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DIRECT PLANTLET REGENERATION FROM AXILLARY BUDS OF *MORUS ALBA* L. (GRAFTED, VAR - WHITE)

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Plantlet regeneration from axillary buds of mature trees of *Morus alba* (grafted var. White) has been achieved. This variety holds a promise in the semi-arid regions of Rajasthan where it is found to perform well in dry and warm climatic conditions. The cultures were successfully established and maintained *in vitro* on Murashige and Skoog medium supplemented with Kn/BAP (2.0-5.0 mgl^{-1}), IAA/NAA (0.1-0.5 mgl^{-1}), Adenine sulphate (25 mgl^{-1}), NH_4NO_3 (10-20 mgl^{-1}) and Arginine (50 mgl^{-1}) as the source of extra nitrogen. Multiple shoots (9-10) were produced within six weeks. These shoot segments on subculture produced multiple shoot. Rooting was obtained on MS medium fortified with IBA (0.5-3.0 mgl^{-1}). Further experiments are in progress for hardening and acclimatization for field transfer of the plantlets. This method with further optimization will help in the large scale production of plantlets.

Key Words : *In vitro, Morus alba*

Mulberry (*Morus alba* L.) is a good sized, fast growting tree of tremendous economic importance. The leaves of the tree are used in sericulture industry to feed the larvae of silk worm (*Bombax mori*). Besides this, the fruits are also sweet and palatable with medicinal properties.

A great deal of heterozygosity exists in this taxa. The plant is however propagated by the usual vegetative methods like graftings and stem cuttings. These processes have their limitations. The cuttings of some highly nutritive varieties are very difficult to root. Tissue culture provides an alternative method compared to conventional methods of vegetative propagation. The *in vitro* culture of cells, tissues and organs offer an unparalleled opportunity for tree improvement (Karnosky, 1981, Arya & Shekhawat, 1986, Jain & Dutta, 1992). Here we report direct plantlet formation from the axillary buds of *Morus alba.*

MATERIALS AND METHODS

The elite trees of grafted *Morus alba* with white sweet fruits were identified. This variety is known to perform very well in semi arid regions of Rajasthan. The explant (nodal segments with axillary buds) was collected from the mature tree growing in the nursery of the University of Rajasthan campus, Jaipur.

After removing the leaves the twigs were kept under running tap water for 15 min. Nodal segments (1-2 cm long) containing axillary buds were used as initial explant. The explant was washed with tap water consisting of a few drops of Extran (a mild commercial detergent) and subsequently rinsed thoroughly with distilled water. The explants were treated with chilled antioxidant solution consisting of Citric acid (750 mgl^{-1}), Ascorbic acid (500 mgl^{-1}), and polyvinyl-pyrrolidone (500 mgl^{-1}) for one hour. They were then surface sterilized with 0.1% $HgCl_2$ solution for 5-7 min followed by several rinses with sterile distilled water and were kept in sterile antioxidant solution just before transfer. The explants were then inoculated on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with growth regulators Kinetin/Benzyl amino purine (Kn/BAP) (3.0-5.0 mgl^{-1}), and Indole acetic acid/Naphthalene acetic acid (IAA/NAA) (0.1-0.5) mgl^{-1} adenine sulphate AS (25 mgl^{-1}), NH_4NO_3 (10-20 mgl^{-1}) and antioxidants i.e. Ascorbic acid (50 mgl^{-1}) Citric acid (75 mgl^{-1}) and PVP (50 mgl^{-1}). The pH of the media was adjusted between 5.8-6.2 before autoclaving it at 15 lbs/sq. inch pressure for 15 min. The cultures were maintained in culture chamber at 26±2°C, a rhythmic cycle of 16 hrs light (2500 lux) and 8 hrs dark period and relative humidity of 55%.

The shoots thus formed were subcultured on shoot multiplication medium (B) which contained BAP (2 mgl^{-1}) + IAA (0.5 mgl^{-1}) AS (25 mgl^{-1}) antioxidants, and additional sources of Nitrogen viz. arginine (50 mgl^{-1}) and L-Glutamine (1 mgl^{-1}) and NH_4NO_3 (1.65 gl^{-1}).

