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## Effect of seed pre-treatment with different concentrations of gibberellic acid (GA<sub>3</sub>) on seed germination and seedling growth of *Cassia fistula* L.

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### Abstract

An experiment was conducted during the year 2015-16 at Biotechnology-cum- tissue culture centre, OUAT, Bhubaneswar, India to study the effect of GA<sub>3</sub> pre treatment (i.e. 700, 710, 720, 730, 740, 750, 760, 770,780,790,800 ppm) respectively and a control (without any treatment) on seed germination and seedling growth in *Cassia fistula* L. seeds. Seeds treated with GA<sub>3</sub> at 760 ppm significantly enhanced the germination percentage (56.66%), mean daily germination (3.77), peak value (12.75), germination value (49.60), plant height (30.00 cm), number of leaves (15.66), root length (24.66 cm). Fresh biomass (1.32 g) and dry biomass (0.52 g) at 45 DAS. Hence it may be concluded that seeds pre-treated with GA<sub>3</sub> at 760 ppm play an important for obtaining better quality seedlings of *Cassia fistula* L.

**Keywords:** *Cassia fistula*, GA<sub>3</sub>, germination, seedling

### Introduction

India is endowed with a treasure house of natural flora and fauna. It is estimated that there are more than 45,000 plant species in India. It is estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine (Danish *et al.*, 2011) [4]. Among these 866 species are considered to be the potential medicinal plants in Indian trade. India officially recognizes over 3000 plants for their medicinal value. World health Organization reported that the traditional medical practitioners treat about 85 per cent of patients in India (WHO survey, 1993), which shows the significant valuable relation between local people with medicinal plants. The WHO estimated that 80 per cent of the population of developing and under developed countries depends on traditional medicines. Among which India's contribution is 15-20 per cent and in India about 2000 drugs used are of plant origin. Demand for medicinal plants is increasing because of having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor. A numbers of modern drugs have been isolated from natural sources and many of these isolations were used based on the nature of illness in traditional medicine (Nayak, 2013) [15]. Traditional systems of medicine have become a topic of global importance. Current estimates from the WHO suggest that, in many developing countries, a large proportion of populations rely heavily on traditional practices. *Cassia fistula* L. is a member of the family Fabaceae known for its characteristic bunches of beautiful yellow flowers and grows throughout India. The tree is a native plant of India, naturalized in Africa, West Indies and South America. It has attained importance as an ornamental and avenue plant (Arora, 1988) [1]. This species has been introduced in different plantation programs like agro-forestry, community forestry, social forestry, village and farm forestry in different parts of Bangladesh (Khan *et al.*, 2006) [11]. It is a fast-growing tree which reaches 30 to 40 feet in height. The well-spaced branches are clothed with pinnately compound leaves, with leaflets up to eight inches long and 2.5 inches wide. These leaves will drop from the tree for a short period of time and are quickly replaced by new leaves. In summer, Golden-Shower is decorated with thick clusters of showy yellow blooms which cover the slightly drooping branches. The blooms are followed by the production of two-foot-long, dark brown, cylindrical pods which persist on the tree (Gilman and Watson, 1993) [7]. Its leaves, stem, bark and pods having antibacterial and antifungal activity (Duraipandiyam and Ignacimuthu, 2007) [6]. The pods of *C. fistula* L. are straight cylindrical pods, 20-60 cm long and 1.5-2 cm in diameter.

