

Seed Storage Behaviour of *Tinospora cordifolia*

C ANILKUMAR*, CR CHITRA, S BINDU AND G RAJKUMAR

Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Thiruvananthapuram, Kerala 695 562
*canildeepa@yahoo.com

ABSTRACT: Studies were conducted on *Tinospora cordifolia*, [Indian tinospora or Guduchi], a highly sought-after medicinal plant and fast disappearing species from wild habitat, for analyzing the seed storage behaviour for the purpose of *ex-situ* conservation; keeping bio diversity conservation in view. Sensitivity of seeds dried to different moisture levels, with respect to germinability, revealed desiccation/drying tolerance up to 8.5% moisture. Apart from this, chilling sensitivity to sub zero temperature also indicated intermediate nature. Control seeds with 16.9% moisture recorded viability for two weeks whereas, seeds dried to 7.8% moisture stored at 20°C retained 80% viability for six months.

Key words: *Tinospora*, Moisture content, Germination, Desiccation, Viability, Storage

Tinospora cordifolia (Willd.) Miers ex Hook.f. & Thoms [Indian tinospora or Guduchi] (*Menispermaceae*) is a large deciduous, extensively spreading, climbing shrub found throughout the tropical parts of India and China, up to 300m altitude [1]. As an important drug of Indian System of Medicine (ISM) *Tinospora* is in use to improve the immune system and body resistance against infections [2]. *T. cordifolia* is threatened with extinction due to its indiscriminate collection and excessive use for commercial purpose to meet the requirements of pharmaceutical industry [3].

Normal propagation of the species is through seeds, though a limited success is reported via stem cuttings [1, 4]. Micro propagation of the species from mature stem cuttings remained problematic owing to the poor explant responses and browning of the shoots with profuse callus formation at the cut ends [3]. In this context, a study on seed storage and viability of *T. cordifolia* seeds is relevant and hence was initiated proactively for germplasm conservation and sustainable use.

MATERIALS AND METHODS

Fresh and ripe fruits of *T. cordifolia* were

collected during three consecutive years i.e. 2011, 2012 and 2013, from Thiruvananthapuram Forest Division (Lat - 8°45' and 8°47' N; Long - 77°1' and 77°4' E). Fruits are sessile, convex, dorsally ridged, ventrally flat with a sub terminal stylar scar and are single seeded. Reddish drupe fruits of 0.5-0.7 cm length and radius of 0.106 ± 0.017 cm were taken. Average weight of 100 fruits was 55 ± 0.01 gm. Seeds are reniform, split in the middle with rough surface and creamy white colour though some are black. They measured 0.683 ± 0.07 and 0.543 ± 0.05 cm in length and breadth respectively with 100 seeds weighing 11.2 ± 0.003 gms. Seeds were extracted from fruits by threshing, and were thoroughly washed in running tap water. Using floatation method, healthy looking submerged seeds were used for the experiment. Apart from this, seeds from green fruits (unripe), orange fruits (partially ripe) and red fruits (ripe) were also collected and tested for germination after documenting phenological and morphological details and estimating moisture content for standardized maturity indices.

The initial moisture content of seeds was determined on fresh weight basis [5]. Germination tests were conducted in wet rolled paper towels

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placed in a seed germinator maintained at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 80% RH, without light. Storage experiments were carried out with seeds dried to different moisture levels in a desiccator containing silica gel, kept at laboratory conditions ($28 \pm 2^{\circ}\text{C}/60\%$ RH). Seeds kept under ambient laboratory conditions ($28 \pm 2^{\circ}\text{C}/60\%$ RH) served as control. Dried seeds were exposed to 80% RH for 24 hours prior to germination tests, to prevent imbibitional injury. To study the combined effect of seed moisture and temperature on seed viability, seeds with different moisture contents were stored in polycarbonate bottles kept at different temperatures (30, 20, 10, -10, -20 and -196°C) for one week. Cryo stored seeds were wrapped in aluminum foils before directly placing in liquid nitrogen for one week; and afterwards were thawed for 5 minutes in 40°C water bath. Subsequently, long-term storage was arranged at temperatures ranging from -20, -10, 10, 20 and 30°C with seeds having 7 % moisture; and seed viability was monitored at 3 months interval [5]. Effect of GA_3 pre-treatment (50 and 100ppm) was also studied and germination speed was calculated as germination percentage on each day of completion of germination. Data were subjected to ANOVA followed with LSD test at 0.05 level.

RESULTS AND DISCUSSION

Physical Purity of seeds was $71 \pm 1.1\%$. When tested for germination, 90% of seeds with 16.9% initial moisture, germinated in a span of 6-67 days without any dormancy.

