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LONG-TERM *IN VITRO* MAINTENANCE OF OIL PALM (*Elaeis guineensis* Jacq.) CLONES THROUGH AXENIC ROOT CULTURES

Keywords: root culture; *Elaeis guineensis*; *in vitro* root elongation

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Studies were carried out to determine whether oil palm clones could be maintained long-term in vitro by means of root cultures. It was found that isolated root explants excised from axenic ramets could be maintained in culture for at least 18 months by repeated subculture. Root explants 3 cm in length produced more axillary roots than 1 cm root explants. Root growth was best in basal liquid medium containing modified MS nutrients supplemented with 1.5 g.L^{-1} of activated charcoal. A full strength modified MS medium supported root tip elongation and production of axillary roots better than a half strength nutrient medium. Root explants with root tips (RT) multiplied by root tip elongation and production of axillary roots whilst roots devoid of root tips (RS) multiplied by production of axillary roots only. Root tip segments (RT) sustained growth better than RS segments during subculturing, but there was a decline in the growth vigour of the new explants in the subsequent subcultures.

INTRODUCTION

The oil palm, *Elaeis guineensis* Jacq., a perennial monocot, with an economic life of 25 years, is propagated sexually. Because of its long economic life, establishment of clones of oil palm with desirable qualities has potential economic and agronomic advantages. Vegetative propagation of oil palm through

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somatic embryogenesis was reported by Jones (1974), Rabechault and Martin (1976), Ahée *et al.* (1981) and Paranjothy and Rohani (1982). In the early eighties, oil palm clones were produced on a large scale by Unilever for field testing (Corley *et al.*, 1982). The plants were reported to be normal. However, plantings from later batches showed floral abnormalities (Corley *et al.*, 1986). Paranjothy *et al.* (1993) also indicated that the frequency of incidence of abnormalities increased with subculturing.

Large scale production of clonal shoots relies on polyembryogenic cultures which are multiplied and maintained *in vitro* by repeated subculture. In view of the fact that tissues maintained in culture for longer periods tend to produce abnormal plants (Paranjothy *et al.*, 1993), attempts were made to find a means of maintaining clones *in vitro* without relying on prolonged maintenance of polyembryogenic cultures for shoot production. Chaturvedi *et al.* (1991) and Bhat *et al.* (1992) used excised roots for long-term culture as an alternative means of plant germplasm conservation. Chaturvedi *et al.* (1991) reported that root explants excised from 8+ and 12-year old root cultures of *Solanum khasianum* retained their totipotency, regenerating shoot buds which developed into fully grown plants in soil. They did not detect any variations in these plantlets. In line with this concept, studies were undertaken to examine the feasibility of maintaining oil palm clones *in vitro* using excised root explant cultures derived from axenic ramets.

MATERIALS AND METHODS

Basal medium

The basal medium was made up of Murashige and Skoog (MS) (1962) macro- and micro- nutrients, Y3 vitamins (Eeuwens, 1976), 0.1 $g.L^{-1}$ myo-inositol and 1 $g.L^{-1}$ glutamine. For a solid medium, 7 $g.L^{-1}$ of Sigma agar was added. Where indicated, 1.5 $g.L^{-1}$ of activated charcoal (AC) was also added. The sucrose levels are indicated in the results section, below. The medium was adjusted to pH 5.7 before autoclaving. Fifty ml of media were poured into 100-ml Erlenmeyer flasks each covered with a polypropylene sheet secured at the neck with rubber bands. The media were autoclaved at 1.05 $kg.cm^{-2}$ for 15 minutes.

Root cultures were incubated for 24 hours in the dark. The temperature of the dark room was maintained at 28°C ± 1°C. Liquid cultures were agitated on a rotary shaker at 100–110 rpm.

