Development of an efficient *In Vitro* regeneration protocol for *Salvadora persica* L. through seed germination

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Salvadora persica L. plants showing relatively high salt tolerance were used for micro propagation and regeneration of plantlets from seed cultures. Present investigation has been focused on efficient *in vitro* propagation protocol for this valuable plant species. *In vitro* plant regeneration is essential for the rapid multiplication of disease-free planting materials and is an imperative for the application of biotechnology tools to plant breeding and genetic improvement. The present study demonstrates *In vitro* regeneration of *Salvadora persica* L. multiplication derived from a seed for regeneration on MS medium supplemented with different concentration and combinations of auxin and cytokinins. Best results were shown by shoot regeneration from seed explants. The highest micropropagation was induced from seed in a combination of KIN (2.0 mg/L) with IBA (0.5 mg/L). The absence of light was favorable for seed germination. The seed germination, as well as shoot initiation, was higher in shoot tip than cotyledon for plant regeneration in *Salvadora persica* L. It reduces the multiplication time and potentially offers an efficient system for regenerating and propagating plants with relatively high genetic uniformity. *In vitro* regeneration consequently will promote the application of plant tissue culture technology in the areas of genetic transformation.

Keyword: IBA, KIN, Mg/L,

Introduction:

Salvadora persica L. (Miswak) belongs to family Salvadoraceae is a facultative halophyte, because it occurs in both non saline to very highly saline habitat. It is a potential source for seed oil and identified as a predominant species in highly saline habitats of coastal and inland black soils. The tender twigs, leaves and roots have many pharmaceutical applications as they contain salvadoricine, salvadourea, dibenzyl thiourea,

Rutin, quercetin, trim ethylamine, thioglucoside, Potash, chloine etc. (Dr. Sujata Mathur., 2013). The tissue culture technique is widely used for sustainable conservation and utilization of valuable secondary metabolites in rare and endangered medicinal plants, particularly those with difficulties in their traditional propagation, such as *S. persica* L. Traditionally, the plant is employed in folk medicine for oral hygiene, dental care, cough, asthma, scurvy, rheumatism, ulcers, and piles curing (Manar S. Fouda., 2021).

Seed, a term applied to the ripened ovule of a plant before germination. Seed is a fertilized ovule or a plant propagule comprising of a living embryo in which growth has been suspended, usually supplied with their own food reserves and protected by special covering layers. In essence, seed is a miniature plant because it is responsible for its regeneration and ultimately for its reproduction success (Khurana and Singh, 2001).

The seed is structural and physiological devices to fit it for its role as a dispersal unit and is well provided with food reserves, which sustain the young plant until a self-sufficient autotrophic organism is established. In nature, plants perpetuate either by seeds or by vegetative means. An angiospermic seed may appear very simple externally but possesses a complex ecophysiology for its further resumption of growth, primarily in germination. The seed is a highly dehydrated structure and is metabolically quiescent. The cotyledons of many plants function as primary photosynthetic organs. From variability studies point of view very few reports are available on the presence of genetic variability for seed traits. Each species of plant has its specific period of viability a seed sown after the period of optimum viability may produce weak plants or may not germinate. In arid zone, probably in no other habitat, a seed is subjected to such scrupulous environmental conditions as in the arid ecosystem where an acute shortage of water is a primary limitation in the germination of seeds.

Generally large seeds were found to germinate faster and more completely than smaller ones and produce seedlings whose initial growth was greater since bigger seed size produces quick, uniform germination and better seedlings (Moles and Westoby, 2004). Stated that various environmental factors that differ among habitats such as temperature, humidity, light, soil characteristics, dispersal syndromes, germination time, densities of competing plants, herbivore, fungi, etc. affect the production and selection of different seed

sizes. So, an investigation on the various seed parameters for improving them would be useful. In the present studies, an attempt has been made to study the seed morphology & effect of different pretreatments to enhance seed germination under laboratory conditions in *Salvadora persica* L. of the Indian arid zone in the year 2014-2016.

Materials and Methods:

Viability of seed:

The morphological parameters such as seed shape, size, colour and weight were taken. The shape and colour of seeds were observed under dissecting microscope and by naked eyes. Length and thickness were measured with the help of Vernier caliper. The volumes were calculated by using water displacement method. Seed density was calculated by using the formula- Seed weight (g)/ Seed volume (ml).

Collection of seeds:

Seeds of *S. persica* L. were collected from three different sites nearby Chhatrapati Sambhajinagar district. In *S. persica* L. seeds were collected from the matured fruits from red flowers bearing plants at all sites.

Surface sterilization of explant:

All seeds were washed with tap water twice in laboratory, followed by 70% ethanol for 30 seconds and then surface sterilized of with $HgCl_2$. Surface sterilization of explant was carried out in laminar air flow. Seeds were rinsed with sterile distilled water followed by 0.3% Mercuric chloride ($HgCl_2$). Finally, all these seeds were inoculated on MS medium aseptically.

Culture media:

For seed induction of media (MS) Murashige and Skoog (1962) was used for seeds germination of *Salvadora persica* L. Seeds were inoculated on MS medium supplemented with IBA and KIN for shoot induction. MS medium fortified with 3% sucrose and gelled with 3 gm/L clerigar and the pH was adjusted to 5.6-5.8. The media was sterilized in an autoclave under 15 psi and 121°C.

