

## Hormonal Effect on Germination and Seedling Development of *Hura crepitans* Seeds

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**Abstract:** To establish propagation test and nursery establishment protocols for *Hura crepitans*, Various concentrations and timing regimes were performed on the seeds of *Hura crepitans* using growth hormones; Thiourea, Gibberellic acid and Coumain. Gibberellic acid pretreated seeds recorded high cumulative germination (100%) and germination energy (95%). Similarly, high percentages were recorded for Thiourea. However, Coumarin showed poor germination as well as recorded high percentage of abnormal seedlings (90%), a poor option for nursery establish of *Hura crepitans*. Ranked mean for the germinated seedlings was significant at  $p < 0.05$  for Thiourea and Gibberellic acid treatments

**Key words:** *Hura crepitans*, seed germination, seedling development, hormones pretreatment

### INTRODUCTION

*Hura crepitans* (family Euphorbiaceae) grows up to 16 m high with a wide spreading crown branching down. The young branches are spineless while the matured boles are thickly crowded with short conical spines. It is commonly grown as shade trees (Keay, 1989).

The rounded hard shelled fruits are about the size of an orange and have as many deep furrows as there are cells, each containing a single flattened seed.

At maturity, the fruit ripen and when exposed to a dry atmosphere, it burst with great force, accompanied by a loud, sharp crack like the report of a pistol, for which reason, it is often called the Monkey's Dinner-bell (Clarke, 2002).

Germination studies are fundamental to any plant multiplication scheme, more so where such plant shows poor response to vegetative propagation methods (Eze and Orole, 1987). Such studies are primarily carried out to obtain information on the field and planting values for different seed lots (ISTA, 1996).

Growth regulators have been established as promoters of germination in seed exhibiting innate dormancy (Gill *et al.*, 1990). These growth regulators often perform such functions at low concentrations. However, unlike physical and most chemical scarification methods, hormonal pretreatments are not seed coat targeted. Seed germination and development cannot be isolated from the process of dormancy as it is a crucial factor determining the surviving and hence continuity of any seed bearing plant species. The process itself is influenced by the

parental and embryo in relation to the prevailing environmental conditions. Simpson (1990) reported that treating seeds with chemical promotes metabolic activity and induces germination. Plant hormones are therefore extremely important for the regulators of seed dormancy and germination (Koorneef *et al.*, 2002; Finkelstein, 2004). However, little is known of the interconnected key molecular processes controlling dormancy and germination in response to hormonal and environmental cues (Kucera *et al.*, 2005).

This present study was carried out to determine the most suitable possibility of germinating and establishing *Hura crepitans* through seeds.

### MATERIALS AND METHODS

Seeds of *Hura crepitans* were collected from Benin-City, Edo State, Nigeria between March and June 2005. Ripe pods were dried and stored at ambient temperature in Kilner jars before the commencement of the study in October 2006. Seed were surface sterilized by stirring in 0.1% Mercuric Chloride for 1 min and rinsed thoroughly thereafter in several changes of distilled water.

Seed were soaked for 5, 15, 25 min in varying concentrations (10 mg L<sup>-1</sup>, 0.1 mg L<sup>-1</sup>, 0.01 mg L<sup>-1</sup> and 0.00 mg L<sup>-1</sup>) of gibberellic acid, thiourea, Coumarin accordingly.

During treatment the seeds were stirred for uniform contact in the hormonal solution and subsequently were thoroughly washed after the time lapses with distilled water before being put up for germination in Petri dishes.

**Germination:** A set of untreated seeds served as control. The treated and untreated seed (control) were allowed to imbibe distilled water in glass Petri dishes lined with filter paper, following a randomized design of five replicates of 10 seeds each for the different pre-treatments under the prescribed exposure time. Germination was recorded daily. A radicle length of 3 mm served as criterion of germination. The experimental set-up was allowed to stand under continuous florescent light at bench level. After germination, seedlings were transplanted at a planting depth of 3 cm into black Polythene bag measuring 20×20×20 cm filled with white river sand. Each Polythene bag represented the different pre-treatment. Watering was done daily with Harris culture medium.

