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Callus development - a possible aid in rooting avocado cuttings

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SYNOPSIS
With adult greenwood avocado cuttings, the differentiation of vascular tissue within the callus on the base of the cuttings, resulting in root formation (vascular cambium), is more likely to occur. Rooting is favoured by a synergistic interaction existing between callus tissue and an auxin. It seems that the treatment of cuttings with an auxin should be done after callus formation and differentiation.

INTRODUCTION

After stem cuttings have been made and placed under environmental conditions favourable for rooting, callus usually develops at the basal end of the cuttings. Callus is an irregular mass of parenchyma cells in various stages of lignification. This callus growth arises from young cells in the region of the vascular cambium, although various cells of the cortex and pith may also contribute to its formation. In most cases, the formation of callus and the formation of roots are independent of each other (Hartmann & Kester, 1975). In some species, however, (eg Hedera helix) (adult phase), adventitious roots have been found to originate in the callus tissue itself which has formed at the basal end of the cuttings. In such cases, callus formation is a precursor of adventitious rooting (Girouard, 1967).

With semi-hardwood adult avocado cuttings (green wood), callus development mainly occurs during the first 60 days in a mist propagation unit, with a gradual increase until 150 days (Bourdeaut, 1970; Ernst, 1978). According to Kadman & Ben-Ya'acov (1965) and Ernst (1978), it generally takes about three to four months before root initiations can be observed.

Kadman & Ben-Ya'acov (1965) could find no connection between the formation of callus and the rooting of cuttings. Sometimes the roots appear directly from the stem tissue, with or without any callus development on the cuttings (Young, 1961; Kadman & Ben-Ya'acov, 1965). According to Kadman& Ben-Ya'acov (1965), roots usually penetrate through the callus. Hendry& Van Staden (1982) found the roots appeared to arise from the callus tissue adjacent to the stem. No anatomical confirmation of the above-mentioned could however be found in literature.

Young (1961) stated that the abundant formation of callus tissue on the base of cuttings from mature trees, indicates that considerable cell division took place. It is therefore suggested that some root initiating substance or organiser is needed in addition to cell division. No clarity exists, however, in literature whether any form of differentiation within

the callus tissue (avocado cuttings) takes place or not. The aim of this study was therefore to determine whether any connection exists between callus formation and root development on the basal end of mature avocado cuttings.

MATERIALS AND METHODS

Several factorial experiments were laid' out in a mist propagation unit with the purpose of rooting semi-hardwood leafy avocado cuttings (adult greenwood). The different treatments consisted of different auxins (irldolebutyric acid (IBA) and indoleacetic acid (IAA)), auxin concentrations (0, 0,5, 1 and 1,5 per cent), auxin carriers (water, talc, ethanol and acetone), carrier concentrations (0 to 100 per cent ethanol or acetone) and three different cultivars (Fuerte, Duke 6 and Duke 7). In one experiment, the rooting medium was aerated in an attempt to determine the effect of oxygen on callus and eventual root development.

Cuttings were examined repeatedly every 60 days for at least 240 days, but no longer than 400 days. During sampling, root development, callus development, defoliation, cutting dieback and the number of dead cuttings were observed. Graphs, to illustrate the tendency of the different variables over the rooting period, were drawn up.

During the rooting period stem material was sampled, killed and fixed in formalin aceto alcohol (FAA) to be surveyed anatomically. The samples consisted of different stages of callus and root development at the base of the stem cuttings.

As described in detail by Ernst (1984), tertiary butyl alcohol was used for dehydration of the stem material after which wax impregnation followed. The sections (10 to 12 pm in thickness) were made with a rotary microtome. Haupt adhesive was used for mounting the sections on the slides, which were stained in Chlorazol Black E. The coverslips were mounted permanently on the slides with Canada balsam. All photographs were taken with a Reichert Diavar photo microscope.

Abbreviations, of which the definitions are as follows, were used in figures for the sake of convenience.

