MICROPROPAGATION OF SELECTED CHESTNUT GENOTYPES BY TEMPORARY IMMERSION SYSTEM (TIS).

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INTRODUCTION

The aim of this study was to micropropagate selected chestnut genotypes in BIOREACTORS by using a Temporary Immersion System, with the objective of reducing production costs and improve plant quality for acclimatization.

As chestnut cultures are prone to hyperhydricity, the control of this disorder, as well as control of fungal contamination in bioreactors, were the main challenges of this study.

MATERIAL AND METHODS

Culture systems:
Conventional culture in semisolid medium with agar (SS) and 3 types of **bioreactors**: Units designed by Vivero de Ourense of TRAGSA, and commercial reactors RITA® (www.vitropic.fr) and PlantForm® (www.plantform.se) (**Fig. 1**).
MS (Murashige and Skoog 1960) medium with half-reduced nitrates and GD (Gresshoff and Doy 1972), supplemented with 0.05 -0.1 mg/l Benzyladenine (BA) and 3% sucrose. Agar (0.7 %) was used to gellify SS medium.

Plant material:
8 clones of chestnut of mature origin, hybrids of *Castanea sativa* x *C. crenata* and *C. sativa* x *C. mollisima*, selected by ink- resistance (*Phytophthora cinnamomi*).

Parameters tested:

1) **Supporting material**: felt and mineral wool cubes (**Fig. 2**).
2) **Nº immersions**.
3) **Explant type**: apical and medium segments, and basal segments with basal callus **(Fig. 3)**.
4) **Culture system** (semisolid -SS- and temporary immersion system-TIS-).

Evaluation:

- Quality of the new shoots, **inversely** proportional to percentage of hyperhydric shoots (**% HS**).
- Multiplication rates: nº segments/explant (**NS**), Longest shoot length per explant (**LS**) and % rootable shoots (**% RS**; those longer than 3-4 cm with an actively growing apex).

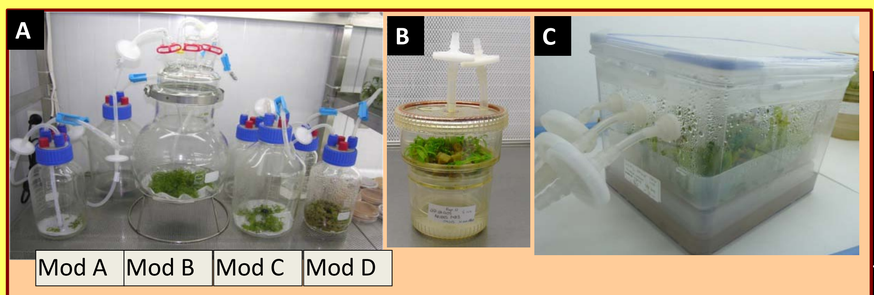


Fig. 1: Bioreactors used. A: Designed by TRAGSA. B: RITA© system, C: PlantForm©.

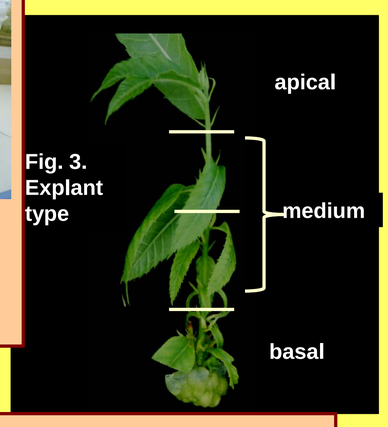


Fig. 3. Explant type

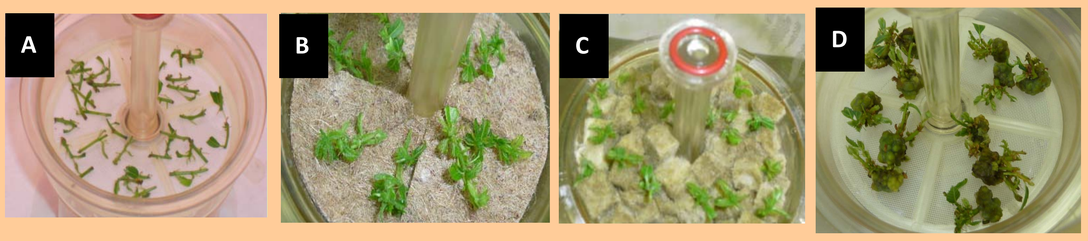
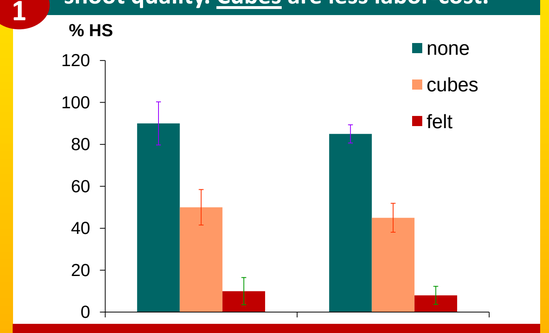


Fig. 2: A, B, C: Apical and medium segments without supporting material (A), with felt (B) and mineral wool cubes (C). D: Basal segments with callus.

