

ATTEMPTS AT PROPAGATION OF AVOCADOS USING VARIOUS TECHNIQUES

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Progress Report

OPSOMMING

Die doel van die navorsing was om avokado plante wat vry van sonvlekviroid is te produseer en vermeerder. Saailinge wat in die groeipuntenting prosedure nou gebruik word was nie onder steriele omstandighede gekweek nie. Die groeipuntenting prosedure wat met sitrus gevolg word is hier gebruik, met die verskil dat plantmateriaal nie gesteriliseer was nie. Oksidasie van die avokado weefsels is vertraag met die toediening van askor-bien / citroen suur oplossings. Uitdroging was vertraag deur die aanwending van lanolienpasta oor die hele groeipunt. Hoewel kallusgroeï duidelik was op die groeipunt verwyderde stam, het die oorgeplante groeipunt nie oorleef nie,

Blaarsteggies is in voorplantingskabinette by 'n temperatuur van 25°C geproduseer, Syknopgroeï op die steggies het eers begin na wortelformasie.

'n Indikasie van die suksesvolle oorplanting van groeipunte na Miller se medium was duidelik nadat blaar primordia op die oorgeplante groeipunte begin groei het.

SUMMARY

The objective of the research was to devise means for obtaining avocado plants free of sunblotch virus and for rapid multiplication of such plants. Seedlings to be used in the shoot-tip grafting procedure were raised under non-aseptic conditions. The technique for shoot-tip grafting of citrus was followed except that all procedures were carried out under non-aseptic conditions. Oxidation of avocado tissue was retarded by application of ascorbic/citric acid solution. Dessication was retarded by the application of lanolin paste over the whole stem tip. Although callus growth of the decapitated root-stock stem was evident, none of the transplanted stem tips survived to grow.

Leaf cuttings were produced in propagation boxes placed in hot water baths held at 25°C. Axillary bud growth commenced following the outgrowth of roots.

Some indication of the success of the transfer of shoot-tips onto Miller's medium was gained from the resumed growth of these shoot-tips.

The primary objective of this research project was to devise a means of obtaining avocado plants free of sunblotch virus. The shoot-tip grafting technique was to be

employed and propagation of virus-free material was to be carried out using tissue culture techniques or using cuttings of vegetative material.

SHOOT-TIP GRAFTING

The shoot-tip grafting procedure makes use of the fact that shoot meristems and adjacent tissue sometimes remain free of virus despite infection of other parts of the plant with virus. By transplanting such virus-free meristems onto virus-free seedlings, it is often possible to produce disease-free plants which are not physiologically juvenile, but are capable of flowering and fruiting following an induction period.

Certain problems were encountered in attempting this technique on avocados. Seed germination was generally slow and erratic. Initially difficulty was experienced in obtaining seed. The Citrus and Subtropical Fruit Research Institute and John da Graca, University of Natal, were helpful in providing seed of Duke and Fuerte cultivars.

Early attempts at germinating seeds under aseptic conditions were not highly successful and caused unnecessary loss of seed when fungus contamination caused rotting. It was found that grafting need not be carried out under aseptic conditions as the sunblotch viroid is not airborne. Seed was found to germinate adequately in sand : peat moss : sawdust (2:1:1 v/v) mixture at 30°C constant. Once seed had germinated it was transferred to a dark chamber where etiolated stems were allowed to develop before grafting was performed, as with shoot-tip grafting of *Citrus*.

Stem tips (Plate 1) were gathered from glasshouse-grown plants which were just beginning a growth flush. Newly formed leaves and larger leaf primordia were removed and the apex, plus about three primordia, were excised under a dissecting microscope using a special scalpel which held small razor blade segments. Seedlings were decapitated about 80 mm above soil level and an inverted T-cut was made about two to three mm below the incision. The bark flaps were lifted on either side of the vertical cut and the excised apex was placed against the cambium and was held in place by the bark flaps (Fig. 1a, Plate 2).

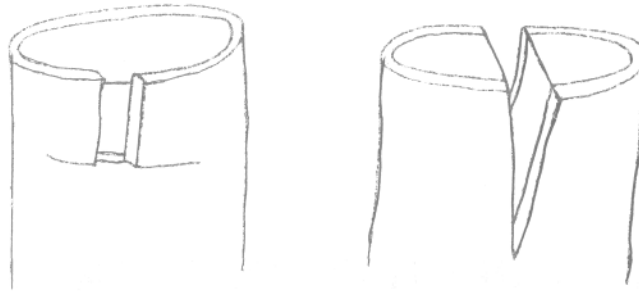


FIG. 1: Diagrammatic representation of incisions into avocado seedling rootstock stems for shoot-tip grafting

Within a few hours of the first attempts, it was clear that oxidation of both the rootstock stem and the transplanted meristem was such that growth at these new cut surfaces was probably impossible. Accordingly cut surfaces of the rootstock were dipped into a solution of ascorbic acid ($200 \text{ mg } \ell^{-1}$) and citric acid ($300 \text{ mg } \ell^{-1}$) and the meristem material was wetted with this solution. A glass beaker was placed over the protruding stem in order to maintain a high relative humidity around the newly cut surfaces (Plate 3). Dessication still resulted and the method was not considered suitable.

