

BIOLOGY AND IN-VITRO GERMINATION BEHAVIOUR OF CITRULLUS COLOCYNTHIS (TUMBA) SEEDS AFTER PRETREATMENTS

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ABSTRACT

Citrullus colocynthis is an important medicinal plant of the Indian Thar desert known as food of scarcity. The present paper deals with seed biology and germination behaviour under controlled laboratory (*in-vitro*) conditions. Results revealed that one-year-old seeds exhibited 86.67% viability as compared to fresh ones (43.33%), which indicates that seeds require resting period for maturity. Fresh and one-year-old stored seeds showed 6.67 and 66.67% germination, respectively under *in-vitro* conditions. The germination percentage can be enhanced significantly when seeds were pretreated with 2 minutes of concentrated H_2SO_4 and presoaked in 25 and 50mg L^{-1} concentrations of NH_4NO_3 for 24 and 48h, respectively.

INTRODUCTION

Citrullus colocynthis (Linn.) Schrad. (Tumba; Family: Cucurbitaceae) is considered to be medicinally important because its fruits are used as a purgative, while roots in jaundice, ascites, urinary diseases, rheumatism, abdominal enlargements, coughs and asthmatic attacks of children (Anon, 1992). Seed germination and early seedling growth are considered the most critical phases for establishment of any species and thus it is often emphasized that tolerance of seeds to various stresses during germination should be determined. Such information may be important for understanding the adaptive strategies of seeds for germination followed by seedling growth (Sen, 1977; Devilliers *et al.*, 1994). Thus the information on seed germination is important because it contributes to a better understanding of certain biological concepts such as plant reproductive strategies, life history traits, adaptation to habitats and physiological processes. Germination of the seeds depends on the environment in which it is present. Studies on seed biology and germination behaviour play a crucial role in life history of Indian Thar desert plants. Hence, the present study was aimed to study the seed biology and germination behaviour of *Citrullus colocynthis*, an important medicinal plant of the Indian Thar desert. The objective of the investigation was to assess the morphological parameters of seeds and their germination behavior with the influence of various pretreatments such as concentrated sulphuric acid and ammonium nitrate (NH_4NO_3) under *in-vitro* conditions.

MATERIALS AND METHODS

Seed biology characteristics

The mature fruits of *C. colocynthis* were collected from Circuit House, Jodhpur (7km away in north-east direction from the University Campus) during September-October 2008-2010. The mature fruits were cleaned and stored in plastic containers with parad tablets/BHC powder to protect them from insects. Seed morphological parameters such as shape, size, colour, weight, volume and density were measured. The shape and colour of seeds were observed under dissecting microscope. The seed size (length and breadth of 20 seeds) was measured with the help of vernier caliper. The seeds volume and density were measured as per standard methods and calculated for 100 seeds. Seed output and reproductive capacity were calculated as per Salisbury (1942). The seed viability was tested by the tetrazolium method as suggested by Porter *et al.* (1947).

In-vitro seed germination behaviour

The seeds were first cleaned and treated with 0.1% $HgCl_2$ for 30 seconds and then kept under running tap water for 3-4h. Afterwards, different pretreatments were provided to fresh as well as one-year-old seeds for enhancing germination percentage and improvement in seedlings growth. The pretreatments were: conc. H_2SO_4 (2, 5, 10 and 15 minutes) and mechanical scarifications; GA_3 (presoaking for 24 and 48h in 2, 5, 10, 25 and 50 mg L^{-1}); and presoaking for 24 and 48h in 10, 25, 50 and 100 mg L^{-1} concentrations of $Ca(NO_3)_2$, KNO_3 , NH_4NO_3 and $Co(NO_3)_2$. The best results are obtained

in acid and mechanical scarifications and 25 and 50 mg L⁻¹ concentrations of NH₄NO₃ presoaked for 24 and 48h. Hence, data only for these pretreatments are mentioned in Table 2.

