

Seed Storage Behaviour of *Tinospora sinensis*

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ABSTRACT *Tinospora sinensis* propagates through stem cuttings and seeds. Desiccation sensitivity and effect of storage conditions on seed germinability shows that seeds are sensitive to freezing temperature. Optimum storage at 10°C and desiccation tolerance up to 6.8 per cent moisture content indicate the intermediate nature of seed storage. Storage of seeds with 6.8 per cent moisture content at 10°C in hermetically sealed containers is recommended for retaining the viability of seed for a longer period.

Key words: *Tinospora sinensis*, desiccation, intermediate, viability, storage

Tinospora sinensis (Lour.) Merr. (Menispermaceae), is a deciduous climbing shrub distributed amongst India, Sri Lanka, Pakistan, Nepal, Bangladesh, Myanmar, China, Thailand, Vietnam and Cambodia. This species is now sparingly found at an altitude of 600-1000 m of the Peninsular Indian semi-evergreen western Ghat forests and its over exploitation attributed the assigned "Near Threatened" status for Kerala region [1]. This monoecious species flowers during December to February and set fruits during January to May. Drupaceous spherical fruits (8 x 7 mm in diameter) are in a cluster of 1-3, turn orange yellow to red, when ripe. This taxon propagates through stem cuttings and seeds. Presently their natural habitats are being destroyed due to several anthropogenic factors. Considering the above, study was initiated to standardize seed storage conditions for germplasm conservation and its sustainable use.

MATERIALS AND METHODS

Fresh ripe fruits of *Tinospora sinensis* were collected from Ponmudi forest range of the southern-western Ghats (Latitude - 8° 45' and 8° 47'N and Longitude 77° 4'E) during three consecutive years - 2002, 2003 and 2004. Average weight of 100 fruits was 57.418±0.98 gm. Each fruit bears a single semi-lunar shaped seed. Seeds

were extracted from fruits and thoroughly washed with water. Submerged seeds were used for the study. Seed surface is rugose, rough and greyish-white in colour. Hilum is at the ventral plane surface and a mid rib encircles the seed. Average weight of 100-seeds was 11.72±0.12 gm. Seed length, breadth and thickness were 0.5024±0.84 cm, 0.838±0.02 cm and 0.638±0.02 cm, respectively. Seeds were dried in laboratory at ambient conditions (28±2°C/60% RH) in a desiccator (filled with silica gel) up to 75 days. The initial moisture content was determined on fresh weight basis [2]. Seeds with different moisture content were collected (19±0.3, 9.3±0.6, 6.8±0.5, 5.4±0.4, 4.4±0.2) at different periods. The seeds kept open under laboratory ambient conditions served as control. Germination studies were conducted in wet rolled paper towels placed in a seed germinator without light (30±2°C/80% RH). Desiccated seeds were conditioned to 80 per cent RH for 24 hours prior to germination test, to avoid imbibitional injury.

To test the effect of moisture content and storage temperature, seeds with different moisture content were stored in polycarbonate bottles under different temperatures (30°C, 20°C, 10°C, 0°C and -10°C) for one week. Seed viability was assessed following ISTA rules [2]. Long-term storage studies on seeds with 6.8±0.5 per cent moisture content were

conducted at six different sets of storage conditions. Seed viability was monitored at an interval of every three months up to one year. Data were subjected to ANOVA followed with a LSD test at 0.05 level.

RESULTS AND DISCUSSION

With the availability of water, seeds became saturated in three hours. As the initial moisture content is comparatively low (19.1%), the water absorption rate is high and most of the seeds germinate (without dormancy) in about two weeks. Germination percentage was maintained up to 45 days from seed desiccation with 6.8 per cent moisture content (Table 1), whereas, significant reduction in germination (60% and 34%, respectively was noticed in 65 and 75 days of seed desiccation). On 60th day of seed desiccation, the moisture content in seed was 5.4 per cent with 60 per cent germination. Subsequent desiccation, up to 75 days, reduced the germination drastically to 34±2.5 per cent with 4.4±0.2 per cent moisture content. The time taken for completion of germination was 21.4±0.25 days, whereas, in the earlier cases it was 14-15 days (Table 1). Seeds were found to be desiccation tolerant up to 5.4±0.4 per cent moisture content; on further desiccation to 4.4±0.2 per cent moisture content, viability was lost, attributed to an intermediate nature of seeds, as described by Hong and Ellis in certain citrus species [3].

