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ROYAUME DU MAROC

AL AWAMIA

REVUE DE LA RECHERCHE AGRONOMIQUE MAROCAINE

- Tissue Culture
- La fumure phospho - azoté du riz
- Contribution du centre d'agriculture de SETTAT
- Agrotechnie des principales legumineuses fourgères

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Review of Date Palm (phoenix dactylifera L.) Tissue Culture

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ABSTRACT

Various laboratories in the world have made attempts to propagate date palm, coconut and oil palms by tissue culture techniques ; success has been achieved with both date and oil palms.

In reviewing date palm tissue culture, the classification followed will be that of behaviour and relevant techniques of tissue culture as a whole from a perspective of their eventual applications to date palm.

Date palm plantlets may be produced through either ; asexual embryogenesis, i.e. initiation and germination of somatic embryo from callus, or organogenesis, i.e. rooting and division of shoot tips and lateral buds. This review explains the background to the cloning methods applied to the date palm and explores the wide range of results obtained with embryo culture, meristematic tissues (shoot-tips and buds) and highly differentiated somatic tissues (leaf, stem, inflorescence and root sections). The browning of tissues and media in date palm tissue culture is also reviewed.

INTRODUCTION

Palms are a much neglected plant group in terms of understanding their development and vegetative propagation potential, yet they are economically important in tropical and sub-tropical regions. Date palms, Phoenix dactylifera L., are usually produced by juvenile palms. The rapid propagation, as well as propagation from a mature specimen, is impossible due to the limited number of offshoots produced and the fact that offshoot production is limited to a certain period in the palm's life span. Seed propagation of

date clones and cultivars is impractical for several reasons. Half of the progeny will be males and half will be females. Further there is no certain way to detemine the sex of progeny nor their fruit or pollen quality prior to flowering (3). Also, seeding females usually produce late maturing fruits of variable and generally inferior quality to that of established clonal trees (56). The bayoud disease (Fusarium oxysporum f.sp. Albedinis) had killed ten million palms in Morocco by 1958 (32), including the greater part of the vigorous and productive palms of the best commerical varieties, such as Nedjool and Bou Fegouss. Some resistant clones are found in areas where palm plantations have been destroyed. The use of these resistant varieties is the most pratical means of controlling the disease (24).

The success of propagating monocotyledons in vitro has been limited to relatively few herbaceous species (5,7). Similarly, most dicots, successfully tissue cultured, have also been the herbaceous types (23). It has been postulated that in woody plants the ability to regenerate plantlets using tissue culture techniques was lower in comparison to herbeceous plants (33). In palms, until ten years ago, little success was achieved in inducing and maintaining good callus (42, 46, 50). Aside from the induced natural vegetative propagation means previously discussed, palms cannot be rapidly propagated to meet demand. Plant tissue culture techniques have been employed to clone a wide range of plants (28) and economically important palms e.g., coconut (12), oil (37) and date palms (42, 51, 52).

The application of tissue culture techniques to date palm would enable : 1) Distribution in large numbers of genetically uniform date palms. 2) Bayoud resistant cultivars, or males having superior pollen with useful metaxenic characteristics to be rapidly propagated. 3) Clones to be propagated from elite cultivars already in existence, or from the Fl hybrids of previously select, and seed-only originated palms, as well as from strain selected from yield.

EMBRYO CULTURE

Introduction.

Embryo culture involves excising an embryo-aseptically from the seed and planting it in a sterile nutrient medium (21). Embryo culture has suggested to have several potential applications in plant research. It is used to save embryos that fail to develop naturally in the fruit or seed, or grow out embryos from interspecific hydridization where defective endosperms are common (18, 22). Also embryo culture may be used to reduce lengthy dormancy periods due to physical and/or chemical inhibitors present in the fruit or seed (21). Excised embryos cultured in vitro, free from these inhibitors, usually germinate immediately. Isolated embryos were also chosen as explant material in metabolic studies (38). The culture of isolated embryo segments may be useful to study the development of the primary meristems, organogenesis and the interactions between different organs (36). The culture of embryo outside the seed was first performed with crucifers (19). It has since become a routine procedure.

