

## Effects of Sulfuric Acid and Hot Water Pre-Treatments on Seed Germination and Seedlings Growth of *Cassia fistula* L.

Amira Sh. Soliman and Mohamed S. Abbas

Department of Natural Resources,  
Institute of African Research and Studies, Cairo University, 12613 Giza, Egypt

**Abstract:** This experiment is separated into two parts: dormancy breaking methods for *Cassia fistula* seeds using different pre-treatments and seedlings growth investigation during the two seasons of 2009/2010 and 2010/2011. The seeds were acid scarification by  $H_2SO_4$  (36N) for 2 minutes and hot water treatment (60, 80 and 100°C for 3, 6 and 9 minutes). Germination indices were determined. In the second part seedlings were kept in greenhouse to superior quality seedlings from different treatments and after 60 days growth parameters were also recorded in both seasons. The results showed that acid scarification for 2 minutes and then soaking in hot water at 100°C for 6 minutes was the best method for breaking dormancy of *Cassia fistula* which resulted in an increased germination percentage to 96% and gave highly quality of golden shower seedlings.

**Key words:** *Cassia fistula* • Sulfuric acid • Hot water • Germination indices • Growth parameters

### INTRODUCTION

*Cassia fistula* is an ornamental tree commonly known as the golden shower and it is one of the 400 different species that comprises the genus *Cassia* and belongs to the subfamily Caesalpiniaceae, of family Fabaceae or Leguminosae [1]. *C. fistula* a semi-wild Indian Labernum, native to India, the Amazon and Sri Lanka has become extensively diffused in various countries including Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil [2]. Some investigators reported the medicinal values of this plant such as antibacterial [3], antiparasitic [1], hypoglycemic [4], Antitumor [5], Antifertility [6] and antioxidant properties [7]. Also, *Cassia fistula* is an important source of naturally occurring bioactive compounds [8]. Seed dormancy is the most limiting factor for plant propagation. However, the blocking of water access into the seed is the most common cause of delay in seed germination [9]. *Cassia* spp. suffers from dormancy owing to the presence of water impermeable thick seed coat that prevents water and oxygen from reaching and activating the embryo, or because of the presence of germination-inhibitor chemical compounds and they require specific treatments for breaking

dormancy [10]. Hence, acid scarification ( $H_2SO_4$ ) for different periods was the most effective treatment in softening the seed coat of *Cassia* seeds [11, 12].

Therefore, the objective of this study was to use  $H_2SO_4$  but decrease its harmful effect for the embryo by decreasing the period that the seeds coat of *cassia fistula* exposed to it and interaction with hot water treatments to determine treatment that promote maximum germination and produce superior quality seedlings.

### MATERIALS AND METHODS

This study was conducted at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and Studies, Cairo University, Giza, Egypt.

**Plant Materials:** Pods of *Cassia fistula* were obtained from Faculty of Agriculture, Cairo University, Giza, Egypt, during the two seasons of 2009/2010 and 2010/2011. At the first of February, pods were crushed to release the seeds, which were tested for germination, in both seasons. To control fungal infection during germination, seeds were surface sterilized in 20% Clorox for ten minutes.

**Treatments and Growth Conditions:** The pre-sowing seed treatments used in the experiment were as follows:

- The seeds were scarified with H<sub>2</sub>SO<sub>4</sub> (36N) for two minutes. After that seeds were washed thoroughly in tap water to remove any trace of acid. Then the seeds were dropped into hot water at 60°C for 3 (T1), 6 (T2) and 9min (T3).
- The seeds were scarified with H<sub>2</sub>SO<sub>4</sub> (36N) for two minutes. After that seeds were washed thoroughly in tap water to remove any trace of acid. Then the seeds were dropped into hot water at 80°C for 3 (T4), 6 (T5) and 9 min (T6).
- The seeds were scarified with H<sub>2</sub>SO<sub>4</sub> (36N) for two minutes. After that seeds were washed thoroughly in tap water to remove any trace of acid. Then the seeds were dropped into a hot water at 100°C for 3 (T7), 6 (T8) and 9min (T9).

