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In Vitro Callus Induction studies in *Salvadora persica* L.

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Abstract: ***Present investigation has been focused on efficient in vitrocallus induction protocol for Salvadora persica L. plant. Leaf, stem node and shoot tip explants from healthy plant were used as source of explant for callus induction. Maximum callus proliferation was obtained on Murashige and skoog medium supplemented with 2, 4D. Callus induction differs according to nature of explant and concentration of growth hormone used. At the concentration of 1.0 mg/L of 2, 4 D massive callus was achieved using leaf as an explant whereas same result was achieved at the concentration of 1.5 mg/L and 2.5 mg/L concentration of 2, 4 D using stem node and shoot tip explant respectively. Three weeks old callus was used for shoot regeneration.***

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

1. Introduction

Salvadora persica L. (Miswak) belongs to family Salvadoraceae is a facultative halophyte, because it occurs in both nonsaline 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, di-benzyl 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).

Medicinal and chemical properties

Miswak (*S. persica* L.) is one of the most commonly used medicinal plants for oral hygiene among global Muslim community. *S. persica* L. is found to be a multipurpose plant and possesses several Agro-pharmaceutical applications. Toothbrushes prepared from the roots and small branches of *S. persica* L. to be highly useful as maintainer of teeth. Plant possesses anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities. It has great medicinal use in the treatment of nose troubles, piles, scabies, leucoderma, scurvy, gonorrhea, boils 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. Showed that the dental loss in adults is very low in the countries where Miswak is used widely.

S. 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 RajagopalK., 2013).*S. 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 *S. 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 *S. persica* L. leaves revealed benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and β-caryophyllene as important constituents. (Abhishek Gupta., 2015).

2. Materials and Methods

Surface sterilization of explant:

Explants leaves and stem node were collected from different localities ofAurangabad region. All explants were washed with tap water twice in laboratory, followed by 70% ethanol for 30seconds 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 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.

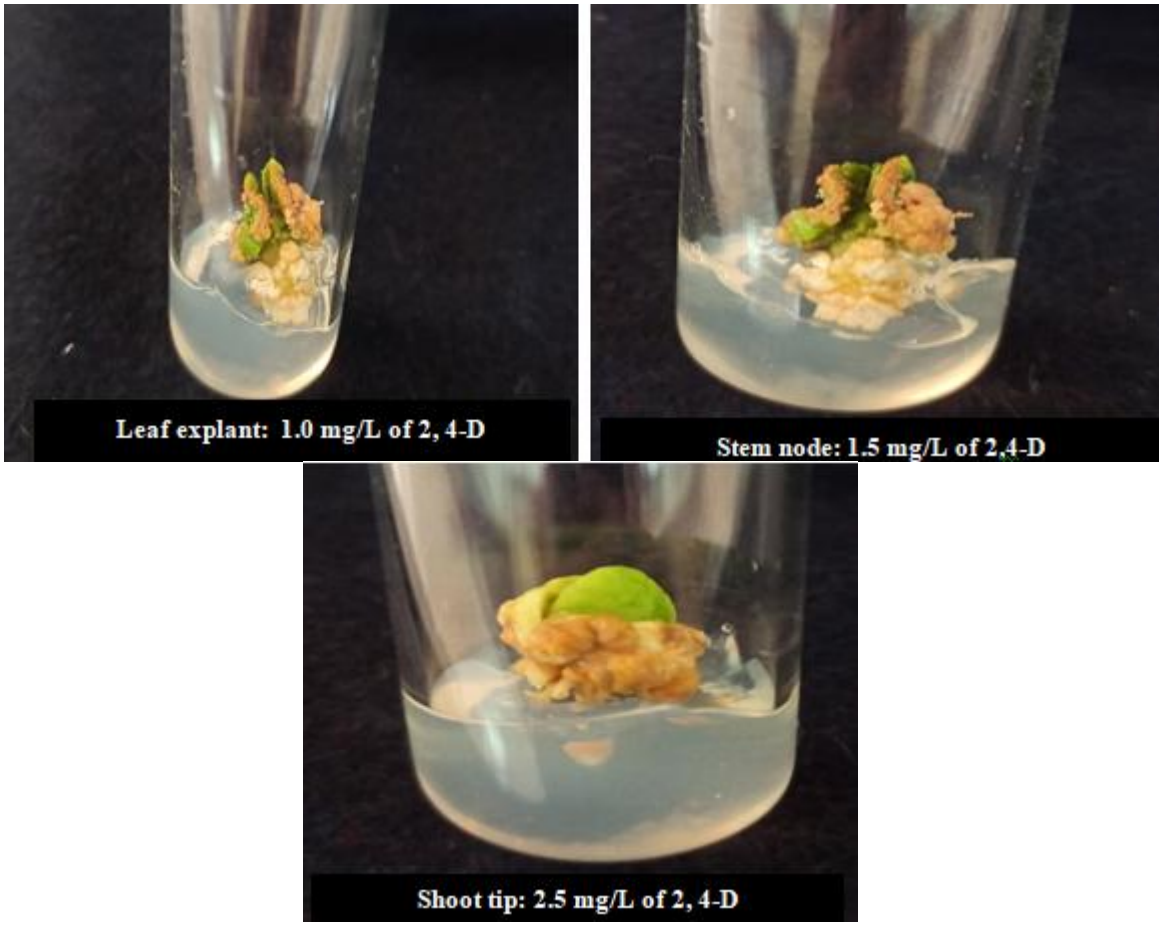
3. Results and Discussion

One of important step in plant tissue culture is 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 achieved 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 were 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 were 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 sub-cultured 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/ Somatic embryo*
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	--	--	--	--
Shoot tip	0.5	--	--	--	--
	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.



4. 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 extraction of secondary metabolites as well.

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