Regeneration of plantlets from date palm embryo-explants. Callus initiation and embryoid induction was first observed by Rabéchault (35) working with oil palm embryos. Reuveni (40) reported that callus and roots developed from the date palm embryo cotyledonary sheath tissue in media containing NAA. This callus continued to proliferate and to differentiate roots when subcultured if a piece of the cotyledonary sheath was present. Ammar & Bendadis (1) established organogenic callus from date palm cotyledonary sheath of zygotic embryo germinated in vitro.

Reynolds & Nurashige (44) cultured embryo explants of Chamaedores costaricana Oerst, Howeia forsteriana Becc., and Phoenix dactylifera in vitro. Green date palm fruits, harvested two to three months after pollination were planted in a medium enriched with 2,4 -D, and a creamy-colored grainy callus was subsequently developed. Transfer of this callus to an auxin-free medium resulted in the development of numerous asexual embryos. Mature zygotic embryos cultured in nutrient media containing charcoal with high auxins levels, 10 and 100 mg/1 NAA, also produced nodular callus (40, 52, 59). Repeated culture resulted in formation of plantlets. Tisserat & Demason (53), described plantlet formation from date palm tissue cultures. The development of asexul embryos from callus closely paralleled excised zygotic embryo germination in vitro (Gf. Fig. 1). There is a similarity in morphology of the asexually derived and the zygotically attained embryos in date palms (43,53).

Zaid and Tisserat (62) had performed a survey study to determine excised embryo callus production. In the Arecaceae. Embryo excised from mature seeds of 38 species were cultured on modified Murashige and Skoog medium containing 3g L-1 activated charcoal ; with 100 mg L-1, 2,4-dichlorophenoxyacetic acid and 3 mg L-1 N6- (2 - isopentyl) adenine. Embryo cultures from 18 of these species produced prolific callus after repeated reculturing for six months (Cf. Fig 2). Zaid and Tisserat (63) had also cultured embryos of date palm to follow their development. The sequence of germination is show in Figure 3.

CULTURE OF DATE PALM MERISTEMATIC TISSUES

A. Introduction

When comparing shoot-tips and lateral buds in vitro versus culturing other explant sources, the following advatages become apparent :

1) Shoot-tip and lateral bud are protected by bud scales and leaves, and are usually easier to surface-sterilize than root or stem explants (26).

2) By culturing shoot-tips or buds, an entire shoot is already present, thus only root induction being required to produce a whole plantlet (27,58).

3) The cells of the shoot-tips and buds are more uniformly diploid than those derived from less meristematic regions (20,29). Presumably, plantlets derived from naturally meristematic regions are likely to be clonal and generate faster than other explant sources.

A distinction is made between bud and apical meristem cultures (58). Lateral bud culture involves the growth of an entire rudimentary vegetative shoot. Apical meristem culture, ideally involves only. the excision and growth of apical dome of the shoot usually less than 0,1 mm in diameter and 0,25 mm in length, sometimes with, though preferably without, a few leaf primordia attached (8). In contrast to culturing herbaceous angiosperm shoot apices, few woody angiosperm shoot-tips has been established in vitro (9,39).

B. In vitro culture of date palm shoot-tips

Schroeder (46) and Staritsky (50) employing date and oil plams

respectively, cultured schoot-tips in vitro with some success. However, most of excised shoot-tips either failed to grow or showed no root differentiation.

Reuveni et al (42) found that growing tip cutures of date palm responded irregularly to growth regulators, but optimal leaf development occured when media contained 0,1 mg/1 NAA and 0,01 mg/1 kinetin. Callus occasionnally formed at the cut surface of the tip, particularly in dim light, when low concentrations of auxin and/or cytokinin were present.

Generally this callus was very short lived and its subculture was unsuccessful (40, 41).

El Hannawy & Wally (15° observed some bud differentiation in date palm cultures. They reported that by adding 200 mg/l "fermentol" to MS medium containing 1,0 mg/l auxin and kinetin, and using an incubation temperature of 3520,60 percent bud differentiation occured. Scharma et al (47) using date palm shoot-tips reported limited success in their development due to the browning of the tissue and media. Tisserat (51,52) culturing date palm shoot-tips found that a high auxin concentration of 10 and 1000 mg/I NAA and 2?4 D caused a reduction in the culture weight, and inhibition of shoot growth, but promoted the formation of yellow-white nodular callus. These nodules were precursors to asexual embryos. Transfer of callus to nutrient medium containing lower levels of auxins such as 0,1 mg/ l NAA or 2,4 D allow shoot development from tips to occur. Male and female shoot-tips were found to grow equally well. Root initiation was infrequent and did not appear to be related to the nutrient medium composition.

