RESEARCH ARTICLE

Promotion of seed germination in *Corallocarpus epigaeus* (Rottler) Hook. f. –A critically endangered medicinal plant, and relevance to conservation

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ABSTRACT

An effective protocol is developed for the germination of *Corallocarpus epigaeus* seeds. The seeds were collected from intact plants. Various reasons are promoting seed dormancy in this species. Such dormancy was broken and achieved 84% *in vitro* and 70% *in vivo* seed germination. This enhanced percentage of germination is enabled for the conservation of this critically endangered plant. This is the first report on successful *in vitro* and *in vivo* germination of *Corallocarpus epigaeus* seeds.

Keywords: Dormancy; Gibberillic acid; Immature embryo; Perlite; Phenolic substances.

INTRODUCTION

Germination is the first and foremost developmental stage in the life cycle of a plant to generate a new population and the ability to accomplish this task is a prerequisite to start cycle (Bewley, 1997). In natural conditions, the seed does not germinate readily if exposed to sun, as the radical is liable to dry up under such circumstances. Therefore it is necessary to adopt suitable methods to enhance the rate of seed germination drastically and elicited quality seedlings with better survival rate in the field.

Natural regeneration in the species *Corallocarpus epigaeus* is very less (30%) due to aborted embryos, hard seed coat, prolonged seed dormancy, consisting of phenolic substances and fungal infection. These factors are basis for the species to become rare and threatened.

Right now there is no exact report on *in vivo* and *in vitro* seed germination in this threatened species. Therefore, there is an urgent need to develop a reproducible protocol for efficient seed germination in order to overcome and nullify the drawbacks and conserve this species. The objective of the present study is to enhance the efficiency of seed germination of *in vivo* and *in vitro* in *C. epigaeus*.

MATERIAL AND METHODS:

Dry fruits of *Corallocarpus epigaeus* were collected from the plants growing in distinctive geographical areas of Warangal and Nalgonda districts of Telangana State, India.

***In vivo* seed germination:**

The mature fresh fruits of *C. epigaeus* were collected during November-December, 2010 and 2011 from Elakalabai village, Athmakur Mandal, Nalgonda District, Telanagana state, India. The fruits were dried under sunlight for 5 days and seeds were separated. These seeds were washed with running tap water and allowed to air dried.

The experiments were also carried out to know the effect of different soil types like red soil and a mixture of Peat + Perlite + S and (l : 1: 1) and GA_3. Seed age was also taken into consideration to observe and to explore the rate of seed germination and number of days required for germination of seeds in *C. epigaeus*. Then replicates used to take average parent of seed germination.

***In vitro* seed germination:**

Mature seeds were collected and allowed to air dried for 5-6 days and then washed under running tap water followed by sterilized with 0.1 % $HgCl_2$, for 3-5 minutes and were washed thoroughly with sterile distilled water thrice. After soaking in distilled water for 8 hrs, seed coat was removed through sterilized forceps and scalpel and de-coated seeds were sterilized with 0.1 % (w/v) $HgCl_2$ for 30 seconds and washed three times with sterile distilled water. These de-coated seeds were inoculated on different forms of MS media such as MS basal solid and MS basal liquid media supplemented with GA_3 (5.0mg/l). The pH of medium was adjusted to 5.7 with either 0.1 N HCI or O.l N NaOH. The medium was dispensed into different culture tubes and autoclaved at 121°C under 15 lbs pressure for 15-20 minutes. The de-coated seeds were inoculated on the paper bridges made of whatman No.1 filter paper partly immersed in liquid medium. All these cultures were incubated at 25±2^{0}C under 16h/8h photoperiod provided with the light intensity of 2000 Lux by white fluorescent tubes observation were recorded from 5 days of inoculation. After seed germination percentage was calculated following the method of Gharineh *et al.,* (2004). **Gp= (NG/NT)×100** (**Gp**=Germination percentage, **NG**= Number of germinated seeds, **NT**=Total number of seeds sown).

$$\textit{Germination percentage} = \frac{\textit{No. of seeds germinated}}{\textit{Total No. of seeds placed on the medium}} X100$$

