



Research article

Effect of antibiotics on *in vitro* seed germination responses in *Adansonia digitata* L.: A valuable medicinal tree

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[Accepted: 15 December 2019]

Abstract: The aim of this work was to investigate the effect of Antibiotics on germination responses *in vitro* on mature seeds of *Adansonia digitata*. Three antibiotics namely Cefotaxime (100–500 ppm), carbenicillen (100–500 ppm), streptomycin (100–500 ppm) were tried to assess their influence on seed germination. Antibiotic stock solutions were added to the autoclaved basal medium ½ Woody Plant medium (WPM) with 0.8 mg l⁻¹ Benzylaminopurine (BAP) and 4% sucrose. Mature seeds without seed coat were used as explant. The seeds were sterilized using 70% ethanol for 30seconds and 4% sodium hypochlorite for 10 minutes followed by incubation for 30 days in light with photosynthetic photon flux of 6 μmol m⁻² s⁻¹. 16 / 8 hour photoperiod. 30 seeds were used for each treatment and the experiment was repeated thrice. 76.6% germination (maximum) was observed in 300 ppm cefataxime treated medium followed by 44.4% at 300 ppm streptomycin and 56.6% at 300 ppm of carbenicillen. The use of streptomycin as an antibiotic did not have a stimulatory effect on seed germination. Percent seed germination was very less as compared to cefataxime and carbenicillen. The study was carried out to develop the most suitable protocol for seed germination *in vitro* and further regeneration studies on this aesthetically important valuable tree species. However, more variables like the effect of Photoperiod, culture vessels, age of explant etc. on *in vitro* seed germination have to be standardized to generate a complete protocol.

Keywords: Micropropagation - Cefataxime - Streptomycin - Woody plant medium - *Adansonia digitata* - Seed germination - Medicinal.

[Cite as: Singh S & Parasharami V (2019) Effect of antibiotics on *in vitro* seed germination responses in *Adansonia digitata* L.: A valuable medicinal tree. *Tropical Plant Research* 6(3): 488–492]

INTRODUCTION

Woody plants represent a vast array of types represented by both angiosperms and gymnosperms. In general, their asexual propagation is more difficult than herbaceous species. This holds true for forest trees also. Popularly known as Baobab, Monkey Bread tree, chemist tree and Upside-down tree, *Adansonia digitata* L. is widespread in drylands of sub-Saharan Africa (Wickens & Lowe 2008). Few cities like Lucknow, Hyderabad, Sagar, and Allahabad have three to four plants reported so far. Lady Irwin College, New Delhi has a very old huge size *A. digitata* in its campus. Bijapur has two plants. One of them is reported to be about 359 years old with 7 metres height and 9.2 metres girth. Bhopal has about 7–8 plants growing as wild in various locations. Being the largest succulent plant in the world it grows up to a height of 25 metres or more and trunk with diameter of 10–12metres (Wickens 1982). The trunk usually tapers and is bottle shaped with branches distributed irregularly. The hollow trees provide reservoirs of fresh water, with water storage capacities, range from 1000–9000 litres per tree (Tukur 2010). It is reported that every part of the tree has numerous medicinal, non-medicinal uses (Singh *et al.* 2013) and is economically important in terms of pharmaceutical and nutraceutical indicators; still its distribution is very limited in Indian sub-continent. Wickens (1982) reported that natural regeneration is very poor and *in vitro* tests give low germination rate, *i.e.* 0–10% for untreated seeds. The seeds seem to have seed coat induced dormancy (Etejere *et al.* 1984). Furthermore, baobab seeds have very hard seed coats and germination is usually less than 20% (Danthu *et al.* 1995). In nature, dormancy is broken by

passage through the digestive system of mammals (Esenowo 1991).

The noteworthy range of available literature dealing with traditional and medicinal uses of *A. digitata* reflects the extent of attention this genus receives in science (Kempe *et al.* 2018) but it is unfortunate to record that the tree is an endangered species and very few efforts are being made all over the world for its regeneration (Singh & Rai 2017).

