Seed germination, dormancy breaking techniques of *Citrullus colocynthis* (L.) Schrad plant

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Abstract

In the present study, dormancy and germination requirements were investigated in seeds of *Citrullus colocynthis* (L.). Seeds were subjected to 10 pretreatments in the present study. These pretreatments included hot water (100° C) followed by the seeds placed between double layered filter paper moistened with 5 ml of distilled water and kept at 28 - 30 °C, sulphuric acid (98% v/v), for 30 min followed by the seeds placed between double layered filter paper moistened with 5 ml of distilled water and kept at 28 – 30° C, and sulphuric acid (98% v/v), for 5, 10, 15, and 20 min after which seeds were placed between double layered filter paper moistened with 5 ml of distilled water and kept at alternating temperatures 20 / 25° C (12h / 12h). In the last four pretreatments, *Citrullus colocynthis* (L.) seeds were subjected to10 and 20 mg/l GA_3 followed by moistening with 5 ml of distilled water, 10 mg/l GA_3, or 20 mg/l GA_3. Concentrated sulphuric acid was partly-successful in dormancy-breaking. Moreover, germination increased at pretreatments and treatments of various levels of gibberellic acid. The highest germination rate and percentage were obtained at concentration of 10 mg/l GA_3.

Keywords: Citrullus colocynthis; dormancy; GA_3; H_2SO_4

Abbreviations:
GA_3: Gibberellic acid

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Introduction

Citrullus colocynthis belongs to the family Cucurbitaceae. This plant is commonly known as bitter apple or bitter cucumber (Abdel-Hassan et al, 2000). The plant which is native to dry areas of North Africa, is scattered throughout the Sahara, areas of Morocco, Egypt and Sudan, eastward through Iran to India and other parts of tropical Asia. It has been known since biblical times and cultivated in the Mediterranean region, especially in Cyprus and in India for many centuries (Duke, 1983).

Citrulls colocynthis is a small perennial creeping herb with prostrate or climbing stem, bearing smooth spherical fruits which are mottled green when young and somewhat yellow when ripe (Shah and Qadry, 1985). For centuries,

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humankind has been totally dependent on plants as source of carbohydrates, proteins and fats for food, and shelter. In addition, plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, flavors, fragrances, colors, biopesticides and food additives (Rao and Ravishankar, 2002). *C. colocynthis* leaves contain cucurbitacin A, B, C, and D, α-elaterin, and probably other constituents (Tannin-Spitz et al, 2007; Al-Yahaya et al, 2000). Roots contain a-elaterin, hentriacontane, and saponins. The oil contains oleic, linoleic, myristic, palmitic, and stearic acids. Seeds contain the phyto sterolin (ipurand), 2 phytosterols, 2 hydrocarbons, a saponin, an alkaloid, a polysaccharide or glycoside, and tannin (Duke, 1983).

It is estimated that, 75% of the world's population relies on plant for traditional medicine (Rao and Ravishankar, 2002). This establishes the importance of *Citrullus colocynthis* as it is used in folk medicine by people in rural areas as a purgative, antirheumatic, antidiabetic, and also as a remedy for skin infections (Tannin-Spitz et al, 2007; Duke, 1983). Being a source of substances with anticancer properties, this plant also plays an important role in cancer treatment (Tannin-Spitz et al, 2007). In addition, immature fruit and seed extracts showed a broad spectrum of antimicrobial activities (Marzouk et al, 2011).

The seeds of colocynth show strong dormancy (Koller et al, 1963), our study aimed at determining the treatments capable of stimulating and enhancing germination.

