International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

In Vitro Multiplication of Salvadora Persica L.

Pramod Fuke[1], Sangeeta Ahuja[2], Narayan B Pandhure[3]

[1]Research Scholar, Department of Botany, Maulana Azad College, Aurangabad, India
Corresponding author: *fuke.pramod[at]gmail.com*

[2]Professor, Department Botany, Sir Sayyed College, Aurangabad, India

[3]Professor, Department of Botany, Tissue Culture Laboratory, Dr. Babasaheb Ambedkar Marathwada University Aurangabad, India

Abstract: ***The present investigation was aimed to develop protocol for rapid micropropagationofSalvadora persica L.a medicinally and economically important desert tree. The leaf explants were inoculated on MS (Murashige and Skoog, 1962) medium along with different concentration of growth hormones. 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 with2.5 mg/L BAPand 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 ofshowed satisfactory rate of multiplication using leaf explant.***

Keywords: MS, IAA, BAP, KIN, mg/L.

1. Introduction

Medicinal plant *Salvadora persica* L.belongs to family Salvadoraceae.It is commonly known as tooth brush tree which is branched and evergreen small tree.*Salvadora*is distributed mainly in tropical regions of Asia and Africa. The plant contains several biologically active compounds such as alkaloids, flavonoids, steroids, volatile oils, terpenoids, saponinsand carbohydrate. Traditionally, the stem of *S. persica* L. plant is widely used as toothbrush in the Middle East Africa and India to relieve toothache. Leaves are used as mouthwash, purgatives and for treatment of 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).

Medicinal and chemical properties

S. persica L. is known to contain several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates(Sunil Kumar *et al*.,2012).The stems of *S. persica* L. was rich in benzyl isothiocyanate (52.5%), benzyl nitrile (38.3%), carvacrol (3.3%), benzaldehyde (2.5%), aniline (0.7%) and naphthalene (0.6%)(Hilal Ahmad and Rajagopal K.,2013).The seed is rich source in oil and contains lauric, myristic and palmitic acids. Its oil has high potential for making soaps, candles and to be used as a substitute for coconut oil. The root contains elemental gammamonoclinicSulphur, benzyl glucosinolate, salvadourea (a urea derivative), m-anisic acid and sitosterol. Benzyl isothiocyanate which is isolated from the root, exhibits antiviral activity (Anthoney Swamy T and Lasiti T. Timothy.,2015). There are many medicinal uses of miswak in oral hygiene. The unique chemical components, fibers proved the effect to periodontal status, caries, antimicrobial, cleanness, whitening, calculus removal, and so on. (Erlina Sih Mahanani and Samantha Victoria Samuel.,2007).

2. Materials and Methods

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 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 ($HgCl_2$).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.

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.

Culture condition:

After the inoculation culture bottles were transferred to culture room under16 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

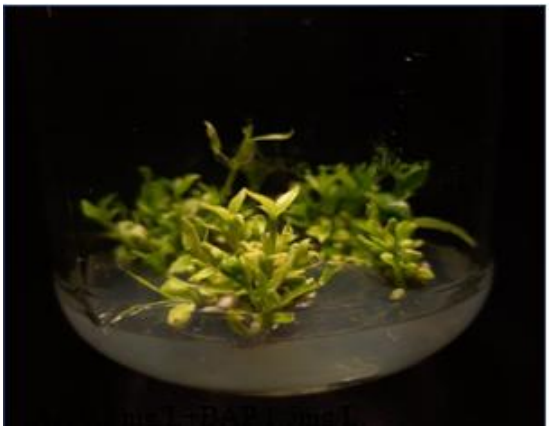
Standard protocol for surface sterilization of explant was analyzed by trial-and-error method. Surface sterilization of leaf and stem node explant were tried with 0.1-0.3% of $HgCl_2$ for 3- 5minutes duration. The maximum microbe's free cultures and high regeneration percentage were recorded at 0.1% for leaf and 0.2% of $HgCl_2$ for stem node explant during the present study. The hormones free MS