The hot water was changed when it cold. For the control, the seeds were sown without any treatment (T0). Treated seeds were planted in 9-cm sterile petri dishes in 5 replicates with 10 seeds for each replication lined with two Whatman No.1 filter papers and moistened with 10 ml of sterile distilled water to ensure adequate moisture for the seeds. Then petri dishes were covered with lids, but not sealed and were maintained in incubator (Memmert, Model, ICP 400-800, made in Germany) at 25/15°C day/night, 12/12 h. light/dark. Data on germination were recorded on daily basis for a period of two months from the date of sowing and the final germination percentage was calculated at the end of the experiment after 60 days. Germination was considered when the radical was visible [13].

**Measurements:** The experiment end at 1<sup>st</sup> April 2010 and 2011, in the two seasons, respectively, germination indices were determined: time to germinate as the time for the first germination to appear (T.G.), germination period as the time between the first germination and the end of germination (GP) according to Berrie [14], germination percentage was calculated according to the equation of ISTA [15]:

$$\text{Germination percentage} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds sown}} \times 100$$

Germination Speed (GS) was also calculated using the following Equation of Maguire [16]: Germination Speed (GS) = No. of seeds germinated/Days of first count +---+

No. of seeds germinated/days of final count. The seedling vigor index (SVI) was calculated according to Hangarter [17] following formula:

$$\text{The seedling vigor index (SVI)} = \frac{\text{Seedling length (cm)} \times \text{Germinated percentage}}{100}$$

**Seedlings Growth Conditions:** Under greenhouse conditions, seedlings (5 cm) were transplanted into plastic vials (8 cm) diameter, filled with a mixture of sand and clay (1:1) during the period of the experiment, (in the first and second seasons, respectively). The seedlings were harvested 60 days after cultivation and the following growth parameters; plant height (cm), root length (cm), number of leaves, total dry weight (g/plant) and leaf area (cm<sup>2</sup>) were recorded in both seasons.

**Statistical Analysis:** A randomized complete design was adopted for the study. There were ten treatments (including the control) with six replications for each treatment and ten seeds were tested for each replication. The data of the germination and the growth parameters were subjected to statistical analysis of variance. The means were compared using the "Least Significant Difference (L.S.D.)" test at the 5% level, as described by Little and Hills [18].

## RESULTS AND DISCUSSION

### Germination Indices

**Germination Percentage:** Generally, there was a significant increase in germination percentage as water temperature increased. There was significant difference ( $P \leq 0.05$ ) in germination percentage between different treatments. However, data in Table 1 and Fig. 1 indicated that the maximum germination percentages (96 and 92 %) were noticed in seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (100°C) for 6 minutes (T8) followed by seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (100°C) for 3 minutes (T7) which gave 91% and 88% without significant difference in first and second seasons, respectively. On the contrary, the minimum germination percentages 50% and 54% were recorded in control (T0) and seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (60°C) for 3 minutes (T1), respectively. The positive effects of H<sub>2</sub>SO<sub>4</sub> scarification and soaking in hot water on germination percentage of *C. fistula* was also found by Zarchini *et al.* [19] who found that the