Plant tissue culture systems are always under threat of microbial invasions be it from any source *i.e.* contaminants from air, the researcher, explant and the growing tissue cultured plant too. Da Silva *et al.* (2003) reported the use of antibiotics to eliminate unwanted contaminants from tissue culture. It was found out that kanamycin and streptomycin sulfate eliminate the contaminating bacteria in micropropagation of *Guadua angustifolia* Kunth. (Nadha *et al.* 2012). Till date, literature survey shows limited reports on clonal propagation of *A. digitata*. This study utilizes three antibiotic agents and was carried out to develop the most suitable protocol for seed germination in controlled conditions while eliminating bacterial and fungal contamination in this valuable tree species. However, more variables like effect of Photoperiod, culture vessels and light intensity etc. have to be assessed and optimized to generate a complete protocol.

MATERIAL AND METHODS

Collection of samples

Fully mature ripe fruits (Fig. 1) were collected in December–January from Mandavgarh, District Dhar, Madhya Pradesh, India between 22° 36'0" N and 75° 18'0" E. The fruits were mechanically broken and seeds were separated from the fruit pulp (Fig. 2).



Figure 1. *Adansonia digitata* L.: **A**, Occurrence of majestic plant in Mandavgarh, District Dhar, Madhya Pradesh, India; **B**, Mature fruits hanging upside down appearing as Dead Rats.



Figure 2. *Adansonia digitata* L.: **A**, Mature Ripe fruit with seeds embedded in white powdery pulp; **B**, Seeds separated from pulp and seed coat removed.

Preparation of medium and explant

½ WPM (Woody Plant medium) with 0.8 mg l⁻¹ BAP and 4% sucrose was prepared and autoclaved for 20 minutes at 121°C and 15 lbs. Calculated amount of the three test antibiotic stock solutions was added and the medium was poured into sterile culture vessels. The mature seeds were separated from fruit pulp and washed in tap water followed by detergent wash in labolene. 1% Bavistin treatment for 30–60 minutes was given followed by rinsing with sterile water thrice. Mature seeds were nicked to remove the hard seed coat, (Fig. 2B) surface sterilized with 4% NaOCl and 70% ethanol for 30 seconds under strict aseptic conditions.

Cultural conditions

30 seeds were used for each treatment and the experiment was repeated thrice. The seeds were incubated for 30 days in light with photosynthetic photon flux of 6 μmol m⁻² s⁻¹. 16/8 hour photoperiod was maintained throughout the course of incubation. Percent germination and percent response (growth) was recorded.

RESULTS

It was observed that antibiotics too affect the process of germination (Fig. 3). 76.6±3.35% germination (maximum) was observed in 300 ppm cefataxime treated medium followed by 44.4±5.09% at 300 ppm streptomycin and 56.6±6.65% at 300 ppm of carbenicillen (Fig. 4). It was observed that increase in cefataxime concentration from 100–300 ppm resulted in a sharp increase in % germination but at higher concentration (500 ppm), % germination was reduced. Carbenicillen at 300 ppm showed increased germination but above 300 ppm, seed germination was inhibited (47.7±7.69%). The use of streptomycin as an antibiotic did not have a stimulatory effect on seed germination. Percent seed germination was very less as compared to cefataxime and carbenicillen. Percent responsive seedlings were highest (69.9±3.35%) in cultures with cefataxime at 300 ppm followed by 63.2±5.77% with 300 ppm carbenicillen (Fig. 5).

