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DORMANCY AND *IN VITRO* SEED GERMINATION OF *CORYNANDRA CHELIDONII VAR. PALLAE* (CLEOMACEAE) AN ENDEMIC AND ETHNOMEDICINAL HERB

Subhash Sirangi and Ajmeera Ragan*
Plant systematics laboratory, Department of Botany, Kakatiya University, Warangal-506009, Telangana, India

***Corresponding Author Email:** raganajmeera@gmail.com

ABSTRACT

Seed germination is a critical physiological process in the life cycle of a plant. Corynandra chelidonii var. pallae is a perennial and seeds fail to germinate lead to rapid extinction of the species. Seeds contain ornamentation with tough seed coat and most of the seeds were abortive at immature & even mature stage of embryos these made the plant less propagative. It is a rare ethnomedicinally important herb restricted to Pakala lake of Warangal District of Telangana. In view of its medicinal importance the species is being over exploited hence there is an urgent need for its conservation before they get extinct. We adopted many methods to break the seed dormancy such as preheating at 35-40^0 C temperature, pre-chilling at 0-5^{0}C temperature for various periods and also tried paper boat technique but all were futile. Seed were soaked in 1-5 mg/ml concentrations of GA3 and photoperiod maintained. At 18/6 LD photoperiod and 2 mg/ml GA3 seed germination was 45 %. In the present study we enhance the percentage of seed germination in vitro and observed effect of ($GA_{3)}$ of various concentration and influence of physical factors like Photoperiod. We also observed plants' unique nature that after germination; the cotyledons and seedlings remain green for several days in aqueous condition that indicate the aquatic nature of plant.

KEY WORDS

Corynandra, Photoperiod, MS medium, germination percentage.

INTRODUCTION

A new variety of *Cleome chelidonii* was described from the littoral waters of Pakhal reservoir (Pakhal Forest Reserve, Warangal district, northern Telangana). (Reddy & Raju, 2001). This herb is commonly called *adavi avalu* and the seeds are used as condiment. These species is having ethnomedicinal, ecological and economic importance. *Cleome chelidonii* and *Cleome viscosa* contain glucocapparin and glucocleomin Callus and suspension cultures of *Cleome chelidonii* produced glucosinolates (Songsak & Lockwood2004). *Cleome chelidonii* is generally known to be used for the treatment of colic, dysentery, headache, otitis and rheumatism (Kirtikar & Basu, 1991). Recently, *Corynandra* Schard. ex Spreng., the earlier name for the segregated genus *Arivelia* Raf. of Cleome (Cleomaceae) was reinstated (Cohrane & Itis, 2014). Accordingly, *Cleome feline* L.f., *Cleome flava* (Banks ex DC.) and *Cleome viscoasa* L.were transferred to *Corynandra*. Hence, *Cleome chelidonii* L.f. is to be called *Corynandra chelidonii* (L.f) Cocharane & Iltis. The local endemic taxon *Cleome chelidonii* var. *pallae* becomes *Corynandra chelidonii* var. *pallae* (reddy & raju) V.S. Raju, ined**.** (V.S. Raju,per. comm.) which grows as perennial in the local water bodies with 3-5-6 foliate leaves, pink flowers and 2-3 inch pods. It is a rare endemic and with sparse seed set; its population is on decline and it is not seen at pakhal over the past three years. Germination is the first developmental step in the life cycle of a plant to produce a new generation and the ability to accomplish
h 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 improve the rate of seed germination and produce quality seedlings with better survival rate. So, it needs to be conserved and hence an attempt is made.

MATERIALSANDMETHODS

In vivo seed germination

The mature fresh pods of *Corynandra chelidonii* var. *pallae* were collected from the littoral zone of Pakal lake, Warangal district, Telangana, India. Plants were grown in the Research Field, Department of Botany, Kakatiya University, Warangal. The pods were dried under sunlight for 5 days and seeds were separated. These seeds were washed with running tap water, followed by sterilization with 0.1% $HgCl_2$. These sterilized seeds were washed thoroughly with sterile distilled water thrice. Later these were treated with different concentrations (1-5mg/L) of auxins GA_3/ IAA/ IBA/NAA individually for different durations (48, 72 hrs.) separately. After the treatment, the seeds were transferred into the earthenware pots containing soil and vermiculite in the ratio 3:1 for their germination. 25 seeds were sown per pot and three replicates were maintained for each treatment and duration. Control was also maintained for each concentration and duration.

In vitro seed germination

The sterilized seeds with 0.1 % (w/v) $HgCl_2$, for 5 minutes, were washed thoroughly with sterile distilled water thrice. These seeds were inoculated on MS (Murashige and Skoog, 1962) containing different concentrations (15-30g/L) of sucrose separately without growth regulators. The pH of medium was adjusted to 5.7 with either 0.1 N HCl or 0.1N NaOH before addition of agar. Medium was solidified with 0.8% (W/V) Difco bacto-agar. The medium was dispensed into different culture bottles/vessels and autoclaved at 121^0C under 15 lbs pressure for 15-20 minutes. The seeds were gently inoculated on the medium. We also adopted many methods to break the seed dormancy such as preheating at 35-40^0 C temperature, pre-chilling at 0-5^0C temperature for various periods and also tried paper boat technique but all were futile. Then the seeds were soaked in 1-5 mg/ml cons. of GA3. Four replicates with each of 50 seeds were maintained. First sample was kept under 24 hr dark, 2nd sample at 20/4 LD and 3rd sample 6/18 LD and 4th at 18/6 LD photoperiod maintained. All these cultures were incubated at 25±2^0C under 16h/8h photoperiod provided with the light intensity of 40 µ mol $m^{-2}s^{-1}$ by white fluorescent tubes. Paper bridge method also tried.

