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Effects of different treatments on seed germination and breaking seed dormancy of *Citrullus colocynthis* an endangered medicinal plant

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Abstract

Citrullus colocynthis plants often have a longer period of dormancy because of the strong seed coat. To reduce the dormancy period and improve seed germination, in the present study we evaluated the significant effects of external conditions including temperature, light and humidity under *in vitro* conditions. The seeds are treated with acid and boiling water for different time intervals. 50% decorticated seeds were obtained by removing the lignified outer coats from the seeds. Intact embryos and decoated seeds were selected and separated directly into half-strength MS medium, paper boats in culture tubes, Petri dishes covered with Whatman No. 1 filter paper, and solid-strength MS medium covered with nylon mesh. In all treatments, the mechanical approach is ideal for breaking seed dormancy in *C. colocynthis*.

The scarification technique results in 92% of seeds germination, compared to 43% and 56% for the remaining acid and hot water treatments respectively. Present research methods help to break and overcome seed dormancy in plants with hard seed coats.

Keywords: *Citrullus colocynthis*, seed germination, acid treatment, dormancy, decoated seeds

Introduction

Citrullus colocynthis is a medicinally important endangered cucurbit it is found across the world's arid regions. It has a broad spectrum of therapeutic applications and is widely utilized in Ayurvedic and folk medicine (Dasari *et al.* 2020) [6]. *C. colocynthis* mainly contains glycosides, cucurbitacins (colocynthin and colocynthetin), Cucurbitacins A, B, C, D, E (Marzouk *et al.* 2022; Chawech *et al.* 2015) [15, 5], Cucurbitacins I, J, K, and L (Sturm *et al.* 2009) [23], cucurbitacin glycosides, flavonoids and flavones glycosides (Delazar *et al.* 2006) [6]. The cucurbitacin, glucoside combination (1:1) inhibited growth of ER (+) MCF-7 and ER (-) MDA-MB-231 Human breast cancer cell lines (Tannin-Spitz *et al.* 2007). Dried fruit pulp of *C. colocynthis* is used to treat indigestion, gastroenteritis and intestinal parasites. *C. colocynthis* has many pharmacological activities, including anti-diabetic, anti-malarial, anti-spermatogenic, anti-inflammatory, anthelmintic, anti-microbial, anti-oxidant, Hypolipidemic, anti-diarrheal and anti-cancerous (Hussain *et al.* 2014; Barghamdi *et al.* 2016; Dhakad *et al.* 2017; Kamran *et al.* 2018) [11, 3, 12].

C. colocynthis fruit is smooth, spherical in shape and contains more than hundred seeds. It is yellow-green fruit which becomes marble with yellow stripes at maturity (Ramakrishna *et al.* 2015) [18]. The seeds are embedded in the pulp; each of the three carpels contains six seeds. The seeds are gray in color, 3mm wide and 5mm long. The matured seed coat is thick dark brown and lignified underneath it a thin, transparent membrane encloses the embryo. The fruit is indehiscent and the seeds are liberated only by breakage or decay of the pericarp (Sperber *et al.* 2017) [22]. Production of *C. colocynthis* plant is very difficult because of hard seed coat and dormancy period of seed leads to very poor germination. Therefore, production of these plants needs to face a lot of problems. In wild flora, seed dormancy is very common for survival purposes under unfavorable environmental conditions (Baskin and Baskin 2004; Geneve 2003) [4].

Dormancy of seeds is a distinctive feature of the arid zone plants. It is essential to perform some treatments for eradicating the obstacle of germination. Menon *et al.* (2014) [16] performed different pretreatment methods in *C. colocynthis*, followed by manual scarification using sandpaper and incubation at room temperature to overcome seed dormancy (Menon *et al.* 2014) [16].

Few researches shown that, mechanical scarification by sand paper and acid treatment are given good result in seed dormancy in plants like *Acacia auriculiformis* (Azad *et al.* 2011) [2], *Parkia biglobosa* (Aliero 2004) [1] and *Ulex europaeus* (Sixtus *et al.* 2003). In *C. colocynthis*, Kshan Sahoo and Kesara performed pretreatment with $\text{Ca}(\text{NO}_3)_2$, KNO_3 , NH_4NO_3 and $\text{Co}(\text{NO}_3)_2$ to prevail over the seed dormancy (Sahoo and Kasera 2012). Similarly, pretreatment of concentrated sulfuric acid (H_2SO_4) broke the seed dormancy of *Leptadaenia reticulate* (Kasera and Shukla 2003) [13] and *Hibiscus hamabo* (Wang *et al.* 2012) [25]. In some cases like *Commiphora wightii* gave good results in low concentrations of KNO_3 , NH_4NO_3 and $\text{Co}(\text{NO}_3)_2$, whereas high concentrations of same treatments reported low dormancy breakage (Lal and Kasera 2014) [14].

