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In the present investigation "in vitro regeneration of *Aloizia chinensis* (Osbeck) Merr." two methods of tissue culture technique were tried. (a) Direct regeneration of multiple shoots from axillary buds through micropropagation and (b) indirect regeneration of plantlets through callus culture.

6.(a). Direct Regeneration Of Multiple Shoots From Axillary Buds Through Micropropagation:

Shoot tips and nodes bearing axillary branches were collected from the selected plants (from the section No. 7(a) and 8(b) of Mati Hill Division of Nanoroni Tea Estate of Tezpur in Central Assam) from first flush period (indicating actively growing season). Such axillary buds were initiated into aseptic cultures for micropropagation.

Thus micropropagation through in-vitro culture of *A. chinensis* based on selection of suitable explants, screening and finding the appropriate media for initial establishment of the explants, shoot proliferation and rooting including transfer of the rooted plantlets onto the soil and their acclimatisation were finally achieved. The striking points during the culture were briefly discussed below.
Basal medium (BM) employed during the cultures were the Murashige and Skoog's basal medium (MSBM) and Vandersmissen et al.'s basal medium (VBM) and White's basal medium (WBM). The pH of the medium was adjusted at 5.7 and the medium was solidified with 0.8% agar. MSBM was found to be better than B5BM for multiple shoot formation, while WBM was best for rooting.

Browning of the media or tissue was circumvented by repeated transfer (2 to 5 times) of the explants onto the fresh medium, while contamination was reduced up to 35% by treating the explants with 0.05% HgCl₂ solution for 15 minutes.

*in-vitro* multiplication of shoot tips or nodal explants were studied with relation to various concentrations (0, 1, 2, 3, 4 and 5 mg/l) of the two cytokinins B5 or Kn, with or without addition of 0.25 and 0.50 mg/l of B5. B5 was found to be most effective stimulator of axillary shoot growth and proliferation.

To find out the optimal level of B5 concentration in MSBM and B5BM for proliferation of nodal explants, six different concentrations (0, 1, 2, 3, 4 and 5 mg/l) of B5 were investigated. It was found that only unfolding of leaf was observed in the MSBM alone however, (a) increasing the B5 concentration up to 2 mg/l resulted in increased shoot bud multiplication rate and (b) increasing the B5 concentration beyond 2 mg/l, resulted in callus formation at the basal end and stunted growth of the shoots. At 2 mg/l of B5,
successive transfer of culture onto fresh medium (also containing 2mg/l of BA) produced 8-12 axillary shoots from each explant in 3 months period. Thus, large scale multiplication became possible by this procedure.

The studies on the effect of GA₃ supplement at two concentrations of 0.25 mg/l and 0.50 mg/l in MSBM, only 0.25 mg/l showed good result in presence of 1 mg/l of IAA producing 10-12 shoot buds, but with 2 mg/l of BA highest number of 6-9 shoot buds formation was possible. Higher concentration of GA₃ (0.5 mg/l) however, showed no effect on multiple shoot production. Presence of 0.25 mg/l of GA₃ increased the growth of the newly produced shoots by 0.5 to 1.0 cm bigger in size compared with individual cytokinin. GA₃ has no inhibitory effect on callus formation of the explants.

Repeated subculture (after 5 to 7 subcultures) in MSBM containing 2 mg/l of BA resulted in stunted shoot growth. However, transfer of these isolated buds onto MSBM containing low level of BA (1 mg/l) resulted in elongation of shoot buds which facilitated the initiation and further growth of roots when transferred to a rooting medium.

For induction of roots, isolated shoots with 2-3 unfolded leaves were cultured in different concentrations of IBA in 1/2 strength of MSBM and full strength of WBM. Good results were obtained in WBM supplemented with 1 mg/l of IBA or 1 mg/l of IBA in combination with 0.1% activated charcoal in 1/2 strength of MSBM. After rooting and accli-
matization the plantlets were successfully transferred to the soil and established in the field condition.

