Grafting of Breadfruit (*Artocarpus altilis*) Using Breadnut (*Artocarpus camansi*) as Root Stock

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Keywords: Sri Lanka, cleft graft, vegetative propagation

Abstract

Breadfruit, *Artocarpus altilis*, is popular in Sri Lanka and it is believed to have been introduced in the 14th century during the Portuguese era. Since it is seedless, it must be vegetatively propagated, usually by root cuttings or root suckers. A successful propagation method is needed to multiply and conserve superior accessions. A study was conducted at Horticultural Crops Research and Development Institute, Gannoruwa to assess the success of cleft grafting using three seedling age classes (30, 45 and 60 d) of breadnut (*Artocarpus camansi*) as root stock. Randomized Complete Block design was used with 60 plants per treatment with three replicates. Preliminary observations were also made on seasonality and the seed germinability of breadnut. Both species have a similar production pattern with the main season May-July and a minor season September-November. Average germination rate of breadnut was 98% and seeds germinated in 10-15 d. The success rate of grafting was 83% and significant (P=0.05) when seedlings were grafted at 45 d. The grafted plants were ready for field planting in 3 months and fruiting commenced 2½-3 yrs after planting. The trees have low branching habits, were early bearing and the average yield of first harvest was 10 fruits.

INTRODUCTION

The origin of the breadfruit, *Artocarpus altilis* (Parkinson) Fosberg, is reported as Micronesia and Polynesia (Ragone, 1997). It is believed to have been introduced to Sri Lanka in the 14th century during the Portuguese era. Breadfruit is a widely grown, nutritious tree fruit. It is more important as a subsistence crop than as a commercial crop in most areas of the world, especially in the Pacific islands, where it is an important staple crop. The fruit is typically roasted or boiled, and occasionally fried as chips. Breadfruit grows well on different types of soils with sufficient depths and drainage. Regular annual rainfall of 200-300 cm and high humidity of 70-90% are preferred. The tree is well suited to hot, humid tropical lowlands and temperature up to 35°C. The tree grows well under direct sunlight and it is seasonal in production.

Genetic erosion of many clonally propagated traditional crops, including breadfruit is a serious problem in the Pacific islands. Although it is an important staple crop, the cultivation and use has decreased in the past 50 years, and replanting has not kept pace with the losses incurred throughout the Pacific by drought, storm damage, natural attribution and other factors (Ragone, 1997). Absence of successful propagation method may be one of the reasons.

The breadfruit shows morphological variability ranging from true seedless fruits to fruits with numerous seeds. A related seeded species (breadnut, "kamansi," "kelur") is known as "Kos Del" in Sri Lanka. The rind of breadnut, *A. camansi* Blanco, is covered with fleshy prickles and the fruit has very little edible pulp with numerous seeds. Seedless breadfruit, called "Rata Del" is more widely used by Sri Lankans than breadnut. Many good selections have been found and listed by extension staff If the Department of Agriculture. Collected samples showed much variation in size, shape and taste. Therefore it is necessary to have a successful propagation method to multiply and conserve these accessions.

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Seedless breadfruit must be propagated by asexual or vegetative means (Rowe-Dutton, 1976). The most popular methods in the Philippines are marcotting and root cutting (Otanes and Ruiz, 1956). In Sri Lanka it is usually propagated by root cuttings or root suckers. The success rate is very low and removal of roots is detrimental to the mother plant. Different propagation techniques are needed. Wester (1914) and Padolina (1931) reported that grafting or budding breadfruit onto breadnut failed. However, both of these species have been successfully propagated by inarching and budding using breadnut and jackfruit as rootstock. In India the seedless types are propagated by root suckers, root cuttings and air layering of root suckers. Root cutting is the commercial method and the success rate was 90% when horizontally planted (Veeraragavathatham et al., 1996). Studies were initiated at the Horticultural Crops Research and Development Institute (HORDI), Gannoruwa to propagate breadfruit by cleft grafting using breadnut seedlings as the rootstock. Preliminary observations were also made on variation of breadfruit seasonality and seed germinability of breadnut.

