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"MICROPROPAGATIONSTUDIES INMELIA AZADERACH L."

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Abstract:

Melia azederach is a fast-growing, deciduous tree that can reach a height of around 45 meters in closed, moist forests, though it is much smaller in the cooler and drier regions in which it is sometimes grown and where is much more likely to be just 10 - 15 metres tall. The straight, cylindrical bole can be free of branches for up to 20 metres, with no buttresses. The tree is generally deciduous, but some forms in the humid tropics are evergreen. A very important tree for local people, providing a wide range of medicinal applications; an insecticide and repellent; a useful timber and many other commodities. It is often cultivated in many parts of the tropics for these many useful qualities. It is planted for re-reforestation in its native areas, where it is a fast growing though short-lived species. It is often planted as a street tree and ornamental, though it has the disadvantage of dropping a lot of leaf-litterDuringthe present investigation efforts have been made to evaluate standard protocol for induction of callus in Melia azederach. Explants were taken from elite plant and aseptically inoculated on MS medium supplemented with various concentrations of 2, 4-D alone and in combination with KIN/BAP 2,4-D. Best results were obtained on 0.5 and 1.0 mg/L of 2,4-D with stem, node as explant for induction of callus and its maintenances.

Keywords: -In vitro,Melia azederach, callus, regeneration.

Introduction:

The plant is a fast-growing, deciduous tree that can reach a height of around 45 meters in closed, moist forests, though it is much smaller in the cooler and drier regions in which it is sometimes grown and where is much more likely to be just 10 - 15 metres tall. An aqueous extract reduces the intensity of asthmatic attacks. The leaf juice is anthelmintic, antilithic, diuretic and emmenagogue. A decoction is astringent and stomachic. A decoction is used to treat diarrhea.The leaves are used externally to treat skin conditions such as scabies and itch. A decoction is used as a gargle to treat tooth problems and strengthen the gums. The leaves are harvested during the growing season and can be used fresh or dried. The flowers and leaves are applied as a poultice in the treatment of neuralgia and nervous headache. The stem bark is anthelmintic, astringent and bitter tonic. It is used as a tonic in India. It can be harvested at any time of the year and is used fresh or dried.

The root bark is emetic, emmenagogue, purgative and vermifuge. It is highly effective against ringworm and other parasitic skin diseases. It can be harvested at any time of the year and is used fresh or dried. The fruit is antiseptic and febrifuge. The pulp is used as a vermifuge. The fruit is harvested in the autumn when it is fully ripe and can be used fresh or dried. The seed is ant rheumatic: It is used externally. A gum that exudes from the tree is considered by some to have aphrodisiac properties. This plant should be used with caution, preferably under the supervision of a qualified practitioner. Excess causes diarrhea, vomiting and symptoms of narcotic poisoning.

Agro forestry Uses:The tree is planted as a pioneer species in northern Thailand in reforestation projects to restore native woodland - it is planted in degraded woodland and open areas in a mix with various other species that all have the ability to grow fast; produce dense, weed-suppressing crowns; and attract seed-dispersing wildlife, particularly birds and bats.It is grown as a shade tree in coffee and abaca (Musa textilis) plantations (Gnana et al., 2013).

Materials and Methods:

Surface sterilization of explant:

Explants viz. leaves and stem node were collected from different localities of University campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. All explants were washed with tap water twice in laboratory, followed by 70% ethanol for 30seconds and then surface sterilized of with HgCl2. 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 explant 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 stem node and leaf explants of Melia azederach. Stem node and leaf were inoculated on MS medium supplemented with 2,4-D, BAP and IAA for callus induction.MS medium fortified with 3% sucrose and gelled with 3 gm/L clarigar 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 $HgCl_2$ was 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 explant. Surface sterilized explants were inoculated on MS medium supplemented with various concentrations of 2, 4-D alone and in combination of KIN and BAP. All combination of growth regulators were found more or less potent for induction of callus.

It could be revealed that, higher concentration of 2, 4-D has potential to induce profused callus. Callus induced with these concentrations was whitish to greenish colour with friable nature. Maximum rate of callus induction were recorded on 2.0 mg/L of 2, 4-D using leaf as explant. However it was found maximum at 2.0 mg/L and 4.0 mg/L of 2, 4-D using stem node as explant with 93.33% of callus induction frequency.

Leaf, stem and node explants also shown formation of callus on MS medium supplemented with 2, 4-D in combination of BAP. Moderate callus was induced with whitish yellow colour which was friable in nature. Highest frequency of callus induction was recorded on 1.0 mg/L of BAP in combination with 2.0 mg/L of 2, 4-D with 46.66 and 53.33 % of callus induction frequency by using leaf and stem node as an explant respectively. Callus induction was also tried with 2, 4-D in combination of KIN but poor, yellowish and friable callus was developed. Maximum induction of callus was revealed by 4.0mg/L of 2, 4-D along with 2.0 mg/L of KIN with 33.33 and 40.00 percent of callus induction frequency. However lower concentration of growth regulators were found less effective for induction of callus or poor type of callus induction was achieved. These induced calluses were subcultured on MS medium with

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different concentrations of hormones to achieved miropropagation.

Table 1: Effects of different concentration of PGR's on Callus Induction of Melia azederach L.

Growth regulators (mg/L)			Leaf explant		Stem node explant	
BAP	2, 4-D	KIN	Colour of callus	frequency of callus induction (%)	Colour of callus	Callus induction frequency (%)
--	0.5	--	Yellowish	26.66	--	--
--	1.0	--	Whitish	33.33	Whitish	33.33
--	1.5	--	Whitish	33.33	Whitish	60.00
--	2.0	--	Greenish	73.33	Greenish	93.33
--	2.5	--	Whitish	53.33	Greenish	80.00
--	3.0	--	Greenish	66.66	Greenish	86.66
--	3.5	--	Whitish	60.00	Greenish	86.66
--	4.0	--	Whitish	53.33	Greenish	93.33
0.5	1.0	--	Yellowish	20.00	Yellowish	26.66
1.0	2.0	--	Whitish	46.66	Whitish	53.33
1.5	3.0	--	Whitish	40.00	Whitish	46.66
2.0	4.0	--	Whitish	33.33	Whitish	33.33
--	1.0	0.5	Yellowish	20.00	Yellowish	20.00
--	2.0	1.0	Yellowish	26.66	Yellowish	20.00
--	3.0	1.5	Yellowish	20.00	Yellowish	26.66
--	4.0	2.0	Whitish	33.33	Whitish	40.00

Percentage response on three separate experiments, each based on a minimum of five replicates.

Plate.1. Photo plate for callus induction and micropropagation in Melia azederach





Conclusion

Medicinal plants are voraciously collected for treatments of many disorders. Theses 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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