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SHOOT TIP GRAFTING OF AVOCADO FOR VIRUS AND VIROID ELIMINATION

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OPSOMMING

*'n Tegniek is gedeeltelik ontwikkel vir groeipuntenting (GPE) van avokado met die oog op virusen viroiduitskakeling. Ongeveer 40 tot 50% suksesvolle entings word verkry deur alie snitte aan die ge-etioleerde onderstammetjie en lootjie onder water uit te voer; die materiaal vir 24 uur ondergedompel tehouen daarna die enting uit te voer. Direk na enting word onoplosbare polivinielpyrrolidoonpoeier (PVP) rondom die basis van die geénte groeipunt geplaas. 'n Vloeibare, half-sterkte Murashige en Tucker-medium word vir die kweek van die plante gebruik. Tot dusver is daar egter nog net **in vitro**-gekweekte saailinge as bron van groeipunte gebruik. Twee maande na GPE word die lootjies van suksesvol-geënte plante op gevestigde saailinge in die glashuisgeënt vir verdere ontwikkeling. Bykomende studies worduitgevoer om die tegniek te verfyn veral sover dit die groeipunt betref.*

SUMMARY

*A technique has been developed for shoot tip grafting (STG) of avocado with the aim of virus and viroid elimination. Approximately 40 to 50% success was obtained by performing all cutting of etiolated rootstock and shoot underwater; keeping the material submerged for 24 hours and performing the grafting thereafter. Insoluble polyvinylpyrrolidone (PVP) powder was placed around the base of the grafted shoot tip. The plants were cultured in a liquid half strength Murashige and Tucker medium. So far, only **in vitro** grown seedlings have been used as a source of shoot tips. After two months the shoots of successfully grafted plants were grafted in the glasshouse onto established seedlings. Additional studies are being done to refine the technique especially as far as the shoot tip is concerned.*

INTRODUCTION

In many ornamental and vegetable crops viruses have been eliminated by means of meristem culture. This technique, however, proved to be generally unsuccessful in fruit trees. In 1972 Murashige *et al.* reported the elimination of a virus from citrus by the technique of STG. The technique was refined (Navarro, Roistacher & Murashige, 1975; de Lange, 1978) and at present is routinely applied in many countries.

In several studies efforts were made to apply this technique in avocado. According to researchers at Riverside, California, excessive callusing proved to be a problem (P.R.

Desjardins; pers. comm., 1980). As far as known these workers have not yet published any results on their techniques. In 1982 it was reported that they had 20 shoot tip grafts that were still alive out of several hundred attempted the previous year and that the routine achievement of successful grafts had not been realized (Desjardins, Drake & Semancik, 1982).

In South Africa attention has been paid to this problem by several workers (Hendry & van Staden, 1 982; Nel & Kotzé, 1 982 & 1 984; Nel, Kotzé & Snyman, 1983).

Similar studies were initiated at the CSFRI, Nelspruit this year. The work was considered essential especially in view of the intended avocado improvement programme (Partridge, 1984). The presence of several viruses and a viroid has been indicated in the avocado. The elimination of such pathogens is a prerequisite in clones to be released to nurseries under this scheme.

METHODS

Culture medium

Improved germination was found on a paper bridge over a liquid medium as compared to an agar-medium. A half-strength Murashige and Tucker (1969) medium containing 5% sucrose at pH 5,7 was used.

Rootstocks

As the present phase of the work is concerned with the technique of STG only, no attention was paid to virus or viroid status of rootstocks or shoots.

Embryos were aseptically dissected from mature Fuerte seeds (Fig. 1) after removal of integuments and sterilization of seeds. The sterilization was achieved by first shaking the seeds in a weak Teepol solution followed by dipping in a 1% sodium hypochlorite solution for 30 min. and washing three times in sterile water. The cotyledons were gently separated, and the embryos dissected out and placed on filter paper bridges on the nutrient medium in culture tubes.

The embryos were incubated in the dark at 27ºC. After approximately two weeks the shoots were 10 to 20 mm long and ready for grafting. The seedlings were cut to leave 10 to 15 mm long hypocotyls. Bracts at the embryonic axis and axillary buds on the hypocotyls were carefully removed. These surgical steps were all performed while the material was submerged in water.