MATERIALS AND METHODS

Ripe breadnut fruits were collected from trees growing in the HORDI research field. The seeds were extracted and weight, length and circumference was recorded for 100 seeds. The seeds were planted in polybags (15 × 20 cm) filled with the mixture of sand, topsoil and dried cow dung with a 1:1:1 ratio. Number of days to 50% germination was recorded. Breadfruit scions were cleft grafted to the breadnut seedling rootstock at three different age (30, 45 and 60 d) classes. A Randomized Complete Block design was used with 60 plants per treatment with three replicates. The scion wood was collected from superior mother plants. Bud wood was collected 2 m after fruits had been harvested from the tree. The root stock was decapitated 15 cm above soil level and split to a depth of 2 cm. The scion was inserted and tied using grafting tape. Grafted plants were enclosed in a high humidity chamber (polythene cover) for 1½ ms (Fig. 1A) to prevent evaporation followed by 20 d in propagators and were then kept under shade nets (70%) for another 20 d. The success rate was recorded after removing the polybags. Three ms after grafting the successful plants (Fig. 1B) were established in the field and records were taken on flowering, fruiting and yield.

RESULTS AND DISCUSSION

Seeds are large with an average weight, length and circumference of 7.3 g, 2.9 cm and 7 cm, respectively. The rate of germination was 98% and seeds germinated in 10-15 d. Table 1 shows the success rate of breadfruit grafted on different age classes of breadnut rootstock. The success rate was 83% and significant (P=0.5) when seedling were grafted at the age of 45 d. The percentage success was low on seedlings at 30 and 60 d due to tenderness and maturity of the stem, respectively. The grafted plants were successfully grown in the field and fruiting commenced 2½ -3 years after planting. The plants have low branching habits and were early bearing (Fig. 2). The average number of fruits per tree at first harvest was 10. Cleft grafting proved to be a simple and effective method of rapidly propagating seedless breadfruit and this method is recommended to mass multiply and distribute selected cultivars.

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Tables

Table 1. Percentage of successful cleft grafts of breadfruit on different age classes of breadnut rootstock.

Age of rootstock (days)	Success rate[1] (%)
30	45 b
45	83 a
60	25 c

[1] The value denoted with a different letter is significantly different (P=0.05); LSD = 9.87; CV = 15.1%

Figures

Fig. 1. Breadfruit scion cleft-grafted to a breadnut rootstock. A. Grafted plant covered with polybag for 1½ months. B. Grafted plants ready for field planting 3 months after grafting.

Fig. 2. Grafted breadfruit trees began bearing fruits in 2½-3 years.

In Vitro Regeneration of *Artocarpus camansi* and *Artocarpus altilis*

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Keywords: breadfruit, breadnut, "chataigne," propagation, plant tissue culture, Caribbean

Abstract

A protocol for the in vitro regeneration of *Artocarpus altilis* (breadfruit) and *Artocarpus camansi* ("chataigne," breadnut) is outlined. Factors influencing culture establishment, production of microshoots, rooting of microcuttings and acclimatization of breadfruit, and "chataigne" plantlets are discussed. The addition of 2 g/L polyvinylpyrrolidone (PVP) significantly reduced oxidative browning of explants of both species. Aseptic cultures of mature meristem and shoot tip explants, juvenile shoot tip and nodal explants were successfully established after exposure to antibiotic and fungicide pretreatments and antibiotic medium treatments. Shoot tip and nodal seedling explants of "chataigne" were established on MS supplemented with 4.4 µM BA and mature meristem, explants of breadfruit on N5K and N15K macronutrients and MS micronutrients and vitamins with BA supplied at 4.4 µM. Shoot multiplication of both species was achieved by subculturing secondary shoot tip and nodal explants on medium which consisted of N30NH$_4$ macronutrients, MS micronutrients and vitamins, supplemented with zeatin 2.2 µM which was more effective than BA at this stage. IBA supplied at 10^{-6} M enhanced in vitro rooting of breadnut microcuttings when compared to IAA and NAA at similar concentrations. Breadfruit microcuttings rooted on basal medium as well as on multiplication medium. Acclimatization of plantlets of both species was possible in vermiculite or jiffy pellets under humid conditions. Approximately 40% of the plantlets were successfully hardened under greenhouse conditions. The in vitro regeneration of both of these species has application for micropropagation, germplasm exchange and preservation and crop improvement.

