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TISSUE CULTURE PROTOCOL OF *SALVADORA PERSICA* L.

Pramod S. Fuke, Shilpa G. Chavan and Ashok M. Chavan

Tissue Culture Laboratory, Department of Botany,

Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, India.

Abstract:

The present investigation aimed to develop standard protocol for *in vitro* propagation of *Salvadora persica* L. It is one of the important medicinal plants belongs to family salvadoraceae. Nodal segment explants were inoculated using MS medium along with various concentration of growth hormone. The regeneration of multiple shooting was achieved on MS medium along with 0.5 mg/L of IBA in combination of various concentrations of BAP and KIN. The maximum percentage of regeneration and maximum length of shoot were recorded on 0.5 mg/L of IBA in combination of 2.0 mg/L and 2.5 mg/L of BAP and KIN using nodal segment explant respectively.

Keywords: *In vitro*, MS, *Salvadora persica* L, IBA, BAP, KIN, mg/L.

Introduction:

Plants are natural source of ayurvedic medicines. Plant derived medicines have been a part of our traditional health care system and the antimicrobial properties of plant derived compounds are well documented. Herbal medicines are more effective and less harmful, as they have negligible side effects. They exhibit low mammalian toxicity and can be handled easily. *Salvadora persica* L. belongs to the family Salvadoraceae. In Ayurvedic system of medicines *Salvadora persica* L. is reported to have potent activity for dental complaints. It is also called as miswak tree for the roots and twinges of this tree have been used for teeth cleaning since ancient times. It is one of the most commonly used medicinal plants for oral hygiene among global Muslim community. Moreover, the tree has been used by many Islamic communities as toothbrushes, and has been

scientifically proven to be very useful in the prevention of tooth decay, even when used without any other tooth-cleaning methods (Ahmad Hilal and Rajagopal., 2013).

Over the last few years, global awareness on the application of herbal therapies to treat diverse conditions has been rising expansively to their encouraging results and scarce adverse effects. As clarified by the World Health Organization (WHO), the majority of the global general population, mostly in emerging countries and societies, hinge on natural medicine and traditional herbal therapies for their focal treatment and health care of various conditions. Consequently, the WHO has inspired developing nations to therapeutic herbs as an additional resource to upsurge the success of health care systems. Amongst the evidence-based plant therapies, positively graded as a "miracle twig" is *Salvadora persica* L (Mekhemar.,2021).

Salvadora persica L. is used traditionally in the treatment of rheumatism, leprosy, gonorrhoea, ulcers, scurvy, tumors and dental diseases. It possesses a number of potential medicinal compounds like Salvadoricine, salvadourea, trimethyl amine, β-sitosterol, di-benzyl thiourea, rutin, thioglucoside, chlorine, potash, Sulphur etc. The ripe fruits of this tree are sweet and edible locally called as Pilu and consumed by rural or tribal population. The seeds of Salvadora yield a pale-yellow solid fat, rich in lauric and myristic acid content which is used in making soaps, illuminants, varnishes, paints as well as in food industry. It is recognized as nonconventional oil seed tree crop. The plant provides a dense shade. It is often lopped for camel and goat fodder. It is grown in plantations or hedges. The seed has high genetic variability, mainly because its cultivation is restricted to dry and saline areas of some countries. Cultivated seedlings and trees must be protected from browsing by animals (Kumar *et al.*,2012).

History of *Salvadora persica* L.:

According to ancient Greek and Roman literatures from the 3500 BC the evolution of the toothbrush may be traced from chewing sticks that were used by Babylonians and to toothpicks that were chewed to help clean the teeth and mouth. During the old days the laws of Manu of ancient Vedic India stipulated that the teeth be cleaned as part of the daily hygienic rituals. The history and the use of "Miswak" as an oral tool as well as the biological effects of *Salvadora persica* L. extracts. Chewing sticks are considered the most popular among all of the dental care tools for their simplicity, availability, low cost and their traditional and religious value. Medical books of ancient India, Susruta Samhita and Charaka Samhita, have also stressed on oral hygiene using herbal sticks. There are various biological properties, including significant antibacterial, antifungal and anti-plasmodial effects in the extract of miswak. During the 2nd century BC, the Greek sophist, Alciphron, recommended a toothpick to clean the ''fi brous residue'' that remained between the teeth after meals. The Greek word, karphos, Alciphron used to describe the toothpick, is roughly translated to 'blade of straw'. The Gospel of Buddhism mentions Buddha receiving a ''tooth stick from the god, Sakka''. The Talmud mentions ''quesem'', a splinter or wooden chip that was ''divided at one end by chewing and biting'' and used like a toothbrush (Sharma *et al.*, 2018).

Distribution and Description of plant:

The genus Salvadora belongs to family 'salvadoraceae'. distributed mainly in the tropical and subtropical region of Africa and Asia (Swamy and Timothy., 2015). It is famously known as the Persian toothbrush tree is broadly distributed throughout the world from the southern region like India, Iran, Iraq, Israel, Egypt, Malaysia, Pakistan to Mauritania in the west region. Also, from the north region of North Africa, Sudan, Ethiopia, Central Africa. It is mainly grown in Saudi Arabia and predominantly seen in the Middle East countries (Rehaman., 2021).