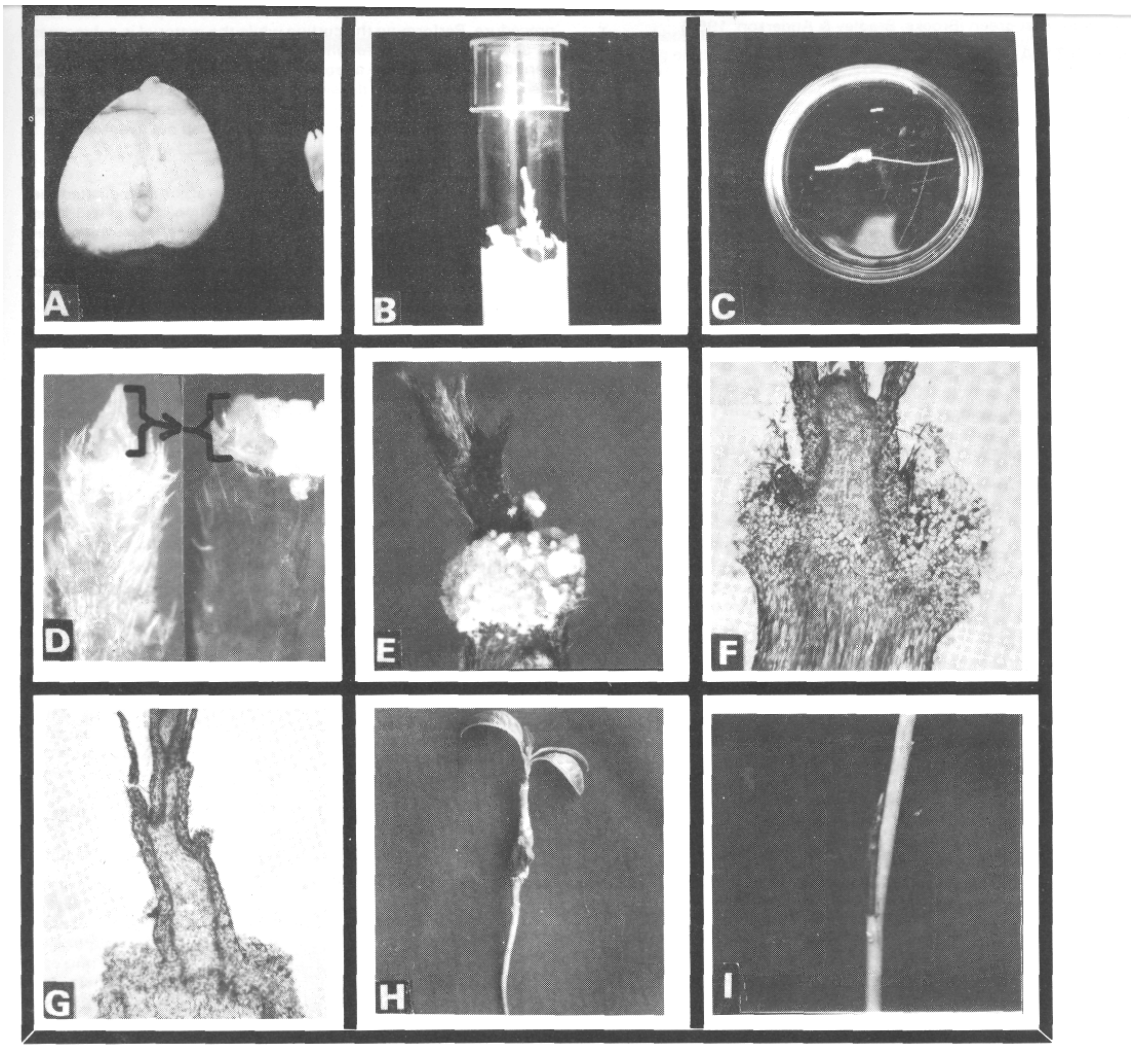

FIG. 1: STG of avocado in the present state of development of techniques.

A: Embyro of mature seed dissected for *in vitro* culture in darkness on a paper bridge over liquid medium.
B: Seedling two weeks after start of embryo culture.
C: Seedling and shoot tip placed in petri-dish with sterile water and cut as shown while submerged and left submerged for 24 h.
D: Tip of *in vitro* grown shoot placed on decapitated rootstock and insoluble PVP placed around grafted shoot-tip. Grafted plants transferred to growth room.
E: Growth of shoot two weeks after STG with PVP powder still covering the rootstock top.
F: Anatomical section – four weeks after STG.
G: Anatomical section – six weeks after STG.
H: STG plant – eight weeks after STG.
I: STG plant grafted on a etiolated seedling in glasshouse.

Shoot tip grafting

Just as in the case of citrus STG, efficient instruments are essential. Of special importance is a Beaver-handle for securing small pieces of razorblades with a cutting edge, and a self-closing tweezers.

After preparing the rootstock and shoot tip, the shoot tip was placed on top of the decapitated rootstock either directly or after varying periods of submergence of the tissues in sterile distilled water.

The shoot tip was placed next to the perimeter of the top surface of the rootstock for

better contact with rootstock cambium. After grafting, sterile insoluble PVP (Polyclar AT) powder was placed around the base of the grafted tip. This chemical has previously been shown to form insoluble complexes with phenols which may thus be removed from plant extracts (Biddington & Thomas, 1973). We have as yet not experimentally evaluated the necessity of the PVP treatment which was included on account of its reported effect on phenolic substances.

