Callus Induction studies in *Salvadora persica* L.

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Abstract:

Salvadora persica L. is commonly known as the miswak. Is a multipurpose tree with immense ethno-ecological importance and is commonly used for varied medicinal purposes? The plant is used as a source of food, fodder, lipids, gum and resins. In addition, it is also valued for rearing honey bees. Its roots are regularly used as tooth brush throughout the country. Besides these the plant is also grown to provide shelter-belts and windbreaks for agriculture crops. It is distributed throughout the arid and semi-arid ecosystem of world and helps in reclamation of sand dune habitats and saline soils in arid ecosystems. However, its population suffers from serious environmental problems such as deforestation, soil degradation, loss of biodiversity and unsustainable livelihoods. *Salvadora persica* L. has high medicinal, economic and ecological values in arid and semiarid ecosystem. Nevertheless, its conservation status is highly threatened and detailed ecological study of *Salvadora persica* L. is suggested to conserve its remaining population Several different classes of compounds were previously isolated from various parts of *Salvadora persica* L. of which the main groups are Trimethylamine, Salvadrin, Chloride, Fluoride, Silica, Sulfur, Mustard, Vitamin C and a small amount of Saponin, Tannin. It is effective on Gonorrhea, Bronchial asthma, Tympanites, Misperistalsis, Mouth problem, Piles, Dysuria and for Rejuvenation in ayurveda.

During the present investigation efforts have been made to evaluate standard protocol for induction of callus in *Salvadora persica* L. Explants were taken from elite plant and aseptically inoculated on MS medium supplemented with various concentrations of 2, 4-D. Best results were obtained from different concentration of 2,4 D leaf 1.5 and 2.0 mg/L, Nodal segment 2.0 and 2.5 mg/L, and Shoot tip 2.5 and 3.0 mg/L opened best result for callus induction.

Keywords: *In vitro*, *Salvadora persica* L, callus, regeneration, mg/L, 2,4 D

Introduction:

Salvadora persica L belongs to family Salvadoraceae. In Indian subcontinent this family is represented by only one genus with two species like *Salvadora persica* L. and *Salvadora oleoides* Decne. Recently, a new species *Salvadora alii* has been described from Sindh, Pakistan. *Salvadora persica* L. is a popular chewing stick commonly known as 'miswak' and is one of the most popular medicinal plants throughout the Indian subcontinent as well as the wider Muslim world. *Salvadora persica* L. has antiurolithiatic properties. It is used for centuries as a natural toothbrush its fibrous branches have been promoted by the World Health Organization for oral hygiene. The plant has been used for the preparation of a number of medicinally important products such as abrasives, antiseptics, astringent, enzyme inhibitors and fluorides. *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 viz. salvadoricine, salvadourea, trimethyl amine, β-sitosterol, di-benzyl thiourea, rutin, thioglucoside, chlorine, potash, Sulphur etc. Besides its medicinal potentialities, it is also suitable in agroforestry systems as a wind break and helps in land reclamation. The ripe fruits of this tree are sweet and edible locally called as Pilu and consumed by rural and 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 (Kumar., *et al*.,2012).

Salvadora persica L. is commonly known as miswak the toothbrush tree it 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. Chewing sticks that are made from the roots, twigs, or stems of *Salvadora persica* L. are commonly used in the Middle East as a means of maintaining oral hygiene. Studies indicate that *Salvadora persica* L. extract is somewhat comparable to other oral disinfectants and anti-plaque agents such as triclosan and chlorhexidine gluconate, if used at a very high concentration (Bayati and Sulaiman., 2008).

Description and distribution of plant:

Salvadora persica L. is a member of the plant family Salvadoraceae. It is mainly distributed in dry and subtropical regions of Africa and the Middle East, as well as the Indian subcontinent. The fresh leaves, twigs and roots of its small tree can be added to the daily diet and are applied in traditional herbal therapy for asthma, scurvy, cough, rheumatic illnesses, oral hygiene and other conditions. The use of *Salvadora persica* L. originates in the Pre-Islamic and Islamic era, as it was introduced by Arabic and Islamic societies as a predecessor of toothbrushes to clean the teeth and promote a good oral hygiene. The advantageous properties of *Salvadora persica* L regarding dental and oral health can be explained by its mechanical action if used for brushing in addition to its pharmacological active constituents. These encompass chemically active substances as tannins that inhibit glucosyltransferase enzyme to diminish plaque and periodontal diseases, and resins that protect against dental caries Moreover the antimicrobial, anti-inflammatory and antioxidant activities of *Salvadora persica* L. have been ascribed to several detectable substances in its natural extracts such as potassium and sodium chloride, as well as salvadorine, vitamin C, Salvadourea, silica, Saponins and different minerals (Mekhemar *et al*., 2021).