The pod is dark green when young, turning dark brown to black when mature. The ripe pod contains dark color sweetish pulp and numerous yellowish-brown seeds. The pods should be collected when ripe and carefully dried. The best pods are those which do not rattle when shaken. These possess the most pulp. The pulp of ripe pods and mature leaves are known to have laxative drug property (Gritsanapan, 2010) [8]. The leaf extract *C. fistula* has potent mosquito larvicidal property (Mehdi *et al.*, 2011) [14]. *Cassia fistula* bark extracts have good correlations with the therapeutic use of its bark in the treatment of inflammatory conditions by practitioners of Ayurvedic system of medicine. Plants which belong to Fabaceae family are rich in flavonoids and bio flavonoids are known for their anti-inflammatory and antioxidant activities (Ilavarasan *et al.*, 2005) [10]. The high exploitation is generally not counter balanced by propagation efforts, hence that decreased the *Cassia fistula* L. population. Seed dormancy is the most limiting factor for the plant propagation. However, the blocking of water access into the seed is the most common cause of delay in seed germination (Ballard, 1973) [2]. *Cassia* sps. 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 (Ramamurthy *et al.*, 2005). Keeping in view of the importance of the crop and its propagation by seeds, the present experiment was designed with the objective to identify the proper concentration of GA<sub>3</sub> for seed pre-treatments effects on seed germination and seedling growth of *Cassia fistula* L.

### Materials and Methods

The investigation was carried out at the nursery of Biotechnology-cum-Tissue Culture Centre, OUAT, Bhubaneswar, Odisha, India during the year 2015-16. Seeds were collected from candidate plus trees identified in Bhubaneswar during the month of July 2015. The nursery area is located at 20° 15' North latitude 85° 52' East longitudes and at the altitude of 25.9 meters above mean sea level. It experiences typical tropical weather conditions and succumbs to the heat and cold waves that sweep in from north India. The summer months from March to May are hot and humid, and temperatures often shoot past 45° C in May. The south west monsoon lashes Odisha and in June, July and August receive, the maximum rainfall, which may average over 220 mm a month. Pleasant weather conditions prevail during November in Bhubaneswar, but December and January face the chilly winds from the North and North-east at average speeds of 7 miles/hour. Temperatures drop to approximately 15° C during these months. The seeds were then pre-treated with GA<sub>3</sub> of different concentration separately each for 24 hours as per the treatments (i.e. 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 ppm) respectively and a control (without any treatment). Seeds then were sown in nursery poly pot prepared with soil, sand and FYM planting mixture of ratio 2:1:1, at the rate of 10 seeds per replication. Seeds were monitored for 15 days, observations basing on the number of seeds germinated, the different parameters have been shown here following under mentioned methodology was calculated

### Germination percentage

Germination percentages were calculated as the number of seed sown and number of seeds germinated express in percentage.

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

### Mean daily germination (MDG)

Mean daily germination was calculated as the average daily germination (ADG) or per day germination and was calculated (Czabator, 1962) [3].

$$\text{MDG} = \frac{X}{N}$$

X=Final germination percentage

N=Number of days in the standard germination test.

### Peak value

Peak value was calculated as the maximum MDG reached at any time during the period of the test (Czabator, 1962) [3].

$$\text{PV} = \frac{\text{Final germination percentage}}{\text{No. of days required to reach the peak value of germination}}$$

### Germination Value

Germination value is an index combining speed and completeness of seed germination. Daily germination counts were recorded and G V was calculated (Czabator, 1962) [3].

Where GV= PV×MDG

PV=Peak value of germination.

MDG= Mean daily germination.

GV= Germination value.

Seeds were monitored for 45 days of sowing for seedling growth to record plant height (30 DAS/45 DAS) i.e., Number of branches, Number of leaves (30 DAS/45 DAS), root length (cm); fresh biomass (g) and dry biomass (g) were recorded. Randomly five seedlings from each replication were marked for observation. The experiment was designed in completely randomized design (CRD) and replicated thrice. The data observed were subjected to statistical analysis (Panse and Sukhatme, 1967) [17]. The data were transferred from where ever required before suitability of ANOVA analyzed in statistical package SAS version 7.0.

