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MICROPROPAGATION OF MEDICINALLY IMPORTANT CLIMBER: *TYLOPHORA INDICA* Merrill. FOR FUTURE DEMAND

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ABSTRACT:

Tylophora indica Merrill. (Damvel) is an important medicinal climber of India. It is also available on many hedges of North Gujarat zone. Plant has high demand for its medicinal values .It help in treatment of many diseases like **cough, asthma, bronchitis, dysentery, diarrhea, wounds, ulcer, hemorrhoids, malignant tumor, and leukemia etc. Beside this all most all the parts of plant are important** so it is up rooted and exploited highly for obtaining medicine. This destructive harvesting of the plant and many other threats like deforestation etc. limited the plant population in present days so, extra care for its multiplication is needed along with its sustainable use. Present work of micropropagation by using basal MS media supplemented with different dosages of hormones were standardize for developing an efficient micropropagation protocol for this important climber which will fulfil future demand.

KEY WORDS: *Micropropagation, Climber, Tylophora indica, Future demand.*

INTRODUCTION:

Tylophora indica Merrill (*T. Asthmatica*) belongs to family Asclepiadaceae with common name damvel is a very important climber .The plant is a small, slender, much branched, under shrub, or twining, pubescent herb, perennial twining climber with long and fleshy roots. Stems are elongate and glabrous but not much branched. The roots have a sweetish taste turning acrid, aromatic odor and a brittle fracture. It has many medicinal values and it is distributed throughout India and mainly found in the plains, forests, hilly slopes and outskirts of the forest. It is also popular and most exploited climber of the Gujarat.

Medicinal value of plant

The most useful parts are leaves and roots. The leaves and roots have emetic, cathartic, laxative, expectorant, diaphoretic and purgative properties. It has also been used for the treatment of allergies, cold, dysentery, hay fever and arthritis. It has reputation as an alternative and as a blood

purifier, often used in rheumatism and syphilitic rheumatism. Root or leaf powder is used in diarrhea, dysentery and intermittent fever. Dried leaves are emetic diaphoretic and expectorant. The roots are suggested to be a good natural preservative of food. It is traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis. It also seems to be a good remedy and pharmaceutically used common product is prepared from its roots, leaves, stem contain several alkaloids including tylophorine ($C_{24}H_{27}O_4N$), tylophorinine ($C_{24}H_{25}O_4$) and anticancerous tylophorinidine ($C_{22}H_{22}O_4N$)[5,6,7]. so, it is exploited largely in traditional medicine as anti-psoriasis, seborrheic, anaphylactic, leucopenia and as an inhibitor of the Schultz-Dale reaction. (Prajapati *et. al*., 2003).

Need for the Study

This plant has been traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma, bronchitis, rheumatism, and dermatitis. The roots of this plant are the main source for medicine so the plant is uprooted largely and exploited on large scale for that purpose of pharmaceutical value. So, it is rapidly disappearing from several zones. Looking at future demand of this plant there is an urgent need to develop a suitable and reliable method of its multiplication. The *in vitro* propagation techniques for many other medicinal plants were periodically developed by using various Plant Growth Regulators (PGRs) and are found to be useful (Afolayan *et. al*., 2004; Rout *et.al*., 1999 and Bhavisha *et.al.,* 2003). So, present work has been conducted to develop an efficient and reproducible method for rapid multiplication of *Tylophora* through micropropagation techniques.

MATERIALS AND METHODS:

Shoot apex, Nodal zone with axillary buds and disease free leaf were selected as explants for callus development. Explants were washed with biological detergent tween 20 which was followed by sterilization with sodium hypochloride for 2 minutes followed by 0.5% mercuric chloride for 3-4 minutes then repeated wash with water to remove traces of sterilants. Murashige and Skoog's medium (1962) supplemented with hormones 2,4-D, BAP and kinetin at different concentrations were used separately and in combination for callus induction in photoperiodism of 8hrs of light and 16hrs of darkness. After eight weeks the *in vitro* developed calli were transferred to MS medium supplemented with BAP, IBA, IAA and kinetin separately and in different combinations for shoot induction. Later the shoot developed calli were transferred for root development of half strength MS salts along with various auxins like IBA and NAA separately and in combinations. Data on formation of callus and shooting and rooting were recorded periodically along with photographs for morphogenesis studies on *Tylophora*.