Table 1. Effects of desiccation of seeds on germination

Period of desiccation in days	Moisture content % ± SE	Germination % ± SE	Time taken for complete germination in days ± SE
0	19.0±0.3	90±4.5	14.8±0.20
13	9.2±0.6	86±4.0	14.4±0.25
45	6.8±0.5	86±2.5	13.4±0.25
60	5.4±0.4	60±3.2	15.4±0.25
75	4.4±0.2	34±2.5	21.4±0.25
	LSD-1.25	LSD-10.04	LSD-0.23

Viability test of seeds with 19 to 4.4 per cent moisture content stored under different temperatures 30° to -10°C are given in table 2. Seeds with moisture content (19%) stored under 30°, 20° and 10°C showed acceptable percentage

of germination (around 80%) throughout and not at 0° and -10°C. Extended exposure to temperatures like, 10°C was detrimental to many tropical recalcitrant seeds like, *Myristica malabarica* [4]. In *T. sinensis*, 88 per cent germination from one week stored seeds (with 19% moisture content) under 10°C confirmed that they are not chilling sensitive and are more adaptive to wider range of physical features. With 9.2 per cent seed moisture content, almost same level of germination was noticed in all conditions up to 0°C, whereas, only 25 per cent germination was noticed in seeds stored under -10°C. Seeds with 6.8 per cent moisture content retained 74 per cent viability even in freezing temperatures (Table 2).

Table 2. Effects of moisture content and storage temperatures on germination in one-week stored seeds

Moisture content % ± SE	Germination (%±SE) in one-week stored seeds under different storage temperature(s)				
	30°C	20°C	10°C	0°C	-10°C
19.0±0.3	84±2.5	88±3.7	88±3.7	74±2.5	42±2.0
9.2±0.6	80±3.2	82±3.2	78±3.7	76±2.5	52±3.7
6.8±0.5	92±3.7	90±2.0	88±3.7	74±2.5	74±2.5
5.4±0.4	72±3.7	60±3.2	56±2.5	44±2.4	54±2.4
4.4±0.2	32±3.7	20±3.2	22±2.0	14±2.4	18±2.0
LSD-1.25	LSD-10.1	LSD-9.1	LSD-9.5	LSD-7.2	LSD-7.7

Seeds stored in open condition (control) remain viable up to 6 months, whereas, in polycarbonate bottles and polyethylene bags, seeds retained viability up to 2 and 6 months, respectively. At 20°C and 10°C in polycarbonate bottle, seeds remained viable up to 9 months (66.7±5.8% and 76.7±5.98%, respectively). At 0°C, the seeds lost viability within one month. The present investigations showed that optimum storage temperature was 10°C with 6.8 per cent moisture content (Table 3).

Tinospora sinensis seeds do not show the characters of either true orthodox seeds or recalcitrant seeds as described by Roberts [5]. An abrupt loss of seed viability on seed desiccation below 5.4 per cent moisture content showed a

Table 3. Percentage germination of seeds stored with 6.8 ± 0.5% moisture content under different set of storage conditions

Storage condition	Days of storage	Moisture content % ±SE	Germination % ±SE	Time for the completion of germination days ±SE
Control	0	6.8±0.5	86.0±2.5	13.4±0.3
Laboratory ambient (30°C/60%RH)	93	6.6±0.1	86.7±5.8	15.0±2.0
	186	6.5±.06*	66.7±5.8*	15.0±2.0
Control	277	6.0±.06*	33.3±5.8*	21.0±4.0*
Laboratory ambient Polycarbonate bottle (30°C/60%RH)	93	7.9±.06*	36.7±5.8*	11.0±1.0
	186	8.0±1.0*	16.7±5.8*	10.3±3.2
Laboratory ambient Polyethylene bag (30°C/60%RH)	93	7.3±.06*	80.0±10.0*	14.3±3.2
	186	7.6±.06*	76.7±5.8*	9.0±2.0
	277	6.8±.05	43.3±5.8*	19.0±4.0*
Controlled (Seed bank condition):	93	7.9±.06*	86.7±5.8	11.0±2.0
Polycarbonate bottle (20°C/20%RH)	186	7.8±.06*	86.7±5.8	7.6±1.2*
	277	6.9±.06	66.7±5.8*	19.6±3.7*
10°C Polycarbonate bottle	93	6.9±.06	86.0±5.8	9.0±2.0
	186	6.6±.06	86.7±5.8	10.7±4.5
	277	7.2±.06	83.3±5.8*	13.2±2.0
	367	8.3±.06	76.7±5.8*	14.3±3.2
0°C Polycarbonate bottle	93	7.5±.06	0	

negative correlation between longevity and freezing temperatures. Similar findings were shown with papaya seeds, in which 6.5 per cent moisture content had no substantial effect on seed germination, but further drying to 4.2-5.3 per cent lowered germination by 6-10 per cent [6]. Gamene et al. [7] reported that in neem seeds (*Azadirachta indica*) when moisture content was reduced to 6.4 to 4.8 per cent, there was a significant loss of germination, showing intermediate seed storage behaviour [7]. Intermediate seeds of *Cinnamomum camphora* showed desiccation tolerance up to 6 per cent moisture content and remain viable up to 5°C, though cannot withstand sub-freezing temperatures [8].

In the case of *Tinospora sinensis*, the seed

characters like, sensitiveness to lower temperature, optimum storage at 10°C, desiccation tolerance up to 6.8 per cent, etc. indicate intermediate seed storage behaviour [9]. For safe seed storage, it is recommended to desiccate seeds to 6.8 per cent moisture content and store under 10°C in hermetically sealed containers.

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