### Results and Discussion

The result obtained during the present course of investigation was carried out to visualize a significant influence of different concentration of GA<sub>3</sub> on seed germination of *Cassia fistula* seeds and seedling growth. The increase in the concentration were increased the rate of germination and then there was a decline. Mean value of germination percentage showed maximum (56.66%) in 760 ppm GA<sub>3</sub> with concentration where as lowest germination percentage (10.00%) was recorded in control seeds. The increased germination percentage in GA<sub>3</sub> treated seeds might be attributed to fact that the GA<sub>3</sub> helps in breaking the seed dormancy which results in early and enhanced seed germination due to the diffusion of endogenous auxin and gibberellins like substances (Gurung *et al.*, 2014; Singh *et al.*, 2016) [9]. GA<sub>3</sub> enhanced seed germination, because it might have antagonized the effect of inhibitors present in aonla seeds reported by Kumari *et al.*, 2007 [12]. The increase in germination percentage with GA<sub>3</sub> might be due to involvement of GA<sub>3</sub> in the activation of cytological enzymes along with increase in GA<sub>3</sub> cell wall plasticity and better water absorption (Padma *et al.*, 2013) [16]. All GA<sub>3</sub> treatments were effective for increasing seed germination when

compared with the control. GA<sub>3</sub> caused stimulate germination power, relative growth rate and earlier beginning production with germination time. The mean value of mean daily germination of seeds was maximum (3.77) in seeds at GA<sub>3</sub> with concentration 760 ppm and minimum (0.66) in seeds of control. The mean daily germination follows the same trend of result as germination percentage. Value of mean daily germination depends on final germination percentage and number of days in the standard germination test (which is constant for each treatment i.e. 15 days). The peak value of germination follows the similar trend of mean daily germination. Peak value was found maximum in (12.75) because of high ratio of final germination percentage and number of days required to reach the peak value and this may be due to maximum germination percentage and less number of days required to reach the peak value. Peak value was found minimum (2.49) in seeds of control which may be due to more number of days required to reach the peak value. The germination value of seeds was maximum at GA<sub>3</sub> with concentration 760 ppm (49.60) and minimum (1.64) in seeds of control. Results shows similar pattern as germination percentage. Germination value depends on product of mean daily germination and peak value. The increase in the concentration of GA<sub>3</sub> increased the growth of the seedling and then there was a steady decline. Analysis of variance revealed significant difference in seedling height, number of branches, number of leaves, root length, fresh biomass and dry biomass over time (P=0.05). The growth attributes and biomass were influenced by the different concentration of GA<sub>3</sub>. At 30 DAS highest plant height (29.66 cm), Number of branches (3.66),

Number of leaves (15.33) were recorded in T<sub>8</sub> (760 ppm). The minimum number of branches (1.33), leaves (5.00) were recorded at T<sub>1</sub> (Control). In case of 45 DAS highest plant height (30.00 cm) and number of leaves (15.66) were recorded in the similar treatment as in 30 DAS in T<sub>8</sub>. Lowest plant height (16.00 cm) and number of leaves (10.00) were recorded in control. GA<sub>3</sub> treated seeds might be attributed to fact that the GA<sub>3</sub> helps in breaking the seed dormancy which results in early and enhanced seed germination due to the diffusion of endogenous auxin and gibberellins like substances (Gurung *et al.*, 2014) [9]. All GA<sub>3</sub> treatments were effective for increasing in growth of seedling when compared with the control. This may be due to activated amylase which digested the available carbohydrate into simpler sugar, so that energy and nutrition were easily available to faster growing seedling. Increase in plant growth due to GA<sub>3</sub> treatment (Lee *et al.*, 1999; Rout *et al.*, 2016). Highest root length (24.66 cm) was recorded in T<sub>8</sub> and lowest (9.33 cm) were recorded in T<sub>1</sub>. Here increase in length of root was recorded in the similar treatment to which has boosted the growth of the seedling above ground level, it might have given the same effect for the below ground i.e. in the root. With regards to fresh biomass significantly highest (1.32 g) was recorded in T<sub>8</sub> and lowest (0.27 g) in control. In case of dry biomass highest (0.52 g) were recorded in T<sub>8</sub> and lowest (0.09 g) in control. Plant growth regulators in overcoming the harmful effect on growth may be due to the change in endogenous growth regulators (Thomas *et al.*, 2005) [22]. It has been confirmed that seed pre-treatment with GA<sub>3</sub> promotes the growth (Taiz and Zeiger, 2010; Dillip *et al.*, 2017) [5, 21].