Salvadora persica L. is a slow growing, evergreen perennial halophyte capable of growing under extreme conditions, from very dry environments to highly saline soils. It is a shrub or a small tree which grows up to 10 meters in height and a girth of 3 feet. Main trunk is errect, more than one foot in diameter, with profusely branched, wide crown of crooked, straggling and drooping branches. Young branches are green in colour. Bark is slightly rough, grayish brown on main stem. Leaves are opposite, entire, succulent, petiolate, fleshy, oblong elliptic to almost circular, 3x7cm, light to dark green, with 5-6 pairs of main nerves. New leaves are produced during April, which on maturity become thick and leathery. Leaves shed from late December to January. Flowers are small, greenish yellow in axillary and terminal panicles, sessile or sub-sessile, bisexual and tetramerous. Small greenish-white flowers are produced in January to April. The fruit is yellow and ripens in the months of May and June. Mature fruits are spherical or

globose drupe with persistent calyx, smooth, fleshy, 5-10 mm in diameter, pink to scarlet and single seeded. Seeds turn from pink to purple-red and are semitransparent when mature. Seeds are dispersed by birds, animals and man after they eat the fruit (Swamy and Lasiti Timothy., 2015).

Medicinal and chemical properties:

There are many traditional uses reported for the *Salvadora persica* L. It has mainly been used in dental care to cure toothache and gum diseases and for cleaning teeth. Additionally, it is used to relieve boils, chest diseases, gonorrhea, headache, spleen troubles, stomachache, and ulcer. The root infusion can also be used to increase milk production in lactating women. Leaves of the plant are being utilized as a mouthwash and in treatment of chest pain, cough, tooth and gum problems, body pain, backache, piles, stomachache, stomatitis, and wounds. The bark latex is beneficial to subside skin sores. The seeds are taken as a tonic, and the seed oil is rubbed to treat joint pain, lumbago, and rheumatism. It has also been used to relieve edema, fever, malaria, and worms. The plant juice is used by women as a female contraceptive. The uses of stem and roots of *Salvadora persica* L. in dentistry could be correlated to the presence of many phytochemical constituents such as tannins that inhibits glucosyltransferase enzyme to reduce plaque and gingivitis. The resins of the chewing stick of the plant protect against dental caries by forming a layer on enamel. Salvadorine isolated from the chewing stick of *Salvadora persica* L. exerted bactericidal effects and stimulated the gingiva. Recently, five α-amylases were isolated from the roots of the plant which showed good affinity towards the substrates such as starch and glycogen (Bratty *et al*., 2020).

Miswak contains important phytoconstituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides in addition to Sulphur, organic Sulphur compounds and lignan glycosides. Pharmacological studies indicated that *Salvadora persica* L. plant possess anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, antipyretic, astringent, diuretic and bitter stomachic activities, anticonvulsant and Antiulcer activity, hypoglycemic effect and it reduced body weight. Miswak has great medicinal use in the treatment of nose troubles, piles, scabies, leucoderma, scurvy,

gonorrhea, spleen disorders, boils, sores, gum disease, stomachache and toothache, to treat hook worm, venereal diseases, for teeth cleaning, in rheumatism, cough and asthma, to lower cholesterol plasma levels, reestablishment of the components of gastric mucosa and as a laxative (Abou-Zaid *et al*., 2015).

Materials and Methods:

Surface sterilization of explant:

Explants like leaves, Nodal segment and shoot tip were collected from different study area of Aurangabad district. All explants 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. Explants were rinsed with sterile distilled water followed by 0.3% Mercuric chloride ($HgCl_2$). Finally, all these explants were dissected in to small pieces and inoculated on MS medium aseptically.

Culture media:

For induction of callus Murashige and Skoog media (MS) (1962) was used for leaf nodal segment and shoot tip explants of *Salvadora persica* L. Leaf, nodal segment and shoot tip were inoculated on MS medium supplemented with 2,4-D for callus 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:

One of important step in plant tissue culture was surface sterilization of explants. Keeping this point in view different concentration ranged from 0.1-0.3% of $HgCl_2$ were tried. Minimum contamination and higher rate of survival rate was archived on 0.1% of