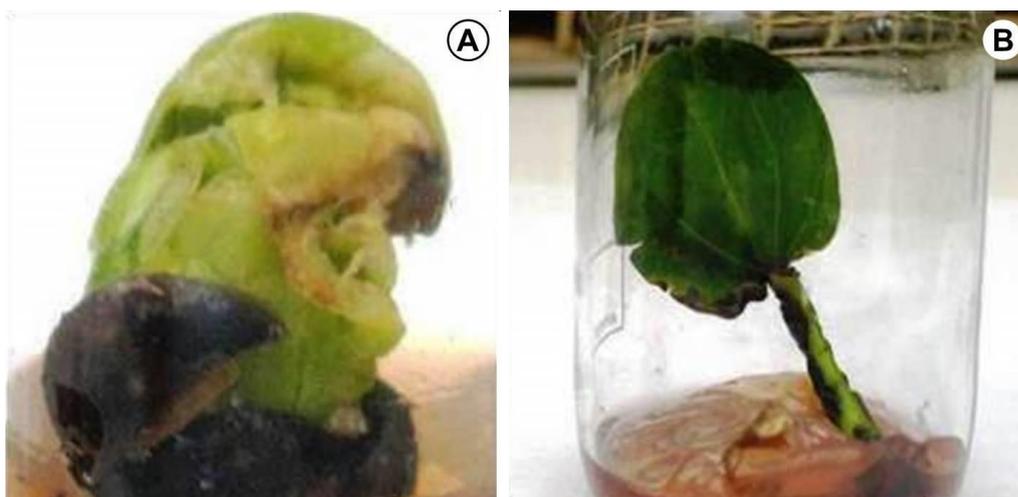


Figure 3. *Adansonia digitata* L.: A, Initiation of germination in vitro; B, Opening of cotyledonary leaf in vitro.

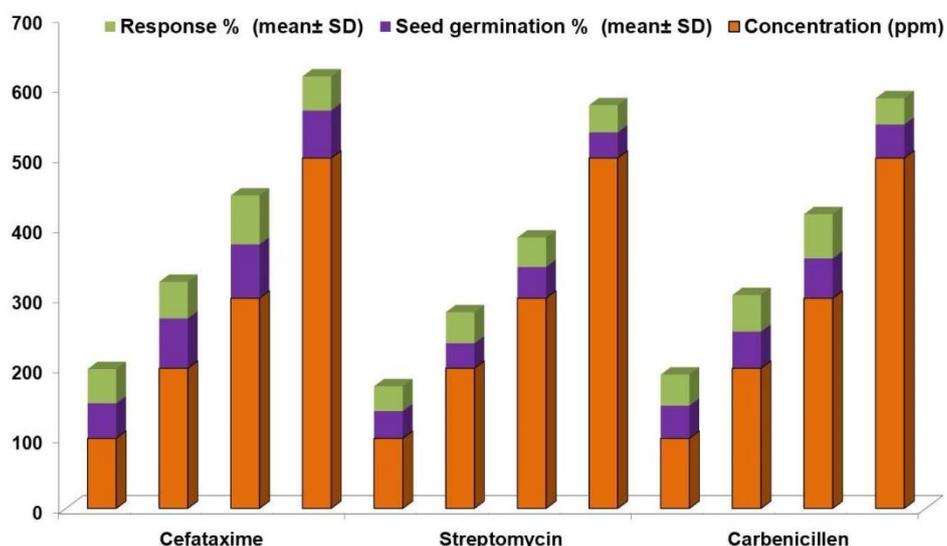


Figure 4. Effect of Antibiotics on Seed germination in vitro.

