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The *in Vitro* Seed Germination Protocol for *Ephedra Foliata*

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Abstract – Ephedra foliata Boiss, family Ephedraceae, a gymnosperm shrub, It bears dioecious flowers and semi-transparent berry like fruits. Dried stems are used to cure bronchitis, asthma, influenza and hay fever. The seeds were treated with 0.4% Bavistin, 0.2% streptomycin and 0.1% $HgCl_2$ and cultured on full and half strength MS medium supplemented with different concentration of GA_3, BAP, Kinetin and NAA alone and in combinations. Within the range evaluated, highest percentage (55% and 30%) of the seed germination was obtained with MS half strength medium containing 2mgl^{-1} GA_3 and combination of 2mgl^{-1} BAP + 0.2mgl^{-1} Kinetin after 3 weeks of inoculation. No germination was observed from seeds cultured on MS full strength medium. The seeds were unable to germinate on vermiculite. The present study first time reveals the in vitro seed germination protocol for this plant.

Keywords: In Vitro Germination, Ephedra Foliata, Vermiculite, MS Medium, Growth Regulators.

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INTRODUCTION

Ephrdra foliata Boiss. (Ephedreace) commonly known as Somlata and unthphog in north western part of India. The plants are dioeciously woody shrubs with 3-5 m long trailing or scandent stem, having subverticillate or fascicled, thin, persistent branches with 2.5–10 cm long internodes. Leaves usually 3, linear setaceous, shortly connate at base, stem performs the function of assimilation and female plants bear semitransparent, nutritious edible berries (female cones) having sweet taste due to the fleshy bracts. These fleshy bracts are important during the food scarcity in arid regions (Meena B et al 2019). From the ancient time, decoction of whole plant is used to cure asthma, fever, snake bite, stomachic, blood purification, dropsy, worms and as cardio tonic (Silori et al. 2005; Quattrocchi 2012). It also retains antimicrobial and hepatoprotective properties (Alqasoumi and Abdel-Kader 2012; Bissa 2015) and high nutraceutical value (Kamboj 2000). But unsustainable exploitation, large scale habitat abolishing, disinclined growth rate, poor rejuvenation, foraging and other man made dilemmas have caused enormous reduction in its natural populations. Therefore, *E. foliata* has now become a rare or endangered species from a vulnerable category in its natural habitats (Kharin 2002; Joshi et al. 2013) Therefore conservation is primary need for existence of this species. Most of *in vitro* work carried on to *Ephedra* related to alkaloid content of callus (Khanna and Uddin 1976, 1977; Ramawat and arya 1977).

Survey of literature reveals that no attempt has been made to develop *in vitro* seed germination protocol of *Ephedra foliata.* Present report deals with design the protocol for *in vitro* seed germination.

MATERIAL AND METHODS

Ephedra foliata Boiss. seeds were collected from mature plant growing in Botanical garden of Department of Botany, Kurukshetra University, Kurukshetra. The seeds were washed under running tap water followed by 0.2 % solution of Tween twenty a detergent for five minutes. The surfaces of seeds were sterilized in 0.4% bavistin and 0.2% streptomycine solution for 25 minutes followed by three times rinsed with DDW. Prior to inoculation seeds were surface sterilized with 0.1% $HgCl_2$ solution for two minutes and then repeatedly washed in DDW.

Explants were cultured on Murashige and Skoog basal medium supplemented with 2% sucrose, different combinations of plant growth regulators and solidified with 0.7% Agar-agar was used. The medium free from PGR's acted as control. The pH of medium was adjusted to 5.7-5.8 with 0.1 N HCL and NaOH before autoclaving at 121° C for 15 minutes. The cultures were reserved under fluorescent light at 25° C ± 2 with a 16 hour photoperiod.

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RESULTS

Just not many ponders beforehand have inspected the seed germination of *Ephedra foliata* and next to no data is accessible for the generation of quality planting material by means of tissue culture. The best germination response of *Ephedra foliata* with complete development of root and shoots was seen as on MS medium with various combinations of growth hormones.