RESULTS AND DISCUSSION

***In vivo* seed germination:**

The germination of seeds *in vivo* was recorded at different time intervals and the data was recorded (Table-1). Primarily the seeds were sown in the red soil (T1) and the germination percentage (18%) was reported after 16 days of sown, in T2 (Perlite:Peat:sand 1:1:1) moderate percentage (34%) of germination was achieved, but the germination was incited two days earlier than the T1. Highest percentage of germination (70%) was observed in T3 ($GA_3$10.0mg/l) within 10 days of inoculation (Fig.1 a and b) and the seedlings were also elongated rapidly. Higher seed germination percentage (60%) was recorded on T4 (12days young seeds) after 12 days of shown in red soil. However, the seedlings were weak with light green colour, whereas seeds did not show any response of germination in T5 (1 year old seeds) even after 20 days of inoculation. When the seed coats were removed and allowed for germination, 10% of germination was observed in T6 after 14 days of inoculation (Table-1).

***In vitro* seed germination:**

In vitro technique was also employed to determine the various aspects for assessing the rate of seed germination. 66% of seed germination was achieved on T7 (MS basal liquid medium) after 12 days of inoculation. Swelling of seeds was started after 3 days, and radical and plumule were emerged out after 6 days of inoculation. Significantly highest percentage (84%) of seed germination was achieved in T8 after 8 days of inoculation (Fig.1 c and d). However, the seeds without seed coats (T9) did not favour the seed germination percentage as T8. Only 56% of seed germination was reported in T10 after 16 days of inoculation and produced long thin roots into the medium and elongated shoots above the solid medium. 68% of seed germination was recorded in T11 (GA_3 5.0mg/l) after 14 days of inoculation and 72% of germination was noted in T12 treatment after 12 days of incubation (Fig.1 e and f). And longer shoots were developed in T12 when compared to all the above treatments (Table-1).

Table 1 : *In vivo* and *In vitro* Seed germination of *C. epigaeus* on various treatments

S.No	Treatment (T)	Percentage of Response of *C. epigaeus* seeds	
	In vivo	***Percentage of response***	***Days of germination***
1	T1 (Red soil) (Control)	18	16
2	T2 (Pertile:Peat:Sand)	34	14
3	T3 (GA_3 10mg/l)	**70**	**10**
4	T4 (10 days seed)	60	12
5	T5 (1year seeds)	NR	NR
6	T6 (SWtC)	10	14
	In vitro		
	Liquid medium (Paper bridge)		
7	T7 MS basal liquid medium	66	12
8	T8 MS + GA_3 5mg/l	**84**	**08**
9	T9 MS + GA_3 5mg/l (SWtC)	52	08
	Solid medium		
10	T10 MS basal solid medium	56	16
11	T11 MS + GA_3 5mg/l	68	14
12	T12 MS + GA_3 5mg/l (SWtC)	**72**	**12**