Materials and Methods

Mature dry fruits of *C. colocynthis* were collected from desert in the outskirts of Ahvaz, Iran. In the laboratory, seeds were extracted from the fruits. The seeds were then surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water prior to any treatment. All germination experiments were conducted using five replications of 10 seeds per each treatment. Table 1 depicts the pretreatments and treatments in the study. The first pre-treatment included hot water (100° C) after which seeds were placed between double layered filter paper moistened with 5 ml of distilled water and kept at 28 - 30° C. The next pretreatment involved sulphuric acid (98% v/v), for 30 min after which seeds were placed between double layered filter paper moistened with 5 ml of distilled water and kept at 28 – 30° C. The next four pretreatments involved sulphuric acid (98% v/v), for 5, 10, 15, and 20 min after which seeds were placed between double layered filter paper moistened with 5 ml of distilled water and kept at alternating temperatures 20 / 25° C (12h / 12h). In the last four pretreatments, 200 seeds were put into two petri dishes (100 seeds each) and 10 and 20 mg/l GA_3 were added to the first and second dish, respectively. After 24 h, the seeds from each petri dish were placed between two double layered pieces of filter paper. The seeds from the first dish were then divided into two groups (each containing 50 seeds). The first group was moistened with 5 ml of distilled water and the second group with 10 mg/l GA_3. The seeds from the second dish were also divided into two groups (each containing 50 seeds), the first group was moistened with 5 ml of distilled water and the second group was moistened with 20 mg/l GA_3. All these seeds were kept at alternating temperatures 20 / 30° C (12h / 12h). The control seeds were moistened with distilled water for 24 h. After 24 h, the seeds were placed between two double layered pieces of filter paper at 30° C (12 h) and 20° C (12 h).

Table 1
Pre-treatments

Pre-treatment Solutions	Moistener
Hot water (100 °C)	Distilled water
(24 h)(98% v/v), for 30 min	Distilled water
Sulphuric acid (98% v/v), for 5 min	Distilled water
Sulphuric acid (98% v/v), for 10 min	Distilled water
Sulphuric acid (98% v/v), for 15 min	Distilled water
Sulphuric acid (98% v/v), for 20 min	Distilled water
GA_3(10mg/l) (24 h)	GA_3(10 mg/l)
GA_3(10mg/l) (24 h)	Distilled water
GA_3(20mg/l) (24 h)	GA_3(20 mg/l)
GA_3(20mg/l) (24 h)	Distilled water
Distilled water (24 h)	Distilled water (control)

Germinated seeds were counted and removed every 24h for 10 days. A seed was considered germinated when the tip of the radicle had grown free of the seed coat. The statistical groups were arranged based on completely randomized design with five replications and the obtained data were analyzed using Duncan's test with the least significant difference (LSD) for all pairs set at $P<0.05$.

Results

In our experiment after applying hot water to the seeds after 6th week, we did not get any result. Kept with H_2SO_4 (95% v/v) for 30 min the seeds did not germinate and therefore the statistical test was not performed. The partly-successful dormancy-breaking treatments were observed during scarification of seeds by means of their soaking in concentrated sulphuric acid for 5, 10, 15, and 20 min. The germination started after nine days of incubation and the obtained germination percentage was 2%.

The successful dormancy-breaking treatment took place only when GA_3 had been applied. Fig. (I) shows the total number of seedlings that emerged at the end of the germination experiments when different concentrations of GA_3 were used. The response was dependent on the concentration of applied GA_3. The highest germination percentage and rate were obtained at GA_3 concentration of 10 mg/l. Pre-treatment of seeds with the GA_3 concentration of 10 mg/l and subsequent application of the same concentration of GA_3 for germination increased both the germination percentage and rate, significantly ($p<0.05$) in comparison with the control. At higher concentration of GA_3, germination decreased. The total number of seedlings in the high GA_3 concentration was less than the number of seedlings compared with low GA_3. Nevertheless, the concentration of GA_3 20 mg/l increased both the germination percentage and rate ($p<0.05$) compared with the control (Fig. II).

