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Enhancing *Cordia myxa* germination and seedling growth through breaking seeds dormancy techniques

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Abstract

The current study was carried out in Agricultural Research Station of Al-Marashda, Qena Governorate, A.R.C., Egypt, during seasons of 2023 and 2024. The aim of study was to explore the influences of different pre-germination treatments on seed germination and seedling growth characteristics of *Cordia myxa* L. The experiment was arranged in a completely randomized block design with 9 treatments, three replications for each treatment. The pre-germination treatments were control (un-treated seeds); soaking in tap-water for 24 and 48 h; soaking seeds in previously boiled water for 24 h at ambient temperature; soaking in GA_3 2000, 4000 and 6000 ppm for 24 h; mechanical scarification (abrasion seed coat with sand paper) and immersion seeds in concentrated sulphuric acid (H_2SO_4) for 5 min. The obtained results showed that the highest germination (G%, DGS, MGR and VI) and seedling growth characteristics (stem length, root length, stem diameter, number of leaves and fresh and dry weights of shoots and roots) of *C. myxa* was attained when soaking seeds in GA_3 at rates of 4000, 2000 and 6000 ppm for 24 h, respectively. The minimum values were shown in untreated seeds. The present investigation concluded the possibility of the *Cordia* trees species to be responded to breaking seed dormancy using 4000 ppm GA_3.

Key words: seed dormancy, pre-germination treatments, germination parameters, early seedling growth, *Cordia myxa*.

Introduction

For around thousands of years, woody plants were utilized in many purposes as the basis of the traditional medicine systems, source for the potent and powerful drugs, as ornamental trees and for timber production [1]. Even the developing countries have initiated the adaptation of the traditional medical practice to be the integral part of their culture. Woody plants were still remains as the primary source of supply of various important drugs and minerals in the orthodox medicine today [2]. Plants are being exploited for its active compound which is responsible for their biological function. Of the important and promising trees is *Cordia myxa* L., which is on the list of threatened species [3]. *C. myxa,* a member of Boraginaceae family, is a deciduous tree with medium

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size but the tree keeps its leaves for most of the year [4]. The fruit is edible and its taste is sweet [5]. *C. myxa* trees are found in the temperature and tropic distracts of the world. It is native to India and Pakistan, naturalized in the south of Iran, Iraq, various oases in Arabia, some Mediterranean regions and in the north of tropical Africa. Its leaves extract is used to treat burns, wound, and stomach pain and have a strong antioxidant efficacy. It is popularly used for chest treatment and urinary infections and as an anthelminthic, diuretic, astringent, demulcent and expectorant agent [4].Pre-germination treatments are employed for breaking seed dormancy in many species. Knowledge of seed biology and physiology helps in identifying the nature of specific seed problem and probable pre-germination treatment for overcoming seed dormancy [6]. Physical dormancy in seeds differs between species, stage of maturity and degree of drought, so pre-germination treatment must be adjusted consequently. Physical seed dormancy may be overcome by mechanical scarification of the seed coat by nicking, piercing, chipping, filing with the help of knife, needle, hot wire burner, abrasion paper [7], hot water or acid treatments [8, 9]. Pre-germination treatments are used to ensure faster and uniform germination rate. Pre-germination treatment methods in *Cordia dichotoma* are employed for breaking seed dormancy and are applied not only to ensure the rapid germination but it also decreases labor, cost and time for nursery production. Effective propagation and seedlings establishment are the initial and basic requirements for sustainable management for these species. So, maintenance and sustainable development for these rare species has become indispensable. Germination in some species is sometimes difficult due to seed coat and the dormant embryos. However, seeds often fail to germinate even under favorable conditions of moisture, oxygen and soil. To overcome these problems, several methods as mechanical scarification, soaking in water and acids are used for treating seeds prior to sowing [10]. Seeds of *C. myxa* treated with gibberellic acid at 500 ppm promote germination [11]. Therefore, the present investigation was carried out with the aims to enhance seeds germination and seedlings growth of *C. myxa* as rare tree species in Egypt by applying various pre-germination treatments.