SWtc: Seed without coat ; SWC: Seed with coat

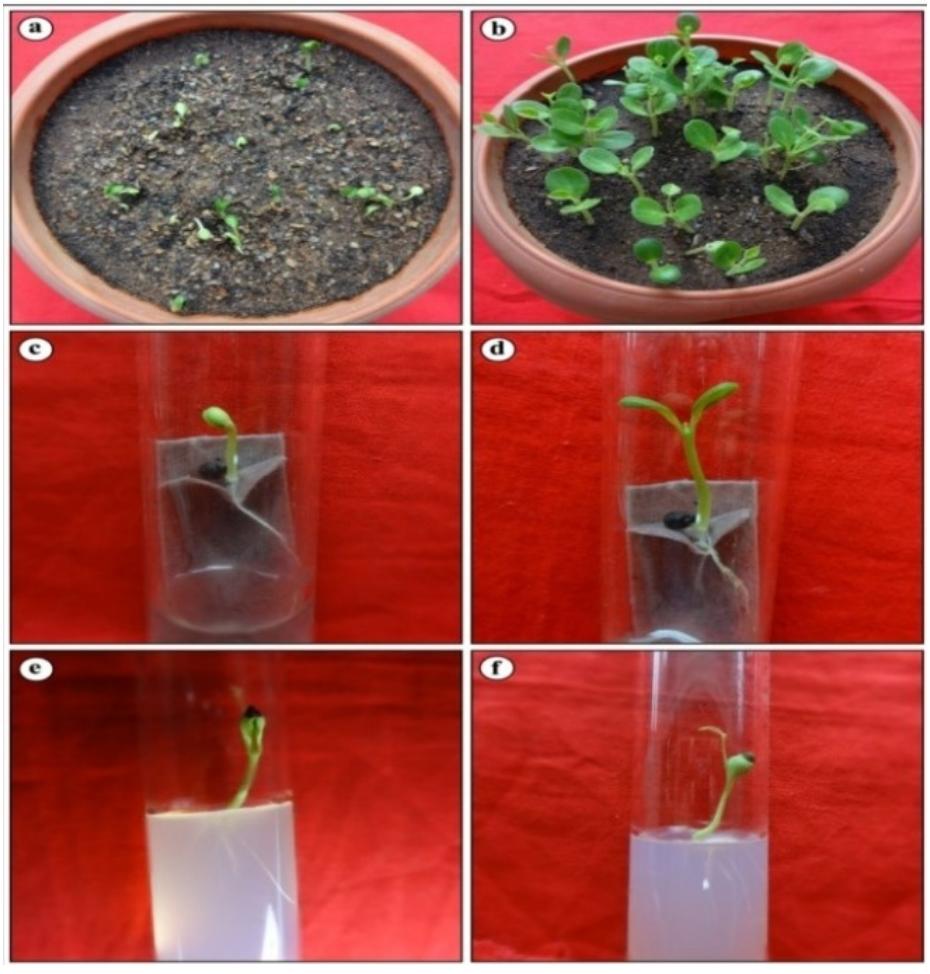

Fig-1: *In vivo* and *in vitro* seed germination of *Corallocarpus epigaeus*.
a & b: *In vivo* seed germination from treated seeds with GA_3 (10mg/l) after 10 (a), 14 days (b) respectively.
c & d: Seed germination after 8 days (c) and after 12 days (d) on MS medium (liquid)+GA_3 (5mg/l) with seed coat (SWC).
e & f: Seed germination after 12 days (e) and after 16 days (f) on MS medium (solid)+GA_3 (5mg/l) without seed coat (SWtC).