Fig. 1: Callus induction

Fig. 2: Shoot initiation

Table- 1: Callus induction of *Tylophora* through shoot apex, leaf and node explants

Note: + = slow growth, ++ = moderate growth, +++ = rapid growth

Type of Explants	Types of Hormone	Conc^{n} of Hormone	Total no. of Explants	Explantswith Callus Form.	Callus development in Week				Frequency growth response (in %)	Mean
					2nd	4th	6th	8th		
Shoot Apex	2,4-D	2mg/l	5	-	-	-	-	-	0	0
	2,4-D+ Kinetin	1:1 mg/l	5	4	-	+	++	+++	80	0.80± 0.447
	2,4-D+BAP	1:1 mg/l	5	3	-	-	+	++	60	0.75± 0.5
	BAP	1mg/l	5	2	-	+	++	+++	40	0.6±0 .548
Leaf	2,4-D	2mg/l	5	2	-	+	+	++	40	0.6±0 .548
	2,4-D+ Kinetin	1:1 mg/l	5	-	-	-	-	-	0	0
	2,4-D+BAP	1:1 mg/l	5	4	+	+	++	+++	80	0.80± 0.447
	BAP		5	-	-	-	-	-	0	0
Node	2,4-D	2mg/l	5	2	+	+	++	++	40	0.6±0 .548
	2,4-D+ Kinetin	1:1 mg/l	5	-	-	-	-	-	0	0
	2,4-D+BAP	1:1 mg/l	5	3	-	+	++	+++	60	0.75± 0.5
	BAP	1mg/l	5	-	-	-	-	-	0	0

Table 2: Influence of various hormones in MS salts on shoot development

Hormone for Shooting	Conc. Mg/ml	Shoot development	% Frequency of growth response	Mean
Kinetin + IAA	1mg +0.2mg	-	0	0
	2mg + 0.2mg	+	25	0.25±0.5
BAP +IAA	1mg + 0.2mg	+	25	0.25±0.5
	2mg+ 0.2mg	+++	75	0.75±0.5
Kinetin + IBA	2mg + 0.5mg	-	0	0
	2.5mg + 0.5mg	-	0	0
	3mg +0.5mg	++	50	0.5±0.577
BAP + IBA	2mg + 0.5mg	-	0	0
	2.5mg +0.5mg	++	75	0.75±0.5
	3mg + 0.5mg	+++	100	1.00±0.00

Note: + = slow growth, ++ = moderate growth, +++ = rapid growth

Table 3: Influence of MS salts along with various hormones for root development.

Plant growth regulators (mg/l)		Days taken for the Emergence of roots	Root development	% Frequency of rooting	Mean
IBA	NAA				
0.5		15-16	+	25	0.25±0.5
1.0		12	+++	100	1.00±0.00
1.5		9-12	++	75	0.75±0.5
	0.5	14-16	+++	75	0.75±0.5
	1.0	13	++	50	0.5±0.577
	1.5	12	++	50	0.5±0.577
0.5	0.5	13	+++	100	1.00±0.00
1.0	1.0	11	++	50	0.5±0.577

Note: + = slow growth, ++ = moderate growth, +++ = rapid growth

RESULTS AND DISCUSSION:

In node and leaf both explants callus induction was reported after 2nd week but it was little later in shoot apex explants. Within four weeks, the entire explants turned in to mass of soft, green and friable callus. Callus development was noted in all the explants in most of the hormones in most

of the concentrations. Best result for callus induction were obtained in 2,4-D+ kin for shoot apex and 2,4-D+BAP for node and leaf. After callus development the callus were further transferred for shoot development. Best result for shoot development was obtained in 3mg/l BAP+ 0.5 mg/l IBA. 1mg Kin+0.2mgIAA, 2mg Kin+0.5mg IBA, 2.5 mg Kin+0.5mg IBA and 2mg BAP+0.5mgIBA showed no results for the shooting. The shoot regenerated callus after transfer in to rooting media it showed best results in 1mg/l IBA and in 0.5mg/l+ 0.5mg/l IBA+NAA in combination similar results were reported by Sayeed *et.al.,* 2005 in *Gloriosa* and in other medicinal plants Wala and Jasrai,2003. *Tylophora* is very sensitive to higher doses of hormones so its organogenesis and *in vitro* development is possible in particular combination of hormones at right stage of development.

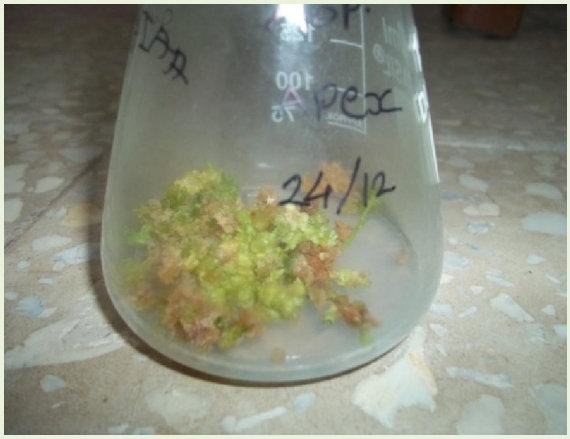

Fig. 3 : Multiple Shoot initiation

Fig. 4 : Shoot formation

Fig. 5 : Root development

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