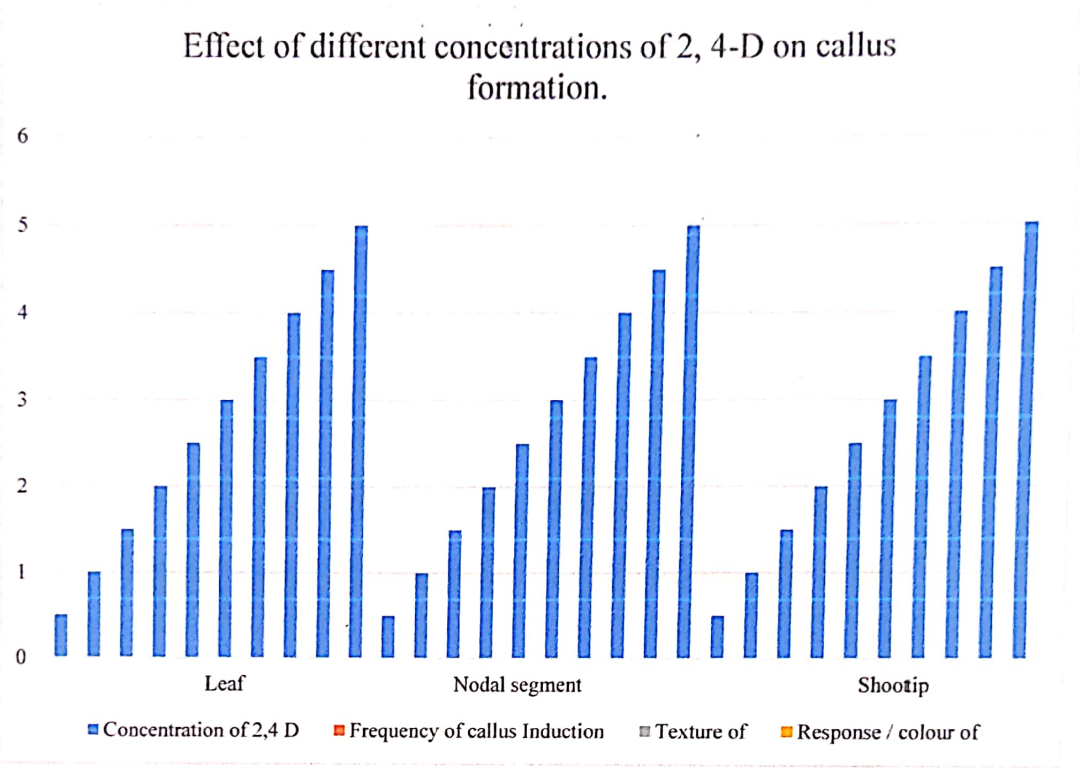
$HgCl_2$ for leaf explants whereas 0.3% of $HgCl_2$ for nodal segment and shoot tip explant. Surface sterilized explants were inoculated on MS medium supplemented with various concentrations of 2, 4-D. All combination of growth regulators were found more or less potent for induction of callus.

It could be revealed that, higher concentration of 2, 4-D has potential to induce profuse callus. Callus induced with these concentrations was whitish to greenish colour with friable nature. Maximum rate of callus induction was recorded on 2.0 mg/L of 2, 4-D using leaf as explant. However, it was found maximum at 2.0 mg/L and 2.5 mg/L of 2, 4-D using nodal segment as explant. Leaf, Nodal segment and shoot tip explants also shown formation of callus on MS medium supplemented with 2, 4-D. Moderate callus was induced with whitish yellow colour which was friable in nature. Highest frequency of callus induction was recorded on 1.5 and 2.0 mg/L 2, 4-D and 2.0 and 2.5 mg/L 2,4 D with callus induction frequency by using leaf and nodal segment as an explant respectively. Maximum induction of callus was revealed by 2.5 and 3.0 mg/L of 2, 4-D using shoot tip as explant. However lower concentration of growth regulators was found less effective for induction of callus or poor type of callus induction was achieved. These induced calluses were subculture on MS medium with different concentrations of hormones to achieved micropropagation. Similar kinds of result were reported by Kakde *et al.*, 2015 and by Jamdhade and Pandhure., 2016. It is revealed that growth hormone 2,4 D incorporated with MS media exhibits rapid callus induction in *Salvadora persica* L.