**Seedling development:** The height of transplanted seedlings was recorded at 3 days intervals. Seedlings were grouped into vigor categories based on germination and seedling height. The vigor index after Abdul-Baki and Anderson (1973), germination value after Czabator (1962), speed of germination, germination energy, germination percent after Maguire (1962). High vigor and low vigor after Idu and Omonhinmin (2001). Seedling evaluation was followed as outlined by Idu and Ogidiolu (2000). The total experiment period was 36 days.

Variance analysis of treatment height was performed using the Least Significant Difference (LSD) multiple range tests co-efficient of variance, standard error and critical difference at 5% were statistically evaluated for the various germination parameters.

## RESULTS

The germination experiment using hormones gave an high germination percentage of 100% using GA<sub>3</sub> of 10 mg L<sup>-1</sup> at 15 min. Ninety five percent germination was recorded for 1 mg L<sup>-1</sup> GA<sub>3</sub> (15 min) pre-treated seeds, 0.001 mg L<sup>-1</sup> Thiourea (15 min) pre-treated seeds and 10 mg L<sup>-1</sup> Coumarin (15 min) pretreated seeds. The energy of germination gave high values of 95% with GA<sub>3</sub>, Thiourea and Coumarin pre-treated seeds. However, this germination energy had lower percentages. It was as low as 20% in 0.1 mg L<sup>-1</sup> Coumarin (5 min) pretreated seeds and in control (Table 1). To measures the ease with which the germination took place, germination speed was taken germination speed of 4.34 was the highest for 10.001 mg L<sup>-1</sup>. Thiourea (15 min) pre-treated seeds. 7.27 was the next for 10 mg L<sup>-1</sup> GA<sub>3</sub> (15 min) pre-treated seeds At the peak of germination 83.4 value of germination was recorded for 0.001 mg L<sup>-1</sup>. Thiourea (15 min) pre-treated seeds. The control had a low germination value of 26 (Table 2).

The seedling development was estimated using the vigor class. High vigor class of 42 with 1 mg L<sup>-1</sup> GA<sub>3</sub> (15 min) pre-treatment seeds was recorded (Table 1 and 2). Vigor category (Table 3) showed the various rank for the seedling height. Mean followed by the same letter are not significantly different at 5% LSD. The 7 value of Coumarin was found to be non-significant at p<0.05 while the F-value of GA<sub>3</sub> and Thiourea were significant at p<0.05. Coumarin pre-treatment 0.01 mg L<sup>-1</sup> (15 min) had high (90%) abnormal seedlings. A vigor index of 1866.60 was calculated for 0.01 mg L<sup>-1</sup> Coumarin (25 min) pretreated seeds.

Table 1: Percentage germination and germination energy of hormones pretreated seeds

