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Research paper

Optimization of Plant Growth Regulators for *In Vitro* Shoot Multiplication of *Salvadora persica* L.

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ABSTRACT

The present investigation was undertaken to develop an efficient protocol for the rapid micropropagation of *Salvadora persica* L., a medicinally and economically important desert tree. Leaf explants were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of plant growth regulators. The stem explants were excised from plant and inoculated on MS medium supplemented with 0.5 mg/L of IAA along with different concentrations of BAP and KIN. The frequency of shoot regeneration from stem node was affected by different concentrations of auxins and cytokinin. Length of shoot recorded was maximum on 0.5 mg/L IAA along with 2.5 mg/L BAP and 3.0 mg/L of KIN using stem as explant. However, at 1.5 mg/L of BAP and 2.0 mg/L of KIN concentration of showed satisfactory rate of multiplication using leaf explant.

1. Introduction

Salvadora persica L., a medicinally important species belonging to the family Salvadoraceae, is commonly known as the toothbrush tree or miswak. It is a small, branched, evergreen tree widely distributed in the tropical and subtropical regions of Asia and Africa. The plant is rich in a variety of biologically active phytochemicals, including alkaloids, flavonoids, steroids, terpenoids, saponins, volatile oils, and carbohydrates, which contribute to its diverse pharmacological properties. Traditionally, the stems and twigs of *S. persica* have been extensively used as natural toothbrushes in the Middle East, Africa, and the Indian subcontinent for maintaining oral hygiene and relieving toothache. In addition, the leaves are used in traditional medicine as a mouthwash, purgative, and remedy for respiratory ailments such as asthma and cough. (Suman Kumari and Narender Singh.,2012). *S. persica* L. is endangered medicinal plants has always been a bottleneck as most of the medicinal plant are over exploited and appropriate conservation measures are not applied timely and effectively leading to their mass extinction (Mayank Tripathi.,2016).

1.1 Medicinal and Phytochemical Properties

Salvadora persica L. is a medicinally important plant that contains a diverse array of bioactive phytochemicals, including volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and

carbohydrates, which are responsible for its wide range of pharmacological activities (Sunil Kumar *et al*., 2012). Phytochemical analysis of the stem has revealed the presence of benzyl isothiocyanate (52.5%) as the predominant constituent, followed by benzyl nitrile (38.3%), carvacrol (3.3%), benzaldehyde (2.5%), aniline (0.7%), and naphthalene (0.6%) (Hilal Ahmad and Rajagopal K., 2013). The seeds are a rich source of edible oil containing significant amounts of lauric, myristic, and palmitic acids, making the oil suitable for industrial applications such as soap and candle manufacture, as well as a potential substitute for coconut oil.

The roots contain several biologically active compounds, including elemental gamma-monoclinic sulphur, benzyl glucosinolate, salvadourea (a urea derivative), *m*-anisic acid, and β-sitosterol. Benzyl isothiocyanate, isolated from the roots, has been reported to exhibit potent antiviral and antimicrobial activities (Anthoney Swamy T. and Lasiti T. Timothy, 2015). Owing to its unique phytochemical composition and fibrous structure, *S. persica* (miswak) has been extensively employed as a natural oral hygiene aid. Numerous studies have demonstrated its efficacy in improving periodontal health, preventing dental caries, reducing microbial load, promoting tooth whitening, removing dental calculus, and maintaining overall oral cleanliness (Erlina Sih Mahanani and Samantha Victoria Samuel, 2007).

2. Materials and Methods

2.1 Preparation of explant and sterilization

The explant like leaf, stem node was collected from young healthy plant of *S. persica* L. from different localities of Chh Sambhajinagar 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 ($HgCl_2$). Leaf explant is sterilized with 0.1% whereas stem node 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 cut in to small pieces and inoculated on MS media.

2.2 Culture media

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 2.5 to 3 % of clerigar for solidification and pH was adjusted to 5.6-5.8. The media were steam sterilized in an autoclave under 15 psi and 121°C.

2.3 Culture condition

After the inoculation culture bottles were transfer to culture room under 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.