Salvadora persica L. is a large, well-branched evergreen shrub or small tree having soft whitish yellow wood, bark is of old stems rugose, branches are numerous, drooping, glabrous, terete, finely striate, shining, and mostly white. Leaves of *Salvadora persica* L. somewhat fleshy, glaucous, 3.8–6.3 by 2–3.2 cm in size, elliptic lanceolate or ovate, obtuse, and often mucronate at the apex, the base is usually acute, less commonly rounded, main nerves are in 5–6 pairs, and the petioles 1.3–2.2 cm long and glabrous. The flowers of *Salvadora persica* L. greenish yellow in colour in axillary and terminal compound lax panicles 5–12.5 cm long, numerous in the upper axils, pedicels 1.5–3 mm long, bracts beneath the pedicels, ovate and very caducous. Calyx is 1.25 mm long, glabrous, cleft half-way down, lobes rounded. Corolla is very thin, 3 mm long, deeply cleft, persistent, lobes are 2.5 mm long, oblong, obtuse, and much reflexed. Stamens are shorter than corolla, but exserted, owing to the corolla lobes being reflexed. Drupe is 3 mm in diameter, globose, smooth and becomes red when ripe (Khatak *et al.*, 2010).

Traditional Uses:

The leaves of *Salvadora persica* L. are eaten as a vegetable in the eastern tropical Africa and are used in the preparation of a sauce, and tender shoots and leaves are eaten as salad. Leaves are bitter in taste, corrective, deobstruent, astringent to the bowels, tonic to the liver, diuretic, analgesic, anthelmintic, useful in ozaena and other nose troubles, piles, scabies, leukoderma, lessening inflammation, and strengthening the teeth. Leaves are pungent and are considered in Punjab as an antidote to poison of all sorts and in south of Bombay as an external application in rheumatism. The juice of the leaves is also used in scurvy. The fruit of *Salvadora persica* L. are sweet and edible. A fermented drink is reported to be made from the fruits.

Fruits possess deobstruent, carminative, diuretic, lithontriptic, and stomachic properties and are used in biliousness and rheumatism. In Sind it is believed that fruits have a good effect on snake bite. Root bark is used as a vesicant and is employed as an ingredient of snuff. A paste of the roots is applied as a substitute for mustard plaster and their decoction is used against gonorrhea and vesical catarrh. A decoction of the bark is used as a tonic in amenorrhea and the dose of the decoction is half a teacupful twice daily and as a stimulant in low fevers and as an emmenagogue. Stem bark of *Salvadora persica* L. is used as an ascarifuge and also in gastric troubles. Seeds have bitter and sharp taste. They are used as purgative, diuretic and tonic seed oil is applied on the skin in rheumatism (Gautam *et al.*, 2012).

Materials and Methods

Preparation of explant and sterilization:

The explant nodal segment was collected from young healthy plantlets of *Salvadora persica* L. from different study area Aurangabad region. All these explants were washed with running tap water for 5 minutes, followed by 70 % ethanol for 1 minute and finally with distilled water for 3 minutes. Surface sterilization of explant was carried out by washing with sterile distilled water for 3 minutes followed by various concentration of mercuric chloride ($HgCl_2$) explant nodal segment with 0.2 % of $HgCl_2$. It was followed by two subsequent rinses with sterilized double distill water in laminar airflow. All these explants were cuts in to small pieces and inoculated on suitable media.

Culture media and Culture condition:

All experiments of investigation were tried on MS media (Murashige and Skoog, 1962) supplemented with various concentration of growth regulators. MS medium was fortified with 3 % sucrose and clerigar for solidification respectively and pH was adjusted to 5.6-5.8. The media were steam sterilized in an autoclave under 15 psi and 121°C. After the inoculation culture bottles were transferred to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes light and temperature at 25±2°C. At least 5 replicates raised for each treatment and data were recorded in table.

Results and Discussion:

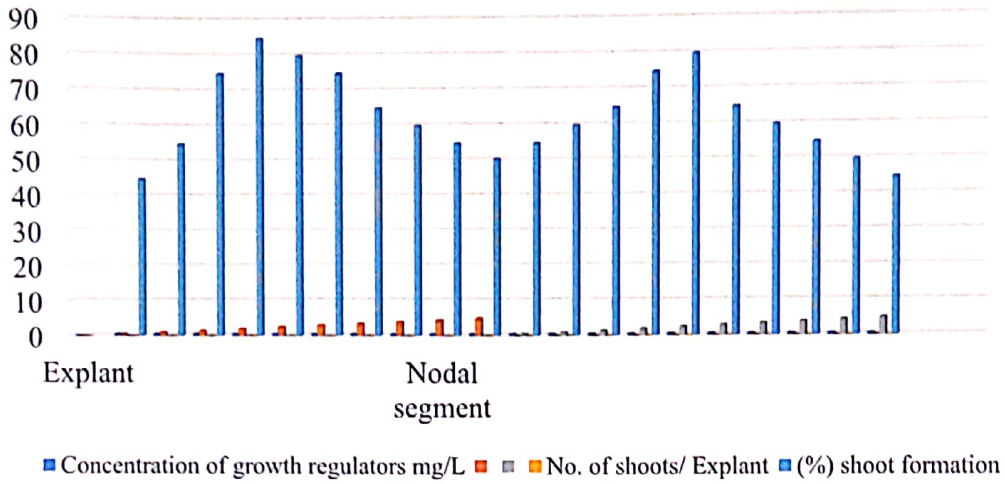
Standard protocol for surface sterilization of explant was analyzed by trial-and-error method. Surface sterilization of explant node segment were tried with 0.1-0.3% of $HgCl_2$ for 3- and 5-minutes duration. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.2% of $HgCl_2$ for nodal segment explant during the present study. Shoot regeneration was achieved from nodal segment explant from BAP and KIN in combination of 0.5 mg/L of IBA. Lower concentration of BAP was found effective to induced shoot regeneration however higher concentration revealed poor result of shoot regeneration similar kind of result was also recorded. The maximum shoot induction percentage along with shoot length was recorded from 0.5 mg/L of IBA in combination of 2.0 mg/L of BAP and 2.5 mg/L of KIN with 85.00% and 80.00% regeneration using nodal segment explant respectively. Similar kinds of result were reported by Kumar *et al.*, (2013). Standard protocol for shoot regeneration was also develop by Ramar *et al.*, (2011) revealed that growth hormone BAP incorporated with MS media exhibits rapid multiplication of *Salvadora persica* L.

Effect of various concentrations of auxins (IBA) in combination with cytokinins (BAP and KIN) for multiple shoot formation from nodal segment as explant.

Explant	Concentration of growth regulators mg/L			No. of shoots/ Explant	(%) shoot formation
	IBA	BAP	KIN		
Nodal segment	0.5	0.5	--	7.68±0.226	45.00
	0.5	1.0	--	7.81±0.228	55.00
	0.5	1.5	--	7.92±0.235	75.00
	0.5	**2.0**	**--**	**8.06±0.273**	**85.00**
	0.5	2.5	--	7.86±0.248	80.00
	0.5	3.0	--	7.71±0.234	75.00
	0.5	3.5	--	7.54±0.224	65.00
	0.5	4.0	--	7.38±0.217	60.00
	0.5	4.5	--	7.24±0.218	55.00
	0.5	5.0	--	7.02±0.205	50.55
	0.5	--	0.5	7.28±0.101	55.00
	0.5	--	1.0	7.51±0.104	60.00
	0.5	--	1.5	7.78±0.096	65.00
	0.5	--	2.0	7.86±0.092	75.00
	0.5	**--**	**2.5**	**8.04±0.092**	**80.00**
	0.5	--	3.0	7.88±0.086	65.00
	0.5	--	3.5	7.68±0.086	60.00
	0.5	--	4.0	7.48±0.066	55.00
	0.5	--	4.5	7.28±0.066	50.00
	0.5	--	5.0	7.14±0.050	45.00

Values represent the mean ± SE and percentage response on three separate experiments, each based on a minimum of five replicates.

Effect of various concentrations of auxins (IBA) in combination with cytokinins (BAP and KIN) for multiple shoot formation from nodal segment as explant.



Effect of various concentrations of auxins (IAA) in combination with cytokinins (BAP and KIN) for multiple shoot formation from nodal segment as explant.

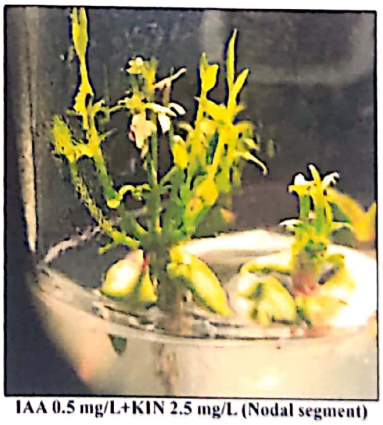
IAA 0.5 mg/L+BAP 2.0 mg/L (Nodal segment)

IAA 0.5 mg/L+BAP 2.0 mg/L (Nodal segment)

IAA 0.5 mg/L+BAP 2.0 mg/L (Nodal segment)

IAA 0.5 mg/L+KIN 2.5 mg/L (Nodal segment)

IAA 0.5 mg/L+KIN 2.5 mg/L (Nodal segment)



IAA 0.5 mg/L+KIN 2.5 mg/L (Nodal segment)

Conclusion:

A reproducible and efficient micropropagation protocol has been developed using nodal segment of a mature *Salvadora persica* L. The best *in vitro* shoot induction response was obtained on 2.0 mg/L of BAP and 2.5 mg/L of KIN for *in vitro* shoot multiplication. The significance of the present protocol is further increased as the *in vitro* maintained shoots provide a regular supply of shoots for rooting and field transfer without any demand for fresh explants material. This simple regeneration system developed for *Salvadora persica* L. from nodal segment in the present study can be used for large scale rapid multiplication of this important medicinal plant *Salvadora persica* L.

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