INTRODUCTION

Breadfruit, *Artocarpus altilis* (Parkinson) Fosberg and "chataigne" or breadnut (*Artocarpus camansi* Blanco) belong to the Moraceae family. The seeded "chataigne" is a close, wild relative of breadfruit (Zerega et al., 2004). Breadfruit is propagated vegetatively by root cuttings and root suckers whereas "chataigne" is propagated by seed. Breadfruit is an important year-round food source in the Caribbean (Roberts-Nkrumah and Badrie, 2005). The potential of breadfruit as a crop has not been realized primarily due to plant factors such as slow propagation rates of traditional methods, seasonality of bearing, short fruit shelf-life and tall habit of the plant making harvest difficult (Ferguson, 1982).

Plant tissue culture techniques allow mass clonal propagation, germplasm conservation and exchange and improvement of crop species (Trigiano and Gray, 2005). In vitro technologies are increasingly important for preserving diversity, particularly of vegetatively propagated species such as breadfruit, jackfruit and "chataigne" that produce recalcitrant seeds which do not store well (Towill, 2005). As such the development of protocols for in vitro culture and preservation of breadfruit and "chataigne" are necessary. Tissue culture propagation of breadfruit was first reported by Rouse-Miller and Duncan (2000), and of "chataigne" by Rouse (1993). Successful in vitro propagation of *Artocarpus heterophyllus* Lam. (jackfruit), another member of the Moraceae family, using seedlings (Rahman and Blake, 1988) and mature tissues (Amin and Jaiswal, 1993) has been reported. This paper outlines in vitro regeneration studies on "chataigne" and

breadfruit with emphasis on establishing aseptic cultures, axillary shoot production, rooting and acclimatization.

MATERIALS AND METHODS

Donor Plants and Explants

The shoots of 6-9 week-old "chataigne" seedlings grown in sand, and juvenile breadfruit root suckers formed on root segments, buried in sand in a propagator, were collected from the greenhouse. Before sterilizing, the laminar portion of the leaves were removed and the stems cut into single- and two-node segments and shoot tips (shoot meristem and 2-3 subjacent leaf primordia). After sterilization, meristem (1-2 mm), shoot tip (3-5 mm) and nodal (5-15 mm) explants were prepared and incubated singly on nutrient medium.

Terminal shoots 60-90 cm long and 30 cm long pieces of semi-hardwood and hardwood stems were collected from a mature 'Yellow Heart' breadfruit accession held at the University of the West Indies. Before sterilization, the leaves of the terminal shoots were removed before subdividing the stems into single- and two-node stem portions and shoot tips which consisted of the shoot apex and two to three subjacent nodes enclosed within bracts. Meristem (1-2 mm), shoot tip (3-5 mm) and nodal (5-15 mm) explants were prepared following sterilization and incubated singly on nutrient medium.

The basal one-third of the semi-hardwood and hardwood stem cuttings were immersed in a forcing solution in 600 ml jars which consisted of 300 mg/L oxine citrate and 2% sucrose and 10 mg/L benzyladenine (BA). Forced buds (2-8 mm), which formed at the nodes within 4-6 w, were sterilized and used as explants.

Surface Sterilization

The explants were kept under running tap water to remove latex from the tissue. The material was washed with liquid detergent for 1 m and then rinsed in running tap water for 5 m followed by distilled water. A 70% ethanol-sterilization step was applied for 30-60 s. The tissue was sterilized in the laminar flow hood by soaking in a solution of 10% household bleach (5.2% available chlorine) and 2-3 drops of Tween20 for 10 m. The sterilant was rinsed off with three changes of sterile distilled water.

Culture Conditions

All cultures were incubated at 26 ± 2°C and exposed to a 16 h photoperiod at 667 $\mu w\ cm^{-2}$ light intensity.

Medium

Basal medium for shoot initiation consisted of Murashige and Skoog (MS) (1962) 3% sucrose whereas modified MS (½ strength macronutrients) and 2% sucrose was used for rooting of microcuttings (in vitro regenerated shoots). The media was solidified with 0.7-0.8% Difco Bacto-agar. The pH of all media was adjusted to 5.7 ± 0.1 prior to autoclaving at 108 kPa and 121°C for 20 m. Culture containers used were 25 × 150 mm tubes and Magenta GA-3, vessels with 10 ml and 50 ml aliquots of medium, respectively, and closed with plastic caps.