When seeds of different maturity indices were tested for germination, those from green fruits did not germinate, while 30% from orange fruits and 90% from red fruits germinated. A gradual increase in fruit weight was observed during ripening. Seed weight and moisture content had a negative correlation with germination (Table 1). This can be attributed to the loss of moisture during development as the endosperm becomes hard, along with seed's ability to prolong viability by inbuilt desiccation mechanism. Physiological maturity was morphologically reflected with a distinct colour change of fruits from green to yellow and then to red. These observations on ripening indices also conform to the reports in 2008 in the same species [6].

Normally, fresh seeds registered a maximum of about 90% germination in 67 days. Among the fresh seeds pretreated with GA_3 (50 and 100ppm) for 24 hours, those treated with GA_3 -50 ppm registered 100% germination within 24 ± 1.18 days while that of GA_3 -100ppm exhibited 80% germination within 40.8 ± 1.78 days. GA_3 -50ppm increased the speed of germination (Table 2).

In the desiccation study after seven days, though the seed moisture was reduced to 9.8% from 16.9% (Table 3) 81% viability was maintained. On further reduction of moisture to 8.5%, germination was reduced to 70%. Below 7.8% moisture, germination fell drastically to 53% implying intermediate nature of seeds [7].

Table 1. Dimensions, moisture content and germination percentage of seeds having different maturation

Nature of fruit	Fruit radius (cm)	Fruit weight (g)	Seed length (cm)	Seed breadth (cm)	Seed weight (g)	Moisture content(%)	Germination (%)	Time taken to complete germination (days)
Green fruits	0.966 ± 0.07	0.525 ± 0.01	0.652 ± 0.09	0.532 ± 0.12	0.10 ± 0.002	22.61 ± 0.45	Nil	Nil
Orange fruits	0.981 ± 0.08	0.559 ± 0.02	0.65 ± 0.09	0.516 ± 0.06	0.095 ± 0.001	20.79 ± 0.23	30 ± 1.7	53 ± 2.3
Red fruits	0.103 ± 0.15	0.57 ± 0.01	0.626 ± 0.15	0.51 ± 0.05	0.09 ± 0.001	17.79 ± 0.57	90 ± 2.9	67 ± 4.5

Table 2. Gibberelic acid treatment

GA ₃ concentration	Germination (%)	Time for the completion of germination (days)	Emergence Index	Germination Speed
50 ppm	100 ± 0	24 ± 1.18	25.8 ± 1.06	4.18 ± 0.02
100 ppm	80 ± 1.88	40.8 ± 1.78	16.8 ± 0.97	5.33 ± 0.01

Table 3. Effect of seed moisture on germination

Period of drying (days)	Moisture content % ± SE	Germination % ± SE	Time for the complete germination days ± SE	Emergence Index	Germination Speed
0	16.9±0.17	90±2.9	67±1.9	18±0.71	1.35±0.007
2	13.4±0.75	88±6	37±2.5	21.4±0.51	2.36±0.02
7	9.8±0.37	81±4.4	66±1.7	16.2±0.24	1.24±0.01
11	8.5±0.01	70±3.1	69±1.1	10.3±0.21	0.98±0.03
15	7.8±0.37	53±2.5	70±1.61	10.54±0.27	0.74±0.03
25	5.35±0.19	48±2.5	36.6±0.75	6.84±0.03	1.31±0.02
38	4.2±0.25	48±2.5	38.6±1.1	7.13±0.13	1.23±0.01
	LSD-5.75	LSD-26.95	LSD-20.85	LSD-0.636	LSD-7.33

Means in columns separated by Least Significant Difference Analysis at 0.05 level.

Seeds with different moisture content stored at different temperatures were tested for viability (Table 4). Seed with 16.9% moisture stored at 30°C registered maximum germination of 90%. With lower moisture levels, seed stored at 30°C lost viability considerably.

Seed with 9.8% and 7.8% moisture and stored at 20°C showed 60 and 65% germination respectively. Cryo preserved seeds with 7.8% moisture retained 60% viability. Seeds with 5.4% moisture recorded 70% viability at 20°C, and 60% viability in cryo storage. Seeds with 4.2% moisture retained 60% viability in 20°C and 55% in cryo.

When tested for long term storage, fresh seeds kept as control remained viable for only three months. Seeds with 7% moisture were specifically tested for long term storage. At 20°C and -20°C such seeds retained 80% and 70%

viability respectively for six months (Table 5).