**Table 1:** Effect of GA<sub>3</sub> on seed germination of *Cassia fistula* L

Treatments	Parameters			
	Germination %	Mean daily germination	Peak value	Germination value
T <sub>1</sub> (Control)	10.00	0.66	2.49	1.64
T <sub>2</sub> (700 ppm)	16.66	1.10	1.99	2.27
T <sub>3</sub> (710 ppm)	26.66	1.77	5.87	10.99
T <sub>4</sub> (720 ppm)	36.66	2.44	6.14	14.15
T <sub>5</sub> (730 ppm)	43.33	2.83	4.39	12.92
T <sub>6</sub> (740 ppm)	50.00	3.33	5.60	18.64
T <sub>7</sub> (750 ppm)	53.33	3.55	7.37	26.50
T <sub>8</sub> (760 ppm)	56.66	3.77	12.75	49.60
T <sub>9</sub> (770 ppm)	40.00	2.66	4.32	11.49
T <sub>10</sub> (780 ppm)	33.33	2.22	6.25	15.44
T <sub>11</sub> (790 ppm)	20.00	1.33	2.66	3.54
T <sub>12</sub> (800 ppm)	16.66	1.10	1.40	1.81
C. D. at 5%	7.75	0.51	5.22	17.80

**Table 2:** Effect of GA<sub>3</sub> Seed pre-treatment on seedling growth of *Cassia fistula* L

Treatments	Parameters								
	Plant Height @ 30 DAS (cm)	Number of branch @ 30 DAS	Number of leaves @ 30 DAS	Plant Height @ 45 DAS (cm)	Number of branch @ 45 DAS	Number of leaves @ 45 DAS	Root Length @ 45 DAS (cm)	Fresh Biomass (g)	Dry Biomass (g)
T <sub>1</sub> (Control)	15.66	1.33	5.00	16.00	2.33	10.00	9.33	0.27	0.09
T <sub>2</sub> (700 ppm)	17.66	2.00	8.00	18.00	2.66	11.66	11.33	0.34	0.13
T <sub>3</sub> (710 ppm)	21.33	2.00	8.66	24.00	2.66	12.66	13.66	0.38	0.13
T <sub>4</sub> (720 ppm)	21.66	2.33	11.00	23.66	3.66	12.33	16.66	0.56	0.23
T <sub>5</sub> (730 ppm)	24.00	2.66	12.66	26.33	3.66	13.00	17.00	0.65	0.38
T <sub>6</sub> (740 ppm)	26.33	3.00	14.33	28.66	3.66	14.66	22.66	0.81	0.35
T <sub>7</sub> (750 ppm)	26.33	3.33	15.00	29.00	4.00	15.33	23.66	1.01	0.37
T <sub>8</sub> (760 ppm)	29.66	3.66	15.33	30.00	3.66	15.66	24.66	1.32	0.52
T <sub>9</sub> (770 ppm)	27.33	3.33	13.66	28.00	3.66	14.66	21.00	0.86	0.34
T <sub>10</sub> (780 ppm)	22.33	2.66	11.33	25.00	3.33	12.00	20.66	0.77	0.33
T <sub>11</sub> (790 ppm)	18.33	2.33	7.33	20.33	3.00	10.00	17.66	0.48	0.25
T <sub>12</sub> (800 ppm)	15.33	2.00	6.66	17.66	2.33	10.33	16.33	0.41	0.21
C. D. at 5%	2.60	0.95	1.64	2.29	0.92	1.66	1.94	0.20	0.13

## Conclusion

It was concluded from the findings that there was a significant effect of GA<sub>3</sub> on the seed germination and seedling growth of *Cassia fistula* L. Therefore 760 ppm GA<sub>3</sub> seed pre-treatment is recommended to obtain better germination, growth and quality seedling of this ornamental tree species.

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