Establishment of Shoot Cultures

1. Oxidative Browning. This experiment determined the effect of antioxidant treatments and frequent transfer of explant onto fresh medium on oxidative browning of "chataigne" shoot tip and nodal explants. Two concentrations of each of three antioxidants were included in basal medium as follows: PVP (2.0 and 5.0 g/L), cysteine (.02 and .03 g/L) and ascorbic acid (.05 and 1.0 g/L). PVP and cysteine were added prior to autoclaving and filter-sterilized ascorbic acid was added to sterile medium. A control treatment contained no antioxidants and another treatment involved transferring explants on control

media onto fresh media after 7 d. There were 20 replicates of each treatment and the percentage of surviving explants was recorded after 4 w.

2. Establishment of Aseptic Culture. This experiment determined the effect of antibiotic and fungicide pretreatments on establishment of aseptic cultures.

Donor Plant Pretreatments. Juvenile root suckers were treated weekly with a systemic fungicide (Benlate [50% benomyl] 1 g/L) and streptomycin sulphate (100 mg/L). After removal from the plant, terminal shoots of mature breadfruit and forced buds were treated overnight with fungicide (Benlate [50% benomyl] 1 g/L) and streptomycin sulphate (100 mg/L).

Medium. Basal shoot initiation media was supplemented with 2 g/L PVP and 4.4 µM BA and fungicide and/or antibiotics. The fungicide (Benlate) was used at a concentration of 0.1% before autoclaving whereas the filter sterilized antibiotic cocktail, streptomycin sulphate (100 mg/L), chloramphenicol (30 mg/L) and rifampicin (6 mg/L), was added to cooled sterile medium.

Treatments. Sterilized forced buds (2-8 mm), shoot tips (3-5 mm), meristems (1-2 mm) and nodal explants (5-15 mm) were placed in liquid medium containing fungicide and antibiotics for 24 h before incubating on solid medium. Shoot tip and nodal explants were incubated on solid medium with antibiotic whereas meristems and buds were incubated on medium without antibiotics. Control explants were cultured on solid medium immediately after sterilization. The experiment was repeated three times with 10 replicates per treatment. Established explants were transferred to shoot initiation medium without antibiotics.

Shoot Initiation and Multiplication ("Chataigne" and Breadfruit)

Shoot tip and single nodes collected from 6 week-old "chataigne" plants were induced to produce apical and axillary shoots on MS supplemented with 4.4 µM BA or 2.2 µM zeatin. Shoot multiplication was achieved by subculturing shoot tip and node explants of shoots initiated on shoot induction medium onto Margara (1978) $N30NH_4$ macronutrients MS micronutrients and vitamins, 3% sucrose supplemented with 2.2 µM zeatin.

Root Production

For adventitious rooting of "chataigne," 2-3 cm microcuttings collected after 10-12 subculture passages were cultured on basal rooting medium supplemented with 1-napthaleneacetic acid (NAA), indole-butyric acid (IBA) or indole-3-acetic acid (IAA) at 10^{-6} M. Control treatments contained no plant growth regulator. There were 16 replicates of each treatment and the number of roots, root length and number of rooted cultures were recorded after 28 d. Microcuttings 2-3 cm tall were cultured on basal rooting medium for rooting.

Acclimatization

Breadfruit and "chataigne" plantlets were planted in vermiculite or jiffy pellets in a tray and placed in a small propagator. The propagator was placed in a shaded fog house with a relative humidity of 100% and temperature at 25°C. After one week the propagator was moved to a less-shaded area with intermittent mist; the cover of the small propagator was removed after 8 w.

RESULTS AND DISCUSSION

Oxidative Browning

The oxidation of phenolic compounds released during explant preparation resulted in browning of the explant base and the medium immediately surrounding, leading to explant death. The phenomenon is widely reported in the literature and a range of treatments, such as antioxidants used as presoaks or medium additives and frequent transfer (Broome and Zimmerman, 1978) has been employed. In this study PVP was most

effective in controlling oxidative browning; resulting in 92% and 80% survival of explants cultured on medium supplemented with 5 g/L and 2 g/L, respectively (Table 1). Cysteine had no effect on reducing browning as both concentrations used resulted in 100% loss of explants as did the control treatment (Table 1). Fifty percent of the explants, which were recultured after 1 w, were alive after 4 w, indicating that transfer to fresh medium had a positive effect on explant survival compared to the control. Less than 30% of the explants cultured on medium supplemented with ascorbic acid were alive after 4 w (Table 1). As a result, ascorbic acid was not considered as a viable treatment to reduce oxidative browning. A combination of 2 g/L PVP and transfer of explants or microcuttings after 3 w was employed to control oxidative browning in both "chataigne" and breadfruit cultures.