Seed longevity and storage behaviour are closely related and the viability of such intermediate seeds can be prolonged only to a limited extent by drying [8&9]. This was similar to the desiccation response and intermediate storage physiology reported on the other allied species *Tinospora sinensis* [10]. Similar intermediate storage behaviour was reported in *Aegle marmelos*, wherein seed with 6.4% moisture and 70% germination, upon further reduction of moisture to 4.5%, had reduced germination of 36% [11]. *T. cordifolia* seeds are also sensitive to sub zero temperatures like that of the intermediate seeds of *Coscinium fenestratum* [12]. Seeds of *Zelkova serrata* survived at low moisture; however, viability was lost more rapidly than seeds with higher moisture, confirming the intermediate storage behaviour of seeds [13]. Similarly,

Table 4. Effect of moisture content and storage temperatures on germination after one week storage of seeds

Moisture content % \pm SE	Germination after one-week storage at different storage temperatures.					% \pm SE -20°C
	30°C	20°C	10°C	-10°C	Cryo	
16.9 \pm 0.17	90 \pm 2.9	31 \pm 2.5	45 \pm 1.59	5 \pm 1.22	8 \pm 1.81	7 \pm 1.2
9.8 \pm 0.37	48 \pm 2.5	60 \pm 1.9	21 \pm 1.7	30 \pm 1.7	36 \pm 1.7	15 \pm 4.4
7.8 \pm 0.37	37 \pm 2.5	65 \pm 2.3	50 \pm 1.58	49 \pm 2.9	65 \pm 1.35	60 \pm 3.1
5.35 \pm 0.19	27 \pm 2.5	73 \pm 1.87	40 \pm 1.58	40 \pm 1.58	61 \pm 1.87	58 \pm 3.7
4.2 \pm 0.25	22 \pm 2.5	60 \pm 1.87	48 \pm 2.5	45 \pm 1.7	55 \pm 1.58	38 \pm 1.5
LSD-5.83	LSD-38.76	LSD-6.45	LSD-15.78	LSD-26.01	LSD-5.43	LSD-10.17

Means in columns separated by Least Significant Difference Analysis at 0.05 level

Table 5. Percentage moisture content and germination of *Tinospora cordifolia* (Willd.) seeds with 7.8 \pm 0.5% moisture content stored at different conditions

Storage condition	Days of storage	Moisture content (% \pm SE)	Germination (% \pm SE)	Time for the completion of germination (Days \pm SE)
Fresh seeds	0	16.9 \pm 0.17	67 \pm 1.9	60 \pm 1.6
Room open-(30°C/60 RH (Control))	93	10.7 \pm 0.19	60 \pm 1.6	39 \pm 1.9
	186	10.6 \pm 0.12	31 \pm 1.87	26 \pm 2.3
	277	10 \pm 0.32	Nil	Nil
Room Polyethylene bag (30°C/60 RH)	93	12.16 \pm 0.33	49 \pm 1.87	19 \pm 1.7
	186	12.7 \pm 0.11	40 \pm 1.58	17 \pm 0.91
	277	13.2 \pm 0.15	Nil	Nil
Room Polyethylene bottle(30°C/60 RH)	93	10.9 \pm 0.13	41 \pm 1.87	19 \pm 1.7
	186	10.01 \pm 0.17	30 \pm 1.35	21 \pm 0.95
	277	Nil	Nil	Nil
20°C Polycarbonate bottle (20°C/20 RH)	93	16.16 \pm 0.3	87 \pm 2	64 \pm 1.2
	186	15.5 \pm 0.35	80 \pm 1.6	62 \pm 0.8
	277	9.7 \pm 0.22	59 \pm 1.8	19.4 \pm 0.79
-20°C Polycarbonate bottle (20°C/20 RH)	93	14.5 \pm 0.16	84 \pm 1.8	69.8 \pm 0.9
	186	14.3 \pm 0.34	70 \pm 1.7	64.5 \pm 2.2
	277	9.3 \pm 0.1	36 \pm 2.9	32.7 \pm 1.01
10°C Polycarbonate bottle	93	41 \pm 0.11	40 \pm 1.58	17 \pm 0.91
	186	13.2 \pm 0.15	Nil	Nil
	277	13.5 \pm 0.17	21 \pm 1.87	16.2 \pm 1.18
-10°C Polycarbonate bottle	93	10.53 \pm 1.01	21 \pm 0.19	17 \pm 1.13
	186	11.07 \pm 0.09	Nil	Nil

Starred values of same storage condition are significantly different from control at the level of $P < 0.05$ based on Duncan's Multiple Range Test.

desiccation sensitive seeds of *Cinnamomum camphora* [14] and *Magnolia ovata* [15] were also reported as intermediate due to sensitivity to sub-zero temperatures during short-term storage. Seeds of *Azadirachta indica* and *Khaya senegalensis* [16], *Mimusops elengi* [17], *Eugenia pleurantha* [18] *Mesua ferrea* [19] and *Trema cannabina* [20] are also reported as intermediate based on their desiccation and temperature sensitivity.

Though the longevity of intermediate seeds is quite short, which is a major constraint for biodiversity conservation, the desiccation tolerance of *T. cordifolia* seeds up to 8.5% moisture and extended survival of seeds dried to 7.8% moisture at -20°C for 186 days indicates the possible extendable seed storage in this species.

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