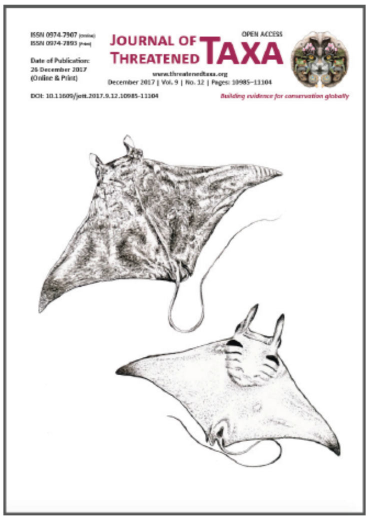

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Short Communication

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Phenology and seed germination of the Indian Screw Tree *Helicteres isora* L. (Malvales: Malvaceae)

Mariappan Muthukumar [1], Thiruppathi Senthil Kumar [2] & Mandali Venkateswara Rao [3]

Department of Botany, Bharathidasan University, Palkalaiperur, Tiruchirappalli, Tamil Nadu 620024, India
[1] muthuksm@gmail.com, [2] senthil2551964@yahoo.co.in (corresponding author), [3] mvrao_456@yahoo.co.in

Abstract: *Helicteres isora* L. (Malvaceae), a medicinally important tree species used in Ayurveda as well as by folklore practitioners, is studied for its phenology, associated problems with the environment and anthropogenic effects. In *Helicteres isora*, flower buds are initiated by the end of July and flowering occurs during the middle of August to late October. Fruits mature during late January to early April. In between various factors like blister beetles, unidentified worms, deficit rainfall, early summer affect the flowering and seed setting, which cause 35–45 % loss. Seed viability of mature seeds were recorded 88.2% at fresh collection and decreases over period of time. In vitro, among the various treatments, acid treated seeds (H_2SO_4, 98.0%; 1 min) resulted in the best germination response of 84.5%. Sterile wet cotton soaked with sterile distilled water was found suitable for germination compared to other substratum, while half-strength MS medium with 2.0% sucrose was suitable for seedling development.

Keywords: *Helicteres isora*, phenology, seed germination.

Helicteres isora L. (Malvaceae) or the Indian Screw Tree has been scripted in the indigenous medicinal systems of India for the treatment of various diseases since time immemorial. Generally, it is habituated as a sub deciduous shrub or small tree based upon the soil type and annual rainfall (Sebastine 1954). It is found profusely distributed all over India including the forests of Andaman Islands. Ayurveda, ethnobotanical records as well as current pharmacological reports revealed that snake bite, dog bite, diarrhoea, diabetes (hypoglycaemic and hyperglycaemic), cancer, microbial contaminations, spasmodic effect and blood related diseases can be treated using different parts of *H. isora* (Kumar & Singh 2014). In addition bioactive compounds like cucurbitacin-B, isocucurbitacin- B, diosgenin, daucosterol, hibifolin, trifolin, rosmarinic acids etc., have been extracted from *H. isora* (Kumar & Singh 2014). Besides having medicinal value, the species yields a dye and its wood is much harder than teak and is being used for building boats, making tool handles, carts, carriages, planks, bows of violin, beams, fencing posts and firewood (Nadkarni & Nadkarni 1976). Many local communities use its fibre to weave bags and make canvas which last long for years (Sebastine 1954), and leaves and juvenile branches are used as fodder (Atluri et al. 2000). The Ministry of Health & Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy-AYUSH owing to its trade demand suggest that conservation efforts and cultivation should be focused on *H. isora*, which has a demand exceeding 100 MT per year (Ved & Goraya 2007).

Santharam (1996) and Atluri et al. (2000) studied the flowering pattern and pollination related phenology. Poor seed dormancy and its natural germination problem were already reported (Badave & Jadhav 1998; Ferdousi et al. 2014), but seed viability and germination studies are not yet experimented properly for easy cultivation practices. The developed protocol for in vitro seed germination and seedling development can be further utilised in tissue culture practices for mass cultivation and sustainable utilisation.

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Materials and Methods

Study area

We studied the *H. isora* population in the Kolli Hills located in the Eastern Ghats of Tamil Nadu, India 11.1667–11.5000 N & 78.2500–78.5000 E with an altitude range of 180–1,415 m. It comprises 503km^2 of which forest covers only 271km^2 (54%); the remaining places are under agriculture and other activities (Geological Survey of India 1976). Three major seasons were categorized for this area, which are monsoon (June–December), winter (January–February) and dry summer (March–May). Annual rainfall varies from 300-1318 mm including both monsoons (southwest and northeast) (Anonymous 1999).

