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In vitro seed germination of *Decalepis hamiltonii* an endangered medicinal plant using gibberellic acid and coconut water

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ABSTRACT

A highly efficient protocol was developed for germination of seedlings from mature seed pods of *Decalepis hamiltonii* Wight. and Arn. It was found that gibberellic acid (GA_3) had a synergistic effect on germination in combination with coconut water. This protocol uses gibberellic acid for germination of seeds, which was more in the presence of coconut water. The maximum number of seedlings per culture with root formation was observed on the medium containing gibberellic acid (0.5 mg/l) full strength media with coconut water (10 %) showed 40% germination, while gibberellic acid (1.5 mg/l) half strength media with coconut water (5 %) resulted in to vigorous and synchronous growth of seedlings up to 50% respectively in comparison with control. The seedlings were kept for acclimatization, and their field survival rate was 50-75 %.

Key words*: Germination, Decalepis hamiltonii, Gibberellic acid, Coconut water, Seedlings.*

Introduction

Decalepis hamiltonii Wight & Arn. (Apocynaceae) is a monotypic species restricted to Southern India and rare in the evergreen forest of Western Ghats and Eastern India (Gururaj *et al.,* 2004). It is used as a culinary spice as its roots are aromatic and highly priced, having a flavouring principle. It is also used as an appetizer, a blood purifier and a preservative (Jacob, 1937; Murti and Seshadri, 1941; Phadke and Gholap, 1994; Wealth of India, 1990). Although vanillin has been synthesized since 1874, natural sources of this flavouring are still in demand and roots of *Decalepis* species can be used as a substitute for vanillin (Shahzad and Sharma, 2014). *D. hamiltonii* has been listed in the IUCN red list of threatened species (Ved *et al*., 2015). Though the plant of *D. hamiltonii* is high in demand but, its population occurs in small patches and declining due to over harvesting in the form of roots (Giridhar *et al*., 2005). *D. hamiltonii* has been sold in market under the name 'Sariva', most of the time as a substitute for *Hemidesmus indicus* (L.) R. Br. a well known ayurvedic medicinal plant. It also has proved its equal in vitro antioxidant potential as a substitute for 'Sariva' (Sandecha *et al.,* 2022).

Germination of plants takes place with a specific condition and that varies from species to species and the use of specific media that gives desired results (Gairola *et al.*, 2011). Storage of seeds in the required condition viz. an optimum temperature, moisture content and correct packaging allows longer storability, the seed viability and vigor.

Therefore, the present study was designed for standardization of media using gibberellic acid and coconut water for seed germination. The present results describe a highly efficient method for micropropagation of plants from seeds of *D. hamiltonii* using gibberellic acidand coconut water. Large scale multiplication may help in commercial cultivation and supply of required raw material to pharmaceutical industries to fulfill their demand.

Materials and Methods

Seeds of *D. hamiltonii* were collected from the cultivation site of Agasti Agroved, Pune, Maharashtra, India. Plant was identified by taxonomist Dr. Suresh Jagtap, Taxonomist, IRSHA, Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India.

No preliminary treatment was given to seeds as they were isolated directly from a pod in sterile form. For germination studies, the experiment was laid out with three replications having 30 seeds each. The seeds were inoculated in media containing gibberellic acid (0.5, 1.0, 1.5 & 2 mg/l and coconut water (5% and 10%) on MS media with different concentrations. Cultures were maintained in a growth chamber at temperature 25±2° C under photoperiod of 8 hours incubated around 4 weeks. Later, the seedlings were washed with sterile distilled water and transferred eventually for primary hardening (2-3 weeks) in sterile coco peat at 28 to 30°C temperature. Further they were transferred for secondary hardening.

Results and Discussion

The Population of *D. hamiltonii* has been declining substantially due to its economic potential and used as a substitute for *Hemidesmus indicus* (L.) R. Br. Considering its potential as a substitute for the ayurvedic drug 'Sariva' and the declining population it is utmost to increase the population by undertaking cultivation as well as using micropropagation techniques (Sandecha *et al.*, 2022). In the present manuscript, a viable plant germination method was optimized for *D. hamiltonii* seeds using gibberellic acid (Plant growth regulator) and coconut water.

Germination was recorded after 4 weeks of culturing, showing callus based indirect germination (Figure 4). The different concentrations of gibberellic acid (0.5-2 mg/l) were used in combination with 5% and 10% coconut water (Table 1). The gibberellic acid concentration of 0.5 and 1.5 mg/l along with 5% and 10% coconut water resulted in 70-75% germination response (Table 2). Coconut water is a natural diuretic with zero additives containing essential electrolytes. Germination of seed with callus was decreased with the increased concentration of gibberellic acid respectively (Figure 1, 5).