Discussion

Hot water was not successful as dormancy-breaking treatment. This response to

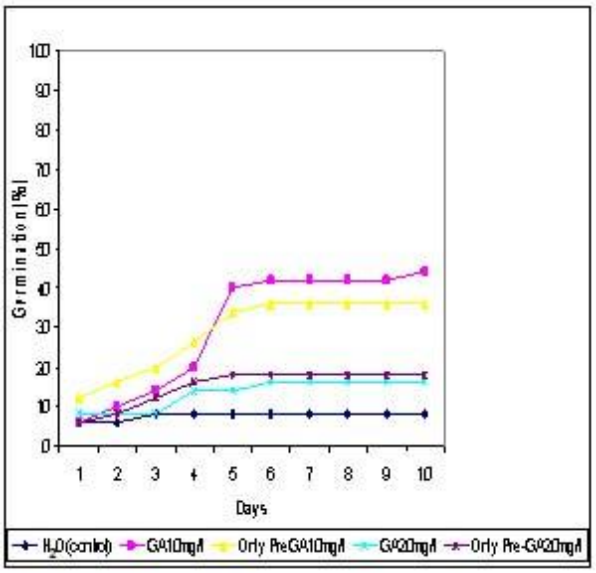

Fig. I. Seed germination rate under various concentrations of GA_3

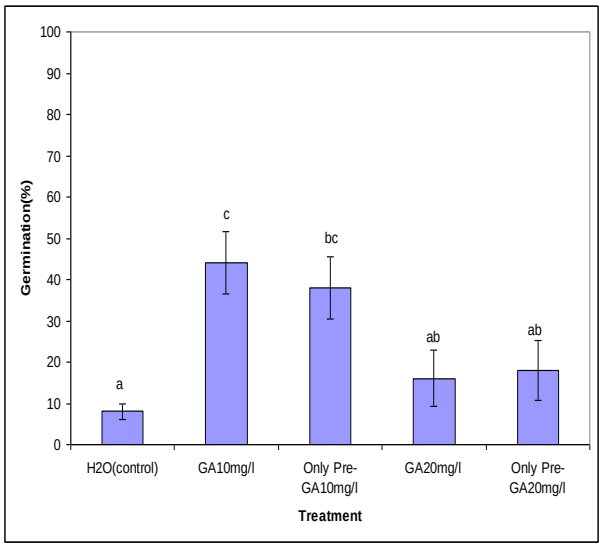

Fig. II. Seed germination rate under various concentrations of GA3 on day 10

the hot water treatments (100° C) confirms the study reported for *C. colocynthis* seeds by Koller et al., (1963). Many chemical substances can completely or partially break dormancy when applied externally to imbibing seeds. The effects of these chemicals are always a function of the concentrations and treatment durations. However, seed germination of some plant species does not respond to them at all (Wang, 1996). Soaking the chemically scarified seeds of *C. colocynthis* in 98% H_2SO_4 for 5-30 min resulted in less or no germination, so this inhibition was imposed by the thick and lignified testa that was

reported for *C. colocynthis* seeds (Koller et al., 1963; Loy and Evensen, 1979). This indicates that *C. colocynthis* seeds have exogenous dormancy. Similar results were reported for *Areca triandra* seeds (Yang et al., 2007).

Acid scarification with H_2SO_4 for 5-20 min. resulted in germination in 2% of the seeds. It is clear that the main action of H_2SO_4 during germination of colocynth seed is directed to the weakening of the seed coat cells, stimulating germination and emergency of the radicle across the endosperm and the seed coat. On the other hand, none of the seeds scarified with H_2SO_4 for 30 min germinated. It can be argued that this was due to strong causticity in such treatments: acid enters the seed, breaks the testa, and then damages the embryo. This finding also confirms the observation of Yang et al. (2007) for *A. triandra* seeds.

In our investigation, cracking seed coat did not show any effect on colocynth seed germination. Therefore, the seed coat is not the single obstacle for germination of *C. colocynthis* seeds. This result is consistent with previous study on *Ferula gummosa* by Nadjati et al. (2006). Thus, we can conclude that endogenous dormancy probably is caused by the embryo immaturity. This conclusion agrees with that obtained for *Opuntia tomentosa* by Olvera-Carrillo et al. (2003).

It is well-known that GA_3 increases and synchronizes seed germination of many plant species (Choudhary et al., 1996) and stimulates cellular elongation and emergency of the radicle across the endosperm and the seed coat (Salisbury and Ross, 2000). GA_3 is widely used to break dormancy of seeds of various plant species (Nadjati et al., 2006).These findings support the results of our study concerning germination process. Indeed, in our experiments all concentrations of GA_3 were effective in promoting germination. Among tested variants of GA_3 treatments, 10 mg/l significantly hastened germination compared to the controls. GA_3 at high concentration (20 mg/l) significantly limits seed germination. The main action of GA_3 during germination of *colocynthis* seed is directed to the weakening of the endosperm cells surrounding the radicle tip (Bakrim et al., 2007). Therefore, the embryo can acquire the strength to break seed coat.

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