Root explants

Normal-looking *in vitro* plantlets of *E. guineensis* with well developed roots were selected (*Figure 1*). Root excision was carried out in sterile petri dishes containing basal nutrient medium to protect the root explants from dehydration. Roots were cut into several segments 3 cm long. The segments included both those devoid of root tips (RS) and those with root tips (RT). Any visible axillary roots on the segments were removed beforehand so that at the end of the study only new root growths (if any) were

Figure 1. Roots of a plantlet used as explants.

accounted for. The actual length of the new root growth was measured during subculture by placing marked centimetre graph paper below the sterile petri dishes in which root excision was made.

Root growth analysis

For root growth analysis, the total number of new axillary roots produced divided by the number of root explants cultured gives the average number of axillary roots per explant. The total length of new axillary roots produced divided by the number produced gives the average length of the axillary roots. The increase in length of root tip in RT segments was determined as total root length less the original explant length.

RESULTS AND DISCUSSION

Selection of root segment size

A preliminary study was carried out to determine whether 1 cm or 3 cm root explants were suitable for use in routine culture of explants. Roots longer than 3 cm were not used because this would have limited the number of explants. Both RT and RS root explants of both sizes were cultured in liquid basal medium containing 30 $g.L^{-1}$ sucrose. The number of explants producing new growths and their lengths were recorded after 3 months in culture.

Axillary root growth was observed in both RS and RT segments cultured in basal liquid medium (*Figures 2A* and *2B*). Besides producing axillary roots, the root tips in RT explants also elongated (*Figure 2A*). The different explant sizes did not show a marked difference in the elongation of the root tips in RT segments (*Table 1*). However, a higher percentage of the 3-cm root explants (both RT and RS) were observed to produce new axillary roots than did the 1-cm explants. The average number of axillary roots produced per explant was also higher in 3-cm root explants, but the lengths were about the same in the two sizes. After the third month the experiment was terminated because the new growths stopped elongating and most of the explants turned brown. Browning of explants in basal medium without AC was also seen in other experiments (see *Effects of solid and liquid media on root growth*).

Since 3-cm root explants produced more axillary roots, meaning that they generated more new explants, this length was chosen for subsequent experiments.

Initiation of new root growth

Effects of ionic strength of MS nutrients on root growth

The effects of ionic strength of MS macro- and micro-nutrients on growth of excised root cultures were investigated. The basal medium for 1/2 × MS had half the strength of MS macro- and micro-nutrients. A full complement of vitamins and amino acids was added to both 1/2 × MS (treatment A) and 1 × MS (treatment B) media (*Table 2*). Both media contained AC and 60 $g.L^{-1}$ sucrose. Observations were made after 7 months of culture.

Table 2 shows that full ionic strength of MS macro- and micro- nutrients (treatment B) supported both root elongation and axillary root growth of RT and RS segments more effectively than did half ionic strength MS basal medium (treatment A). Although the average length of axillary roots in RT and RS explants was longer in treatment A, the number of axillary roots produced was more in treatment B. We preferred to score for the number of axillary roots produced as these roots can be used as the source of RT explants in the subsequent culture for multiplication purposes (see *Culture multiplication and maintenance*).

As a result of this study, full strength MS basal medium was used in subsequent experiments.

Effects of solid and liquid media on root growth

Three basal media were tested for root multiplication *in vitro*. Medium A was made up of solid basal medium supplemented with AC. Medium B was liquid basal medium with AC and medium C consisted of liquid basal medium without AC. All three media contained 60 $g.L^{-1}$ sucrose.

Two clones of root explants were cultured in all the three media. Root explants from clone P99 were

