Use of a Continuous Immersion System (CIS) for Micropropagation of Chestnut in Photoautotrophic and Photomixotrophic conditions

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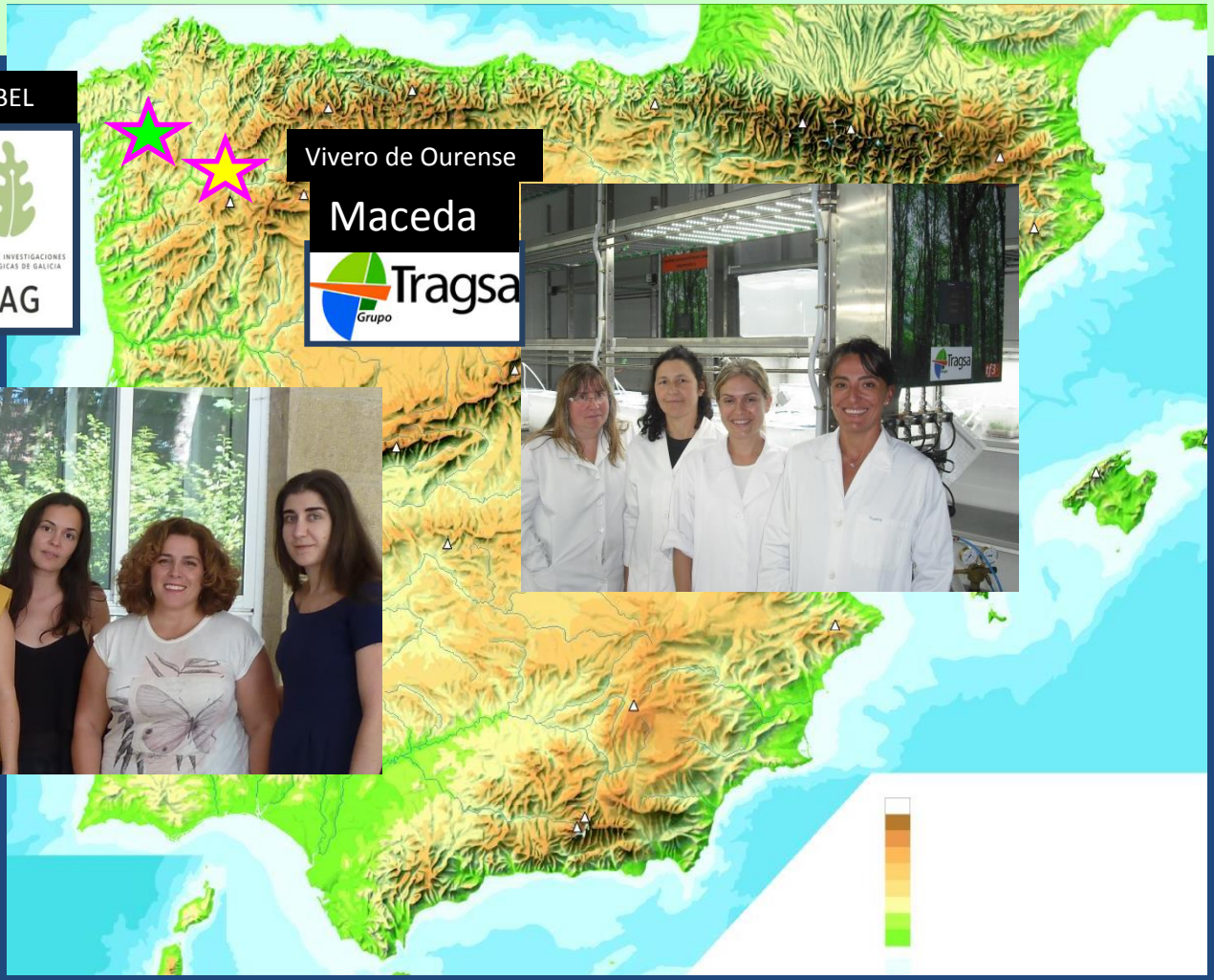
OBJECTIVES:

Improvement of large- scale propagation in liquid media

Application to *Castanea sativa* genotypes with natural resistance to ink disease (*Phytophthora spp*)



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TIS

mporary **I**mmersion **S**ystem

rced Ventilation

pporting material

Bioreactors

TA®

antForm®

signed by TRAGSA

Easy to use

Good results

Vieitez et al. 2007. Protocol for micropropagation of *Castanea sativa* Mill. In: Jain and Häggman (eds.) Protocols for micropropagation of woody trees and fruits. Springer.

Vidal et al. 2014. Comparison of Temporary and Continuous Immersion Systems for Micropropagation of Axillary Shoots of Chestnut and Willow. Acta Hortic (in press).