Pre-treatment	Germination (%)			Germination energy (%)			High vigor			Low vigor		
	Coumarin	Thiourea	GA <sub>3</sub>	Coumarin	Thiourea	GA <sub>3</sub>	Coumarin	Thiourea	GA <sub>3</sub>	Coumarin	Thiourea	GA <sub>3</sub>
0.001 mg L <sup>-1</sup> 5 min	80.00	80.00	70.00	80.00	80.00	65.00	22.00	40.00	5.00	18.00	0.00	30.00
0.001 mg L <sup>-1</sup> 15 min	80.00	95.00	80.00	55.00	95.00	70.00	40.00	27.00	25.00	0.00	20.00	15.00
0.001 mg L <sup>-1</sup> 25 min	50.00	90.00	90.00	45.00	90.00	90.00	20.00	12.00	30.00	2.00	32.00	15.00
0.01 mg L <sup>-1</sup> 5 min	50.00	70.00	80.00	50.00	60.00	80.00	7.00	35.00	27.00	18.00	0.00	12.00
0.01 mg L <sup>-1</sup> 15 min	75.00	60.00	45.00	60.00	60.00	45.00	22.00	17.00	7.00	15.00	10.00	15.00
0.01 mg L <sup>-1</sup> 25 min	90.00	60.00	65.00	95.00	60.00	50.00	30.00	20.00	22.00	15.00	10.00	10.00
0.1 mg L <sup>-1</sup> 5 min	50.00	55.00	75.00	20.00	55.00	70.00	22.00	15.00	27.00	3.00	12.00	10.00
0.1 mg L <sup>-1</sup> 15 min	80.00	90.00	80.00	80.00	90.00	70.00	30.00	7.00	17.00	10.00	37.00	22.00
0.1 mg L <sup>-1</sup> 25 min	85.00	75.00	85.00	80.00	75.00	85.00	25.00	25.00	15.00	17.00	17.00	27.00
1 mg L <sup>-1</sup> 5 min	45.00	65.00	60.00	45.00	60.00	50.00	20.00	27.00	25.00	3.00	5.00	5.00
1 mg L <sup>-1</sup> 15 min	70.00	60.00	95.00	40.00	55.00	90.00	27.00	17.00	42.00	8.00	12.00	5.00
1 mg L <sup>-1</sup> 25 min	85.00	85.00	70.00	85.00	85.00	50.00	20.00	12.00	22.00	22.00	30.00	12.00
10 mg L <sup>-1</sup> 5 min	45.00	85.00	60.00	45.00	85.00	60.00	15.00	20.00	17.00	7.00	22.00	12.00
10 mg L <sup>-1</sup> 15 min	95.00	70.00	100.00	95.00	70.00	95.00	17.00	22.00	30.00	30.00	12.00	20.00
10 mg L <sup>-1</sup> 25 min	55.00	70.00	70.00	55.00	70.00	70.00	17.00	17.00	25.00	10.00	17.00	10.00
Control	80.00			20.00			10.00			27.00		
SE±	4.61	3.32	3.75	5.71	3.62	4.19	1.98	2.28	2.41	2.18	2.84	1.88
CV (%)	25.00	17.00	19.00	36.00	19.00	23.00	34.00	42.00	42.00	71.00	70.00	50.00
CD at 5 (%)	28.56	20.56	23.23	35.77	22.38	21.22	12.25	14.12	14.91	13.48	17.63	11.66

Table 2: Germination speed, vigor indices and percentage abnormal seedlings

Pre-treatment	Speed of germination			Vigor index			Germination value			Abnormal seedling (%)		
	Coumarin	Thiourea	GA <sub>3</sub>	Coumarin	Thiourea	GA <sub>3</sub>	Coumarin	Thiourea	GA <sub>3</sub>	Coumarin	Thiourea	GA <sub>3</sub>
0.001 mg L <sup>-1</sup> 5 min	5.00	3.34	2.96	1590.40	1840.00	898.10	40.00	43.42	32.56	43.75	0.00	78.57
0.001 mg L <sup>-1</sup> 15 min	3.52	8.34	4.55	1618.40	1706.20	1336.00	21.12	83.4	27.30	0.00	52.63	31.25
0.001 mg L <sup>-1</sup> 25 min	1.94	4.33	4.17	1026.50	1507.50	1563.30	15.52	47.63	54.21	10.00	55.56	27.78
0.01 mg L <sup>-1</sup> 5 min	2.00	3.17	5.00	715.00	1471.40	1467.20	18.00	29.68	55.00	90.00	0.00	31.25
0.01 mg L <sup>-1</sup> 15 min	3.14	2.00	2.50	1365.00	933.60	765.00	25.12	8.00	10.00	33.33	0.00	44.44
0.01 mg L <sup>-1</sup> 25 min	5.84	2.67	3.14	1866.60	1122.00	1103.05	58.40	24.03	15.70	22.22	33.33	0.00
0.1 mg L <sup>-1</sup> 5 min	1.86	2.17	3.11	994.00	818.95	1503.75	5.58	19.53	31.10	10.00	18.18	13.33
0.1 mg L <sup>-1</sup> 15 min	2.67	4.67	4.52	1671.20	1393.20	1436.80	42.72	42.03	36.16	25.00	77.78	43.75
0.1 mg L <sup>-1</sup> 25 min	4.28	5.22	5.00	1417.80	1540.50	1213.80	42.80	41.76	35.00	29.41	41.18	47.06
1 mg L <sup>-1</sup> 5 min	1.60	2.76	2.21	882.00	1190.80	1198.20	9.60	22.08	19.89	0.00	0.00	16.67
1 mg L <sup>-1</sup> 15 min	3.97	2.28	3.94	1250.90	1051.80	1757.50	19.85	20.52	59.10	0.00	16.67	0.00
1 mg L <sup>-1</sup> 25 min	3.17	3.84	2.11	1636.25	1482.40	136.50	50.72	49.92	21.10	41.18	52.29	21.43
10 mg L <sup>-1</sup> 5 min	2.83	3.17	1.88	631.35	1394.00	895.20	16.98	0.72	11.28	0.00	23.53	0.00
10 mg L <sup>-1</sup> 15 min	4.50	2.33	7.27	1719.50	1227.10	1779.00	63.00	32.62	58.16	57.89	14.29	30.00
10 mg L <sup>-1</sup> 25 min	2.33	4.34	2.50	1008.15	1242.50	1152.20	20.97	30.38	32.50	18.18	42.86	14.29
Control	6.50			1620.00			26.00			57.14		
SE±	0.33	0.42	0.38	101.68	72.55	109.75	4.67	4.70	4.32	6.55	6.33	5.51
CV (%)	39.00	44.00	40.00	30.00	21.00	35.00	60.00	50.00	50.00	100.00	86.00	80.00
CD at 5 (%)	2.02	2.61	2.34	630.20	449.69	680.26	29.0	29.15	26.75	40.60	39.25	34.12