Materials and methods

The present investigation was conducted at the Agricultural Research Station of Al-Marashda, Qena Governorate, ARC, Egypt (26° 9' N, 32° 42' E.) during two successive seasons of 2023 and 2024. This study aimed to evaluate various pre-germination treatments on *Cordia myxa* L. seeds and their effect on the growth of seedlings. The seeds of *C. myxa* were obtained from Kom-Ombo Tropical Farm, Aswan. The seeds were subjected to the following treatments: control (untreated seeds) (T1); soaking seeds in tap water for 24 h (T2); soaking seeds in tap water for 48 h (T3); soaking seeds in previously boiled water for 24 h at ambient temperature (T4); soaking in 2000 ppm GA_3 for 24 h (T5); soaking in 4000 ppm GA_3 for 24 h (T6); soaking in 6000 ppm GA_3 for 24 h (T7); immersing seeds in concentrated sulphuric acid (H_2SO_4 98%) for five minutes with the quick washing with tap water (T8) and mechanical scarification (abrasion seed coat with sand paper) (T9).

Experimental design:

This experiment was done from 20th February to 20th May during the two growing seasons. Seeds were sown in poly pots size of 20 × 30 cm after pre-germination treatments. Poly pots were

filled with prepared medium of clay loamy soil, its chemical and physical properties was shown in Table (1). Twenty seeds were planted per pot, about 2 cm depth and irrigation was done every two days until full germination. Observations for germination traits were recorded every three days. This experiment was layout as a completely randomized blocks design system including the nine pre-germination treatments with three replicates, each replicate contained three pots.

Table 1: Physical and chemical analysis of the used growing media.

Physical properties	**Soil texture**	**Sand (%)**		**Silt (%)**	**Clay (%)**	
	Clay loam	17.00		36.35	46.65	
Chemical properties	**Organic Matter %**	**HCO_3 (meq/ 100 g soil)**	**SO^{-}_4 (meq/ 100g soil)**	**Soil pH**	**E.C. (mmhose/cm)**	**Ca CO_3 %**
	1.85	0.82	3.86	7.81	1.42	2.31

Collected data:

The data were recorded at the end of the experiment on 20th May, after three months from sowing seeds, and the measurements for germination and seedlings were: Germination percentage (G %), at the end of the germination period the germination percentage was calculated using the following equation by [12]**:**

$$G\%=(\mathrm{Ng}\div\mathrm{Nt})\times 100$$

where G % is germination percentage, Ng is the number of germinated seeds and Nt is the total number of sowing seeds.

Vigor index was calculated using the formulas proposed by [13]**:**

VI= Seedling length × Germination %

Mean germination rate (MGR) was recorded according to [14] as number of days to attain 50% of total germination**.** Daily germination speed (DGS) was obtained by dividing the cumulative germination percentage by the number of days since sowing.

Stem length (cm), root length (cm), leaves number, stem diameter (cm), shoot fresh and dry weight (g), root fresh and dry weight (g) were recoded three months after sowing the seeds.

Statistical analysis:

The data were statistically analyzed for the two seasons according to the procedure outlined by [15]**.** The least significant difference (L.S.D) at 5% was used for comparing the means of treatments. Statistical analysis was performed using Microsoft Office 2010 Excel program.

Results and discussion

The data in Table (2) showed the effect of pre-germination treatments on germination percentage (G %), daily germination speed (DGS), mean germination rate (MGR) and vigor index (VI) of *C. myxa* seeds. There were significant influences among pre-germination treatments in the two studied seasons. The obtained data offered that the maximal values on these traits were recorded with soaking in GA_3 at rate of 4000, 2000 and 6000 ppm for 24 h, respectively. The minimum values of germination characteristics were obtained from untreated seeds, followed by

soaking seeds in tap water for 24 h in the mean of the two studied seasons, respectively.

Table 2. Influence of pre-germination treatments on germination percentage (G %), daily germination speed (DGS), mean germination rate (MGR) and vigor index (VI) of *C. myxa* L. seeds during the two seasons of 2023 and 2024.

