	18.65±0.89
Single seed weight (g)	0.20±0.00
100 seed weight (g)	17.71±0.80
Seed length (mm)	8.43±0.42
Seed width (mm)	6.18±0.38
Seed thickness (mm)	3.71±0.07

Table 1. Morphological characteristics of *Cassia fistula* fruit and seed

Trts.	Seed treatments	Germination percentage (%)	Days for germination (days)
T ₁	Conc. Sulphuric acid (5 min.) + 12 hours water soaking	77.333	19.970
T ₂	Conc. Sulphuric acid (5 min.) + 24 hours water soaking	72.000	17.394
T ₃	Conc. Sulphuric acid (10 min.) + 12 hours water soaking	83.333	14.364
T ₄	Conc. Sulphuric acid (10 min.) + 24 hours water soaking	79.667	13.636
T ₅	Hot water + 12 hours water soaking	56.000	23.212
T ₆	Hot water + 24 hours water soaking	55.333	24.000
T ₇	12 hours water soaking	77.333	44.091
T ₈	24 hours water soaking	33.000	45.636
T ₉	Control (Without treatment)	0.000	0.000
SE(d)		13.869	4.383
CD(0.05)		29.363	9.279

Table 2. Effect of presowing treatment on the seed germination of *Cassia fistula* (60 DAS)

Trts.	Seed treatments	No of Leaves	Stem Girth (cm)	Stem Length (cm)	Root Length (cm)	Fresh Shoot Weight (g)	Fresh Root Weight (g)	Dry Shoot Weight (g)	Dry Root Weight (g)	Vigour Index
T ₁	Conc. Sulphuric acid (5 min.) + 12 hours water soaking	7.333	1.633	32.267	34.300	11.600	5.833	4.353	2.467	5147.83
T ₂	Conc. Sulphuric acid (5 min.) + 24 hours water soaking	8.333	1.533	31.833	32.567	12.600	5.500	4.727	3.067	4636.80
T ₃	Conc. Sulphuric acid (10 min.) + 12 hours water soaking	9.667	2.200	35.100	35.633	13.133	6.800	5.423	3.120	5894.39
T ₄	Conc. Sulphuric acid (10 min.) + 24 hours water soaking	8.667	2.133	33.133	34.200	14.800	7.133	5.063	3.113	5364.22
T ₅	Hot water + 12 hours water soaking	5.000	1.267	27.567	26.400	8.300	5.600	3.843	2.337	3022.15
T ₆	Hot water + 24 hours water soaking	5.667	1.433	26.833	27.800	8.600	4.833	3.613	2.560	3023.01
T ₇	12 hours water soaking	3.000	1.300	26.033	23.667	6.367	6.367	3.257	2.193	3843.45
T ₈	24 hours water soaking	2.667	1.433	24.067	25.700	6.300	4.200	2.743	1.490	1642.31
T ₉	Control (Without treatment)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00
SE(d)		0.471	0.156	1.09	1.063	0.391	0.42	0.179	0.26	107.496
CD(0.05)		0.99	0.328	2.291	2.234	0.822	0.883	0.377	0.547	225.841

Table 3. Effect of presowing treatment on the seed germination of *Cassia fistula* (10 months of sowing)