Table 3: Ranked mean for hormone pretreatments

Pre-treatment (Coumarin)	Ranked mean	LSD (H) <sup>+</sup> mean	Pre-treatment (Thiourea)	Ranked mean	LSD (H) <sup>+</sup> mean	Pre-treatment (GA <sub>3</sub> )	Ranked mean	LSD (H) <sup>+</sup> mean
Control	12.25a	17.15	Control	12.25a	15.31	Control	12.25a	14.97
10 mg L <sup>-1</sup> 5 min	14.03a	18.93	0.1 mg L <sup>-1</sup> 5 min	14.89ab	17.95	0.001 mg L <sup>-1</sup> 5 min	12.83a	15.55
0.01 mg L <sup>-1</sup> 5 min	14.3a	19.20	0.1 mg L <sup>-1</sup> 15 min	15.48c	18.54	0.1 mg L <sup>-1</sup> 25 min	14.28b	17.00
0.1 mg L <sup>-1</sup> 25 min	16.68ab	21.58	0.01 mg L <sup>-1</sup> 5 min	15.56c	18.62	10 mg L <sup>-1</sup> 5 min	14.72b	17.64
1 mg L <sup>-1</sup> 15 min	17.87ab	22.77	10 mg L <sup>-1</sup> 5 min	16.4c	19.46	10 mg L <sup>-1</sup> 25 min	16.46c	19.18
10 mg L <sup>-1</sup> 15 min	18.1b	23.09	0.001 mg L <sup>-1</sup> 25 min	16.75c	19.81	0.001 mg L <sup>-1</sup> 15 min	16.70c	19.42
0.01 mg L <sup>-1</sup> 15 min	18.2b	23.10	1 mg L <sup>-1</sup> 25 min	17.44d	20.50	0.01 mg L <sup>-1</sup> 25 min	16.97	19.69
10 mg L <sup>-1</sup> 25 min	18.33b	23.23	1 mg L <sup>-1</sup> 15 min	17.53d	20.59	0.01 mg L <sup>-1</sup> 15 min	17.00d	19.72
1 mg L <sup>-1</sup> 25 min	19.25c	23.55	10 mg L <sup>-1</sup> 15 min	17.55d	20.61	0.001 mg L <sup>-1</sup> 25 min	17.37d	20.09
1 mg L <sup>-1</sup> 5 min	19.60c	24.50	10 mg L <sup>-1</sup> 25 min	17.75d	20.81	10 mg L <sup>-1</sup> 15 min	17.79d	20.51
0.00 mg L <sup>-1</sup> 5 min	19.88c	24.78	0.001 mg L <sup>-1</sup> 15 min	17.96d	21.02	0.1 mg L <sup>-1</sup> 15 min	17.96d	20.68
0.1 mg L <sup>-1</sup> 5 min	19.88c	24.78	1 mg L <sup>-1</sup> 5 min	18.32e	21.38	0.01 mg L <sup>-1</sup> 5 min	18.34de	21.06
0.001 mg L <sup>-1</sup> 15 min	20.23d	--	0.01 mg L <sup>-1</sup> 25 min	18.7e	21.76	1 mg L <sup>-1</sup> 15 min	18.54de	21.22
0.001 mg L <sup>-1</sup> 25 min	20.53d	--	0.1 mg L <sup>-1</sup> 25 min	20.54f	23.60	1 mg L <sup>-1</sup> 25 min	19.50e	--
0.01 mg L <sup>-1</sup> 25 min	20.74d	--	0.01 mg L <sup>-1</sup> 5 min	21.02f	24.08	1 mg L <sup>-1</sup> 5 min	19.97e	--
0.1 mg L <sup>-1</sup> 15 min	20.89d	--	0.001 mg L <sup>-1</sup> 5 min	23.0f	--	0.1 mg L <sup>-1</sup> 5 min	20.05e	--
F = Ratio = 0.73			F = Ratio = 3.33			F = Ratio = 2.71		