The shoots thus multiplied were kept for rooting on media (C) which was 1/2 MS media supplemented with

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Table 1: Plantlet regeneration media for *Morus alba*

Morphogenetic response		Media
A.	Shoot initiation	MS + BAP 3.0 mgl^{-1} + IAA 0.5 mgl^{-1} + AS 25.0 mgl^{-1} + NH_4NO_3 10 mgl^{-1} + Citric acid 75 mgl^{-1} + Ascorbic acid 50 mgl^{-1} + PVP 50 mgl^{-1}.
B.	Shoot multiplication	MS + BAP 2 mgl^{-1} + IAA 0.5 mgl^{-1} + AS 25.0 mgl^{-1} + NH_4NO_3 1.65 gl^{-1} + L - Arginine 50.0 mgl^{-1} + L - Glutamine 1.0 mgl^{-1} + Citric acid 75.0 mgl^{-1} + Ascorbic acid 50.0 mgl^{-1} + PVP 50 mgl^{-1}.
C.	Root induction	1/2 MS (Inorganic & Organic only) + Sucrose 10 gl^{-1} + IBA 0.5 mgl^{-1}.

IBA (0.5-3.0 mgl^{-1}).

Fifty replicates were used in each set of experiments and they were repeated three times to confirm the results.

RESULTS AND DISCUSSION

Bud break was obtained in 10-12 days on media A (Table 1) and 1-3 shoot were obtained per explant. These shoots were separated and subcultured on shoot multiplication media B (Table 1). 9-10 shoots were harvested in 6 weeks time. The shoot segments were cut and subcultured again to yield the same number of shoots in another 6 weeks on the same fresh medium. The shoots were further transferred to rooting medium C (Table 1). Roots were initiated in the shoot segment in 4 weeks and developed profusely by the end of 6 weeks. Plantlets thus obtained were subcultured on the same liquid medium for hardening and acclimatization. The experiments are in the process of mass multiplications of the propagules.

The mass propagation of most forest and cultivated trees including plantation crops using tissue