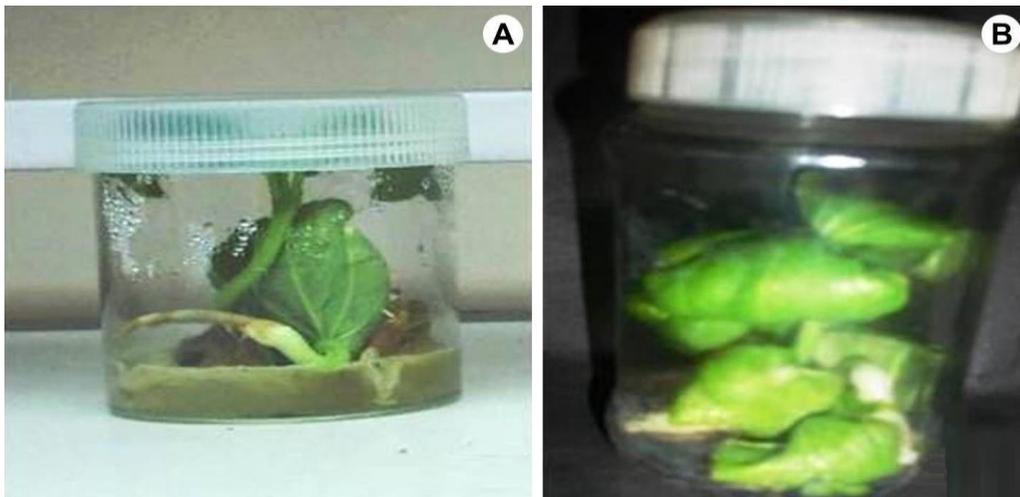


Figure 5. *In vitro* seedling in ½ WPM & 300 ppm cefataxime: **A**, 30 day old; **B**, 60 day old.

DISCUSSION

In the present study, three antibiotics were used to assess their influence on seed germination and subsequent responses in the form of growth of seedlings. These were not used as promoters of growth *in vitro*. Growth was visible even without adding the antibiotics in the culture medium. But their presence has provided safe and sterile ambience for germination & growth. In another study, the seed germination was significantly affected by the age of fruits or seeds collected during the study as well as the seed pre-treatments. *A. digitata* endosperm cultures showed best response when immature seeds from 30–45 days fruits were used as explants (Singh *et al.* 2010). The first research with antibiotics added in culture media involved vanillin and its derivatives (McAlpine 1974, Thurston *et al.* 1979). Throughout the years, efforts have been made for introducing certain antibiotics or antifungals to prevent contamination of plants *in vitro* cultures. Arditti (2008) reported many anti-contaminants which can be used in high concentrations such as 400 mg l⁻¹ ampicillin, 500 mg l⁻¹ carbenicillen, 800 mg l⁻¹ geneticin, 1000 mg l⁻¹ cefotaxime, but most recommended concentration is between 10–50 mg l⁻¹ tetracycline hydrochloride, cycloheximide, neomycin sulfate and rifampicin.

The present study is the first report of its kind where in Cefataxime at 300 ppm gave best results. The fact that antibiotics do not influence process of germination directly is documented (Hillis *et al.* 2011). Furthermore, it is reported that determination of toxicity of antibiotics to plants provides the need of a tiered risk assessment. In a study, chlortetracycline was found to be one of the most toxic compounds at environmentally relevant concentrations, with significant effects observed as low as 1000 µg l⁻¹. Environmental concentrations of chlorotetracycline as high as 7,730 µg l⁻¹ have been observed in swine manure (Kumar *et al.* (2005). *In vitro* cultures, of all kinds, are exposed to contamination risks with different kinds of microorganisms. (Cosma & Vancea 2017) concluded that anti-contaminants, used in concentrations recommended by literature, have prevented infections, but reduced the capacity for germination rate of *Triticale* Wittmarck. Usually, the cultures initiated from explants taken from plant material grown in septic conditions, show a high occurrence of accidental infestations (Cachita-Cosma & Ardelean 2009). Hence, protection to these plants is mainly provided by adding antibiotics (Makovitzki *et al.* 2007).

CONCLUSION

In the present study, percentage responsive seedlings was found to be highest *i.e.* 69.9±3.35% in cultures treated with cefataxime at 300 ppm followed by carbenicillen. Similarly, maximum germination was found to be in cultures treated with cefataxime followed by Streptomycin and carbenicillen. The purpose to study the effect of antibiotics will greatly help in minimizing microbial contaminations along with stimulating the growth of seedlings *in vitro*. Owing to poor natural regeneration and seed coat induced dormancy in this medicinally important tree species; if more variables like Effect of Photoperiod, culture vessel, Age of explant etc. are standardized, this will greatly contribute in generating a protocol for *in vitro* seed germination in this endangered species. Good quality plant material for regeneration studies can be obtained and the plant can be conserved using tissue culture approaches.

ACKNOWLEDGMENTS

The author expresses heartfelt thanks and pays tribute to Late Dr. Shashi Rai for providing immense

guidance in Research. The author extends gratitude to National Chemical Laboratory, Pune for providing Research facilities.

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