Phenology

Plants (5 numbers) were marked and observed at regular intervals (week) from March 2011 to April 2014. Observations were recorded during flowering and fruiting. The matured fruits of about 500g were collected and utilised for the experiments. Each follicle was untwisted to collect the seeds and stored in a glass desiccator for experimental uses and long storage. The seed number and weight of seeds of individual fruit were noted and viability tests were carried out every month from immediate collection to until the end of a 24-month period.

Seed viability and germination

Seed viability was tested through water dipping method and viable seeds were accounted for further germination experiments. Viable seeds were identified and washed under running tap water for 10min to remove debris. A set of 250 seeds were soaked in sterile distilled water for 24 hours in the dark; similarly another set (n=250) was soaked in 50mM GA_3 (dissolved in sterile distilled water) and kept in the dark for 24 hours. A third set of seeds (n=250) was treated by soaking in H_2SO_4 (98.0 %) for 1min. The seeds were decontaminated with commercial Teepol™ (Sigma-Aldrich, India) for 2-3 min followed by ethanol (70.0% v/v) for 30 sec, 3% NaOCl for 1min and 0.1% (w/v) $HgCl_2$ for 5min and finally five times washed with sterile distilled water.

Sterilised seeds were transferred to three different kinds of substratum, half-strength Murashige & Skoog (½ MS) medium, full-strength Murashige & Skoog (1962) (MS) medium and sterile cotton soaked with sterile distilled water to identify the suitable substratum for seed germination and seeding development.

Results and Discussion

Phenology of flower and fruit set

The vegetative growth of the plant is high once the monsoon season sets (Image 1a). Though the onset of the monsoon is early June, the total coverage and regular rainfall for the study area starts from 3–4 weeks of the same month. Flower buds emerge around the third week of July. Flowering starts during the middle of August and is prolonged up to late October (Image 1b). The plant bears the flower for three days; the next day the pollinated ones give up the corolla and anthers, whereas the whole flower is abscised if it is un-pollinated (Image 1c,d). Atluri et al. (2000) report is also consistent with our data except the flowering onset and offset periods as the geographical and climatic situations varies between both the study areas. Fruit development takes around 78±7 days and mature fruits are available from late January to early April (Image 1e). The average length of the mature fruit varies from 5–7 cm. The fruit has five follicles with each one twisted around each other, either on the right hand side or left hand side. But both right side twisted and left side twisted fruits are available on the same plant and the same branch and even in the same fascicle. Each follicle consists 24.29±0.47 seeds and in single fruit 117±1.96 and the total number of seeds weighed around 377.71±4.91 mg.

Factors influencing flower, fruit set and distribution

Survival and distribution of *H. isora* is at high risk as their population is being disturbed through natural as well as anthropogenic causes. We had noticed the blister beetle (*Mylabris pustulata*) feeding on the mature flowers (Image 1f). Earlier Atluri et al. (2000) identified the same species could prevent overall 6% of flowers to form fruits. An unidentified larvae present inside the fruit follicles survives mainly by feeding upon fruit contents (Image 1g,h). Overall 8% of fruits were infested with these unidentified larvae. Fruits persist till the first week of April thereafter fruits start dehiscing to distribute seeds as summer begins. During the survey period we observed that monsoon course was sometimes delayed or recorded deficit rainfall or early withdrawal, similarly climatic course pattern is seriously upset. Our observation over the climate conditions revealed that the summer sets early than expected tenure which causes the fruits to dry prior to full maturation. Early climatic change caused a reduction of atmospheric humidity and increased temperature, which arrests fruit maturation and forces the fruits to get dried. Such dried fruits contain only immature seeds which are generally not able to germinate. These kinds

Image 1. Phenology, problems in fruit setting, distribution and in vitro seed germination of *H. isora*.
a - *H. isora* habitat; b–d - flowering; e - fruits (fresh and dried); f - Blister beetle (*Mylabris pustulata*) eating flower of *H. isora*; g & h - unidentified larvae presents inside *H. isora* fruit follicle feeding seeds; i - trimming of developing branches; j - local tribe in fruit collection; k - seeds from single matured fruit; l - seed germination in cotton bed; m - seedling development in cotton bed; n - seed germination and development in MS medium; o - germinated seeds developed in ½ MS medium.

of anomalies happened to flower by end of season and the developmental stages were hindered by changes in atmospheric climatic conditions unexpectedly. Over 35–41 % of fruits bear immature non-viable seeds. Matured tree branches and most of the newly developing plantlets were regularly trimmed or cut down as in the

Table 1. Seed germination of *H. isora* using various treatments and suitable substratum.