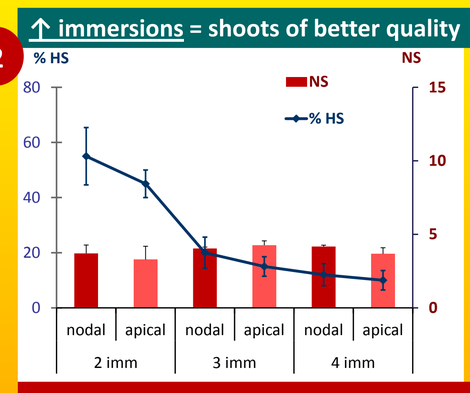
RESULTS

1 SUPPORTING MATERIAL: felt and wool cubes ↑ shoot quality. Cubes are less labor-cost.



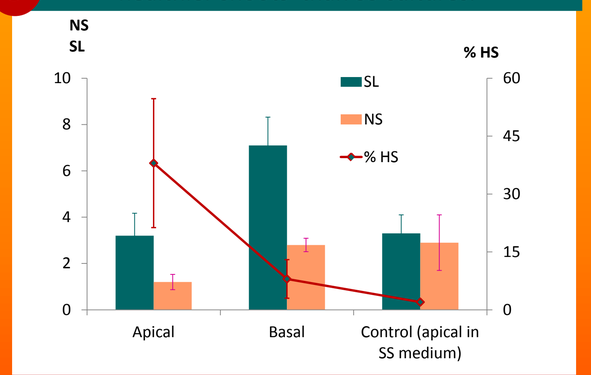
Exp. performed in RITA© with apical and medium segments from 2 clones. BA 0,05 mg/L , 2 immersions/24 h.

2 ↑ immersions = shoots of better quality



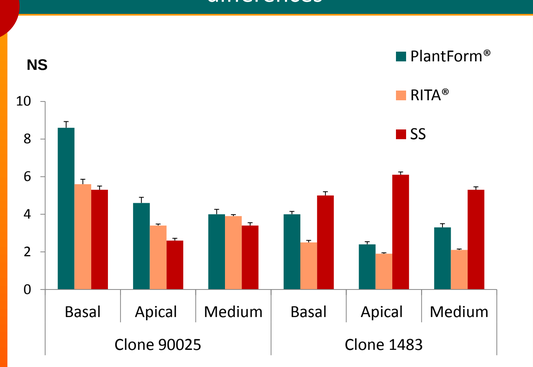
Exp. performed in RITA© with apical and medium segments, wool cubes and BA 0,05 mg/L. Clone 90025.

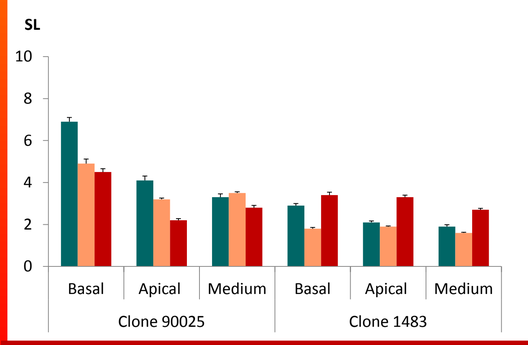
3 Basal explants with callus produce longer and healthier shoots than SS cultures.



Exp. performed in reactors designed by TRAGSA with wool cubes, BA 0,05 mg/L and 3 imm/24 h. Control in SS medium with BA 0,1 mg/L. Clone 111.