It was clear that some local treatment was required in order to protect the newly exposed and delicate tissues at the graft. Accordingly, softened lanolin paste was applied to cover the whole grafted area (Plate 4). This procedure did appear to alleviate the desiccation to some extent but callus growth of the cambium of the rootstock resulted in opening of the lanolin cap and tissues dried out.

A different placement of the meristem was then attempted. After decapitation, a vertical cut was made in the rootstock stem (Fig. 1b) and the apex was held under pressure in the V-cut. Lanolin was applied once again. The growth response in the stem still caused cracking of the lanolin and drying-out ensued. At this stage it is felt that some means of preventing desiccation of this tissue must be employed before the cambiums will grow to form a graft union. It may be possible to place a small agar block over the exposed area, although aeration may limit subsequent growth.

PROPAGATION OF CUTTINGS

Leaf cuttings were collected from glasshouse-grown trees. The cuttings included the leaf and an attached stem segment ($\pm 30 \text{ mm}$ long) and the axillary bud. Old mature leaves and young recently expanded leaves were collected. Where large leaves were sampled, the leaf area was reduced simply by cutting the leaf blade transversely. The basal end of the stem segments were wetted and then dipped into 'Seradix' rooting powder (Indole butyric acid). The leaf cuttings were then placed in a bottom-heated mist

bed ($\pm 30^{\circ}\text{C}$) or into a propagation box. The propagation boxes consisted of water-tight soil containers with clear Perspex lids. These were bottom-heated by placing them in water baths held at 25° and 30°C .

All leaf cuttings formed callus at the basal ends of the stem segments under all conditions of incubation, however, leaves placed in the mist-bed showed a greater propensity for rotting. Only leaf cuttings held at 25°C formed roots (Plate 5). The roots appear to arise from the callus tissue adjacent to the stem. It was only after the appearance of root growth that the axillary bud commenced growth.

This method of propagation is very slow; up to three months are required for root growth prior to bud sprouting. The fact that bud burst only occurred after root growth was evident means that root synthesized hormones may be implicated. The production of rooted cuttings may be hastened following further research into the application of specific hormones and concentrations thereof. The use of etiolated plants may be an area deserving of further experimentation.

MERISTEM CULTURE

The culture of shoot meristems can be used for the rapid propagation of plants and for the production of virus-free plants or stem material. Excision of stem apices was carried out as for the shoot-tip grafting procedure except that excised meristems were placed directly onto agar in a test tube. The agar contained Millers medium (commonly used for the soybean callus bioassay for cytokinins) to which was added 1 mg l^{-1} kinetin and 1 mg l^{-1} naphthalene acetic acid. The avocado stem tips were sterilized in 0,3% sodium hypochlorite for ten minutes and rinsed three times in autoclaved distilled water before the meristems were dissected out and transferred to the medium on a laminar-flow bench.

The sterilization and excision is quick and has permitted all transferred material to survive, free of fungal contamination. Leaf primordia are growing at this stage, but the ultimate success of the technique will depend on whether roots will also grow. Alternatively, grafting of stems onto established seedlings will have to be carried out as for the rapid method with *Citrus* shoot-tip grafted stems.

The technique may provide a means of growing virus-free material in large quantities, based on its simplicity. However, the initial attempts were carried out fairly recently and final evaluation can only follow the successful establishment and indexing of such plant-lets in the soil.

CONCLUSION

The initial outline of the research project appears, in retrospect, to be based on the assumption of rapid successes. A measured degree of success has been achieved but not of the extent where practically useful data are available. If the project was to be continued, there does seem to be scope for intensified research into all three aspects of freeing material of sunblotch virus and propagating virus-free plants. It may be early to evaluate at this stage, but the tissue culture meristem propagation method appears to

be a quick and simple procedure.



