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Micropropagation through leaf cultures of *Agave vera-cruz* Mill.

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SUMMARY

A protocol for micropropagation of *Agave vera-cruz Mill.* through leaf culture has been developed. In vitro grown immature leaf explants were cultured on Murashige and Skoog medium with L_2 vitamins (MMS) containing 3% Sucrose and 0.8% Agar. Callus was initiated on MMS + 2, 4-D (4.52 mM) + BAP (8.8 mM). Highest rate of regeneration was observed when this callus was sub-cultured on MMS + 1AA (17.10mM) + BAP (17.6mM). Shoots of about 5-6 cm were excised along with basal callus from the mother callus and sub-cultured on MMS + IBA (2.52mM) + BAP (8.8mM) + GA_3 (2.5mM) for further proliferation and elongation of shoots. Roots were developed at the base of these shoots after two weeks of culture on the same medium. Regeneration of the plantlets through organogenesis was confirmed by histological studies. Four months old plantlets were transferred to pots containing sand, soil and manure in the ratio of 1:1:1 without any intervening hardening phase. 70 to 80 per cent of survival was recorded.

Key words : *In vitro* leaf, Organogenesis, Micropropagation, *Agave vera-cruz.*

Agave vera-cruz Mill. is long life cycle plant widely distributed in the arid and semi arid places of Karnataka and Andhra Pradesh. It is considered as a true 'Kalpataru' which offers hope of an additional source of income to the farmers, commonly called Grey Aloe of India. Agaves are the source of cordage fibers, sugars and cellulose apart being ornamental (Madrigal-Lugo *et al*., 1990). Agaves are also potential source of several sapogenins, the juice of leaves contain appreciable amount of sapogenins like diosgenin, hecogenin, tigoenin and ginatogenin (Gbolade *et al*., 1985). These are the precursors for the commercial production of steroidal formulations (Blunden *et al*., 1980). Contrary to other *Agave* species, *Agave vera-cruz Mill.* exhibits low vegetative propagation level, hence tissue culture technique might constitute an appealing choice for reintroduction to the natural environment. Successful regeneration and propagation was reported from stem expants of *Agave fourcroydes* (Groenewald *et al*., 1977), bulfil explants *of Agave arizonica (*Powers and Backhaus, 1989), and shoot tip explants of *Agave vera-cruz* (Tejavathi and Gayathramma, 2005). Previous attempts to regenerate plants from in vitro leaf cultures of *Agave sisalana* (Hazra *et al*., 2002) was successful. To the best of our knowledge, this is the first report of successful plant regeneration through organogenesis from immature *in vitro* leaf culture of *Agave vera-cruz Mill.*

MATERIALS AND METHODS

Selection of the explant was based on the previous reports in other systems that embryogenic ability of in vitro plantlet organs is faster than that of intact plants (Shoyama *et al*., 1995). The 4th leaf of 5 month old *in vitro* maintained shoots which were raised on MMS + NAA (5.37mM) + Zea (0.91mM) was selected as explant. They were excised under aseptic conditions and made into segments of 1 sq.cm each. They were dipped in streptomycin for 3 min. and washed with sterile distilled water to remove the traces of antibiotic. Since the morphogenetic responses of the explants to Murashige & Skoog's medium containing L_2 vitamins (Phillip and Collins, 1979) were favourable in terms of time taken for initiation and proliferation, it was decided to use this combination for further experiments and referred to as MMS hence-forth. MMS medium was supplemented with various growth regulators at different concentrations and pH was adjusted to 5.6 prior to autoclaving at 108 kpa. The medium was gelled with bacteriological agar (0.8%) and sucrose was used as carbon sucrose (3%). The cultures were incubated at 25 ± 2^0C under 16 : 8 light and dark regimes.

Histology:

Organogenic calli segments were fixed in FAA (formaldehyde : acetic acid : alcohol) for 48 h and then transferred to 70% alcohol. Ethyl alcohol and n-butyl alcohol series was followed for dehydration before embedding in paraffin wax. Customary paraffin technique was employed for the preparation of slides. Sections were cut at 15mM thick and stained in HEIDENHAIN's

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