Pre-germination treatments	G %		DGS		MGR		VI	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	40.0^{d}	43.3^{d}	0.133^{e}	0.152^{e}	30.0^{a}	28.3^{a}	1739.0^{f}	1883.1^{g}
Tap water 24 h.	41.7^{d}	46.7^{d}	0.142^{e}	0.174^{e}	29.2^{ab}	26.8^{ab}	2087.3^{ef}	2498.8f^{g}
Tap water 48 h.	55.0^{b}	60.0^{b}	0.209^{c}	0.259^{c}	26.3^{c}	23.2^{cd}	3408.7^{cd}	3789.6^{cd}
Boiled water 24 h.	43.3^{d}	46.7^{d}	0.156^{e}	0.183^{de}	27.7^{bc}	25.7^{bc}	2292.3^{e}	2613.5^{f}
GA_3 2000 ppm	70.0^{a}	75.0^{a}	0.305^{b}	0.373^{b}	23.0^{d}	20.2^{de}	5057.2^{b}	5670.6^{b}
GA_3 4000 ppm	75.0^{a}	80.0^{a}	0.376^{a}	0.450^{a}	20.2^{e}	18.0^{e}	6437.5^{a}	6988.2^{a}
GA_3 6000 ppm	56.7^{b}	61.7^{b}	0.240^{c}	0.292^{c}	23.7^{d}	21.2^{d}	3604.7^{c}	4042.1^{c}
H_2SO_4 5 min.	53.3^{bc}	56.7^{bc}	0.207^{cd}	0.235^{cd}	25.8^{c}	24.0^{c}	3110.3^{d}	3365.2^{de}
Scarification	45.0^{cd}	48.3^{cd}	0.166^{de}	0.193^{de}	27.2^{c}	25.0^{bc}	2513.8^{e}	2748.7^{ef}
LSD 5%	8.4	10.3	0.043	0.058	1.9	2.5	489.6	667.6
LSD 1%	11.6	14.2	0.060	0.079	2.7	3.4	674.4	919.5

Data in Table (3) showed the effect of pre-germination treatments on the stem length, root length, stem diameter, leaves number of *C. myxa* as well as shoot and roots resh and dry weights during the two seasons of 2023 and 2024. It is clear that the best results were recorded with soaking in GA_3 at rate of 4000, 2000 and 6000 ppm for 24 h in both studied seasons, respectively. The presented data revealed that the untreated seeds and soaking seeds in tap water for 24 h achieved the least measurements of *C. myxa* seedlings in both studied seasons. The present investigation was carried out to study the influence of pre-sowing treatments on breaking seed dormancy of *C. myxa* seeds. The increment of germinated seeds achieved as a result of applying various pre-germination treatments compared to control. The different pre-germination treatments improved the germination parameters i.e. germination percentage, daily germination speed, mean germination rate and vigor index as well as improved seedling growth parameters as stem and root length, leaves number, and stem collar in this study. The highest values of germination parameters were recorded with soaking seed at rate 4000, 2000 and 6000 ppm of GA_3 for 24 h, followed by soaked seeds in tap water for 48 h. The minimum results were registered with soaking seeds in boiled water for 24 h followed by control seeds.

Table 3. Influence of pre-germination treatments on stem length (cm), root length (cm), stem diameter (mm) and leaves number of *Cordia myxa* L. during the two seasons of 2023 and 2024.

Pre-germination treatments	Stem length (cm)		Root length (cm)		Stem diameter (mm)		Leaves number	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	23.2^{e}	23.0^{g}	20.2^{f}	20.6^{h}	0.27^{cd}	0.29^{g}	7.3^{f}	8.3^{g}
Tap water 24 h.	28.1^{d}	30.8^{f}	22.2^{ef}	22.9^{gh}	0.29^{bcd}	0.31^{f}	7.7^{ef}	8.3^{g}
Tap water 48 h.	33.3^{bc}	34.0^{bc}	28.7^{c}	29.2^{cd}	0.36^{d}	0.35^{d}	10.0^{bc}	11.0^{cd}
Boiled water 24 h.	29.9^{cd}	31.4^{e}	23.2^{e}	24.4^{g}	0.31^{bcd}	0.32^{ef}	8.0^{def}	9.3^{f}
GA_3 2000 ppm	34.9^{b}	36.5^{b}	37.4^{b}	39.2^{b}	0.41^{ab}	0.43^{b}	10.7^{b}	12.3^{b}
GA_3 4000 ppm	45.8^{a}	45.7^{a}	40.0^{a}	41.6^{a}	0.43^{a}	0.47^{a}	12.3^{a}	14.0^{a}
GA_3 6000 ppm	33.7^{b}	35.7^{bc}	30.1^{c}	29.8^{c}	0.38^{abc}	0.39^{c}	10.3^{b}	11.3^{c}