PLATE 1: Flushing avocado shoots which provided apices for shoot-tip grafting and meristem cultures

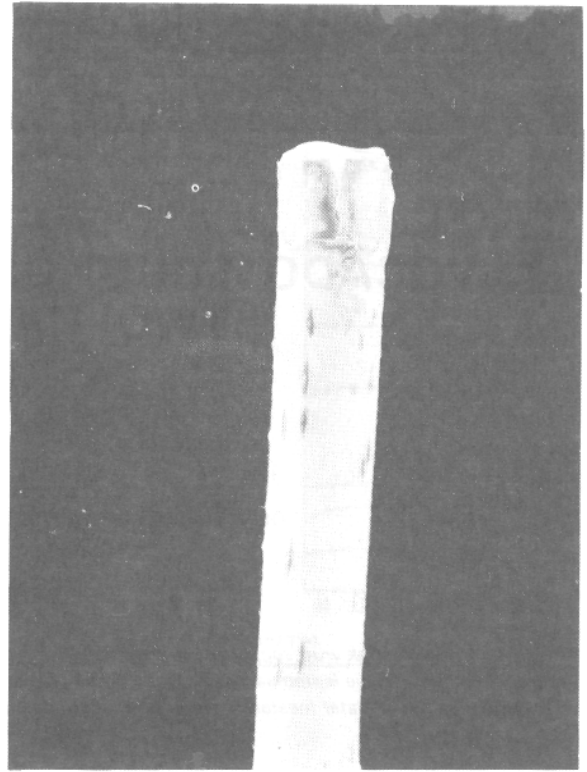


PLATE 2: Magnified view of avocado rootstock stem with inverted T incision

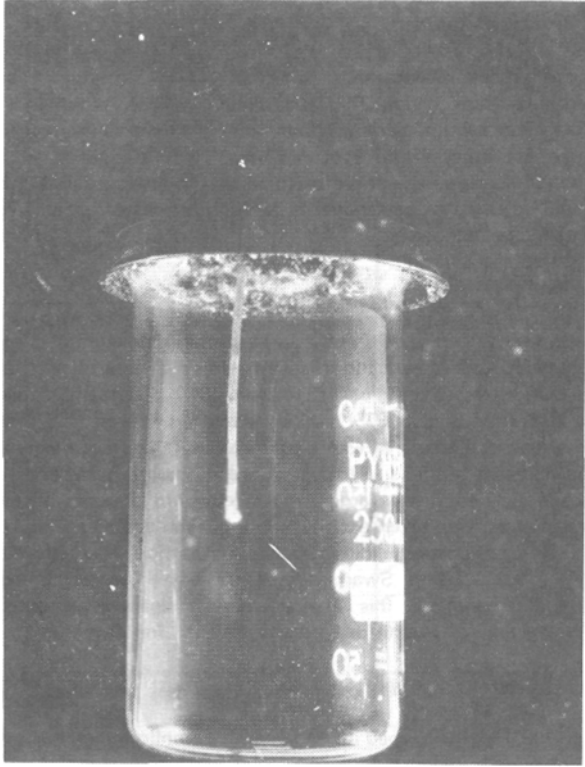


PLATE 3: Shoot-tip grafted seedling covered by a glass beaker

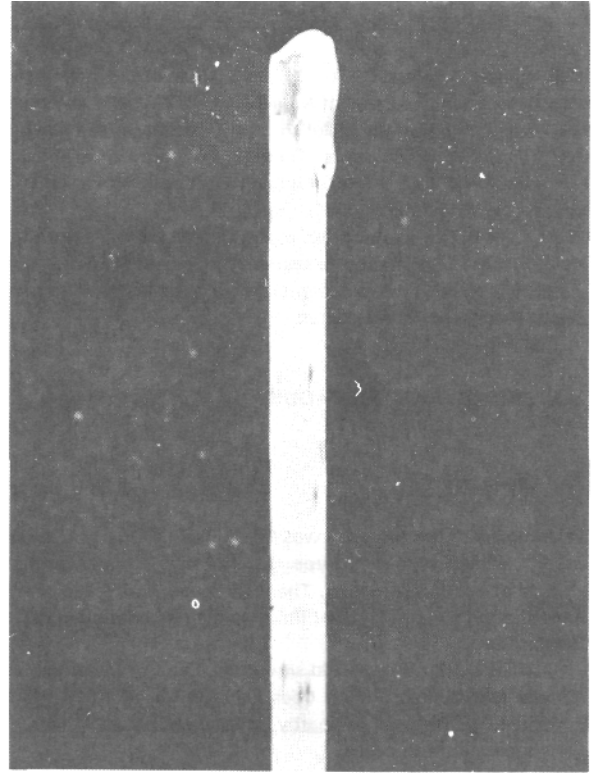


PLATE 4: Lanolin paste covered shoot-tip grafted avocado stem