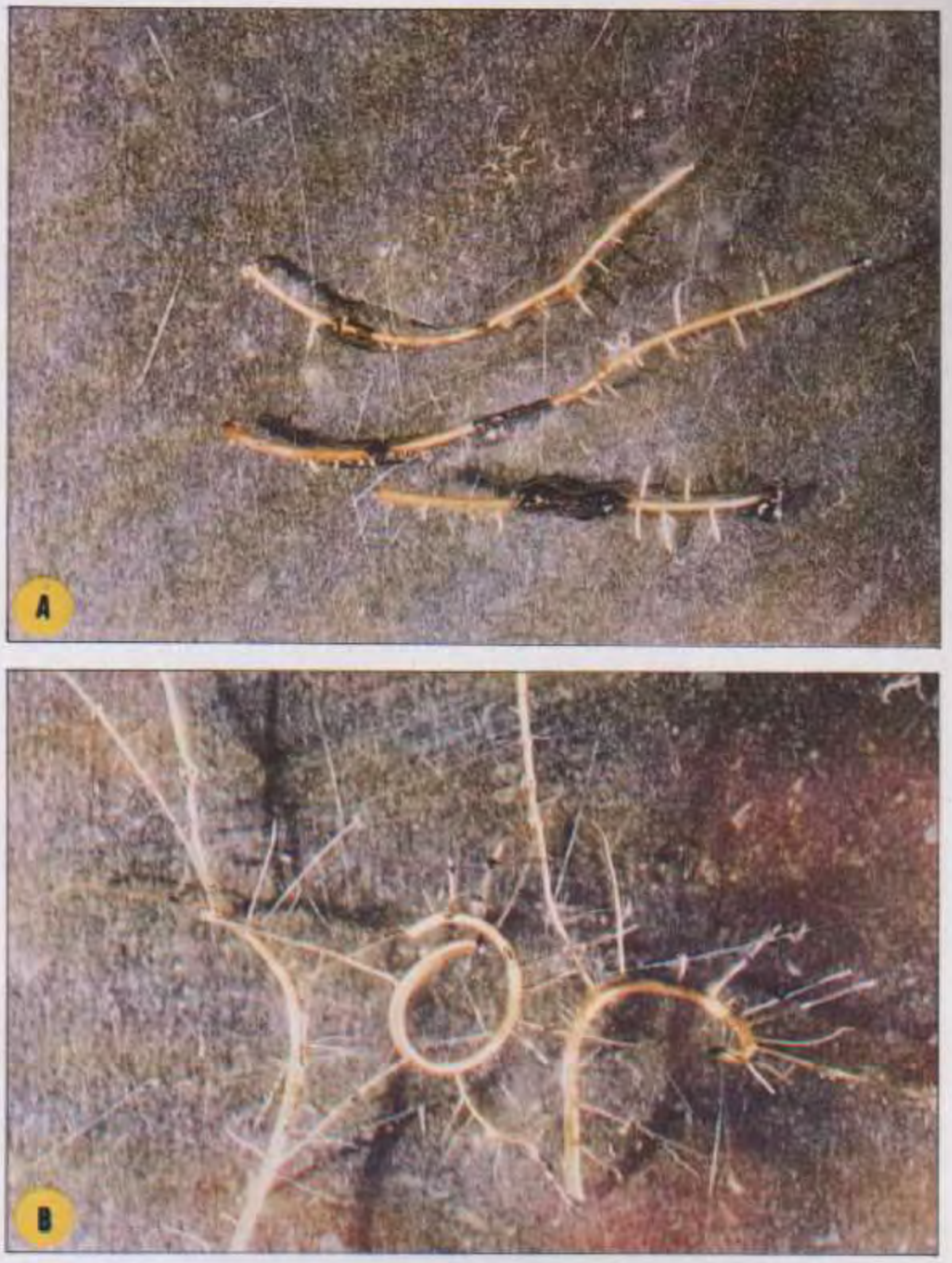

Figure 2. New root growth initiated from root tip and root segment explants; A) root tip (RT) explants and B) root segment (RS) explants.