The plantlets obtained from the investigation were relatively uniform and a complete protocol has been established in all respects to shoot multiplication (response to cytokinins), rooting, acclimatization and finally establishment to the soil.

6.(b). Indirect Regeneration Of Plantlets Through Callus Culture:

For obtaining callus from different parts of the seedlings, the excised explants from leaf, root, hypocotyl and cotyledon were taken from 10 - 12 days old in-vitro grown healthy seedlings. Such explants were cultured with four different auxins individually in MSBM and in combination with three (1,2 & 4 mg/l) concentrations of the two cytokinins (BA and Kn) in MSBM or RgBM.

In the indirect regeneration of plantlets or organogenesis from callus culture of A. chinensis, suitable explants of the seedlings, screening and finding out the appropriate medium for callus induction and proliferation from different organs of seedlings, regeneration of shoots from callus and rooting were determined including transfer of the rooted plantlets to the soil and successful acclimatization of the plantlets were thus finally achieved. The important points of the investigations were summarised below.
Basal Medium (BM) employed during the studies were the Murashige and Skoog basal medium (MSBM) and Bamboor et al. 's Basal Medium (B-BM). The pH of the medium was adjusted at 5.8 and the medium was solidified with 0.6% agar. MSBM was found to be better for callus induction, proliferation and regeneration of shoots from the callus and B-BM showed best result in differentiation of shoot buds from cotyledon callus only.

6.(b).1.1) Callus induction on root explant:

Individually no other auxins except 2,4-D at 1 mg/l and above produced callus on root explants. Callus were off white and friable. IBA at 1 mg/l or above produced secondary and tertiary roots on the root callus.

2,4-D at 2 and 4 mg/l in combination with 1 mg/l of IBA or Kn in MSBM produced callus on the root callus. Callus growth was observed upto 30 - 40 days and then it turned brown and died.

6.(b).1.11) Callus induction on leaf explant:

Excised leaf were cultured in MSBM supplied with four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of four different auxins (IAA, IBA, NAA and 2,4-D). Good callus was developed in the treatments of 1 mg/l and above of 2,4-D (100%), 1.5 and 2.0 mg/l of NAA (75%); 1.5 and 2.0 mg/l of IAA (50%) and 2.0 mg/l of IBA (40 - 50%) with 1 - 2 white roots.
IAA at 4 mg/l in combination with 1 mg/l of BA, callus was developed in 40 - 50% of the leaf explants and with 1 mg/l of KN callus was noticed on 45% of the explants in MSBH. Calli were soft, light green in colour and proliferation of callus was observed upto 50 - 60 days. Then the growth slowly reduced, calli turned brown and died.

IBA at 2 mg/l in combination with 1 mg/l of BA or KN in MSBM, 30 - 35% of the leaf explants produced callus. In 4 mg/l of IBA, callus production did not increase but produced 1 - 2 roots from the callus.

NAA at 2 mg/l with 1 mg/l of BA or KN produced callus on 20 - 30% of the explants in MSBM or BSBN.

2,4-D gave best result in the production of callus on leaf calli, in both the concentrations i.e 2 mg/l and 4 mg/l with 1 mg/l of BA or KN produced callus on 100% of the explants.

Comparatively, except the treatments of 2, 4-D, the lower concentration of cytokinin produced good callus. Increased cytokinin concentration decreased the callus production on leaf explants.

6. (b). 1.11) Callus Induction On Hypocotyl Expn: For obtaining callus from the hypocotyl explants excised hypocotyl were cultured in the MSBM with four different concentrations (0.5, 1.0, 1.5 & 2.0 mg/l) combining with four different auxins (IAA, IBA, NAA and 2,4-D) individually or two concentrations (2.0 and 4.0 mg/l) of
the above four auxins in combination with three concentra-
tions (1, 2 & 4 mg/l) of the two cytokinins (IAA and KN).