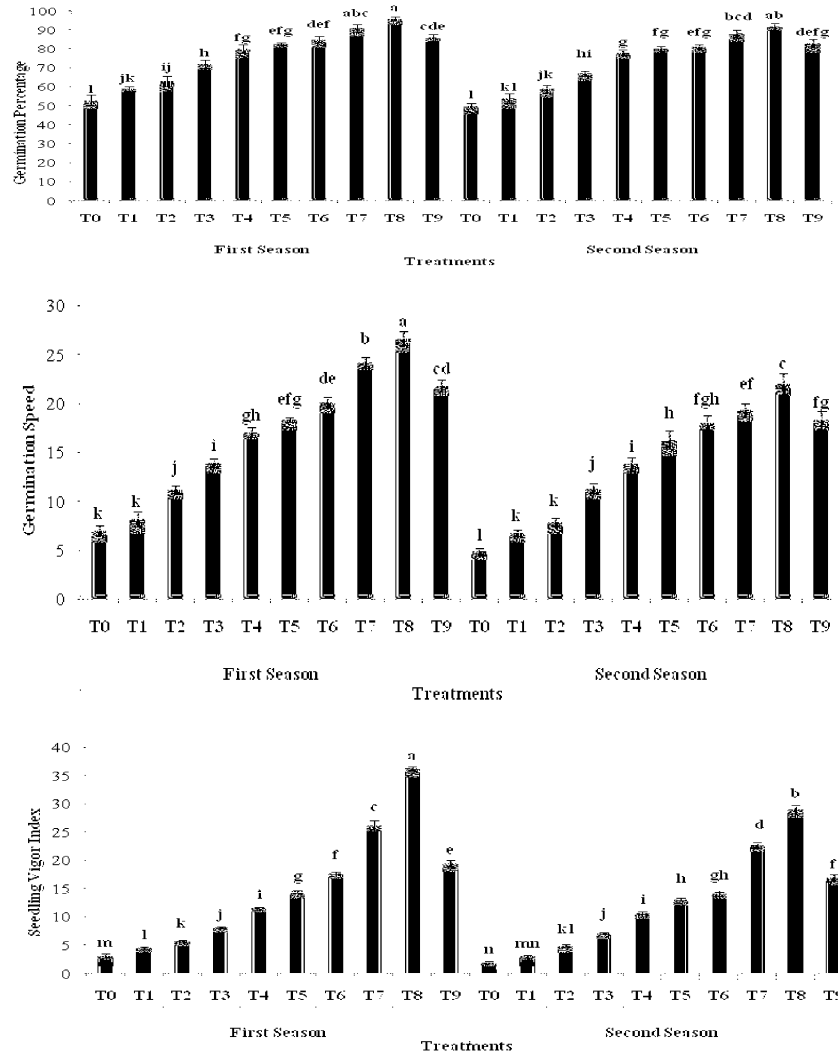


Fig. 1: Effect of different pre-treatments on germination indices of *Cassia fistula* during the two seasons of 2009/ 2010 and 2010/ 2011. Columns labeled with different letters are significantly different at  $P \leq 0.05$ . Vertical bars represent  $\pm SE$ .

Table 1: Effect of different pre-treatments on Germination indices of *Cassia fistula* during the two seasons of 2009/ 2010 and 2010/ 2011.

Treatments	Germination percentage		Time to germinate		Germination period		Germination speed		Seedling vigor index	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
T0	53.00	50.00	19.60	24.80	31.60	35.20	7.04	4.85	3.13	1.95
T1	59.00	54.00	15.60	23.60	27.20	31.60	8.19	6.80	4.50	3.01
T2	63.00	59.00	14.40	21.00	24.40	28.40	11.21	7.89	5.76	4.93
T3	72.00	67.00	12.00	19.60	22.00	26.40	13.96	11.30	8.12	7.10
T4	80.00	78.00	10.00	17.60	20.80	24.00	17.03	13.81	11.55	10.74
T5	82.60	80.00	8.40	15.80	18.80	23.20	18.37	16.28	14.44	13.10
T6	85.00	81.00	7.60	12.00	17.60	22.80	20.13	18.03	17.75	14.11
T7	91.00	88.00	4.40	7.60	14.00	19.20	24.22	19.32	26.24	22.73
T8	96.00	92.00	2.80	3.60	11.60	13.60	26.65	21.96	36.24	28.94
T9	86.00	83.00	7.20	9.20	15.60	22.00	21.81	18.34	19.51	16.95
Mean	76.76	73.20	10.20	15.48	20.36	24.64	16.86	13.86	14.72	12.36
LSD <sub>0.05</sub>	5.29	5.30	2.34	2.30	2.51	2.61	1.49	2.11	1.08	1.24