Table 1. Media composition used for *in vitro* culture of *D. hamiltonii* seeds under control conditions

Component	Culture medium			
	C1	C2	C3	C4
MS	1	½	½	½
Coconut Water (%)	-	-	5	10

Table 2. Effect of media composition on plant germination through embryogenesis in *D. hamiltonii* seeds

No	Media Composition	Germination with callus	Germination without callus
1)	**C1**	41.67	25.00
2)	**C2**	25.00	20.83
3)	**C3**	29.17	66.67
4)	**C4**	62.50	8.33

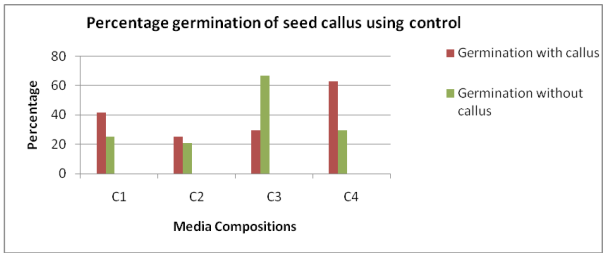


Fig. 1. Responses of seed cultures on different media composition in *D.hamiltonii*

In all treatments of half and full strength gibberellic acid (2 mg/l) media, shoot hyperhydricity was ascertained except in 10% coconut water with (2 mg/l) gibberellic acid (Fig. 2, 5). The maximum response togermination (50%) was observed in half strength MS media of gibberellic acid (1.5 mg/l) in

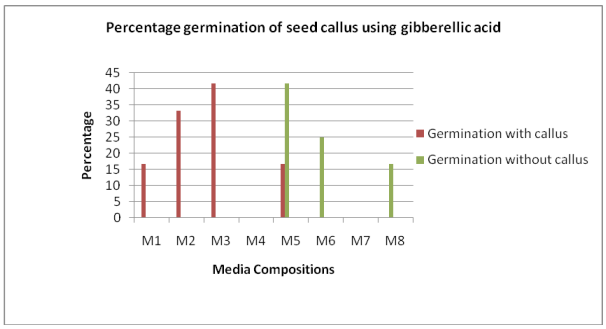


Fig. 2. Responses of seed cultures on media with Gibberellic acid in *D. hamiltonii*

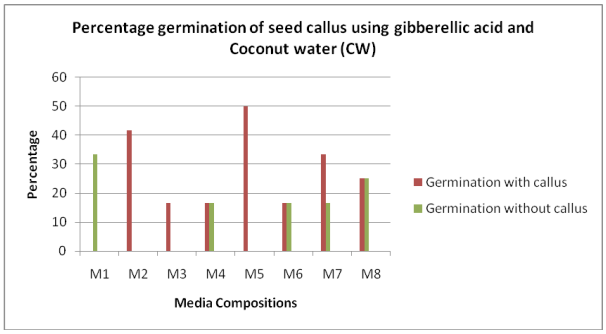
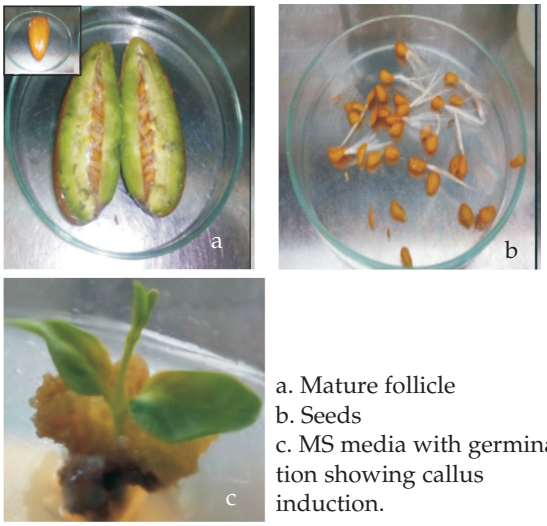


Fig. 3. Responses of seed cultures on media with gibberellic acid and Coconut water in *D. hamiltonii*



a. Mature follicle
b. Seeds
c. MS media with germination showing callus induction.

Fig. 4 (a-c). *In vitro* seed germination in *D. hamiltonii*

Table 4. Effect of media composition on plant germination through embryogenesis in *D. hamiltonii* seeds using different concentration of gibberellic acid

Media Composition	Germination with callus	Germination without callus
M1	16.67	0.00
M2	33.33	0.00
M3	41.67	0.00
M4	0.00	0.00
M5	16.67	41.67
M6	0.00	25.00
M7	0.00	0.00
M8	0.00	16.67

combination with 5% coconut water showing vigorous and synchronous growth. While the best results of germination without callus were seen in half strength MS media (0.5 mg/l) in combination with coconut water (5%) as compared to other media compositions. (Figure 3, 6). Callus induction from seed coat can lead to clonal propagation. Similar results were reported by Origenes (2020) where coconut water was used to soak seeds of Papaya.