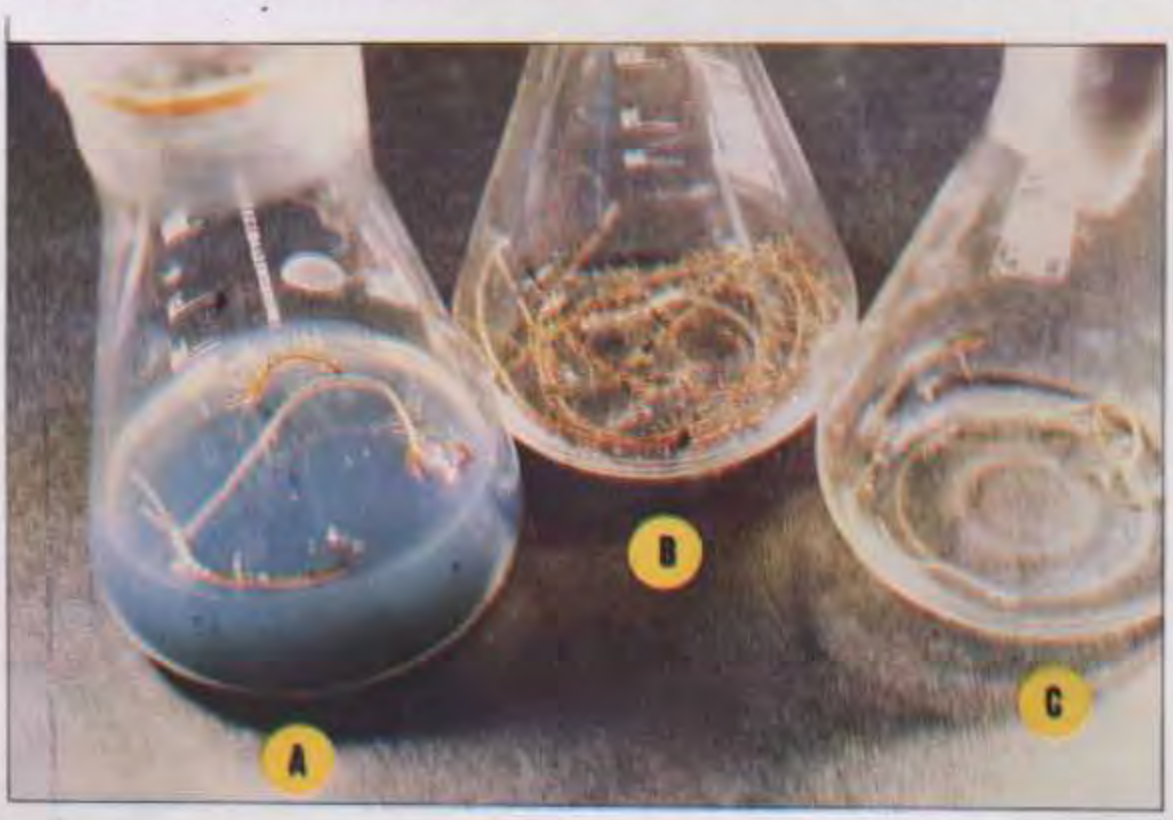

Figure 3. Root elongation in A) solid basal medium + activated charcoal, B) liquid basal medium + activated charcoal and C) liquid basal medium only.

TABLE 1. EFFECT OF ROOT EXPLANT SIZE ON ROOT GROWTH

Explant size	Percentage RT segments elongating	Average length increase in RT (cm)	Percentage producing axillary roots		Average number of axillary roots per explant		Average axillary root length (cm)	
(cm)			RT	RS	RT	RS	RT	RS
A. 1	67	1.0	33	40	1	4	0.5	0.5
B. 3	67	1.0	67	90	2	6	0.5	0.6

Clone: P114.
Number of cultures: 3 RT (segments with root tips) per treatment.
10 RS (segments without root tips) per treatment.

TABLE 2. EFFECTS OF IONIC STRENGTH OF MS MACRO- AND MICRO- NUTRIENTS ON GROWTH OF RT AND RS SEGMENTS

Media	Percentage RT elongating	Average length increase in RT (cm)	Percentage producing axillary roots		Average number of axillary roots		Average axillary root length (cm)	
			RT	RS	RT	RS	RT	RS
A. 1/2 × MS	17.6	5.8	23.5	8.3	3	2	7.9	8.6
B. 1 × MS	70.0	10.1	40.0	33.3	4	8	3.2	4.3

Clone: P75.
Number of cultures: 17 RT and 12 RS cultures per treatment A.
20 RT and 12 RS cultures per treatment B.