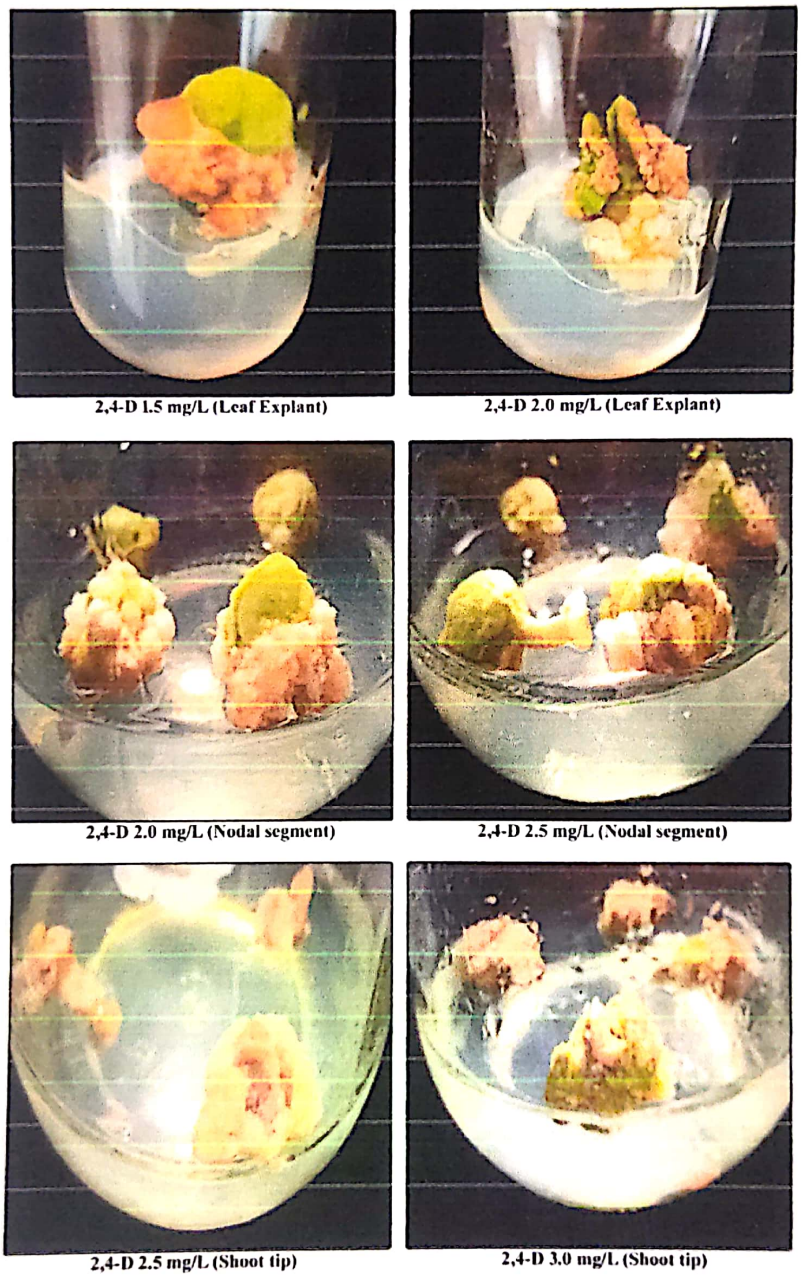
Effect of different concentrations of 2, 4-D on callus formation.

Source of explant	Concentration of 2,4 D (mg/L)	Frequency of callus Induction	Texture of callus	Response / colour of callus	Induction of callus/ Somatic embryo
Leaf	0.5	+	--	Swelling of explant	--
	1.0	++	Friable	Greenish and white	callus
	1.5	+++	Friable	Greenish	callus
	2.0	+++	Friable	Whitish	callus
	2.5	++	Friable	Whitish	callus
	3.0	+	Friable	Whitish	callus
	3.5	+	Friable	Yellowish	callus
	4.0	+	Friable	Yellowish	callus
	4.5	--	Friable	Yellowish	callus
	5.0	--	Compact	Yellowish	callus
Nodal segment	0.5	--	--	--	--
	1.0	--	Friable	Yellowish	callus
	1.5	+	Friable	Yellowish	callus
	2.0	+++	Friable	Greenish	callus
	2.5	+++	Friable	Greenish	callus
	3.0	++	Friable	Whitish	callus
	3.5	++	Friable	Whitish	callus
	4.0	+	Friable	Yellowish	callus
	4.5	--	Friable	Whitish	callus
	5.0	--	Friable	Whitish	callus
Shoot tip	0.5	--	--	--	--
	1.0	+	Friable	Swelling of explant	callus
	1.5	+	Friable	Yellowish	callus
	2.0	+++	Friable	Greenish	callus
	2.5	+++	Friable	Whitish	callus
	3.0	+++	Friable	Greenish	callus
	3.5	++	Friable	Whitish	callus
	4.0	+	Friable	Yellowish	callus
	4.5	--	Friable	Yellowish	callus
	5.0	--	Friable	Yellowish	callus

--No Callus, +Poor Callus, ++Moderate Callus, +++Massive Callus, mean ± SE and percentage calculated by three separate experiment with five replicates.