most germination rates and germination value in *Cycas revoluta* L. germinated seeds were obtained in seeds pretreated with hot water at 100°C for 1 h along with 25% sulfuric acid for 2 h. In addition, Al-Menaie *et al.* [12] who observed that treated seeds of *C. fistula* with H<sub>2</sub>SO<sub>4</sub> scarification followed by dropping in hot water at 50°C resulted in higher germination percentage. On other hand, Aduradola and Adejomo [20] reported that reduced germination percentage for *Erythronphleum suaveolens* seeds soaked in concentrated H<sub>2</sub>SO<sub>4</sub> and attributed it to probable destruction of the embryo by the acid.

**Time to Germinate:** Overall results showed significant differences at the 5% level among different treatments applied. There is a nearly negative relationship between increasing of water temperature and time to germinate of seeds from 24 days to 4 days after sowing. Similar, germination of *Cycas revoluta* L. seed improves after treatment with low concentration of sulfuric acid and hot water with high temperature [19]. However, data in Table 1 revealed that the faster treatment was (T8) 2.80 and 3.60 days after germinated in first and second seasons, respectively. Conversely, the later treatments were control (T0) and (T1) 24.80 and 23.60 days after germinated in the second season. Similar results had been reported by Negi *et al.* [21] who found that more than 95% seeds of *Cassia auriculata* germinated within 1 to 2 days in contrast to 2-3% when they were not treated with H<sub>2</sub>SO<sub>4</sub>. Further, Al-Menaie *et al.* [22] who reported that for *Cassia siamea* H<sub>2</sub>SO<sub>4</sub> scarification at 50°C for 24 hours recorded the highest germination percentage of 72 and for *Cassia roxburghii* H<sub>2</sub>SO<sub>4</sub> scarification at 21°C for 48 hours observed the highest germination percentage of 28 during daily observations for two months after sowing.

**Germination Period:** All tested factors, significantly affected at the 5% level on germination period. The difference between treatments was significant. However, careful observations of data in Table 1 showed that, the shorter germination period 11.60 and 13.60 days were noticed in seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (100°C) for 6 minutes (T8) follow by T7 and T9 in first season. On the contrary, the longer the period of germination 35.20 and 31.60 days was observed in control (T0) and treatment (T1) respectively, in the second season. In this respect, Olmez [23] indicated that the pre-treatment by submersion in sulphuric acid for 1 min should be used

to overcome dormancy of the *Hippophae rhamnoides* seeds. However, Gupta *et al.* [24] improved the seed germination when reported that pre-treated the *Glycyrrhiza glabra* seeds with concentrated H<sub>2</sub>SO<sub>4</sub> for five min improved the germination. Also, Bhuse *et al.* [25] reported that treating the seeds of *Cassia angustifolia* with H<sub>2</sub>SO<sub>4</sub> for 12 min gave highest germination of 72 per cent. On the other hand, Bharatkumar *et al.* [26] could not find any improvement in germination of *Catharanthus roseus* seeds due to pre-soaking treatments for different periods.

**Germination Speed:** Results in Table 1 and Fig. 1 indicated that, treatment with acid scarification and soaking in hot water had significant effect on germination speed at 5% level. However, the maximum germination speed 24.22, 26.65 and 21.81 seed/day were noticed in treatments T7, T8 and T9, respectively, in first season. Conversely, the minimum germination speed 4.85, 6.80 and 7.89 seed/day were recorded in treatments T0, T1 and T2, respectively in second season. This obtained result goes in line with those findings by Karaboon *et al.* [27] who concluded that the best efficiency method for breaking dormancy of *Cassia fistula* seeds was acid scarification method that soaking in concentrated sulphuric acid for 15 minutes. Also, Vivekmittar *et al.* [28] found that *Psoralea corylifolia* seeds recorded higher germination when treated with sulphuric acid for 5 minutes after followed by dipping in water for 4 h and again scarifying with concentrated H<sub>2</sub>SO<sub>4</sub> for 20 minutes.