TABLE 3. EFFECTS OF SOLID AND LIQUID MEDIA ON ROOT GROWTH OF CLONE P99

Media	Percentage RT elongating	Average length increase in RT (cm)	Percentage producing axillary roots		Average number of axillary roots		Average axillary root length (cm)	
			RT	RS	RT	RS	RT	RS
A. Agar + AC	27	1.5	7	20	1	5	0.8	0.9
B. Liquid + AC	42	11.4	17	17	2	3	6.5	5.6
C. Liquid	13	8.0	7	0	1	0	3.0	0

Number of cultures: 15 RT and 15 RS cultures per treatment A and C.
12 RT and 12 RS cultures per treatment B.

observed after four months in culture (*Table 3*). Clone P66 was observed after nine months in culture (*Table 4*).

A higher percentage of RT segments elongated in liquid medium with AC (medium B) than on solid medium with AC (medium A) or in liquid medium without AC (medium C), and this was observed in both clones (*Tables 3* and *4*). The average increase in length of RT segments was also highest in liquid medium with AC for both clones. *Figure 3* shows the growth of root cultures in the three different media.

In general, RS explants appeared to produce axillary roots more readily than the RT explants, especially in the case of clone P66 (*Table 4*). With clone P99 (*Table 3*) it was observed that the RS explants cultured in liquid medium without AC (*Table 3, medium C*) turned dark brown and ceased growing.

Longer culture intervals may have also contributed to the higher percentage of elongation and increase in length of RT explants of clone P66 (9 months) as compared with clone P99 (4 months). Besides this factor, the clonal difference may also have contributed to the different responses.

Root multiplication

Excised root cultures were multiplied by subculturing the new roots, and then transferring them to fresh liquid medium containing AC. *Tables 5* and *6* show how the RT and RS segments of clone P99 (*Table 3*, medium B), multiplied after four months of culture.

Table 5 shows that 5 RT explants elongated and that the increases in length varied from 2.5 to 21.0 cm. During subculture, the new root growths were cut into segments each 2.0–3.0 cm long, giving a total of 5 new RT and 14 new RS explants. Two of the RT segments (root numbers 1 and 4) also produced axillary roots. Root number 1 produced two, measuring 7.0 and 4.5 cm respectively, and root number 4 produced an 8 cm long axillary root. After cutting these axillary roots into 2.0 to 3.0 cm segments, 3 new RT and 4 new RS explants were obtained. Thus, 5 initial RT explants were able to yield a total of 8 new RT and 18 new RS explants.

Multiplication in RS segments was similar to that in RT segments except that RS segments multiplied only by the production of axillary roots only. *Table 6* shows that two RS segments produced a total of 4 axillary roots with lengths varying from 2.5 to 12.0 cm. During subculture, a total of 4 new RT and 6 new RS explants were obtained.

From these examples, it can be seen that multiplication of RT segments depends on root tip elongation and regeneration of axillary roots, while RS segments depend only on regeneration of axillary roots. The number and length of axillary roots in RS and RT explants and the increase in length of root tips in RT segments will indicate the number of new explants to be obtained.

Culture maintenance

In an attempt to maintain excised roots *in vitro*, root explants (≈ 3 cm) from clones P123, P144-1 and P144-2 were cultured in liquid basal medium containing AC and 30 $g.L^{-1}$ sucrose. After 4–5 months of culture, the new root growths were cut into 2–3 cm long explants and then transferred to fresh liquid basal medium.