Effect of different concentrations of 2, 4-D on callus formation



2,4-D 1.5 mg/L (Leaf Explant)

2,4-D 2.0 mg/L (Leaf Explant)

2,4-D 2.0 mg/L (Nodal segment)

2,4-D 2.5 mg/L (Nodal segment)

2,4-D 2.5 mg/L (Shoot tip)

2,4-D 3.0 mg/L (Shoot tip)

Conclusion:

Salvadora persica L. has been a good alternative to the toothbrush in Rural India, since it is inexpensive, and readily available. It has many medicinal properties, and is a traditional practice so common in large percentage of our population. Thus, it can be recommended as an important and effective tool for oral hygiene. *Salvadora persica* L. has strong antiulcer, antifungal, anti-parasitic, antiviral and antibacterial properties. Although crude extracts from various parts of *Salvadora persica* L. have been shown to have medicinal applications from time immemorial, modern drugs can be developed after extensive investigation on modern scientific lines of its bioactivity, mechanism of action, pharmacotherapeutics, toxicity and after proper standardization and clinical trials. Several therapeutically and industrially useful preparations and compounds have also been marketed recently, which has generated enough encouragement among the scientists in exploring more information about this medicinal plant.

A lot needs to be done however, on various biotechnological aspects in this plant. Since, harvesting from the wild, preparation of drugs leads to loss of genetic diversity as well as habitat destruction, for which domestic cultivation can be a viable alternative and may overcome the problems which are common in herbal extracts such as misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants. However, the use of controlled environments via cell and tissue culture route can overcome cultivation difficulties and could be a means to manipulate phenotypic variation in bioactive compounds and toxins as controlled growth systems also make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest with the aim to increase potency, reduce toxin levels and increase uniformity and predictability of extracts. Rapid plant multiplication and improvement though biotechnological methods are limited for *Salvadora persica* L. There has been a significant progress in the use of tissue culture and genetic transformation techniques to alter pathways for the biosynthesis of target metabolites in different medicinal plants however, no attempt has been made in *Salvadora persica* L. in this regard.

Acknowledgments:

Authors are thankful to the Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for encouragement and support for present research work.

References:

Kumar Sunil, Rani Champa and Mangal Manisha (2012) A Critical review on *Salvadora persica* an important medicinal plant of arid zone *International Journal of Phytomedicine* 4 (3) 292-303.

Bayati Firas A. AL and Sulaiman Khudir D. (2008) *In Vitro* Antimicrobial Activity of *Salvadora persica* L. Extracts Against Some Isolated Oral Pathogens in Iraq *Turk Journal of Biology* 32 (2008) 57-62.

Mekhemar Mohamed, Geib Mathias, Kumar Manoj, Radha, Hassan Yasmine and Dorfer Christof (2021) *Salvadora persica* Nature's Gift for Periodontal Health *Antioxidants* 10 (712) 1-20.

Swamy T and Lasiti T. Timothy (2015) Phytochemical and antibacterial evaluation of ethanolic extract of Salvadora persica root extract against selected microorganisms *International Journal of Bioassays* 4 (12) 4658-4666.

Bratty Mohammed Al, Makeen Hafiz A, Alhazmi Hassan A, Syame Sohier M, Abdalla Ashraf N, Homeida Husham E, Sultana Shahnaz, Waquar Ahsan, and Khalid Asaad (2020) Phytochemical, Cytotoxic and Antimicrobial Evaluation of the Fruits of Miswak Plant *Salvadora persica* L. *Hindawi Journal of Chemistry* (2020) 1-11.

Abou-Zaid Atef A, Elbandy .M, Nadir. A (2015) Miswak (*Salvadora persica* L.) Roots as Antibacterial Agent and a Potential Food Bio Preservative *International Journal of Science and Research* (IJSR) 4 (2) 2288-2293.

Kakde Nilesh, Shimple Laxman and Pandhure Narayan (2015) *In vitro* Studies in *Tylophora asthmatica* (Burm.F) Merill *International Journal of Advanced Research in Biological Sciences*. 2 (12) 204–207.

Jamdhade Pranita and Pandhure Narayan (2016) High frequency *in vitro* regeneration via somatic embryogenesis in medicinal plant *Aegle marmelos* (L.) corr. *International Journal of Advanced Research in Biological Sciences*. 3 (1) 7-12.