Mean followed by the same letter are not significantly different at 5 (%) (LSD)

## DISCUSSION

High cumulative germination (100%), germination speed, germination energy recorded for gibberellic acid are consistent with the observations of Idu (1994) and Henrique *et al.* (1999) working with *Bixa orellana* and *Bracharia brizantha* respectively using gibberellic acid pretreatment. Reddy and Khan (2001) also reported a high vigor index for Khirmi (*Mimosa hexandra*) on gibberellic acid of 300 ppm both ways.

Coumarin had a high cumulative percentage germination of 95% at 10 mg L<sup>-1</sup> (15 min) pre-treatment but has most of values less than 50% cumulative percentage germination. Thiourea had germination energies and percentage above 50% and as high as 95% germination percentage at 0.001 mg L<sup>-1</sup> (15 min). Idu and Omonhinmin (1998) also recorded 85 and 30% as the highest cumulative.

Most works in plant hormones clearly indicate that dormancy and germination are under hormonal control (Pillay, 1966). Generally, auxin and gibberellic acid concentrations are relatively high during maturation, but in some species one or both of these hormones show a marked decline at seed maturity thus imposing a conditional rest or dormancy. Therefore, pre-treatment of seeds with plant hormones often terminate the presupposed (Black and Naylor 1959; Hashimoto and Rappaport, 1966ab).

The stimulating inhibitory effects of thiourea and Coumarin may have been due to the action on the storage materials of the seeds, (Phosphate Oxygen ratio) and the coupling action in germinating seeds either directly or indirectly, this view is supported by Jaris *et al.* (1968), Bryant (1985) and Mayer and Poljakoff-Mayber (1989).

Seedling evaluation is of great importance in forestry, horticulture and silviculture. Most often seeds are capable

of germination, however, they may not produce healthy normal seedlings in nurseries. Abnormal seedlings were recorded for 0.01 mg L<sup>-1</sup> Coumarin (5 min) pre-treated seeds. This supports earlier view on inhibitory effect of Coumarin by Idu and Omonhinmin (1998). However, the vigor index recorded for 0.01 mg L<sup>-1</sup> Coumarin (25 min) pre-treatment contravenes earlier observations. Expectedly, Gibberellic acid and Thiourea recorded high vigor indices.

In conclusion, hormonal pre-treatment with Gibberellic acid and Thiourea are ideal for promoting seed germination in *Hura crepitans*, while Coumarin remains inhibitory.

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