C	Callus tissue
Co	Cortex
D	Dead
Df	Defoliation
Db	Dieback
E	Epidermis
P	Phloem
Pe	Periderm
R	Root
S	Stem tissue
Sc	Sclerenchyma
V	Vascular cambium
X	Xylem

RESULTS

Although rooting significantly increased with an increase in IBA concentration from 0 to 1,5 per cent, callus formation decreased. Where ethanol was used as IBA carrier, callus formation decreased even further, with an increase in ethanol concentration. To the contrary, IAA had a mild stimulating effect on callus development at the same IBA concentration range (Table 1).

	Rooting Auxin concentration %					Callus Development Auxin concentration %				
Auxin	0.0	0.5	1.0	1.5	AV	0.0	0.5	1.0	1.5	AV
IBA	8.3	38.3	46.7	45.0	34.6	90.0	83.3	85.0	76.7	83.8
IAA	10.0	30.0	31.7	26.7	24.6	91.7	96.7	93.3	95.0	94.2
AV	9.2	34.2	39.2	35.9	29.6	90.8	90.0	89.2	85.9	89.0

As indicated in Figure 1, the Duke 6 cuttings, treated with IBA or IAA, did not root after 400 days. The callus percentage during this period was less than 10 per cent. Defoliation, cutting dieback and the percentage of dead cuttings, increased significantly over 400 days.

After 400 days, less than 10 per cent of the Duke 7 cuttings (IBA or IAA treated) produced roots (Figure 2). Less than 35 per cent callus formation (poor quality) occurred during the first 60 days of rooting. Defoliation, cutting dieback and the percentage of dead cuttings were less than 15 per cent after 320 days.

Approximately 50 per cent of the Fuerte cuttings (IBA and IAA treated) rooted after 400 days (Figure 3), with the first signs of rooting observed between 60 and 120 days. Callus production after 60 days was ± 70 per cent. During 180 to 400 days, there was a significant increase in defoliation, cutting dieback and the percentage of dead cuttings (Figure 3).

Under aerated (medium) conditions, more than 90 per cent IBA treated Fuerte cuttings produced callus of a good quality (Figure 4). Rooting after 240 days was ± 60 per cent on average for the different IBA treatments. A slight increase in defoliation, cutting dieback and the percentage of dead cuttings could be observed between 180 and 240 days (Figure 4).

In all the experiments, no cutting rooted in the absence of callus tissue. Cutting dieback preceded defoliation up to 120 to 180 days for Fuerte (Figures 3 and 4) and approximately 280 days for Duke 6 (Figure 1) and Duke 7 (Figure 2), after which defoliation was the first sign of cutting deterioration.

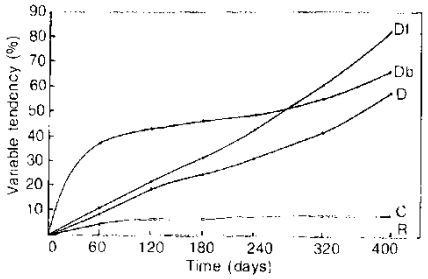

Fig 1 Variable tendencies of Duke 6 cuttings treated with IBA and IAA

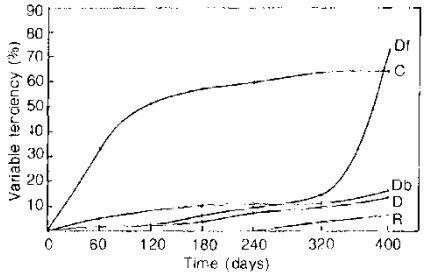

Fig 2 Variable tendencies of Duke 7 cuttings treated with IBA and IAA

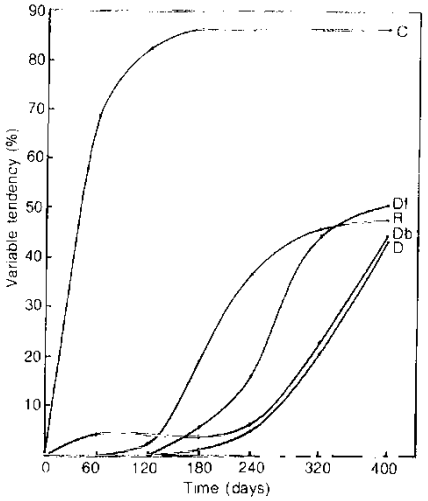

Fig 3 Variable tendencies of Fuerte cuttings treated with IBA and IAA.