**Seedling Vigor Index:** Data presented in Table 1 and illustrated in Fig. 1 showed that, significant differences at the 5% level among different treatments applied. However, the highest seedling vigor index (36.24) was recorded in seeds treated with T8 in the 1<sup>st</sup> season. While, the lowest seedling vigor index (3.01) was recorded in seeds treated with T1 in the 2<sup>nd</sup> season. The positive effects of acid scarification and soaking in hot water on seed germination of golden shower may be due to breaking of seed dormancy, improving of seed coat impermeability and increased imbibition of seeds. These results are in agreement with those obtained by Miranda *et al.* [29] who reported that sulfuric acid may create or enlarge pores in the seed, enabling water to enter the seed and directly contact the embryo and thus accelerate the germination process. Also, Babeley and Kandya [30] found that the increasing in germination percentage and production of vigorous seedlings was observed in *Cassia fistula* seeds when treated with

concentrated H<sub>2</sub>SO<sub>4</sub> for 45 to 90 minutes. Only one percent germination was recorded in control. Furthermore, Karaguzel *et al.* [31] reported that scarifying the seeds in concentrated sulfuric acid (36N) increased imbibition and improved germination, seedling vigor Index and growth characteristics of *Lupinus varius* (Leguminosae), as the duration of scarification time increased up to 16 h at 24°C. On the other hand, Okunlola *et al.* [32] found that mechanical scarification of the seeds of *Parkia biglobosa* may be effective for breaking dormancy and improving the seedling vigor.

### Growth Parameters

**Plant Height (cm):** Significant differences ( $P \leq 0.05$ ) were observed between various levels of sulfuric acid and hot water on plant height (Table 2 and Fig. 2). The results revealed that the tallest plants (28.82 and 37.76 cm) were obtained in seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (100°C) for 3 and 6 minutes respectively, in the 1<sup>st</sup> season. While, the shortest plants (3.92 and 5.58 cm) were obtained in control and seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (60°C) for 3 minutes in the 2<sup>nd</sup> season. Similar results were obtained by Agbogid *et al.* [33] who demonstrated that acid pre-treatment of *Dacryodes edulis* has a highly significant effect of improving seed viability and enhances seedling emergence and seedling growth. Furthermore, Gupta [34] reported that H<sub>2</sub>SO<sub>4</sub> treatment (up to 20%) increased the germination rate (84-86%) and resulted in better seedling survival (80%) in *Asparagus racemosus* when sown in field.

**Root Length (cm):** Root length was differed significantly ( $P \leq 0.05$ ) between various treatments of sulfuric acid and hot water. However, data in Table 2 and Fig. 2 revealed that the longest roots (25.52 and 30.04 cm) were recorded with seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (100°C) for 3 and 6 minutes, respectively, in the 1<sup>st</sup> season, while, the shortest roots (3.12 and 4.84 cm) were observed with control and seeds treated with H<sub>2</sub>SO<sub>4</sub> (36N) for 2 minutes and soaked in hot water (60°C) for 3 minutes in the 2<sup>nd</sup> season. These results are in agreement with those obtained by Anim-Kuapong and Teklehaimanot [35], who found that root length significantly affected by treatments and the longest root was recorded in seeds of *Albizia zygia* scarified by H<sub>2</sub>SO<sub>4</sub> for 5 minutes. While, Bharath [36] reported that significantly higher root length (5.47 cm), shoot length (7.86 cm), seedling

vigour index (949) and seedling dry weight (13.74 mg) was noticed at 25°C in germination of *Catharanthus roseus*.

**Number of Leaves:** Overall results showed significant differences at the 5% level among different treatments applied. Data in Table 2 revealed that the maximum number of leaves (37.40 and 40.20) were recorded with treatments T7 and T8, respectively, in the 1<sup>st</sup> season without significant differences between them, while, the minimum number of leaves was obtained with control treatment (7.80) followed by T1 (6.50) and T2 (11.60) in the 2<sup>nd</sup> season without significant differences between them. Supporting results were obtained by Mabundza *et al.* [37] indicated that scarification of seeds of *Tamarindus indica* L. with 95% H<sub>2</sub>SO<sub>4</sub> for 5 minutes enhanced germination of the seeds, number of leaves and seedlings height. Moreover, hard coat seed dormancy in *Cassia angustifolia* could come by treatment with commercial H<sub>2</sub>SO<sub>4</sub> at 100 ml per kg of seed for 10 minutes [38].