The present experiment was undertaken to establish efficient protocol for seed germination and to overcome seed dormancy for *in vitro* growth of *C. colocynthis* plants for further experiment.

Materials and Methods

C. colocynthis plant seeds were collected from different areas like Godavari River in Basara, Nizamabad, Koonoor river valley in Warangal, Telangana State and Tiruchurapally, Tamilnadu State. Dark brown mature seeds were collected from fully ripe and yellow pulp dried fruits in the month of August. Defective seeds were removed and then the collected seeds were stored in dark condition in polyethylene bags at room temperature. *In vitro* seed germination was tested in the following methods.

- Initially, 15 ml of full strength and half strength MS medium filled separately in petri dishes and glass bottles.
- Single layer and multilayer of Whatman No. 1 filter paper are placed in separate petri dishes on 5ml distilled sterile water and 5 ml half strength MS media.
- Whatman No.1 filter paper was used as paper boat in culture tubes filled separately with 15 ml of half strength MS media and distilled sterile water.
- The seeds were treated with different concentrations of acid at different time intervals.
- The seeds were Immersion in boiling water for different time intervals.
- 50% decocted seeds were obtained by removing the lignified outer layers from the seeds. Intact embryos and decocted seeds were selected and directly plated on half strength MS media, paper boat in culture tubes, Whatman No. 1 filter paper in petri dishes, solid half strength MS media covered with nylon mesh. Grown seedlings were used for further experiments.

Results and Discussion

C. colocynthis seeds germinated in 25 to 30 days in the greenhouse and some seeds took almost two months to germinate. For the *in vitro* plant experiment, it is necessary to get sterile explants. Therefore, it is required to *in vitro* seed germination. The challenging task is intact seed germination in *in vitro* conditions. In *in vitro* conditions half strength and full-strength MS media used in petri dishes and glass bottles separately; for every treatment 50 seeds were allowed to grow in culture room. It was observed that, among the all seeds,

none of the seed was germinated. Intact seeds were unable to get any response. Cucurbit seed is very difficult to grow directly on MS media similarly (Menonet al. 2014) [16], it is fail to grow *C. colocynthis* seeds without any pretreatment.

Intact seeds at rest were tried with Whatman No.1 filter paper in petri dishes with 10 ml distilled water and 10 ml full strength MS media separately and maintained with same till the end of experiment. Similarly, Whatman filter paper used as a paper boat in culture tubes which contains 15 ml half strength MS media or water separately. The results were nullified similarly like previous observation, no seed germination was observed in every trail of the experiment (Table 1). Similarly, in *C. colocynthis* an assortment to intact seeds like contact and daily alteration of temperature, in combination with continuous light, continuous darkness, 48 hr pretreatment of the moist seeds at 5 °C were failed to improve germination (El-keblawy *et al.* 2019) [9].

Various methods were tested to reduce the mechanical resistance of the seed coat such as scarification with concentrated H_2SO_4 and hot water treatment. In each treatment, seeds were treated at different time intervals (30, 60, 120, 180 and 240 sec.) separately. This mechanical dormancy breaking treatment gave promising results for seed germination. Among these two treatments; hot water treatment gave 10% good results over the concentrated H_2SO_4 treatment (Fig 2). In acid treatment, best results obtained in 60 seconds time period with 44% of seed germination. Later germination gradually decreased with increased treatment time. At 240 seconds in acid treatment, absence of seed germination was observed. It's clearly shown that seeds are tolerating concentrated H_2SO_4 treatment maximum at 180 seconds. Seed germination percentage increased from 30 seconds to 120 seconds in hot water treatment, behind increase in the treatment time shown less results. Optimum seed germination in hot water treatment was observed at 120 seconds treatment with 56% of seed germination (Fig 1). Similar way in *C. colocynthis*, when seeds treated in hot water at 90 °C for 10 minutes originated noteworthy increasing with the amount of 40% germinated seeds than the control treatment in germination (Saber *et al.* 2011; Aliero 2004) [19, 1].