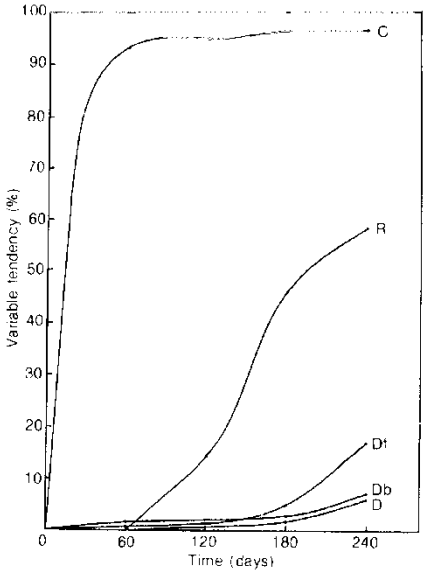

Fig 4 Variable tendencies of Fuerte cuttings (IBA treated) under aerated conditions (medium)

Although root development out of stem tissue was observed (Figure 5), root development out of callus tissue was more likely to occur (Figure 6).

As illustrated in Figure 7, radial division of vascular cambium out of the adult stem tissue into the differentiating callus tissue on the cutting base (usually within the first 60 days) was observed. This was immediately followed by tangential division (between 60 and 120 days) into secondary vascular tissue (secondary phloem and xylem). An advance stage of differentiation could be observed anatomically (Figure 8) between 120 and 240 days after commencement of the rooting experiments.

Although not orderly arranged as in stem tissue, sclerenchyma cells (scattered) were visible. No sclerenchymatic ring could be observed at any stage (Figures 8 and 9).

Adventitious root differentiation in callus tissue took place in the vascular cambium. Roots developed through the sclerenchyma without any difficulty (Figure 9).

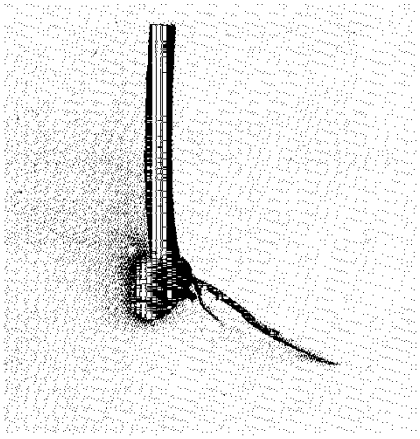

Fig 5 Root development directly from stem tissue

Fig 6 Root development directly from callus tissue

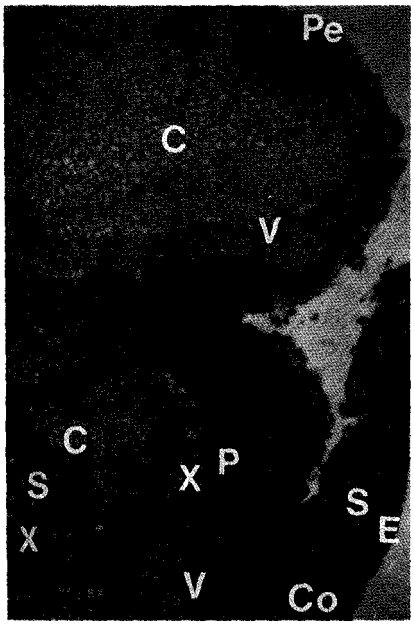

Fig 7 Early stage of callus differentiation in cross section (25x).