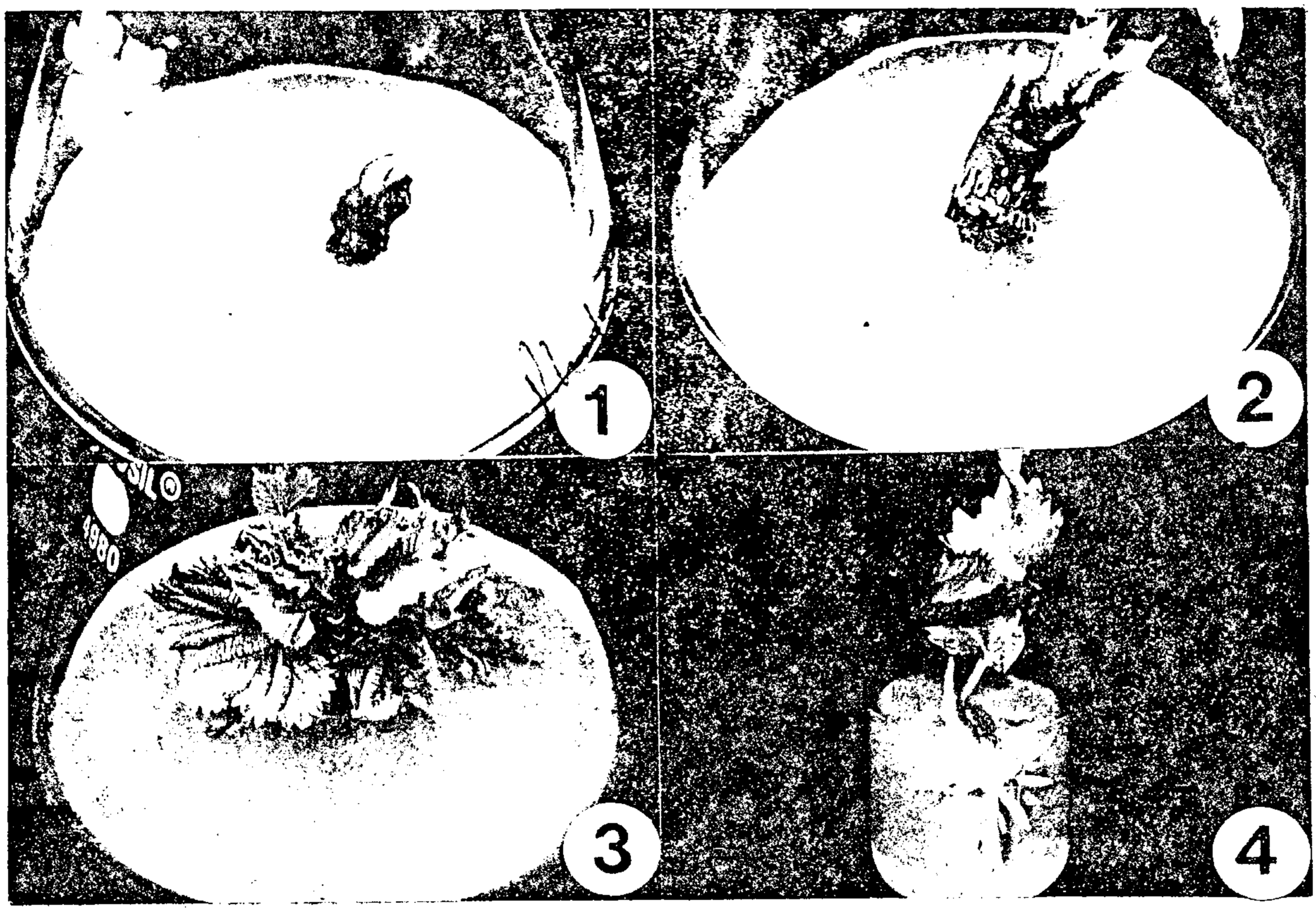

Figure 1-4 : Plantlet regeneration from axillary bud of *Morus alba*
Fig. 1. Shoot bud initiation on MS medium supplemented with BAP 3.0 mgl^{-1} + IAA 0.5 mgl^{-1} + AS 25 mgl^{-1} + NH_4NO_3 10 mgl^{-1} + Citric acid 75 mgl^{-1} + Ascorbic acid 50 mgl^{-1} + PVP 50 mgl^{-1}. Fig. 2. Further development of shoot buds after 2 weeks on the same medium. Fig. 3. Multiple shoots on MS medium fortified with BAP 2 mgl^{-1} + AS 25 mgl^{-1} + NH_4NO_3 1.65 gl^{-1} + L-Arginine 50 mgl^{-1} + L-Glutamine 1 mgl^{-1} + Citric acid 75 mgl^{-1} + PVP 50 mgl^{-1} + Ascorbic acid 50 mgl^{-1}. Fig. 4. A rooted shoot on 1/2 MS medium with IBA 0.5 mgl^{-1} and Sucrose 10 gl^{-1}.

culture technique has been extensively attempted in recent years (Bonga 1977, 1981, Arya *et al,* 1989, Tomar & Gupta, 1988). In the present investigation we have been able to raise complete plantlets from the nodal segments of *Morus alba* (grafted white). This variety of mulberry is known to perform well in semi-arid and arid regions of Rajasthan and large scale production of plantlets of this tree species provides a potential for its plantation in these alien areas where it is scantily found.

While working on this tree species we have come across several problems and have succeeded in overcoming them to a certain extent. It was seen that initially, at bud break, the buds tended to gave rise to inflorescence (3 - 5 in number) along with the leaves. This was reduced to a great extent by the addition of 10 mgl^{-1} NH_4NO_3 in the shoot initiation media. The pre-treatment of the explant with antioxidant solution was found to favour shoot bud induction. This was due to the fact that antioxidants inhibit the leaching of phenolics from the explant which suppresses the growth of the explant. Also BAP (2 mgl^{-1}) with low concentration of IAA (0.5 mgl^{-1}) was found to give better results as compared to the concentrations of Kinetin. Similar results were reported in various genotypes of *Morus* sps. by Jain *et al.* (1990), Horak Kim *et al.* (1985), and Mahtre *et al.* (1985).

Profuse rooting was obtained on MS media fortified with 0.5 mgl^{-1} IBA. In the rooting experiments it was observed that initially a certain amount of callus was formed along with the roots which was minimized/ removed by reducing the amount of sugar to just 1% from the initial 3% and at 1/2 MS inorganic salt concentration. Low concentrations of IBA were found favourable for root induction, as higher concentrations caused callus formation at the base of the shoot. Similar findings were reported by Horak Kim *et al.* (1985). This technique of direct regeneration from nodal segments has a great potential for micropropagation of *Morus alba.* Numerous plantlets have been developed by this method. Further experiments are in progress for field transfer of these plantlets.

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