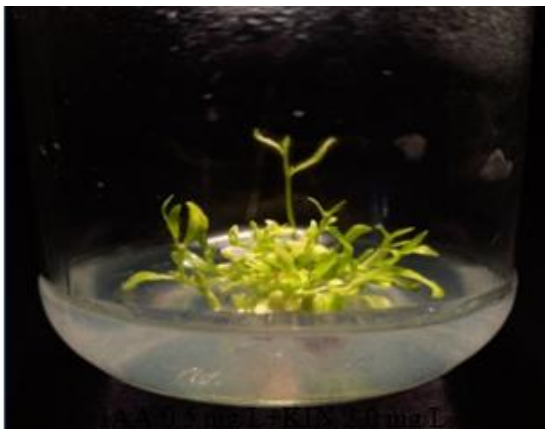
medium was found ineffective to induce callus or regeneration of shoot using both explants leaves and stem node. Shoot regeneration was achieved from both explant from BAP and KIN in combination of 0.5 mg/L of IAA. Lower concentration of BAP was found effective to induce shoot regeneration however higher 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 with90% regeneration along with 4.66±0.116and 4.64±0.107cm 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/Lof BAP and 3.0 mg/L of KIN with 90% of shoot regeneration along with shoot length5.04±0.074 and 5.14±0.092cm 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 2013which revealed that growth hormone BAP incorporated with MS media exhibits rapid multiplication of *S.persica* L.Similar results were reported for *in vitro*shoot multiplicationin *Celosia argentea* L.using various explants (Sachin Nirpane and Narayan B. Pandhure 2018).

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	--	3.86±0.231	70.00
		1.0	--	4.14±0.172	80.00
		1.5	--	**4.66±0.116**	**90.00**
		2.0	--	4.24±0.193	85.00
		2.5	--	3.94±0.116	75.00
		3.0	--	3.84±0.117	65.00
		3.5	--	3.68±0.066	60.00
		4.0	--	3.34±0.102	55.00
		4.5	--	2.92±0.135	45.00
		5.0	--	2.62±0.096	30.00
	0.5	--	0.5	3.46±0.146	60.00
		--	1.0	4.06±0.231	75.00
		--	1.5	4.08±0.162	75.00
		--	**2.0**	**4.64±0.107**	**90.00**
		--	2.5	4.42±0.115	80.00
		--	3.0	3.92±0.066	70.66
		--	3.5	3.78±0.115	65.00
		--	4.0	3.38±0.185	55.00
		--	4.5	3.32±0.203	50.00
		--	5.0	2.82±0.086	35.00