H_2SO_4 5 min.	32.1^{cd}	32.4^{cd}	26.2^{e}	27.2^{d}	0.33^{a-d}	0.34^{de}	9.0^{cd}	10.3^{de}
Scarification	31.2^{cd}	31.7^{de}	24.5^{de}	25.3^{ef}	0.32^{a-d}	0.33^{def}	8.7^{de}	9.7^{ef}
LSD 5%	1.70	2.83	2.35	2.37	0.02	0.03	1.26	0.83
LSD 1%	2.34	3.90	3.24	3.26	0.03	0.04	1.73	1.15

In this concern, treating seeds of *C. sinensis* with GA_3 500 ppm plus 1% KNO_3 resulted in earliest seed germination, maximum seed germination percentage, and subsequent seedling growth as highest leaf area, and maximum leaf fresh and dry weight [16]. Similar studies conducted in *C. biosseri* seeds revealed a high germination percentage by GA_3 treatment [17, 18]. Also, 20–30% seed germination in *C. myxa* and up to 60% in *C. gharaf* was recorded with freshly seeds treated with GA_3 [11]. GA_3 promoted seed germination in *C. gharaf* to the maximum value of 81% [19]. Soaking seeds in gibberellic acid promotes the germination of dormant seeds in many tree species such as, *Citrullus lanatus* [20], *Penstemon digitalis* [21], *Tamarindus indica* [22] and *Elaeocarpus prunifolius* [23]. The present result is in agreement with the obtained of [24], who suggested that increased seed germination was directly proportional to the increase in GA_3 concentrations (500 and 1000 mg/l). However, they stated that at higher GA_3 concentration (1500 mg/l), seed germination of *Passiflora* species reduced. Seed germination was increased due to GA_3 treatment possibly through improved synthesis of hydrolytic enzymes, which were further transported to the endosperm. The enzymes break down the stored food to supply energy required for seed germination [25]. The positive effect of GA_3 may be due to induce cytokinin action and rescinds the germination inhibitor (ABA) which eventually stimulates the biochemical processes of the seed germination [26]. Gas, as an endogenous plant growth regulator, plays a vital role in the seed germination process [27]. Previous studies have suggested that exogenous gas can reduce the minimum effective exposure time of different germination inhibitors and promote germination rate in various plant species [28, 29, 30].

GA_3 pre-treated seeds showed higher germination percentage and other germination parameters compared to those that had been treated with the other treatments. In the present study, immersion seeds in concentrated sulphuric acid improved germination parameters of *C. myxa*. The highest germination characteristics were recorded in concentrated H_2SO_4 for 20 min, while the lowest ones in control seeds of *Cordia dichotoma* [31]. The concentrated sulphuric acid softens the seed coat causing uniform inflow of water and unrestricted expansion of embryo. Also, the positive effects with H_2SO_4 have also been reported in *Quercus leucotrichophora* [32]. The highest germination percentage was recorded in seeds of *Tamarindus indica* and *Melia azedarach* treated by mechanical scarification, followed by immersion in concentrated H_2SO_4 for 20 and 10 minutes [33]. Moreover, GA_3 pre-treatments increased the seedling length and different measurements of *C. myxa* seedlings. Generally, the optimal rate of GA_3 used for seed soaking is 2000 ppm for 24h in *C. myxa* seeds. On the other hand, the optimal GA3 concentration is about 50-150 ppm in different plants [34, 35]. This may be related to the thickness of cordia seed-coat. As a matter of fact, exogenous GA_3 seemed to induce the activation and production of protease and α-amylase in seeds, thus improving the ability of germination in seeds [30, 36].