Seed germination studies were performed in sterilized petridishes lined with single layer of filter paper moistened with distilled water. The experiments were carried out in alternate white light and dark (12h) obtained from 3 fluorescent tubes of 40 watts each fitted at a height of half meter from the petridishes (1000 lux) in seed germinator at 28°C temperature. Each petridish contained 10 seeds in triplicate. In acid scarification experiments, pretreated seeds were tied in muslin cloth and kept under running tap water for 3-4h. For mechanical scarification, seeds were treated with sand paper/emery stone at micropylar end to enhance germination percentage. Seeds were also presoaked for 24 and 48h in 25 and 50 mg L⁻¹ concentrations of NH₄NO₃. Thereafter, the pretreated seeds were placed for germination studies. The observations were recorded daily for a period of 10 days. After 10 days, the germination (%) and root and shoot length of seedlings were recorded. The experiments were performed in triplicate and repeated thrice.

The seedling vigour index (VI) was calculated as proposed by Abdul-Baki and Anderson (1973). Germination value of the seeds, which is an index combining the speed and completeness of the germination was calculated for each treatment using the formula proposed by Czabator (1962).

All above-mentioned experiments were performed in triplicate and repeated twice. The experimental results were subjected to analysis of variance (ANOVA) as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The study of seed biology is important in the life of a desert medicinal plant because of its adaptability to the environment, variability in shape and size so that there will be a long term sustainability in desert environment. The potentiality totally depends on the seed output of a plant. It is an important factor affecting the species composition of a community.

It is evident from Table 1 that seeds were obovate shaped, pale brown coloured having 1.056 cm length and 0.893 cm

Table 1: Morphological parameters of *C. colocythis* seeds

Parameters	Values
Shape	Obovate
Size	Length 1.056 ± 0.432 cm Breadth 0.893 ± 0.232 cm
Colour	Pale brown
Weight of 100 seeds (g)	2.057 ± 0.015
Volume of 100 seeds (cc)	2.30 ± 0.030
Density (gcc ⁻¹)	0.877 ± 0.012
Seed output plant ⁻¹	41580 ± 85.394
Reproductive capacity	27721.3 ± 0.236
Viability	43.33% fresh 86.67% one-year-old

± Standard deviation

breadth. The density of the seeds was 0.877g cc⁻¹. The viability increases up to one year of storage, i.e. fresh seeds showed 43.33%, while one-year-old seeds were 86.67% viable. The production potentiality was 41580 seeds plant⁻¹, whereas 27721.3 was the reproductive capacity. Availability of viable and vigorous seeds at planting time is important for achieving targets of agricultural production because good quality seeds act as a catalyst for realizing the potential of other inputs.

Seeds of many land plants fail to germinate and pass through a phase of dormancy that may be caused by several factors and delay the whole life cycle of plants (Saba *et al.*, 1998). Various pretreatments have been found to improve the phenomena of germination and parameters of seedling growth (Sen and Kasera, 1994; Bose and Sarma, 2000). Dormancy of seeds is a characteristics feature of the arid zone plants which can be overcome by providing some pretreatments. Kasera and Shukla (2003) in *Leptadaenia reticulata* and Mohammed and Sen (1990) in *Trianthema portulacastrum* reported improvement in seed germination after providing different concentrations of conc. H₂SO₄ acid pretreatment. In the present investigation, 2min. conc. H₂SO₄ pretreatment enhanced the seed germination in fresh as well as one-year-old seeds but higher concentrations were detrimental for its growth. Improvement in germination of seeds soaked in conc. H₂SO₄ might be due to dissolution of funicles and formation of deep fissures in hilum and cracked palisade cells, which ultimately might have helped to absorb water and gases to initiate germination (Sheelavantar *et al.*, 1993). Sulphuric acid

Table 2: Effect of different pretreatments on seed germination (%), seedling growth (cm), root/shoot lengths (R/S ratio), Vigour Index (VI) and germination value (GV) in fresh and one-year-old seeds of *C. colocythis*