Establishment of Aseptic Culture

Meristem and shoot tip explants had reduced contaminant levels compared to nodal explants, due in part to the size and position of the explant on the shoot. Overall levels of both fungi and bacteria increased basipetally. The 24 h explant pretreatment reduced fungal contaminant of juvenile shoot tip and mature and juvenile nodal explants, however, bacterial contamination of these explants remained high (Table 2). The explant pretreatment, followed by incubation on medium containing the antibiotic cocktail, significantly reduced bacterial contamination in juvenile shoot tip and nodal explants and mature shoot tip explants. The aseptic establishment of single-node and forced-bud explants from mature trees and stem cuttings, respectively, was not successful as growth of both fungi and endophytic bacteria could not be checked. The results demonstrate that aseptic cultures of meristem, shoot tip and nodal explants of juvenile shoots and meristem and shoot tip explants of mature trees can be established. The use of fungicide explant pretreatments was used successfully by others, such as Haldeman et al. (1987), to decontaminate field explants. Additionally, the two-step process of incubating explants in liquid medium containing antibiotics followed by upright culture on solid medium containing antibiotics was successfully employed by Young et al. (1984).

Shoot Initiation and Multiplication

1. "Chataigne." Both 4.4 μM BA and 2.2 μM zeatin induced shoots on explants. BA 4.4 μM was also used by Rahman and Blake (1988) to induce shoots on jackfruit seedling explants. At the end of the initiation stage 95% of all "chataigne" explant cultures had produced one shoot per explant, except single-node explants which were collected from positions furthest away from the shoot tip; these produced 2-16 buds/shoots per node. Increased axillary growth from cultured nodal explants occupying positions furthest away from the shoot tip on donor plants was reported by Leakey and Mohammed (1985) and may be due to the reduced effect of apical dominance at these nodal positions. Additionally, nodes collected from positions just behind the shoot tip were slower to respond on initiation medium compared to other nodes.

Shoots formed at stage 1 were subdivided and used to initiate shoot production cultures on medium supplemented with 2.2 μM zeatin which produced greater shoot proliferation compared to 4.4 μM BA. The number of shoots obtained per explant increased to a maximum of eight with successive subculture passages. After 10-12 subculture passages, it was necessary to subculture shoots on $N30NH_4$ medium (Margara, 1978) which contained a reduced NH_4^+ ions compared to MS in order to halt the observed decline of the in vitro shoots. The need to use medium with low ionic concentrations of macronutrients for woody species has been widely reported and a formulation for woody plants (WPM) was developed by Lloyd and McCown (1980).

2. Breadfruit. Breadfruit explants, established as outlined above, were observed for morphogenetic response. Swelling of meristems, and swelling, twisting and increase in length of outer bracts and attached leaves of mature, juvenile and shoot tips were observed. However, apical growth leading to shoot development did not occur. Fifty percent bud-burst of juvenile single-node explants was noted. Continued growth of the

excised buds on medium did not result in shoot development and a resurgence of bacterial growth occurred within three weeks of transfer to medium without antibiotics.

A small percentage of breadfruit shoots were initiated with mature meristem tip explants (1-2 mm) on N15K or N5K (Margara, 1978) basal shoot medium supplemented with 4.4 μM BA (Rouse-Miller and Duncan, 2000). Multiplication was achieved as outlined for "chataigne." Established shoots were subdivided and placed on $N30NH_4$ medium (Margara, 1978) supplemented with 2.2 μM zeatin for multiplication (Fig. 1) (Rouse-Miller and Duncan, 2000). Large numbers of microcuttings of both species which could be taken to stage 3 (rooting) were generated after several subculture passages.