3. Results and Discussion

Standard protocol for surface sterilization of explant was analyzed by trial-and-error method. Surface sterilization of leaf and stem node explant were 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 0.1% for leaf and 0.2% of $HgCl_2$ for stem node explant during the present study. The hormones free MS medium was found ineffective to induce callus or regeneration of shoot using both explants leaves and stem node. Shoot regeneration was achieved from both explant from BAP and KIN in combination of 0.5 mg/L of IAA. Lower concentration of BAP was found effective to induce shoot regeneration however higher concentration revealed poor result of shoot regeneration. The maximum shoot induction percentage along with shoot length was recorded from 0.5 mg/L of IAA in combination of 1.5 mg/L of BAP and 2.0 mg/L of KIN with 90% regeneration along with 4.68±0.117 and 4.65±0.108 cm of shoot length using leaf explant respectively. Stem node also revealed induction of shoot. Maximum percentage of shoot regeneration was achieved on 2.5 mg/L of BAP and 3.0 mg/L of KIN with 90% of shoot regeneration along with shoot length 5.05±0.075 and 5.15±0.093 cm respectively. Similar kinds of result were reported by (Suman Kumari and Narender Singh.,2012). Standard protocol for shoot regeneration of miswak plant was also developed by Dr. Sujata Mathur in 2013 which revealed that growth hormone BAP incorporated with MS media exhibits rapid multiplication of *S. persica* L. Similar results were reported for *in vitro* shoot multiplication in *Celosia argentea* L. using various explants (Sachin Nirpane and Narayan B. Pandhure 2018).

Table 1 Effect of various concentrations of auxins (IAA) in combination with cytokinin's (BAP and KIN) for multiple shoot formation from leaf as explant

Explant	Concentration of growth regulators mg/l			No. of shoots/ Explant	(%) shoot formation
	IAA	BAP	KIN		
Leaf Explant	0.5	0.5	--	3.87±0.232	70.00
		1.0	--	4.15±0.171	80.00
		1.5	--	4.68±0.117	90.00
		2.0	--	4.23±0.194	85.00
		2.5	--	3.95±0.117	75.00
		3.0	--	3.83±0.118	65.00
		3.5	--	3.69±0.067	60.00
		4.0	--	3.35±0.103	55.00
		4.5	--	2.93±0.136	45.00
		5.0	--	2.63±0.097	30.00
	0.5	--	0.5	3.47±0.147	60.00
		--	1.0	4.07±0.232	75.00
		--	1.5	4.09±0.169	75.00
		--	2.0	4.65±0.108	90.00
		--	2.5	4.43±0.116	80.00
		--	3.0	3.93±0.067	70.66
		--	3.5	3.79±0.116	65.00
		--	4.0	3.39±0.186	55.00
		--	4.5	3.33±0.204	50.00
		--	5.0	2.83±0.087	35.00

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

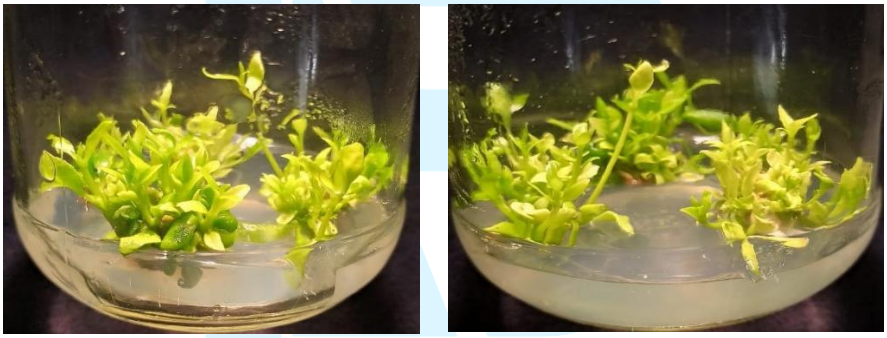

Table 2 Effect of various concentrations of auxins (IAA) in combination with cytokinin's (BAP and KIN) for multiple shoot formation from nodal segment as explant

Explant	Concentration of growth regulators mg/l			No. of shoots/ Explant	(%) shoot formation
	IAA	BAP	KIN		
Nodal segment	0.5	0.5	--	4.09±0.129	70.00
		1.0	--	4.45±0.051	75.00
		1.5	--	4.72±0.087	80.00
		2.0	--	4.87±0.082	85.00
		2.5	--	5.05±0.075	90.00
		3.0	--	4.74±0.059	80.00
		3.5	--	4.53±0.074	75.00
		4.0	--	4.33±0.067	78.00
		4.5	--	4.15±0.051	70.00
		5.0	--	3.95±0.068	60.00
		--	0.5	3.97±0.075	65.00
		--	1.0	4.23±0.038	70.00
		--	1.5	4.53±0.087	75.00
		--	2.0	4.75±0.088	80.00
		--	2.5	4.87±0.093	85.00
		--	3.0	5.15±0.093	90.00
		--	3.5	4.85±0.093	85.00
		--	4.0	4.65±0.110	75.00
		--	4.5	4.33±0.150	70.00
		--	5.0	3.89±0.074	55.00

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

4. Conclusion

Medicinal plants are collected for treatments of many disorders. These plants are exploited at large scale. These plants need to be conserved. If these plants are propagated through modern techniques like tissue culture, then within short time period we can grow large number of plantlets. Present piece of work is useful for developing rapid micropropagation of *S. persica* L. plant.

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