Pretreatments	Germination		Seedling growth				R/S Ratio		VI		GV			
	Fresh	Old	Root length		Shoot length		Fresh	Old	Fresh	Old	Fresh	Old		
			Fresh	Old	Fresh	Old								
Control	6.67	66.67	4.600	2.92	3.20	2.83	1.438	1.032	52.03	38.35	0.742	88.90		
Conc. H ₂ SO ₄ (min.)	2	40.00	83.33	2.283	3.60	1.683	2.55	1.357	1.412	158.6	512.48	40.00	173.58	
	5	6.67	13.33	3.000	3.075	1.85	1.775	1.622	1.732	32.35	64.65	0.89	2.962	
	10	3.33	10.00	2.500	1.74	1.50	1.90	1.670	0.916	13.32	36.40	0.277	2.50	
	15	0.00	3.33	0.00	4.50	0.00	2.10	0.00	2.143	0.00	21.98	0.00	0.157	
Mechanical scarification		16.67	26.67	2.640	2.525	1.62	1.50	1.630	1.683	71.01	107.3	5.558	11.86	
	NH ₄ NO ₃ (mg L ⁻¹ ; 24h)	25	66.67	90.00	4.105	5.104	2.105	2.937	1.950	1.738	414.0	732.7	88.90	202.5
		50	50.00	60.00	2.890	6.972	1.767	3.06	1.636	2.280	232.9	601.9	83.35	90.00
	NH ₄ NO ₃ (mg L ⁻¹ ; 48h)	25	3.33	6.67	0.500	3.03	0.50	2.355	1.000	1.287	3.33	35.92	0.223	2.224
		50	66.67	90.00	4.520	3.63	3.20	3.04	1.413	1.194	514.7	600.3	148.2	405.0
CD	0.315*	0.517*	0.006*	0.031*	0.005*	0.003*	0.002*	0.002*	0.002*	0.518*	0.501*	1.023*	4.382*	

* = Significant at (p < 0.05) level

rendered the seed coat soft causing uniform inflow of water and unrestricted expansion of embryonic parts (Marunda, 1990). Mechanical scarification pretreatment does not show any significant increase in germination percentage.

The effects of KNO_3 , NH_4NO_3 and similar substances on the germination of a variety of seeds have obtained conflicting results. Stimulation or inhibition being obtained depends on the concentration of nitrogen sources and the type of seeds used. In *Withania somnifera*, the lower concentrations of KNO_3 , NH_4NO_3 and $\text{Co}(\text{NO}_3)_2$ promoted the germination and seedling growth, while higher ones retarded these parameters (Swami et al., 2011). Gehlot and Kasera (2011) observed that one-year-old seeds of *Withania coagulans* exhibited cent percent germination with maximum values for root: shoot ratios in 0.75% of NH_4NO_3 pretreatment. In the present studies, fresh and one-year-old stored seeds of *C. colocynthis* showed highest germination, i.e. 90% after providing 25 and 50 mg L^{-1} of NH_4NO_3 pretreatment soaked for 24 and 48h, respectively. Seedling growth is judged by root and shoot ratio. The highest R/S ratio (1.950) value was found in fresh seeds treated with 25 mg L^{-1} and (2.280) in one-year-old seeds with 50 mg L^{-1} NH_4NO_3 soaking for 24h. Vigour index was found to be maximum in 50 and 25 mg L^{-1} of NH_4NO_3 soaked for 48 and 24h, respectively. The highest speedy germination was reported when seeds were pretreated with 50 mg L^{-1} of NH_4NO_3 . The data were significant at ($p < 0.05$) for all parameters.

So the present study reveals that for quicker multiplication of the plant to suffice the emerging demands of pharmaceutical companies, it is needed to apply such kind of chemicals to get more numbers in lesser time. It is concluded that the hard seed coat of this plant need low concentrations of concentrated sulphuric acid for better germination and it has the affinity for nitrates which when supplied through NH_4NO_3 gives remarkable germination results.

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