IAA at its individual treatments in 0.5 mg/l or
above produced callus on 100% of the explants, and on 0.5
mg/l it produced 1 - 2 shoot buds from the upper side of
the cut ends.

NAA at its 0.5 mg/l produced 1 - 2 shoot buds
from the hypocotyl callus and at 1.0 mg/l or above concentra-
tion produced callus on 100% of the explants.

IBA at 1.0 mg/l produced callus on 75% of the
explants and few roots from the distant cut ends of the
hypocotyl explants.

2, 4-D in its every concentration produced callus
on 100% of the explants.

In combination with auxin and cytokinin treat-
ments, hypocotyl explant produced best callus (100% of the
explants) in most of the treatments. In the treatments with
IAA and NAA (at 2 mg/l) with 2 and 4 mg/l of BA, the hypoco-
tyl explants produced single shoot at the upper cut end, but
with the treatments of IBA with 1 mg/l of BA or KN, it
produced 2-3 roots from the distant end. 2, 4-D pro-
duced soft, irritable and white callus but IAA and NAA pro-
duced green and compact callus.

Initially, the calli swelled, sometimes cracked
and then the callus development started from the cut ends
and proliferated to the body of the explants.
8.(d).1.1v).Induction of Callus On Cotyledon Explant:

To obtain callus from cotyledon, the excised cotyledons were made into 2-3 pieces and all the pieces were cultured in the same culture vessels containing MSBM or B5BM supplemented with four concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of four auxins (IAA, IBA, NAA and 2,4-D) or two concentrations (2.0 and 4.0 mg/l) of the above four auxins in combination with three (1, 2 and 4 mg/l) concentrations of the two cytokinins (BA and KN).

Except 2, 4-D and NAA the other two auxins did not give positive result on callus induction on cotyledon explants. At 4 mg/l of 2, 4-D with 1 mg/l of BA and KN, 50% of the explants produced callus in MSBM or B5BM respectively. NAA at 4 mg/l in combination with 1 mg/l of BA or KN produced callus on 80% of the cotyledon explants with 1-10 white roots in MSBM. IBA at 4 mg/l with 1 mg/l of either BA or KN 60% and 50% of the explants produced callus in MSBM. At high (2 and 4 mg/l) concentration of cytokinin with 2 mg/l of IAA or NAA only 10% of the explants produced callus with 1-2 shoot buds.

Before callusing the cotyledon explants swelled first and callus development started from the cut ends and extended towards the body of the explants.
6.(b).2. Regeneration Of Shoot From The Callus:

For differentiation of shoot buds the calli were cultured in the shoot bud regenerating medium containing 0.5 and 1 mg/l of the four auxins (IAA, 1BA, NAA and 2, 4 - D) with three (1, 2 and 4 mg/l) concentrations of two (BA and Kn) cytokinins.

6.(b).2.i. Regeneration Of Shoot From Root Callus:

Root calli have failed to differentiate shoot buds in any one of the treatments. After 20 days of culture root calli showed no development but it gradually turned brown and died.

6.(b).2.ii. Regeneration Of Shoot From Leaf Callus:

The treatments containing 2,4-D failed to differentiate shoot buds from leaf callus in any treatments. IBA at 1 mg/l produced only roots with 1 mg/l of BA or Kn but not shoot buds. IAA at 0.5 mg/l with 4 mg/l of BA produced 1 - 2 shoot buds in 30% of the leaf calli in HSBN and 20 - 25% of the calli in B5BN. 1 mg/l of IAA in combination with BA or Kn in any concentration failed to differentiate shoot buds. NAA at 0.5 mg/l with 2 mg/l of BH
produced 1 - 2 shoot buds in 20 - 25% of the calli in MSBM and 10 - 20% of the calli in B5BM. With 4 mg/l of BA, NAA at 0.5 mg/l produced 1 - 2 shoot buds in 30 - 35% of the calli in MSBM and 20 - 25% of the calli in B5BM. At 1 mg/l of NAA with any concentration of BA or Kn, calli failed to regenerate shoot buds in both the medium. All the four auxins failed to regenerate shoot buds in combination with Kn in MSBM or B5BM.