**Total Dry Weight (g/plant):** There was significant difference ( $P \leq 0.05$ ) in the total dry weight between different treatments as shown in Table 2 and Fig. 2. The maximum dry weight per plant (3.46, 3.70 and 3.38 g/plant) was recorded with treatments T7, T8 and T9, respectively, in the 1<sup>st</sup> season with significant differences between them, while, the minimum dry weight per plant (0.17, 0.49 and 0.82) was recorded with treatments T0, T1 and T2, respectively, in the 2<sup>nd</sup> season with significant differences between them. While, Khan *et al.* [39] reported that 2% effective microorganisms solution could be used for getting maximum seed germination and seedling development of *C. fistula* in terms of germination percentage, shoot, root lengths, vigor index and total dry weight. Furthermore, Gupta *et al.* [40] showed that different treatments like hot water treatment at 70°C for 10 minutes followed by concentrated H<sub>2</sub>SO<sub>4</sub> scarification for five minutes was most effective in breaking the dormancy which was imposed by seed coat in *Abutilon indicum*. However, Al-Helal *et al.* [41] found that seed treatment with H<sub>2</sub>SO<sub>4</sub> was more effective for removal of seed coat dormancy in Senna than mechanical or boiling water or incision of testa.

**Leaf Area (cm<sup>2</sup>):** All tested factors, significantly affected at the 5% level on leaf area. The difference between treatments was significant. Data in Table 2 showed