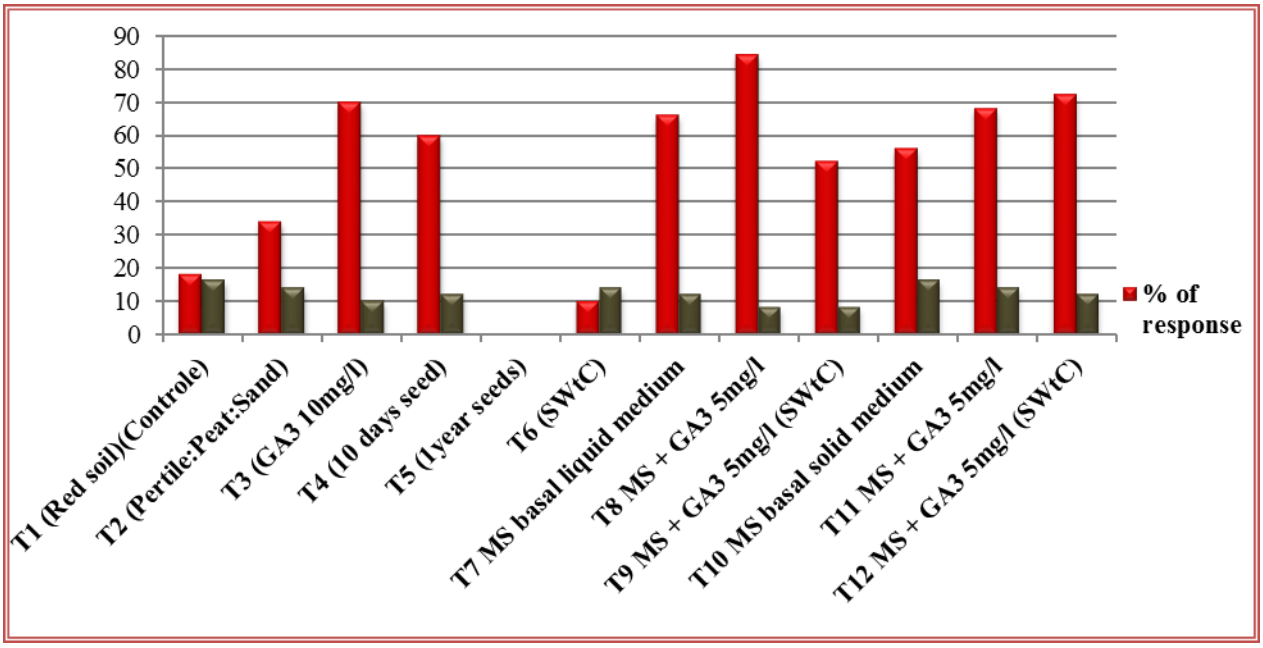

Fig. 2 : Comparison of *in vivo* and *in vitro* Seed germination of *C. epigaeus*.

DISCUSSION:

In the present study, seed germination of *C. epigaeus* was observed both in *in vivo* and *in vitro.* In *in vivo* two different types of soils were used for the germination of seeds. Moreover, moderate percentage response of germination (34%) was noticed in the mixture of Perlite+Peat+Sand (*in vivo* germination) after 14 days of inoculation compared to red siol (T1) where less response (18%) was observed after 16 days of inoculation. The similar effect of Perlite+Peat+Sand (T2) on seed germination was reported in *cyclamen alpinum* (Bueruen and Sahin, 2009). Environment, soil factors and physical factors influence seedlings emergence (Hegarty *et al.,* 1979, Khan *et al.,* 1980, Thomas, 1981, Okusanya, 1978). However, significantly maximum seed germination (70%) was reported when seeds were treated with GA_3 (10.0mg/l) in T3, the first germination was apparent by 8th day and reached a peak after 10th day. Seeds were fresh but dried for 10 days yielded 60% of response after 12 days of inoculation *in vivo*. However, fresh seeds were given less response compared to normal seeds, this might be because of immature embryo which may counteract the germination ability. Similar results were noted in *Withania somnifera* (Pandey *et al.,* 2013). One year old seeds did not respond in germination, it indicates that the increasing the age of the seeds gradually declines in viability of the seed and this leads to failure in seed germination. Seeds without seed coat (SWtC) were exhibited only 10% of germination *in vivo* after 14 days of inoculation. Maximum percentage of seed germination (84%) was achived after 8 days of incubation from seed with coat (SWC) on MS liquid medium added GA_3 (5.0mg/l) than the seed without coat (SWtC). MS solid medium with GA_3 was proved for the best percentage of germination of seeds with SWtC seeds after 12 days of inoculation. Sprouting of seeds was observed after 3 days of culture, and elongation shoots was observed after 12 days with two pair of leaves, besides production of roots. However, from this study maximum percentage (84%) of germination was recorded on MS liquid medium with GA_3 at 5.0mg/l (T8). The seed germination was found to be dependent on the concentration of GA_3 (5.0mg/l) and internal factor of the seed. Similar observations were also reported in Cucurbitaceae species (Nelson and sharples, 1980 and Staub *et al.,* 1989). Seed germination in *Luffa* has been reported by Davis (1997).

Maisalmani and Dharmalingam (2002) reported the highest percentage of germination in GA_3 treated seeds in *Grevellea robusta* compared to $KN0_3$, thiourea and combination of these three. Madhusudan (2011) had also reported that GA_3 showed the enhancement of seed germination in *Wrightia tinctoria* as observed in the present investigations. According to our investigation, significantly maximum percentage of seed germination, with healthy seedlings and early germination were found on paper bridge method

compared to MS solid medium with GA_3 treatment to seeds. Hence, we have used the same method for the use of seedlings for all the experiments in the present investigations. Thus, the present protocol can be used for its conservation and multiplication of the species. Based on this observation, paper bridge method is recommended for enhance of seed germination in *Corallocarpus epigaeus.*

Conflicts of interest: The authors stated that no conflicts of interest.

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