6.(b).2.iii).Regeneration Of Shoot From Hypocotyl Callus:

IAA and NAA at 0.5 mg/l with 4 mg/l of BA produced the best result for differentiation of shoot bud from hypocotyl callus. Both auxins produced 10 - 12 shoot buds in 30 and 75% of the calli in MSBM. IBA also produced shoot buds but to a lesser extent, 2,4-D at 1 mg/l failed to regenerate shoot buds in combination with BA or Kn in any one of the medium. IAA at 0.5 mg/l with 1 and 2 mg/l of BA produced 5 - 6 shoot buds in 70 - 72% of the calli and 5 - 7 shoot buds in 75% of the calli in MSBM and 3 - 4 shoot buds in 40 - 42%, 3 - 5 shoot buds in 48 - 50% calli respectively in B5BM. NAA at 0.5 mg/l with 1 and 2 mg/l of BA produced 3 shoot buds in 40%, 4 - 5 shoot buds in 62 - 65% of the calli in MSBM and 2 - 3 shoot buds in 35 - 40% and 3 - 4 shoot buds in 50% of the calli in B5BM respectively. IBA at 0.5 mg/l with 1 mg/l of BA produced 3 - 4 roots in MSBM or B5BN. While with 2 and 4 mg/l of BA 1 - 2 shoot buds were produced in 20 - 25% and 30 - 32% of the calli respectively. 2, 4-D showed poorest result. At
1 mg/l it showed no differentiation at all and at 0.5 mg/l it produced single shoot bud in 5 - 10% of the calli. with 4 mg/l of BA in MSBM.

6.(b).2.iv). Regeneration Of Shoot From Cotyledon Callus:

2, 4-D at 1mg/l failed to differentiate shoot buds from cotyledon callus with three concentrations (1, 2 and 4 mg/l) of BA or Kn in MSBM or B5BM. But at 0.5 mg/l produced single shoot bud in 10 - 20% of the calli. with 1, 2 and 4 mg/l of BA only in MSBM. NAA at 0.5 mg/l with 1, 2 and 4 mg/l of BA differentiated 1 - 3 shoot buds in 25%, 30% and 40 - 45% of the calli respectively in MSBM and 25%, 30% and 50% in B5BM respectively. At 1 mg/l of NAA with 1, 2 and 4 mg/l of BA produced 1 - 2 shoot buds in 12%, 20% and 25% of the calli in B5BM respectively. IBA at 0.5 mg/l with 1, 2 and 4 mg/l of BA produced 1 - 2 shoot buds in 25%, 27% and 40% of the calli. in MSBM and with 2 and 4 mg/l of BA in B5BM produced 1 - 2 shoot buds in 12 and 25% of the calli respectively. IAA comparatively gave best result in shoot bud regeneration from cotyledon callus. At 0.5 mg/l of IAA with 2 mg/l of BA in B5BM produced 10 - 12 shoot buds in 50 - 60% of the calli. and in MSBM it produced 5 - 7 shoot buds in 60% of the calli. At 1mg/l of IAA in combination with three concentrations of BA produced lesser number of shoot buds.
All the auxins, except 0.5 mg/l of IAA in combination with Kn failed to differentiate shoot buds from cotyledon callus in both the medium.

The shoot differentiated from the callus of different plant parts were successfully rooted (rooting is similar to micropropagation method), acclimatized and transferred onto the soil. The investigation proved that the technique of regeneration of plantlets from callus culture would be possible for obtaining large number of saplings from plant parts of different segmental origin, which may offer further scope for identification of somaclonal variations and related biochemical studies.