Very recently Zaid and Tisserat (60) had cultured Phoenix dactylifera L. shoot tip explants from adult trees, offshoot, seedlings and asexual plantlets on modified Murashige and Skoog nutrient media containing 10 mg/1 NAA. Differential morphogenetic responses were obtainded dependent on the explant type and parent source (Cf. Table 2). The same authors had also determined the action of several auxins and cytokinins on development of Phoenix dactylifera L. seedling shoot tips and apical meristems. Shoot tip explants consisted of the apical dome with two to four leaf primordia and varied in size from 0,5 to 1 mm2. Meristems and tips were cultured on modified Murashige and Skoog medium (30) containing 3 mg L-1 activated charoal, 0,1-300 mg L-1 —naphthaleneacetic acid (NAA), 2,4 - dichlorphenoxyacetic acid, indoleacetic acid, indolebutyric acid, 4 - chorophenoxyacetic acid, N6 - benzyladenine, 6 - furfurylaminopurine or N6 (2 - isopentyl) adenine. Best consistant shoot regeneration occured on nutrient media containing 10 mg L-1 NAA (Cf. Fig. 4). These shoots were recultured on nutrient media,

devoid of charcoal, containing 10 mg L-1 NAA or kinetin to obtain rooting and enhanced shoot development. Best rooting was achieved with 0,1 mg L-1 NAA (Cf. Fig. 5) with 63% of the shoots initiating adventitious roots after the first culture passage. Axillary bud outgrowths were occasionally obtained from shoots cultured on media containing 0,01 and 0,1 mg L-1 NAA only.

In vitro culture of date palm buds. Most buds of date palm were reported to die within the first 30-50 days after planting in vitro (42, 46). Only the largest and most distinctly differentiated buds grew. These buds exhibited leaf expansion and produced additional leaves (41). Organ culture of the date palm was also attempted by Brochard (4), but according to the published report apparently not much success has been achieved, Tisserat (51) and Zaid (59) also investigated the conditions for bud development, and found that in nutrient medium shoot-tips and leteral buds grew equally well on the same medium. Callus cultures have been initiated from axillary buds of 2,4 year old date palm offshoots.Zaid and Tisserat (61) found that subcultured lateral buds callus on nutrient media devoid of charcoal and supplemented with 0,1 mg/1 NAA, produced adventitious plantlets (Cf. Fig. 6). Tisserat and DeMason (53) found that on a medium devoid of 2,4 D and N6 (2-isopentyl) adenine (21P), sectioned buds callus consisted of two distinct types of tissues ; a loose friable tissue and compact aggregates. The friable portion of the callus was composed of large non-meristematic cells and disorganized clumps which were highly vaculated and ranged in diameter from 20-40 µm. This tissue was not involved in embryo formation and was generally found surrounding the aggregate clumps which consisted of densely cytoplamic cells containing few vacuoles and usually were 8-20 Mm in diameter. The formation of vascular bundles wilthin the asexual plantlet at the 8 week old stage corresponded to that found in the zygotic seedling. Embryogenic callus cultures derived from date palm lateral buds were subjeted to : 0,-15, -23,-30 and -196°C for up to 3 months (55). Revived cultures developed callus and plantlets.

CULTURE OF HIGHLY - DIFFERENTIATED SOMATIC TISSUES IN VITRO

1. Leaf cultures. Callus has developed from a seedling date palm leaf (46). This callus gave rise to roots several months later. Similar results were obtained by Reuveni & Kipnis (41). In their study, primordial leaves survived in culture and expanded, especially in the presence of light. The addition of plant growth regulators at concentrations of O.l mg/l and above was injurious to cultured leaves.

Eeuwens & Blake (14) working with date palm leaf found development of root initials to be enhanced by the presence of a low level of gaz and auxins, and by a reduction in either the level of minerals or sucrose. Phoenix leaf petiol explant has initiated roots within 6 weeks when subcultured onto a medium with high levels of auxin (13). Root initiation was not prevented by the presence of high cytokinin or low sucrose levels, but occurred more frequently in media containing high sucrose and reduced cytokinin levels. Poulain et al (34) obtained some callus at the base of young date palm leaves. Buds developed at the insertion zone between young leaves and rachis. Roots were obtained on MS supplemented with a combination of low auxin levels such as 1,2 and 3 mg/l NAA, IBA, and IAA respectively. Scharma et al (47) noted callus from leaf petiols of date palm initiated in media employed by Staritsky (50) or using Eeuwens Y/3 mineral formulation (12).