The grafted plants were placed on paper bridges on a similar medium as that for embryos and kept at 27°C with a 16 hour photo period.

Establishment of successful grafts

Approximately six weeks after STG the leaf blades of the grafts were removed and the sections containing the graft as well as the hypocotyls of the rootstock were grafted onto well-established plants in the glass house (Fig. Il). This graft is performed similarly as that in citrus (de Lange, 1978).

Both etiolated Duke-7 plants with shoots approximately 5 mm in diameter and normally grown Fuerte seedlings with shoots approximately 10 mm in diameter have been used.

Anatomical studies

Material was fixed in a formalin: acetic acid: 50% ethanol mixture (1:1:8 v/v/v), dehydrated in alcohol, cleared in xylol, embedded in paraffin wax, sectioned in 12 /urn sections and stained by Safranin/Fast green (Brooks, Bradley & Anderson, 1950).

RESULTS AND DISCUSSION

Phenolic browning

The anatomical study clearly shows that cutting of the rootstock normally in air, results in degeneration of the distal shoot portion. Even one day after cutting, the top portion was deformed (Fig. 2A) and after six days there was extensive degeneration (Fig. 2H). If the cutting was done under water and the rootstock immediately removed and transferred to the growth room, a vast improvement was found (Fig. 2B & I). Further improvement was found if the rootstocks were left submerged for varying periods of time. Particularly impressive was the condition of the rootstock left submerged for 24 hours and held in the growth room another 24 hours (Fig. 2F). The cut surface was nearly as smooth as that of the Control (Fig. 2G) placed in the fixing medium directly after cutting.

When considering the "six days in growth room "sect ions, it must be borne in mind that the cut surfaces were exposed and some desiccation was unavoidable. If grafted and covered with PVP powder less deformation could have resulted.

As a standard procedure we have chosen 24 hours submergence in all subsequent studies. A very thin cross-section (±0, 5 mm) is cut from the rootstock top just before grafting.

Additional work in this regard is required as far as the shoot tip is concerned. The present procedure is to leave an approximately 5 mm length of shoot tip submerged, and cut the final approximately 0,5 mm long shoot tip just before grafting.

Hendry and van Staden (1982) reported oxidation of tissue after cutting. They tried unsuccessfully to overcome this problem by dipping cut surfaces into a solution containing ascorbic acid and citric acid. In preliminary studies we cut material and left it for varying periods of time in a 1% ascorbic acid solution. This treatment, however, was not as beneficial as our present standard procedure. Performing the cutting while the material is submerged in an ascorbic acid solution and leaving it submerged for some period of time, might be a proposition. Hendry & van Staden (1982) also tried to prevent desiccation of newly exposed tissue by application of soft lanolin paste. Although this treatment alleviated the desiccation to some extent, the tissue dried out eventually.

Positioning of shoot tip on rootstock

Sectioning of a number of unsuccessful grafts has indicated the importance of positioning the shoot tip. When the tip had been placed too far away from the perimeter as happened in the earlier phases of the work (Fig. 3A), the graft died eventually due to unsuccessful union in the central portion of the rootstock upper surface. This can possibly be explained by both the depression that develops in the pith (central position) as well as the absence of contact with meristematic cells in the cambium region.

By leaving the rootstocks and shoot tips submerged for 24 hours and placing the tip in good contact with the cambium, 40 to 50% of grafts were successful.

Establishment of successful grafts

A high mortality rate was found with grafted plants transferred to soil. Similar results were reported from California (Desjardins, Drake & Semancik, 1982).

The indications are that shoot tip grafted plants can be grafted onto established seedlings in the glasshouse with a high rate of success. In the case of etiolated plants the plastic wrapping should not be left on longer than one week, to prevent decay due to fungal growth.

According to preliminary results normally grown, vigorous seedlings in the glasshouse are equally suitable, with the advantage that no provision for etiolation facilities is necessary and that the shoots are more rigid and manageable for grafting.

CONCLUSIONS

By employing the techniques described, a high rate of success in STG is possible. Important procedures were shown to be cutting of the rootstock and tip under water and leaving it submerged for 24 hours and placing the shoot tip near the perimeter of the top cut surface of the rootstock. The problem of failures upon transferring grafted plants to soil was overcome by grafting them onto established seedlings in the glasshouse.

In the initial phases of this work shoot tips were obtained mainly from shoots of *in vitro* grown seedlings. Present studies are now focusing on the use of shoots tips from sun-

blotch infected orchard trees either directly or after *in vitro* growth of such shoots.