As shown in the Fig 2 (a, b) seeds were decoated manually by removing approximately 50% of seed coat. These decoated seeds were used for different treatments by using nylon mesh and Whatman No.1 filter paper along with distilled water and MS media separately. Half strength MS media covered with nylon mesh in petri dishes were used for seed germination (Fig 2c, 2d). Paper boat method performed in culture tubes (Fig 2f). Interesting results were obtained in these treatments and in all experiments, maximum seeds were germinated within 10 to 14 days of time period. Nylon mesh gave 86, 78 and 92 percentage of seed germination respectively. These germinated seeds were transferred to MS media for further growth (Fig 2e). Finally, *in vitro* raised plant acclimatized to vermi composed pot and maintained in the green house (2g, 2h, 2i). In *C. colocynthis*, researchers achieved good results with mechanical scarification over the other treatments (Menon *et al.* 2014; Saber *et al.* 2011; Sahoo and Kasera 2012) [16, 19, 20].

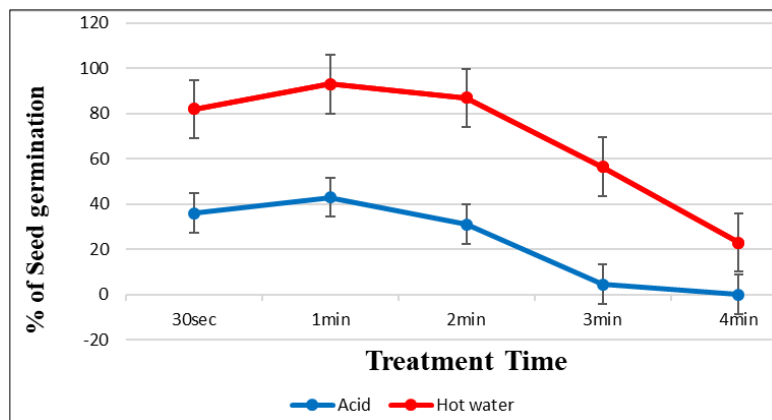


Fig 1: Effect of hot water and acid treatments on seed germination of *C. colocynthis*



Fig 2: *In vitro* seed germination of *C. colocynthis* (a) Full seed coated seeds (b) 50% decoated seeds (c) Seed germination on half strength MS media covered with nylon mesh (d) Initiation of shoots and roots on half strength MS media (e) Germinated seed transformed on to full strength MS media (f) Seed germination on Whatman filter paper boat (g) *In vitro* raised plant acclimatized into sterile vermi composed pot (h) *In vitro* raised plant growing in greenhouse (i) *In vitro* raised plant with fruits.

Table 1: Effect of different treatments on seed germination of *Citrullus colocynthis*

S. No	Treatment	Time	No. of Seed Used each experiment	Avg. No. of Seed germination	% of seed germination
1	Full strength MS Media	30 days	50	0	0
2	Half strength MS Media	30 days	50	0	0
3	Whatmen filter paper				
	With H ₂ O	30 days	50	0	0
4	Whatmen filter paper				
	With MS media	30 days	50	0	0
5	Paper boat with H₂O				
	Full seed	30 days	50	0	0
6	Paper boat with MS				
	media full seed	30 days	50	0	0
7	50% Seed coat removed with nylon mesh	10 to 14 days	50	43±0.33b	82
8	50% Seed coat removed with paper boat in MS media	10 to 14 days	50	39±0.51c	78
9	50% Seed coat removed with paper boat in H ₂ O	10 to 14 days	50	46±0.00a	92

Conclusion

The present study evaluated the effects of different treatments on seed germination and seed dormancy of *Citrullus colocynthis*. Here we used different methods like acid, hot water treatments with various concentration and time intervals and scarification (mechanical method) to remove seed dormancy. Above all the treatments mechanical method is very suitable to remove seed dormancy in *C. colocynthis*. Scarification method was resulted 92% of seed germination, remaining acid and hot water treatments were observed less results i.e., 43 and 56% respectively, the present study shows that scarification method is less harmful to the embryo. In this aspect this process is cost effective and low risk. Hence, this technique is most advisable to compare with other treatments in *C. colocynthis*.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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