Zaid (59), working with date palm leave explants from adult trees, offshoots, seedlings and asexual plantlets found that only subcultured leave callus from seedling and asexual plantlets produced roots. No further morphogenetic response was observed. Rhiss et al (45) and Drira (II) had also obtained some organogenetic callus from offshoot leave sections (Cf. Fig 7).

2. Stem culture Staritsky (50) and Smith & Thomas (49), both working with oil palms, and Eeuwens (13) with coconut and date palm, obtained a white callus on a few stem cultures. Further attempts to subculture this callus failed.

Phoenix stem explants reportedly enlarged considerably in size during the first few weeks of culture (51). Repeated reculture to fresh media resulted in the formation of nonfriable nodular callus. Plantlets were developed from this callus. Poulain et al (34) working with date palm stem tissues also successfully initiated callus.

3. Inflorescence culture. Inflorescences of several species have been cultured in vitro (31). Since 1973, several workers attempted to culture palm inflorescences. Explants of females and male oil palm inflorescences were cultured on a variety of media and usually developed somewhat normally (49).Callus was not obtained.They suggested that explants from immature inflorescences are more likely to callus from mature material. Further, a high auxin level was speculated to be necessary to disrupt normal development. This has subsequently been confirmed in date palm (14).

Date palm ovules, carpel tissue, parthenogenetic endosperm, and the fruit stalk blackened within 24 hours after culturing on nutrient media and subsequently died (41). Also cultures of date palm

floral bud reproductive tissues and especially male anthers, usually turned brown and died after a few weeks in culture (54), de Mason et Tisserat (53) found that in vitro applications of auxins to media dia increase the frequency of visible expanded carpels developing from supposedly date palm male flowers. Furr and Ream (17) tested the effects of temperature in date pollen germination in vitro. Optimal results were obtained at 80°F.

Vestigal female date carpels on surviving male flowers enlarged and became quite prominent (51). White friable callus usually initiated from the floral bud strand (54). In some cases, roots and embryoids were initiated from explants of Cocos inflorescences rachillae (13) and from date palm (52). Roots have not been initiated on inflorescence rachis explants which lack leaf or meristem tissue.

Date palm inflorescence culture was also largely investigated by Drira (11). Morphogenetic responses were found dependent on the origin and physiological stage of the explant.

4. Root culture. Staristsky (50) and Schroeder (46) were the first to investigate root cultures in palms in vitro. Oil palm root and root primordia failed to develop (50). Schroeder (46) observed that date palm root pieces in turn developed secondary rootlets but did not produce shoots. Eeuwens (13) found that isolated roots excised from cultured explants of date and coconut palms continues growth and produce laterals when subcultured on liquid static media. Callus was also reported to form at the roottip region of young date palm seedlings (48,49). This callus had produced leaves and shoots. Other investigators (47) reported no growth for cultured date palm roots. Usually, severe browning and death of the root explants occurred within the first few weeks of culture. However, Zaid and Tisserat (61), obtained some callus from seedling and asexual plantlets roots when callus failed to exhibit any morphogenetic response.

BROWNING OF TISSUES AND MEDIA IN DATE PALM TISSUE CULTURE

During the course of growth and development in vitro, plant tissues not only deplete the nutrients that are furnished in the medium, but also release substances that can accumulate in the cultures. These substances, such as phenols, may have profound physiological effects on the cultured tissues. Date palm tissue cultures, like those of many other palnts, have been commonly observed to release discoloring substances into the medium, which inhibit their own growth. For date, injury through cutting of tissue is

accompanied by secretion of the substance into the medium. The intact organ, as exemplified by embryos or whole leaves on tips do not brown and thus grow well in culture (41). Browning of the tissue and the adjacent medium is assumed to be due to the oxidation of polyphenols and formation of guinones which are toxic to the tissues (25,42).

To minimize browning, Murashige (28), has suggested the presoaking explants in ascorbic and citric acid sollutions and adding them to the culture medium. Zaid and Tisserat (60,61) soaked their date palm explants in an anti-oxidant solution (150 mg/l citric acid and 100 mg/l ascorbic acid) prior to the surface sterilization treatments. Addition of a combination of absorbants including citrate, adenine, glutamine, and PVP retarded browning in date palm explants (45).