Substratum	Soaking in water			GA_3 treatment			Acid treatment		
	Response (%)	Day of response	Seedling development*	Response (%)	Day of response	Seedling development*	Response (%)	Day of response	Seedling development*
MS	17.5±1.60[b]	25–32	+++	18.6±1.21[b]	31–36	+++	45.6±2.12[b]	18–22	+++
½ MS	18.5±1.12[b]	28–35	+++	20.6±0.86[b]	29–35	+++	46.9±1.48[b]	20–26	+++
SCB	**33.7±1.14[a]**	**14–18**	-	**34.1±1.28[a]**	**16–20**	-	**84.5±1.82[a]**	**3–5**	-

* Seedling development; - = poor; ++ = moderate; +++ = good.

process of tourism development for widening roads and/or to build stone walls (Image 1i). Apart from these, local communities collect the fruits and use them for their ethnomedicinal properties, and local vendors collect fruits indiscriminately for selling in local markets (Image 1j). We found that the distribution of *H. isora* was very difficult due to the above mentioned problems.

Seed viability and germination

Seeds were 88.2% viable initially during the collection period and the viability was merely the same for the next six months. Thereafter, it started to reduce and fall down to 24.9% by the 24th month (Fig. 1). Badave & Jadhav (1998) reported poor seed dormancy of *H. isora*. Hence long term storage of seeds is difficult in *H. isora*. Seeds of *H. isora* are not suitable for natural germination as its seed coat is very hard. Ferdousi et al. (2014) results were consistent with our findings. There are very few seedlings germinated in the field while thousands of seeds are dispersed every year. Shriram et al. (2008) germinated the seeds through acid treatment, but it lacks the detail report on percentage of success and suitability of substrate. Viable seeds were experimented for germination and found acid treatment alone the best treatment in terms of germination (84.5 %), when the suitable substratum is provided (Table 1; Image 1l,m). Sterilised wet cotton bed supported well, whereas other two substratum were not suitable even though the seeds got the same acid treatment (Table 1; Image 1n). Over-all sterilised wet cotton is a superior substratum, provides enough liquid matter to initiate germination. The other two substrates, however, do not have free liquid substance to support seed germination. This is due to the very hard nature of seed coat, where the acid treatment scarifies the seed coat and allowed the existing free water to infiltrate through seed coat and imbibe the seeds immediately. Germinated seeds were staged up to cotyledonary leaves and developed radicle in sterilised wet cotton (Image 1m), since it does not have any nutrients essential to its growth pattern. Hence the germinated seeds were transferred to half-strength MS medium for further seedling development (Image 1o). So that the developed seedlings can be further utilised for futuristic approaches like in vitro propagation, genetic analysis etc.

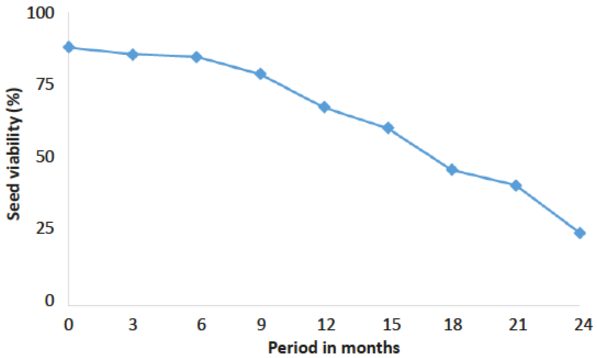

Figure 1. Seed viability of *H. isora* from collection period to 24 month.

Conclusion

The present study provided a better insight into the flowering and fruit setting and its associated problems in *Helicteres isora*. Poor seed setting, low natural seed germination rates and indiscriminate collection of plant materials caused this plant serious stress in the study area. So immediate action has to be undertaken in terms of creating awareness among the local community and concerned government agencies and initiate action for species conservation. In addition, in vitro propagation should be undertaken to mass propagate and reintroduce it to augment the existing population.

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Notes

Book Review