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



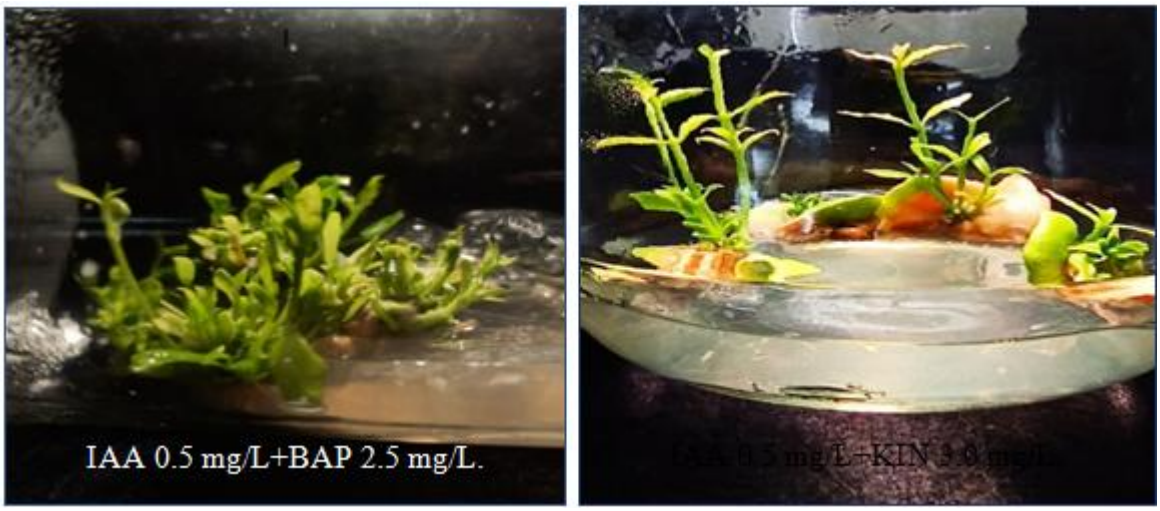


Effect of various concentrations of auxins (IAA) in combination with cytokinin's (BAP and KIN) for multiple shoot formation from nodal segment as explants

Explant	Concentration of growth regulators mg/l			No. of shoots/ Explant	(%) shoot formation
	IAA	BAP	KIN		
Nodal segment	0.5	0.5	--	4.08±0.128	70.00
		1.0	--	4.44±0.050	75.00
		1.5	--	4.72±0.086	80.00
		2.0	--	4.86±0.081	85.00
		2.5	--	**5.04±0.074**	**90.00**
		3.0	--	4.74±0.058	80.00
		3.5	--	4.52±0.073	75.00
		4.0	--	4.32±0.066	78.00
		4.5	--	4.14±0.050	70.00
		5.0	--	3.94±0.067	60.00
		--	0.5	3.96±0.074	65.00
		--	1.0	4.22±0.037	70.00
		--	1.5	4.52±0.086	75.00
		--	2.0	4.74±0.087	80.00
		--	2.5	4.86±0.092	85.00
		--	**3.0**	**5.14±0.092**	**90.00**
		--	3.5	4.84±0.092	85.00
		--	4.0	4.64±0.109	75.00
		--	4.5	4.32±0.149	70.00
		--	5.0	3.88±0.073	55.00

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

Photo plate:



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 tissueculture, then within short time period we can grow large number of plantlets. Present piece of work is useful for developing rapid micropropagationof *S. persica* L. plant.

5. Acknowledgments

Authors are thankful to the Principal, Maulana Azad College, Aurangabad affiliated to Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing to all necessary facilities to carry out this research work.

References

[1] **Suman Kumari and Narender Singh (2012).***In vitro* plantlet regeneration from cotyledonary node explants of *Salvadora persica* L. a medicinally important desert tree. *Journal of Agricultural Technology*.8(5),1839-1854.

[2] **Hilal Ahmad and K. Rajagopal (2014).***Salvadora persica* L. (Miswak) in dental hygiene. *The Saudi Journal for Dental Research* 5, 130–134.

[3] **Suman Kumari and Narender Singh (2012).***In vitro* plantlet regeneration from cotyledonary node explants of *Salvadora persica* L. a medicinally important desert tree *Journal of Agricultural Technology* 8(5) 1839-1854.

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

[5] **Hilal Ahmad and Rajagopal K (2013).**Biological Activities of *Salvadora persica* L. (Miswak) Medicinal & Aromatic Plants 2 (4) 1-5.

[6] **Anthoney 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.

[7] **Erlina Sih Mahanani and Samantha Victoria Samuel (2007).** MISWAK (Salvadora persica) as a Cleansing Teeth Mutiara Medika 7 (1) 38-42.

[8] **Mayank Tripathi (2016).***Salvadora persica*L. (miswak):an endangered multipurpose tree of India *Indian journal of plant science*5(3) 24-29.

[9] **Sachin Nirpane and Narayan Pandhure (2018).***In Vitro* Multiplication in Celosia Argentea L. Var. Argentea *International Journal of Science and Research*7(1)728-730.