Rooting

"Chataigne" microcuttings cultured on 10^{-6} M NAA or IAA and auxin-free medium did not develop roots. However, IBA at 10^{-6} M induced rooting (Fig. 2) in 50% of the cultures with mean root-length of 17.9 cm and mean number of roots 9. Rahman and Blake (1988) reported optimal rooting of jackfruit microcuttings on half MS, 2% sucrose and 10^{-6} M IBA. An equal dose response experiment to determine optimal IBA concentrations and pulse treatment of microcutting bases with IBA at 200 ppm prior to culturing on medium did not improve on the results obtained when microcuttings were rooted on medium supplemented with 10^{-6} M IBA (Fig. 2).

Breadfruit rooted on auxin-free basal root medium and on multiplication medium (Fig. 1), indicating there was in vitro rejuvenation of the mature tissue with successive subculture passages.

Acclimatization

Plantlets of both species were successfully transferred to ex vitro conditions. "Chataigne" (60%) and breadfruit plantlets (40%) were successfully hardened 2 m after they were removed from the in vitro system (Fig. 3).

CONCLUSION

In vitro regeneration breadfruit and "chataigne" plantlets has been demonstrated with implications for propagation, germplasm exchange and preservation and crop improvement. However, the nutritional and plant growth regulator requirement for inducing apical and axillary shoots on primary explants need to be investigated further. Multiplication, rooting and acclimatization procedures also need to be optimized before the benefits of applying this technique to these crops can be realized.

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Tables

Table 1. Effect of antioxidant type and concentration on percentage survival of "chataigne" explants.

Type and concentration of antioxidant or no antioxidant (g/L)	% surviving explants after 4 weeks
PVP (2.0)	80
PVP (5.0)	92
Ascorbic acid (0.05)	23
Ascorbic acid (0.1)	28
Cysteine (0.02)	0
Cysteine (0.03)	0
Recultured explants	50
Control	0

Table 2. Effect of fungicide/antibiotic explant pretreatments and antibiotic medium supplements on the establishment of aseptic cultures of breadfruit.

Explant Type	Mature field plants			Juvenile plants		
	Control	Pretreat[1]	Pretreat + medium suppl.[2]	Control	Pretreat[1]	Pretreat + medium suppl.[2]
	% contaminated explants					
	F/B	F/B	F/B	F/B	F/B	F/B
Meristem	0/20	0/0	-	0/0	0/0	-
Shoot tip	0/40	0/40	0/0	0/40	0/100	0/0
Nodes	100/100	60/100	80/100	60/100	20/100	30/0
Forced buds	0/90	0/80	-	0/80	-	-

[1] Pretreatment with fungicide/antibiotic.
[2] Pretreatment with fungicide/antibiotic and antibiotic medium supplements.
[3] F = fungi; B = bacteria.

Figures

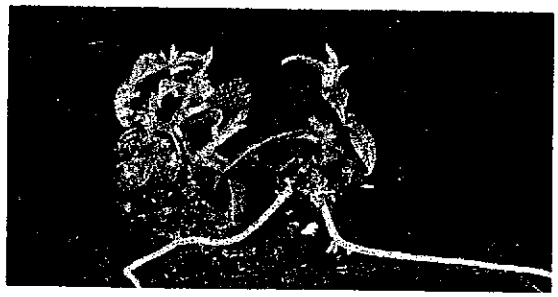

Fig. 1. Breadfruit adventitious shoot cluster produced on shoot production medium and plantlet rooted on basal rooting medium.

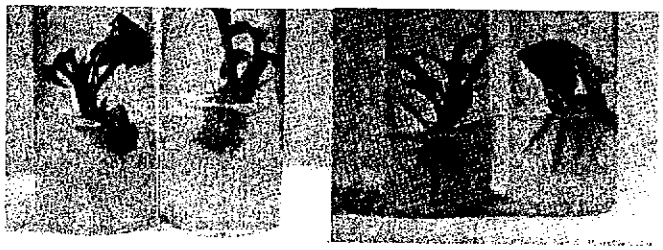

Fig. 2. Microcuttings of "chataigne" cultured on basal rooting medium supplemented with IBA 10-6 M showing root initiation (left) and root development (right).

Fig. 3. Acclimatized "chataigne" plantlets in vermiculite (left) and jiffy pellets (right).