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Development of an Efficient Protocol for In Vitro Callus Induction in Salvadora persica L.

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Abstract:
The present investigation was undertaken to establish an efficient protocol for *in vitro* callus induction in *Salvadora persica* L. Healthy leaf, nodal stem, and shoot tip explants were excised from donor plants and cultured for callus induction. The explants were inoculated on Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D). Callus induction and proliferation varied significantly with the type of explant and the concentration of the plant growth regulator. Among the explants tested, leaf explants exhibited the highest callus induction and proliferation on MS medium supplemented with 1.0 mg L^{-1} 2,4-D. In contrast, maximum callus formation was observed at 1.5 mg L^{-1} and 2.5 mg L^{-1} 2,4-D in nodal stem and shoot tip explants, respectively. The induced calli were friable and actively proliferating, and three-week-old callus cultures were subsequently used for *in vitro* shoot regeneration studies.

Keywords: *Salvadora persica* L, callus cultures, Miswak, organogenesis, halophyte, salt tolerance.

Introduction:

Salvadora persica L. (miswak), a member of the family Salvadoraceae, is a facultative halophyte capable of thriving under both non-saline and highly saline environmental conditions. The species is widely distributed in coastal and inland saline habitats, particularly in saline black soils, where it is recognized as one of the dominant woody plant species. In addition to its ecological significance, *S. persica* is considered a valuable source of seed oil with considerable industrial potential. Various plant parts, including the tender twigs, leaves, and roots, possess significant medicinal properties owing to the presence of a diverse range of bioactive phytochemicals such as salvadoricine, salvadourea, dibenzyl thiourea, rutin, quercetin, trimethylamine, thioglucoside, potassium salts, and chloride compounds. These phytoconstituents contribute to the plant's broad spectrum of pharmacological activities and support its extensive use in traditional and modern herbal medicine (Dr. Sujata Mathur.,2013). The tissue culture technique is widely used for sustainable conservation & 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).

Medicinal and chemical properties:

Salvadora persica L. (miswak) is one of the most extensively used medicinal plants for oral hygiene, particularly among Muslim communities worldwide. The roots and young branches have traditionally

been used as natural toothbrushes due to their mechanical cleansing action and the presence of biologically active phytochemicals that promote oral health. Numerous pharmacological studies have demonstrated that *S. persica* exhibits a broad spectrum of biological activities, including antimicrobial, anti-plaque, anti-inflammatory, analgesic, antipyretic, antioxidant, astringent, stomachic, aphrodisiac & antidotal properties.

In traditional medicine, *S. persica* has been employed in the management of various ailments, including dental caries, toothache, periodontal diseases, nasal disorders, piles, scabies, leucoderma, scurvy, gonorrhoea, boils, venereal diseases, rheumatism, cough & asthma. In addition, extracts of the plant have been reported to reduce plasma cholesterol levels, promote the restoration of gastric mucosal integrity, and exert mild laxative effects. Epidemiological studies have further indicated that populations with regular use of miswak generally exhibit improved oral hygiene, a lower prevalence of dental plaque and periodontal diseases, and reduced rates of tooth loss compared with populations relying solely on conventional oral hygiene practices.

Salvadora persica L. seed oil is useful for the treatment of some skin diseases and joint pain also reported that the plant extract itself has an analgesic effect against heat stimuli, but not the chemical stimuli. In Greco-Arab system of medicine, the fermented juice prepared from the fresh fruits is a strong aphrodisiac agent, and is also used as general body tonic. (Hilal Ahmad and Rajagopal K.,2013). *Salvadora persica* L. have different biological properties, including significant antibacterial, antifungal and anti-plasmodial effects. Phytochemical investigation revealed that it contains alkaloids, glycosides, flavonoids, carbohydrates, tannins, saponins and steroids. It also contains oleic, linoleic, stearic acids, esters of fatty acids and aromatic acids, and some terpenoids. The major components from the essential oil of *Salvadora persica* L. stem have been identified as 1,8-cineole (eucalyptol) (46%), α-caryophellene (13.4%), β-pinene (6.3%), and 9-epi-(E)-caryophellene. The analysis of the volatile oil extracted from *Salvadora persica* L. leaves revealed benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and β-caryophyllene as important constituents. (Abhishek Gupta.,2015).

Materials and Methods:

Surface sterilization of explant:

Explants leaves and stem node were collected from different localities of Chh Sambhajinagar region. 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 (1962) was used for nodal segment, shoot tip and leaf explants of *S persica* L. explant inoculated on MS medium supplemented with 2,4-D callus induction.MS medium fortified with 3% sucrose and gelled with 3 gm/L clerigar and the pH was adjusted to 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. Each experiment set in three set, five of each.

Results and discussion:

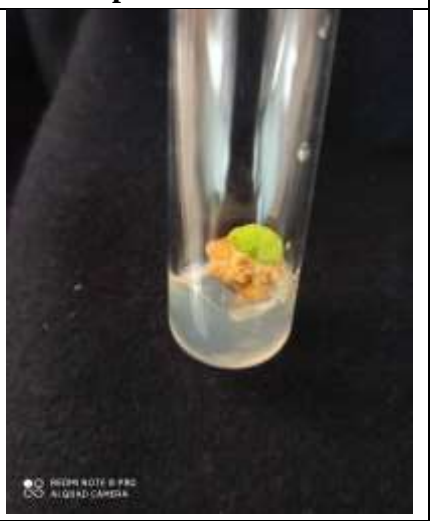
One of important step in plant tissue culture was surface sterilization of explants. Keeping this point in view different concentration ranged from 0.1-0.3% of $HgCl_2$ were tried. Minimum contamination and higher rate of survival rate was archived on 0.1% of $HgCl_2$ for leaf explants whereas 0.3% of $HgCl_2$ for stem node 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 was found more or less potent for induction of callus. It could be revealed that; optimum concentration of 2,4-D has potential to induce profuse callus. Callus induced with these concentrations was whitish to greenish in colour with friable to compact nature. Maximum rate of callus induction was recorded on 1.0 mg/L of 2, 4-D using leaf as explant,1.5 mg/L of 2,4 D using nodal segment, 2.5 mg/L of 2,4 D using shoot tip 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 achieve micropropagation.

Observation Table: 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**
Leaf	0.5	+	--	Swelling of explant	--
	1.0	**+++**	**Friable**	**Greenish and white**	**callus**
	1.5	+++	Friable	Greenish and white	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	--	--	--	--
	1.5	**+++**	**Friable**	**Greenish**	**callus**
	2.0	+	Friable	Yellowish	callus
	2.5	+++	Friable	Greenish	callus
	3.0	++	Friable	Whitish	callus
	3.5	++	Friable	Whitish	callus
	4.0	+	Friable	Yellowish	callus
	4.5	--	--	--	--
	5.0	--	--	--	--
	0.5	--	--	--	--

Shoot tip	1.0	+	--	Swelling of explant	--
	1.5	+	Friable	Yellowish	callus
	2.0	+++	Friable	Greenish	callus
	2.5	**+++**	**Friable**	**Greenish**	**callus**
	3.0	+++	Friable	Greenish	callus
	3.5	++	Friable	Whitish	callus
	4.0	+	Friable	Yellowish	callus
	4.5	--	-	--	--
	5.0	--	--	--	--

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

Leaf	**Nodal segment**	**Shoot tip**
		

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 callus and secondary metabolites as well.

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