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).

$$Gp = (NG/NT) \times 100$$

RESULTS

In vivo seed germination

Seed germination is a critical physiological process in the life cycle of a plant. *Corynandra chelidonii* is an perennial and the seeds fail to germinate. Seeds ornamented with tough seed coat and most of the seeds were found abortive with immature and even mature stage of embryos.

Primarily the seeds were sown in the compost, but the germination percentage was found to be very less (8-10%). The results on *in vivo* seed germination after GA_3/IAA/IBA/ NAA treatment (1-5 mg/L) to seeds at different time intervals (48 and 72 hours) are presented in Table 1 The treated seeds germinated early than the controls in *Corynandra chelidonii* var. *pallae.* The seeds were treated with 2.0 mg/L GA_3 for 72 hours showed maximum percentage of germination (17) compared to other concentrations of auxins (IAA/IBA/NAA).

Table No.1: Effect of GA_3/ IAA/IBA/ NAA on *in vivo* seed germination in *C. chelidonii.*

Concn of PGR (mg/L)	Seeds treated for 48hrs		Seeds treated for 72hrs	
	Days for germination	% of germination	Days for germination	% of germination
Control	10	10	12	10
GA_3				
1	08	10	09	10
2	**08**	**15**	**09**	**17**
3	08	10	09	15
4	06	08	06	08
5	08	08	06	08
IAA	08	NR	09	NR
1	08	NR	09	NR
2	08	NR	07	NR
3	06	NR	06	NR
4				
IBA	07	NR	08	NR
1	07	NR	08	NR
2	07	NR	08	NR
3	06	NR	06	NR
4				
NAA	08	NR	09	NR
1	08	NR	09	NR
2	08	NR	07	NR
3	06	NR	06	NR
4				

Mean ± Standard Error

Control - untreated

In vitro seed germination

Many methods were attempted to break the seed dormancy such as preheating at 35-40^0 C temperature, prechilling at 0-5^{0}C temperature for various periods but all the efforts were futile. The seeds were inoculated on MS basal media containing various levels of sucrose concentrations Table 2. The seeds were unable to germinate in media containing different concentrations of sucrose. The seeds were soaked in 1-5 mg/ml cons. of GA_3. Four replicates with each of 50 seeds were maintained. First sample was kept under 24 hr. dark, 2nd sample at 20/4 LD and 3rd sample 6/18 LD and 4th at 18/6 LD photoperiod. The highest percentage of seed germination was observed in the 4th replicate at 2 mg/ml GA_3.Table 3 explains about the concentration of GA_3 and photoperiod (LD). Paper bridge method also tried but the germination percentage was comparatively less.

Table No. 2: Effect of different cons of sucrose + MS media on *in vitro* seed germination of *C. chelidonii*.

Type of medium	% of germination	No. of days for seed germination	Seedling Height (cm)±SE	
			root length	Shoot length
MS+15gm/L Sucrose	NR	NR	_	_
MS+20gm/L Sucrose	NR	NR	_	_
MS+30gm/L Sucrose	NR	NR	_	_
Distilled Water (paper bridge)	25	10	1.61±0.28	1.08±0.11

Mean ± Standard Error

Table No. 3: Shows effect of seed germination at different Photoperiods (LD) when seeds were presoaked in GA_3 1-5 mg/ ml concentration of GA_3

Concentration of GA_3 (mg/ml)	24 hr dark	20/4 LD photoperiod	18/6 LD photoperiod	6/18LD photoperiod
1	5	10	30	2
2	4	10	**45**	5
3	10	5	25	6
4	10	10	20	3
5	5	12	10	2

Figure 1. a) Plant habitat; b) pods; c) collected seeds; d) *in vitro* seed germination in 2 mg/ml GA_3 at 18/6 LD Photoperiod; e) paper bridge in distilled water; f) *in vivo* seed germination.

DISCUSSION

In the present investigations, seed germination of *C.chelidonii* var.*pallae* was observed in all the concentrations of plant growth regulators *In vivo* and *In vitro*. The seed germination was not affected by the concentration of auxins and the duration of the treatment and also observed that there is no effect on seed germination of *C. chelidonii var.pallae also on MS with 15-30g/L sucrose, paper bridge, pre chilling and pre heating.*

According to our observations, maximum percentage of seed germination, with healthy seedlings and early germination were found on 18/6 LD photoperiod and at 2 GA_3 concentration method compared to different methods, seed germination in Gibberellin (GA) is an essential phytohormone that controls many aspects of plant development, including seed germination, leaf expansion, stem elongation, flowering, and seed

development (Davies, 1995) several studies from recent years have shown that gibberellin is an effective germination stimulator in several species (Giba et al., 1993; Karam and Al-salem, 2001; Çetinbaş and Koyuncu, 2006). *Talinum triangulare* effected by photoperiod (Nwoke 1982). Light-controlled germination has been associated with phytochrome, modulating seed responses to signals that can terminate dormancy and initiate germination (Benech-Arnold et al., 2000). A similar effect has been reported for other species (Cirak et al., 2004; Faravani and Bakar, 2007). In general, absence of light has a negative effect on germination in several species (Cirak et al., 2004; Sugahara and Takaki, 2004. Thus, the present protocol can be used for its conservation and multiplication of the species.

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***Corresponding Author:**
Ajmeera Ragan*
Email: raganajmeera@gmail.com