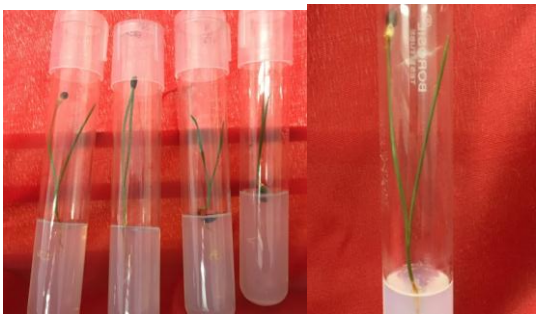
The seeds of *Ephedra* were tested against their germination potential in field and lab conditions. In field only 4-5 % germination was observed in the month of mid-June to end of July. The germinated seedlings showed high mortality rate and only 1 % seedlings survived. While no seed germination observed on vermiculite throughout the year.

Collected Seeds From Plant

***Ephedra foliata* in Botanical garden of Kurukshetra University**

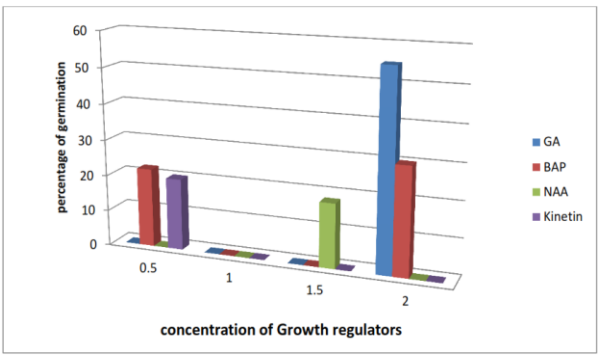
On the other hand 30-55% germination of seeds observed *in vitro* conditions by influence of plant growth regulators. Various combinations and concentrations (0.25-2.5 mg/l) of PGRs were used for this experiment. The best results were found in half strength MS medium supplemented with 2 mg/l GA and 2mg/l BAP + 0.2 mg/l Kinetin. While 1.5 mg/l NAA, 0.5 mg/l BAP and Kinetin alone showed the little germination and less growth of seedlings, which died later. The combinations of PGRs in full strength medium showed no seed germination.

Each experiment had 25 test tubes, five test tubes used as control with three replicates. Control did not show a single seed germination. Maximum germination was observed in half MS supplemented with 2 mg/l GA (55%) with mean seedling length 6.9 ± 0.02 cm, 30% germination was given by 2 mg/l BAP + 0.2 mg/l Kinetin with mean seedling length 6.7 ± 0.01 followed by 0.5 mg/l BAP (22%), Kinetin (18%) and 1.5 mg/l NAA (20%) with mean seedling length 2.1±0.5cm, 2.7±0.3cm and 1.6±0.4cm after the three weeks of inoculation.



***In vitro* germinated seedlings**

The graph shows the effect of PGRs on seed germination.



DISCUSSION

Past *in vitro* investigations of Ephedra seeds which lack the key data for production of quality planting material. The seeds showed maximum germination percentage with GA but the higher concentration of GA inhibits the germination, the same influence of GA, BAP and IBA on seed germination of dragon fruit (Hylocereus undatus) studied (Vishnupriya J. et al 2019) and GA and IAA on seed germination of radish (Raphanus sativus L.) (Neelam D. and Abhishek B., 2019). The seed doesn't germinate below 20ºC and above 45ºC. The surface sterilizing chemicals also affect the germination percentage ie higher concentration of $HgCl_2$ which might kills the embryo due to this seed unable to germinate. On the other hand some chemicals enhance the

germination percentage ie 5% $MgSO_4$ for 15 minutes in Casimiroa (Sanna S. et al, 2006). The plants grown in control conditions showed faster growth than *in vivo* plants. Ephedra is a source of different secondary metabolites and have great medicinal and pharmaceutical value, which may be useful for many industries.

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