As sunblotch viroid can be seed transmitted, rootstocks will have to be raised from seeds of trees free of sun-blotch. As in the case of citrus, instruments will have to be sterilized by dipping in a sodium hypochlorite solution after each surgical operation on infected material, to prevent possible mechanical transfer of pathogens.

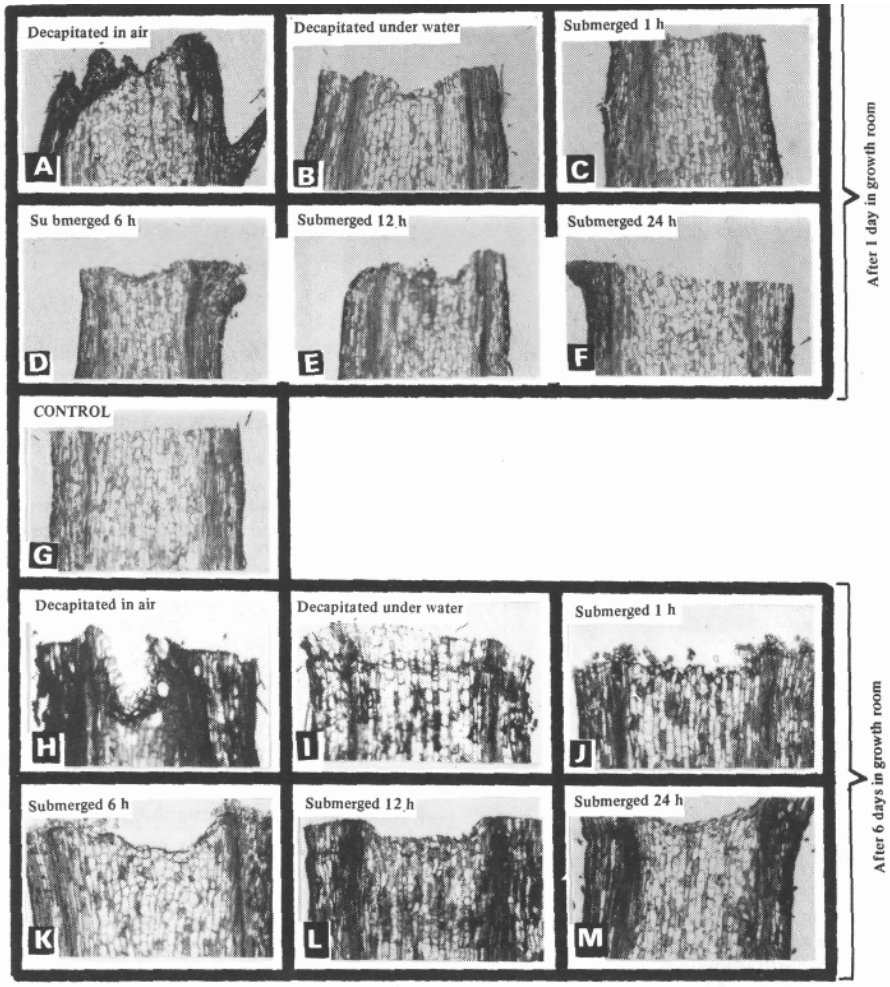

FIG. 2:
Anatomical sections of tops of *in vitro* grown avocado seedlings, decapitated and treated as indicated but not grafted.
Control (G): cut and immediately fixed in FAA. Treatments A & H: cut in air and cultured on growth medium.
All other treatments cut while submerged in water.
Treatments B & I: removed from water directly after cutting. The other treatments kept submerged for varying periods of time before culture on liquid medium.

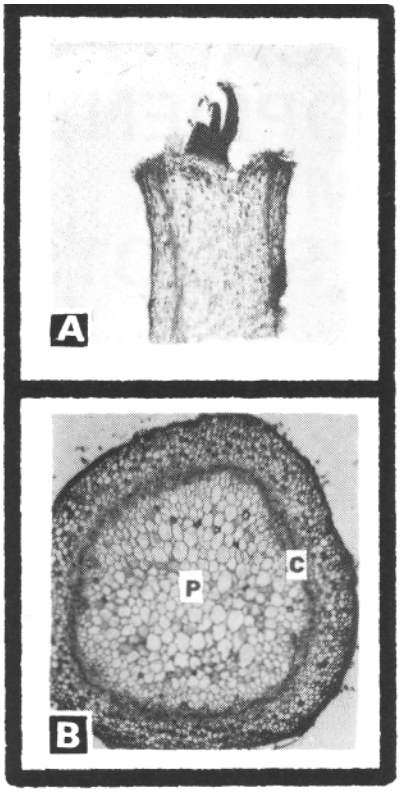

FIG. 3:
A: Unsuccessful union two weeks after STG.
B: Anatomical section of cross-diameter of etiolated *in vitro* grown avocado shoot.
c: cambium
p: pith

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