Initially P123 showed an increase in the number of root explants produced (*Table 7*), which was largely contributed by the number of axillary roots regenerated in RT and RS explants: these became RT explants in the next subculture. The other two clones also produced some growths but they were not as vigorous as P123. After going through the first or second subculture, many new root explants turned brown and stopped growing or produced much thinner growths than the initial explants. Thin roots tend to lose growth vigour (poor elongation and less initiation of axillary roots) and they also brown easily. The poor growth eventually brings down the number of new explants produced in the subsequent subcultures. This experiment was terminated after the fourth subculture (≈ 18 months). Ahée and Duhoux (1994) also reported that excised root cultures of *Faidherbia=Acacia albida* became less vigorous in the successive subcultures. After 5 subcultures, a large number of the roots ceased to grow.

TABLE 4. EFFECTS OF SOLID AND LIQUID MEDIA ON ROOT GROWTH OF CLONE P66

Media	Percentage RT elongating	Average length increase in RT (cm)	Percentage producing axillary roots		Average number of axillary roots		Average axillary root length (cm)	
			RT	RS	RT	RS	RT	RS
A. Agar + AC	73	4.5	0	20	0	1	0	5.1
B. Liquid + AC	93	14.2	13	33	1	2	9.0	5.0
C. Liquid	53	2.4	0	13	0	3	0	3.8

Number of cultures: 15 RT and 15 RS cultures per treatment.

TABLE 5. MULTIPLICATION OF EXPLANTS IN RT SEGMENTS OF CLONE P99

	Root number	Increase in length of root tips (cm)	Number of new explants ≈ 2.0–3.0 cm		Length of new axillary roots (cm)	Number of new explants ≈ 2.0–3.0 cm	
			RT	RS		RT	RS
	1	7.0	1	1	7.0	1	1
					4.5	1	1
	2	8.6	1	2	–	–	
	3	2.5	1	–	–		–
	4	21.0	1	6	8.0	1	2
	5	18.0	1	5	–		
						–	–
Total	5		5	14		3	4

TABLE 6. MULTIPLICATION OF EXPLANTS IN RS SEGMENTS OF CLONE P99

	Root number	Length of axillary roots	Number of new explants ≈ 2.0–3.0 cm	
		(cm)	RT	RS
	1	2.5	1	–
	2	12.0	1	3
		7.5	1	2
		4.2	1	1
Total	2		4	6

TABLE 7. MULTIPLICATION OF ROOT CULTURES

Clone number	Number of initial cultures		Number of new roots produced					
			First subculture		Second subculture		Third subculture	
	RT	RS	RT	RS	RT	RS	RT	RS
P123	3(100)[a]	6(33)	15	3	4	1	9[b]	1[c]
P144-1	6(100)	6(0)	6	0	1	0	0	0
P144-2	3(100)	6(16)	5	1	4	3	2[c]	0

[a]The figure in brackets is the percentage of root segments producing new root growths.
[b]6 of the 9 explants were less than 1 cm in length and therefore were not cultured.
The remaining 3 were subcultured a fourth time.
[c]The root cultures were transferred to the fourth subculture.

This exercise shows that it is possible to maintain excised roots of oil palm *in vitro* but the medium needs to be improved to sustain the new growths for longer periods. The liquid medium supports root elongation better than the solid medium and addition of AC helps to reduce browning (*Tables 3* and *4*). It was observed that RT segments gave a higher percentage of growth than RS segments (*Tables 2, 3, 4* and *7*). The increase in new root explant number is contributed by the elongation of root tips and production of axillary roots. RT segments elongate from the pre-existing root tip meristems while axillary roots regenerate from pre-existing and newly developed root primordia which are found along the root length. RS segments lack root tip meristems; therefore they cannot elongate. However, the root primordia present along their length can regenerate into axillary roots. These axillary roots possess root tip meristems and they become new RT explants in the subsequent subculture. If they elongate more than 3 cm, the axillary roots can yield both RS and RT segments in the next subculture. Further experiments are in progress to optimize the culture media and protocols in attempts to improve the growth vigour of the subcultured root explants *in vitro*.

Shoots derived from excised root cultures must be evaluated in the field for abnormalities, and this is the objective of on-going studies.

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