There are highly significant differences between seedling parameters of *C. myxa* as affected by various pre-germination treatments as tabulated in Table (4). Higher values of fresh and dry

weight of shoot and root of *C. myxa* were coincided with soaking seeds in gibberellic acid at a rate of 4000 ppm, followed by soaking in gibberellic acid at a rate of 2000 ppm. On the contrary, lower values of the fresh and dry weight of shoot and root were recorded with the control, followed by soaking seeds for 24 h in tap-water in the two studied seasons. The pre-sowing treatment with GA_3 improved the seedling growth parameters in *C. myxa*, increment in the seedling growth was observed under the GA_3 at a rate of 4000 ppm. GA_3 pretreatment improved germination rate, germination potential, hypocotyl length and radicle length in *Cannabis sativa* [30]**.** Also treating of *Eriobotrya japonica* seeds with GA_3 (250 mg/l) resulted maximum stem diameter and plant height as compared to control [37]. Similarly, maximum plant height and stem diameter of *Citrus aurantifolia* plantlets was recorded with GA_3 (200 mg/l) seed treatment [38]. The positive effect of GA_3 in seedling growth chiefly due to enhance cell division in the cambium tissue and its rapid cell progeny as proved by [39, 40]**.** The higher weight of the fresh and dry shoots in the GA_3 pre-germination treatment chiefly because of improved the mobilization of water, nutrient uptake, membrane permeability and transportation which leads to higher production of photosynthetic products.

Table 4. Influence of pre-germination treatments on the fresh and dry weight of stem and root (g) of *C. myxa* L. during the two seasons of 2023 and 2024.

Pre-germination treatments	Fresh shoot weight (g)		Dry shoot weight (g)		Fresh root weight (g)		Dry root weight (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	3.83^{h}	4.24^{h}	2.12^{f}	1.88^{e}	1.12^{e}	1.34^{g}	0.67^{g}	0.66^{f}
Tap water 24 h.	4.04^{gh}	5.41^{g}	2.71^{e}	2.95^{d}	1.40^{de}	1.92^{f}	0.97^{f}	0.91^{e}
Tap water 48 h.	6.22^{cd}	6.90^{cd}	3.56^{c}	3.36^{c}	2.01^{c}	3.13^{d}	1.57^{d}	1.48^{c}
Boiled water 24 h.	4.78^{fg}	6.03^{f}	3.08^{d}	2.96^{d}	1.42^{de}	2.11^{f}	1.07^{f}	0.99^{de}
GA_3 2000 ppm	7.63^{b}	8.61^{b}	4.31^{b}	4.22^{b}	3.51^{b}	4.02^{b}	2.01^{b}	1.84^{ab}
GA_3 4000 ppm	10.89^{a}	11.45^{a}	5.73^{a}	5.31^{a}	4.09^{a}	4.52^{a}	2.26^{a}	1.93^{a}
GA_3 6000 ppm	6.83^{bc}	7.43^{c}	3.61^{c}	3.34^{c}	3.12^{b}	3.56^{c}	1.78^{c}	1.69^{b}
H_2SO_4 5 min.	5.70^{de}	6.68^{de}	3.34^{cd}	3.25^{cd}	1.82^{cd}	2.62^{e}	1.31^{e}	1.17^{d}
Scarification	5.00^{ef}	6.34^{ef}	3.1^{d}	2.93^{d}	1.72^{cd}	2.24^{f}	1.10^{f}	1.00^{de}
LSD 5%	0.84	0.55	0.32	0.36	0.45	0.34	0.18	0.19
LSD 1%	1.16	0.76	0.44	0.50	0.62	0.46	0.24	0.27

Conclusion

The better performance of germination and early growth of seedlings was attained when soaking seeds of *Cordia myxa* in GA_3 at rates of 4000, 2000 and 6000 ppm for 24 h, respectively over all other treatments. So, the present result recommends the nursery owners and other organizations for seedling producer in Egypt to apply the optimal treatment (4000 ppm GA_3) for maximum seed germination and better early growth of this tree species.

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