Culture condition:

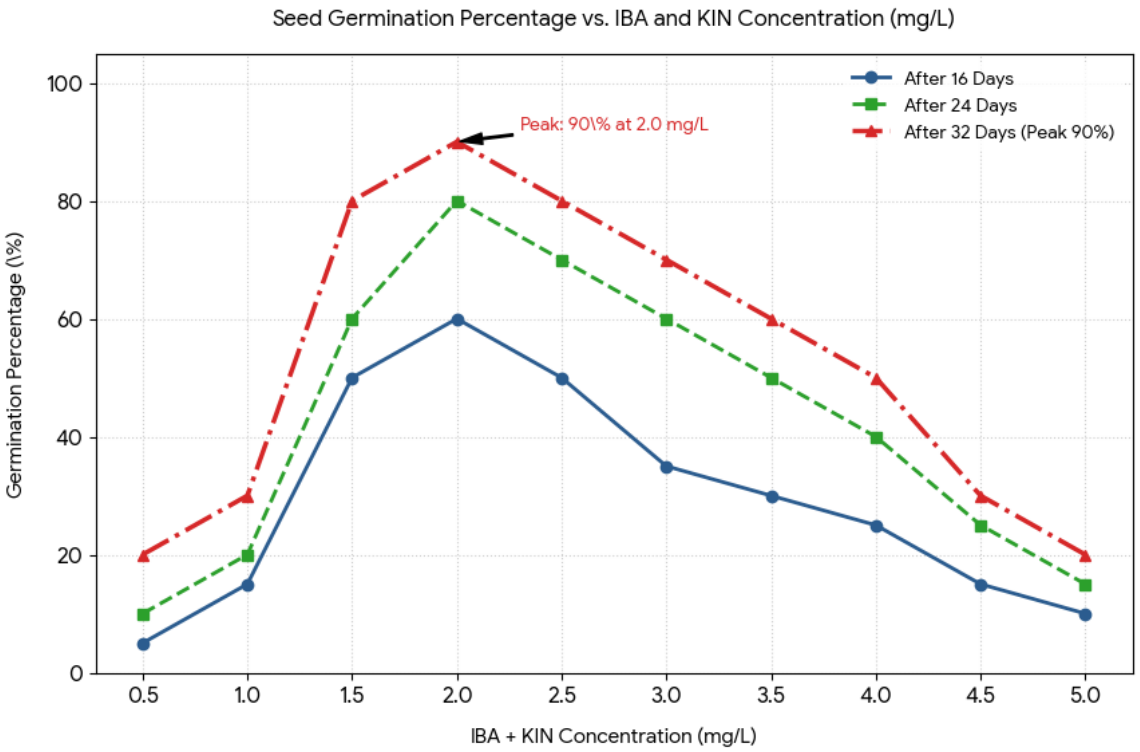
After inoculation culture bottles were transferred to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes light and temperature 25±2°C. Maximum humidity was adjusted with air conditioner.

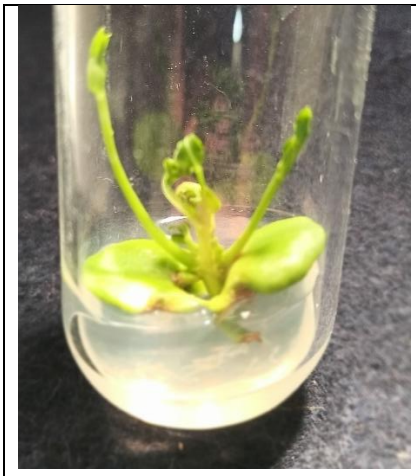
Results and discussion:

Standard protocol for surface sterilization of seeds was analyzed by trial-and-error method. Surface sterilization of seed was tried with 0.1-0.3% of $HgCl_2$ for 3-5 minutes duration. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.2% for seed of $HgCl_2$ for the present study. The hormones free MS medium was found ineffective in callus induction using seed explants. The higher percentage of seed gemination is observed in 0.5 mg/L IBA combination with 2.0 mg/L KIN concentration yielded similar results. Similar kinds of result were reported by Suman Kumari and Narender Singh., 2012. Similar results were reported for *in vitro* seed germination observed in *Celosia argentea* L. using seed explants and growth hormones like BAP, KIN and IAA (Sachin Nirpane and Narayan B. Pandhure 2018). Same result also reported for *In vitro* seed germination of *Solanum virginianum* L. using various seed (Rohit Shete *et al*.,2016).

Observation Table: Effect of IBA in combination with KIN on *in vitro* seed germination.

Source of Explant	**MS medium in combination with IBA and KIN (mg/L)**		**(%) seed germination**		
	IBA	KIN	After 16 days	After 24 days	After 32 days
Seed	0.5	0.5	05	10	20
		1.0	15	20	30
		1.5	50	60	80
		2.0	**60**	**80**	**90**
		2.5	50	70	80
		3.0	35	60	70
		3.5	30	50	60
		4.0	25	40	50
		4.5	15	25	30
		5.0	10	15	20





After 16 days

After 24 days

After 32 days

Conclusion:

Medicinal plants are voraciously collected for treatments of many disorders. These plants are bioreactors. If these plants propagated through modern techniques like tissue culture, raw Material could be utilized for therapeutic purpose. Present piece of work is useful for developing od seed germination from *S. persica* L. plant.

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