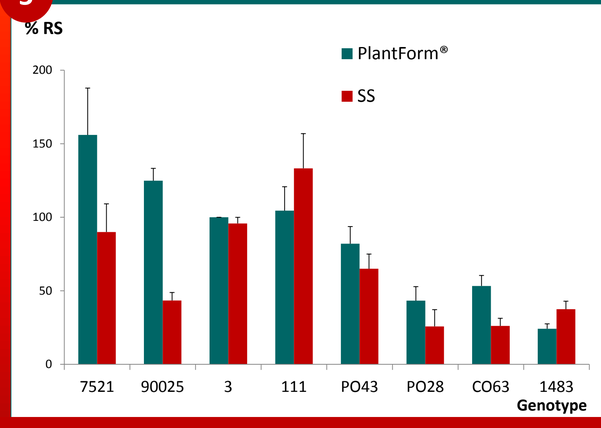
4 PlantForm bioreactors produce longer shoots and higher multiplication rates than RITA©. Genotypical differences





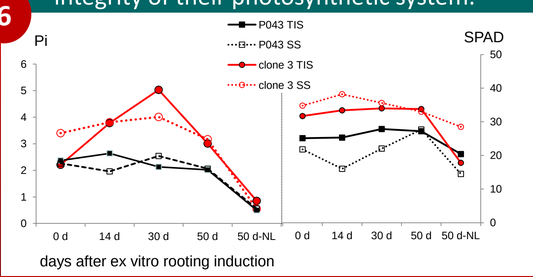
Exp. performed with wool cubes, BA 0,05 mg/L and 4 imm/24h in 2 genotypes, 3 culture systems and 3 explant types. SS medium with BA 0,1 mg/L.

5 Culture in PlantForm © reactors produce more rootable shoots than SS medium in 6 of 8 clones.



Basal explants of 8 chestnut clones were cultured in SS medium with BA BA 0,1 mg/L and in PlantForm© with wool cubes, BA 0,05 mg/L and 4 imm/24 h.

6 Fluorometric levels of chestnut leaves indicate the integrity of their photosynthetic system.



Samples were leaves formed during the in vitro phase. In day 50 new leaves were also measured (50d-NL). Fv/Fm values were ~ 0,82 for all samples.

Other results not shown:
Large vessels were prone to fungal contaminations. PPM did not alleviate the problem as multiplication rates were reduced.

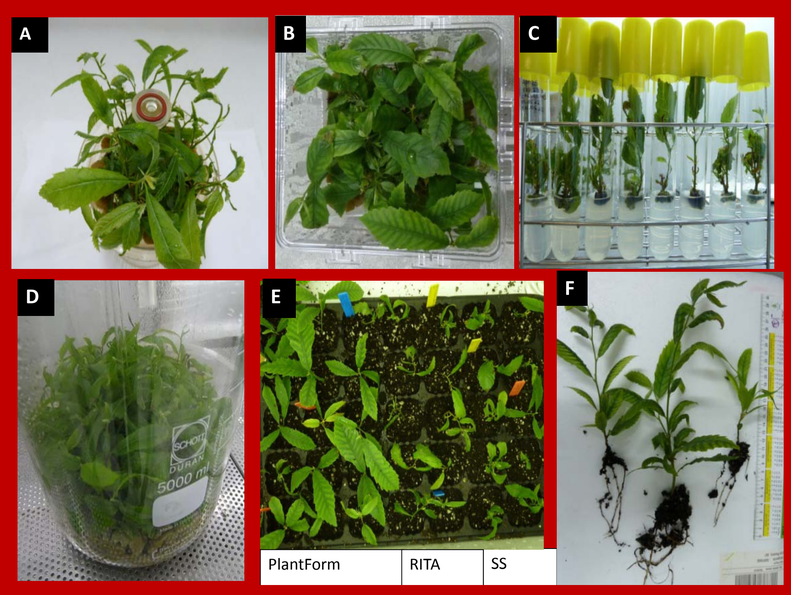


Fig. 4. **Basal explants of clone 90025 cultured in RITA®(A), Plantform® (B) and SS medium (C); basal explants of clone 111 cultured in Mod C (D). Rooting of basal explants of clone 90025 cultured in PlantForm®, RITA® or SS (A), and rooted shoots from Plantform® 5 weeks later (B).**

CONCLUSIONS

1. Axillary shoots of 8 chestnut genotypes have been successfully propagated by a Temporary Immersion System. In 6 clones, multiplication rates and nº of rootable shoots were higher than in semisolid culture.
2. PlantForm© bioreactors produced more, longer and healthier shoots than RITA© vessels.
3. Basal explants with callus produced longer shoots that can be used for rooting, whereas apical and medium segments produced shoots that can be used to maintain the stock.
4. The use of supporting materials as wool cubes, together with 3 or more immersions/24 h prevented or reduced hyperhydricity.
5. Shoots were successfully acclimatized during ex vitro rooting and showed normal photosynthetic capacity.

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