Seedlings which were ready for primary hardening (Figure 7) were further transferred for secondary hardening. First time we have reported *in-vitro* germination of *D. hamiltonii* seeds using coconut water in combination with gibberellic acid (plant growth regulator) resulted into healthy growth.

Similar results for *Decalepis hamiltonii* were also reported by a few researchers using seeds. Utami and Hariyanto (2019) successfully reported in vitro seed germination and development of seedlings for the less available plant, *Phalaenopsis amboinensis*. Herawati *et al*. (2020) published their research by adding coconut water in the media which enhanced the growth and regeneration of *Dendrobium gatton sunray* whereas recently, Vasupen, *et al*., reported the positive results forcoconut water, benzylaminopurine, and naphthalene acetic acid for *in vitro* seed germination *Eulophia flava* (Lindl.) Hook. f.

Table 3. Media composition used for *in vitro* culture of *D. hamiltonii* seeds using gibberellic acid

Component	Culture medium							
	M1	M2	M3	M4	M5	M6	M7	M8
MS	½	1	½	1	½	1	½	1
Gibberellic acid (mg/L)	0.5	0.5	1	1	1.5	1.5	2	2

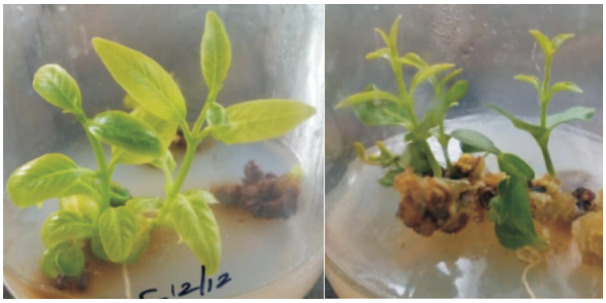
Table 5. Media composition used for *in vitro* culture of *D. hamiltonii* seeds using gibberellic acid & Coconut water

Component	Culture medium							
	M1	M2	M3	M4	M5	M6	M7	M8
MS	½	1	½	1	½	1	½	1
Gibberellic acid (mg/ L)	0.5	0.5	1	1	1.5	1.5	2	2
Coconut water (%)	5	10	5	10	5	10	5	10

a. MS + GA_3 (0.5mg/L) b. MS + GA_3 (1.0mg/L) c. ½ MS + GA_3 (1.5 mg/L) d. MS + GA_3 (2.0 mg/l)

Fig. 5 (a-d). Seed germination on media with Gibberellic acid (GA_3) in *D. hamiltonii*

a. Seed germination with callus induction on MS medium supplemented with GA_3 (0.5 mg/l)
b. Abnormal growth on MS medium supplemented with GA_3 (1.0 mg/l)
c. Germination on half strength MS medium supplemented with ½ GA_3 (1.5 mg/l)
d. Germination without callus induction on MS medium supplemented with GA_3 (2.0 mg/l)



a. MS + GA_3 (0.5 mg/l) CW (10%) b. ½ MS + GA_3 (1.5 mg/l) CW (5%)

a. Seed germination on MS medium supplemented with GA_3 (0.5 mg/l) CW (10%) showing healthy growth.
b. Germination with callus induction on MS medium supplemented with GA_3 (1.5 mg/l) CW (5%)

Fig. 6 (a – b). Seed germination on media with Gibberellic acid (GA_3) and Coconut water in *D. hamiltonii*

Finally, the seedlings kept for hardening were ready to transfer to the field for cultivation. Hardening of *in vitro* raised seedlings or plants enables them to withstand the changes that occur in environmental conditions when planted outside in a nursery for successful establishment.

Table 6. Effect of media composition on plant germination through embryogenesis in *D. hamiltonii* seeds using different concentration of gibberellic acid and Coconut water

Media	Germination with callus	Germination without callus
M1	0.00	33.33
M2	41.67	0.00
M3	16.67	0.00
M4	16.67	16.67
M5	50.00	0.00
M6	16.67	16.67
M7	33.33	16.67
M8	25.00	25.00

Conclusion

The percentage of seedlings establishment of *D. hamiltonii* depended on gibberellic acid in combination with coconut water for successful *in vitro* germination. The present findings will help in the germination of *D. hamiltonii* for its conservation, large scale multiplication, cultivation, and supply of raw material on a large scale to fulfil their growing demands.

Fig. 7. Seedling in acclimatization phase in growth chamber.

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Conflict of interest

The authors declare that they have no conflict of interests.

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