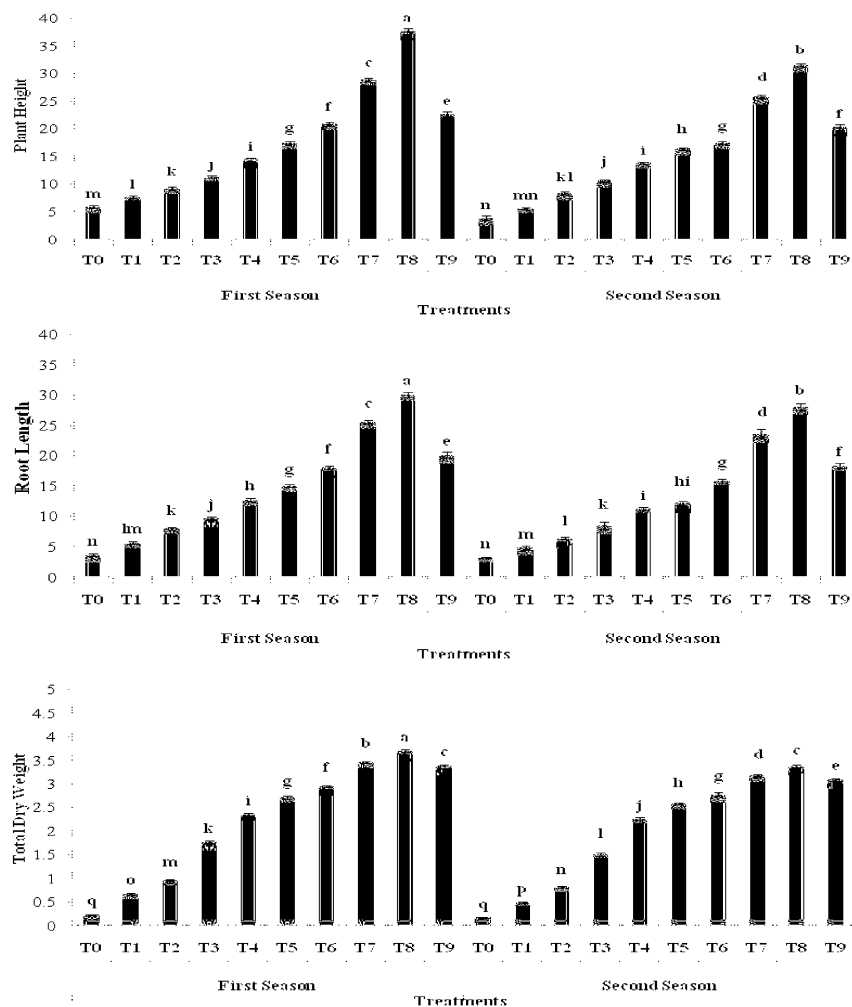


Fig. 2: Effect of different pre-treatments on germination parameters of *Cassia fistula* during the two seasons of 2009/ 2010 and 2010/ 2011. Columns labeled with different letters are significantly different at  $P \leq 0.05$ . Vertical bars represent  $\pm SE$ .

Table 2: Effects of different pre-treatments on growth parameters of *Cassia fistula* during the two seasons of 2009/ 2010 and 2010/ 2011.

Treatments	Plant height (cm)		Root length (cm)		Number of leaves		Total dry weight (g/plant)		Number of leaves	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
T0	5.86	3.92	3.60	3.12	9.20	7.80	0.23	0.17	18.80	15.60
T1	7.62	5.58	5.64	4.84	13.20	10.20	0.67	0.49	25.20	21.20
T2	9.18	8.34	8.00	6.32	16.60	11.60	0.96	0.82	38.80	30.20
T3	11.30	10.60	9.70	8.50	18.60	15.20	1.77	1.51	46.20	36.80
T4	14.46	13.78	12.68	11.20	20.00	18.60	2.35	2.26	52.60	46.20
T5	17.48	16.38	15.02	12.22	24.20	21.40	2.71	2.58	61.00	53.20
T6	20.90	17.40	18.00	15.80	28.80	22.60	2.95	2.77	72.80	67.20
T7	28.82	25.84	25.52	23.60	37.40	32.40	3.46	3.18	89.40	84.80
T8	37.76	31.44	30.04	28.04	40.20	38.20	3.70	3.37	112.60	100.20
T9	22.68	20.40	20.08	18.30	34.60	30.40	3.38	3.09	81.40	78.20
Mean	17.61	15.37	14.83	13.19	24.28	20.84	2.22	2.02	59.88	53.36
LSD <sub>0.05</sub>	0.94	0.83	0.89	1.19	4.19	4.21	0.06	0.08	5.65	6.95

that, the biggest leaf area (89.40 and 112.60 cm<sup>2</sup>) was recorded in T7 and T8, respectively, in the 1<sup>st</sup> season significant differences between them, while the smallest leaf area (15.60 and 21.20 cm<sup>2</sup>) was recorded in T0 and T1, respectively, in the 2<sup>nd</sup> second season significant differences between them. Similar results were reported by Joshi and Pant [42], who indicated that H<sub>2</sub>SO<sub>4</sub> scarification for 2 hours increased growth characteristics of *Canna indica*. However, Mehta and Sen [43] reported that seeds of *Cassia italica* exhibited seed dormancy and pre-treatment with concentrated H<sub>2</sub>SO<sub>4</sub> and mechanical scarification improved germination. Also, treating the seeds of *Rauvolfia serpentina* with hot water (80°C for 5min) and then cooling down to room temperature produced highest germination percentage and better seedling vigour index [44]. On other hand, Verma *et al.* [45] showed that invigoration studies in *Withania somnifera* seeds pretreated with 100 ppm GA<sub>3</sub> resulted in vigorous growth of seedlings under laboratory conditions

### CONCLUSION

It can be concluded that the best method for breaking dormancy of *Cassia fistula* which resulted in an increased germination percentage to 96% and gave highly quality of golden shower seedlings is acid scarification for 2 minutes and then soaking in hot water at 100 °C for 6 minutes.

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