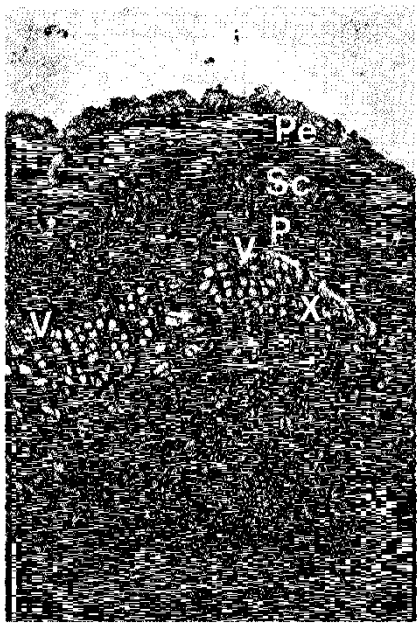

Fig 8 Advanced stage of callus differentiation in cross section (25x).

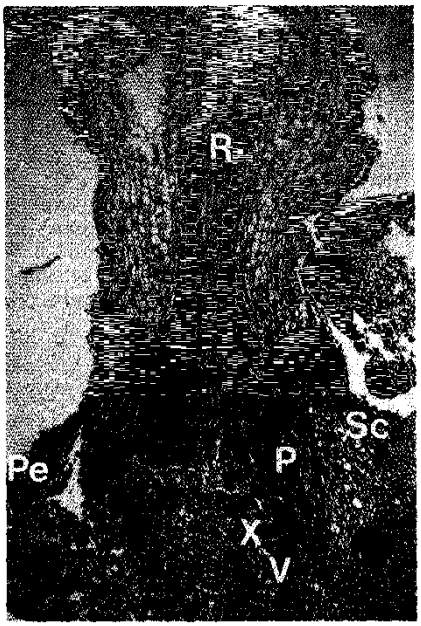

Fig 9 Differentiated root within callus tissue in cross section (25x).

DISCUSSION

From the results it seems that the differentiation of vascular tissue within the callus on the base of the cuttings, resulting in root formation, is more likely to occur with semi-hardwood adult greenwood cuttings (avocado). Root initiation took place in the vascular cambium and developed through the scattered sclerenchyma cells without any difficulty. Thus callus formation is an important precursor of rooting. This is contrary to the findings of Kadman& Ben-Ya'acov (1965), who stated that no connection was found between the formation of callus and the rooting of cuttings. According to Kadman & Ben-Ya'acov (1965), roots only penetrate through the callus.

In accordance with the findings of Ernst (1978), there is no doubt that IBA has a suppressive influence on callus formation, which increases with an increase in both auxin and ethanol (carrier) concentration. In contrast to IBA, IAA tends to have a stimulating effect. Oxygen significantly increases the quality of callus produced, as well as the eventual rooting of the IBA treated cuttings.

Rooting of Duke 6, Duke 7 and Fuerte cuttings was influenced by the quality of the callus produced at the base of their cuttings, as well as the percentage of callus within the first 60 days. Although Reuveni & Raviv (1981) reported a positive correlation to exist between rooting and leaf retention of cuttings, leaf retention (slight defoliation), as in the case of Duke 7 (low callus percentage), does not necessarily lead to high root formation. The primary reason for the low rooting percentage of Duke 7 cuttings seems to be its tendency

to produce insufficient callus. With all three cultivars, defoliation was preceded by cutting dieback, which eventually led to total loss of cuttings. Thus it seems important to have a high callus formation (within 60 days) and low cutting dieback and defoliation (for at least 180 days).

In conclusion, it seems that the treatment of cuttings with an auxin, is more likely to succeed if done after callus formation and differentiation (approximately 60 days) have occurred. The use of other hormones in combination with auxins such as cytokinins, should also be borne in mind in hastening the differentiation process. According to Mastalerz (1977), the stimulatory effect of auxins on root formation does not take place unless the tissues also contain an appropriate concentration of cytokinins. Inasmuch as the pH of the rooting medium can influence the type of callus produced, which in turn can affect emergence of newly-formed adventitious roots (Hartmann & Kester, 1975), pH studies should be included in further experiments.

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