Addition of other absorbants to nutrient media such as dihydroxynaphtalene, dimethylsulfoxide, were ineffective against browning in date palm explants (41,51). Apavatjrut and Blake (2) suggested that browning could be eliminated by a nutritionally balanced medium. Excision of browning explant parts during culture was also advocated to prevent this problem (49).

The use of charcoal is preferred over cysteine and other adsorbants because the latter are often toxic to the plant tissues at higher concentrations (47). Addition of 3 percent charcoal has caused substantial root and shoot growth of Phoenix dactylifera embryos (44,52,57,60,61). Constantin et al. (6) suggested that the phytohormones required for callus growth and shoot development for tobacco are absorbed by charcoal addition. Similarly, Fridborg & Erikson (16), postulated that the addition of charcoal to a culture medium drastically alters the properties of the medium. Hence, growth regulator substances are tested at high levels (e.g. 10 and 100 mg/l) with charcoal included in the nutrient media to obtain beneficial effects on tissues (44,51,57,60,61).

Fig 1 : Comparison of asexual embryos (left) derived from lateral bud callus with excised zygotic embryos (right) at the cotylegon elongation stage. (Source : B. Tisserat, 1979, Jour. of Exp. Bot. 30) (119).

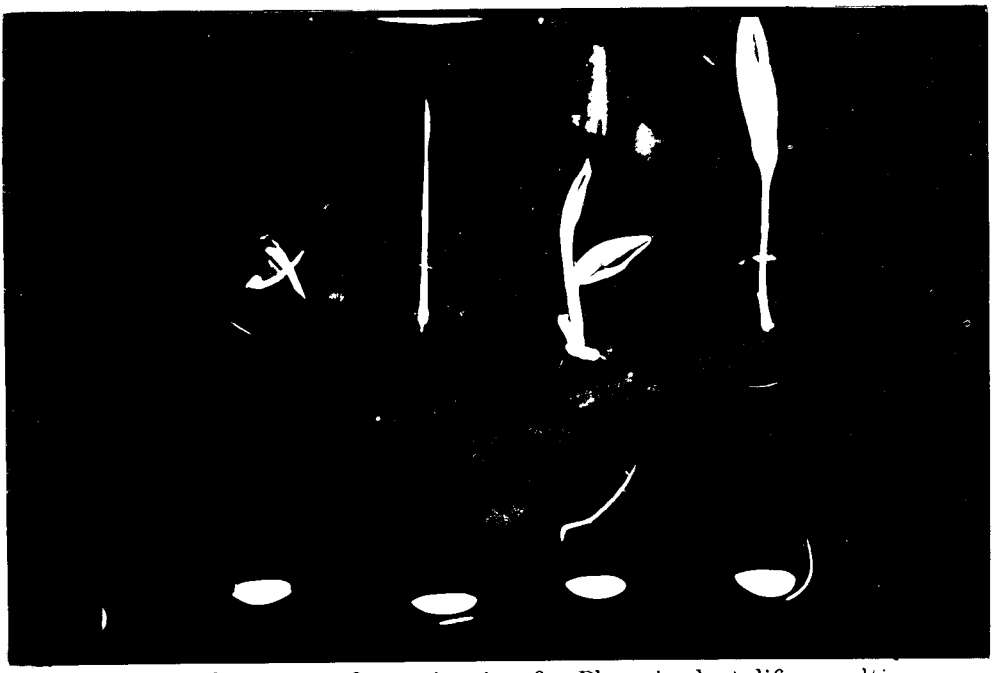

Fig 3 : Sequence of germination for Phoenix dactylifera cultivar Sayer excised embryos cultured on a modified Murashige and Skoog medium containing 0,3 activated charcoal. From left right : earlu cotyledon elongation stage (1-Week old); emergences of first foliar leaf (3-week old); and established seedling in vitro (6-Week old). Note that the cotyledon haustorium is much reduced in size in all stages of seedling development.

Fig 4 : Morphogenetic effect of NAA on date palm shoot tips after 8 weeks in culture. Explants were cultured on a modified Murashige and Skoog nutrient medium containing (from left to right) : 0.0, 0.01, 10, 0.1 and 1.0 mg/l NAA. Note that the center culture containing 10 mg/l NAA produced the largest shoot.

Fig 5 : Comparison of the rooting responses obtained by reculturing dates shoots to media containing 0.01 and 0.1 mg/l NAA. First two cultures to left contain 0.1 mg/l NAA ;second two cultures on right contain 0.1. mg/l NAA. Note that both shoot and root development was enhanced by culture on media containing 0.1 mg/l NAA.

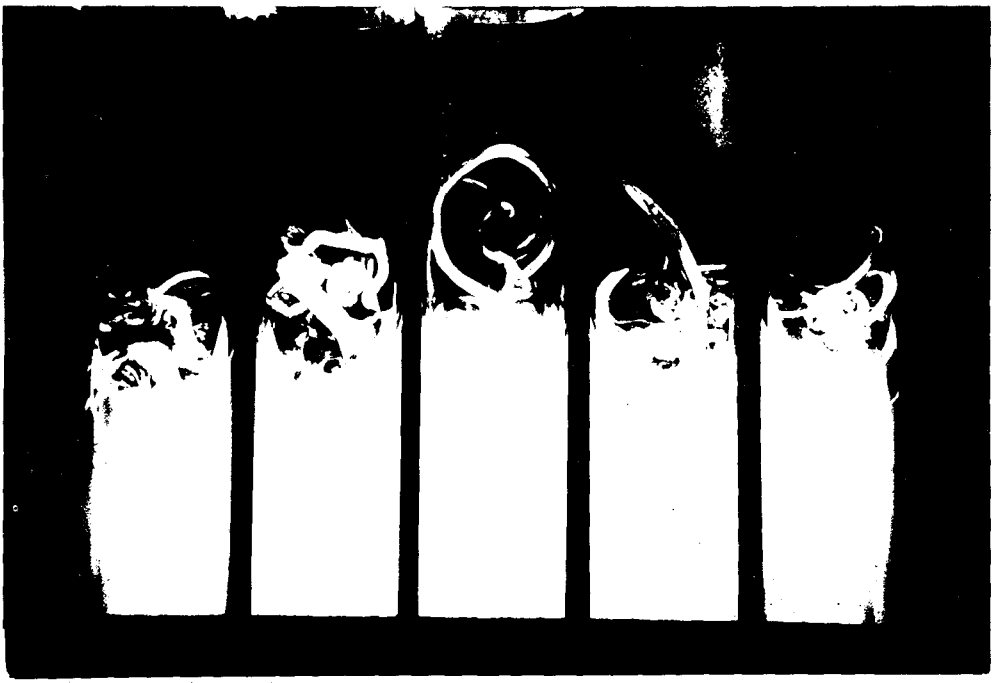

Fig 6 : Examples of organogenetic and embryogenetic responses obtained from date palm shoot tips and leafy bud cultures on basal media containing 0.1 mg L INAA. From left to right : offshoot shoot tip callus and embryoids, lateral bud culture, offshoot shoot tip culture adventitious plantlet from shoot tip calli, and lateral bud calli and ambryoids.

TABLEAU 1 Influence of growth regulators on the growth of date palm shoot tips.

Test levels (mg/l)	Shoot growth (%) Growth Regulator Type					
	NAA	2,4-D	IAA	Kinetin	BA	2iP
0.0	58	50	42	80	58	80
0.1	58	—	—	60	67	60
0.3	67	47	33	40	58	40
1.0	58	53	42	53	50	53
3.0	67	53	33	66	58	66
10.0	75	53	33	73	33	73
30.0	—	67	50	—	33	—
100.0	75	13	—	60	42	60
300.0	58	6	25	53	42	53

TABLEAU 2. Morphogenesis obtained from shoot tip cultures derived from various explant sources. [1]

Exploant sources	Syrvival/ treatment (%)	Shoot growth/ culture (%)	Shoot length/ culture (%)	leaves/ culture	Rooting/ culture (%)
Adult tree	70	85	2.12 ± .71	1.5 ± .5	0
Juvenille offshoot	78	80	2.75 ± .69	2.5 ± .6	0
Seedling	85	100	2.35 ± .65	2.0 ± 0.0	60
Axexual plantlet	95	100	1.67 ± .39	2.2 ± .4	80

1 15-20 cultures employed per treatment ; results taken 8 weeks after planting. Shoot tips were planted on a modified Murashige and Skoog tips were planted on a modified Murashige and Skoog medium containing 10 mg L^{-1} NAA

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