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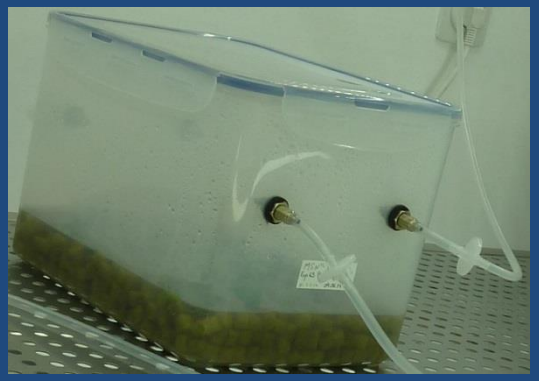
CIS: **C**ontinuous **I**mmersion **S**ystem

Forced Ventilation
Supporting materials



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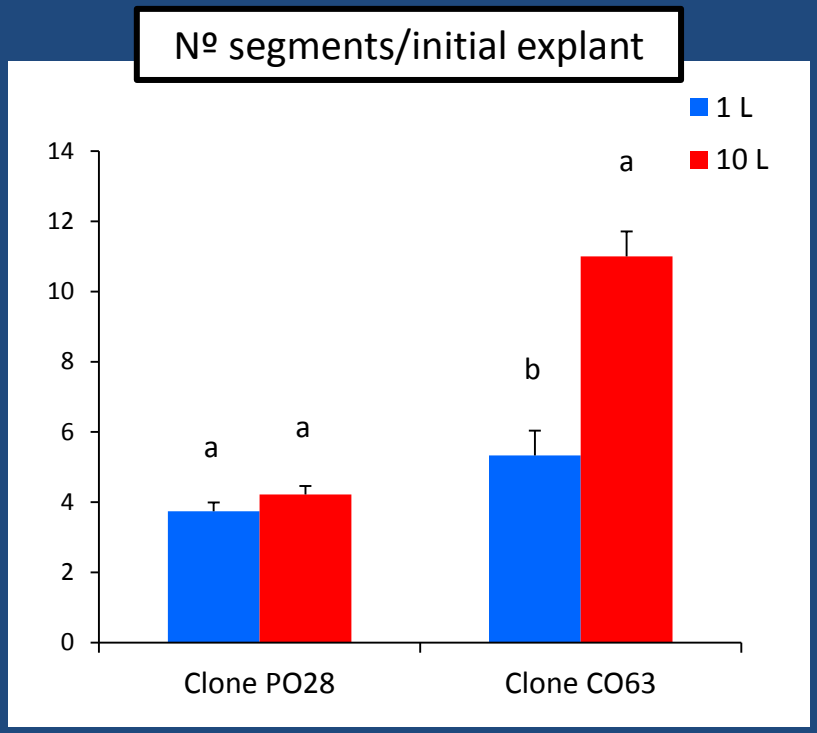
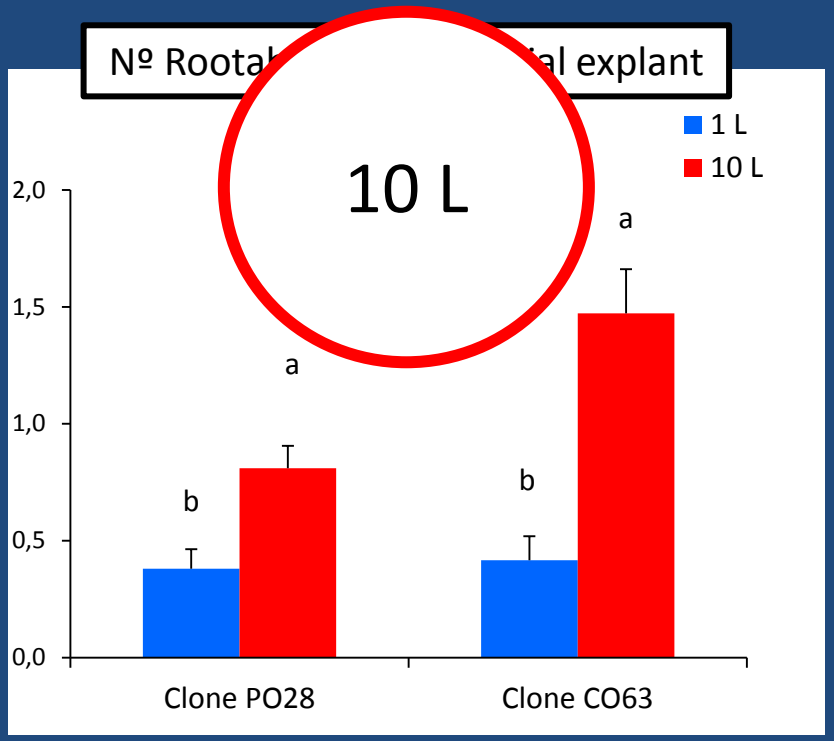
Effect of vessel size: clones CO63 and PO28

Larger vessels → higher proliferation rates





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Effect of supporting material: clone 9002

Rockwool cubes

Perlite

Vermiculite

Glass beads

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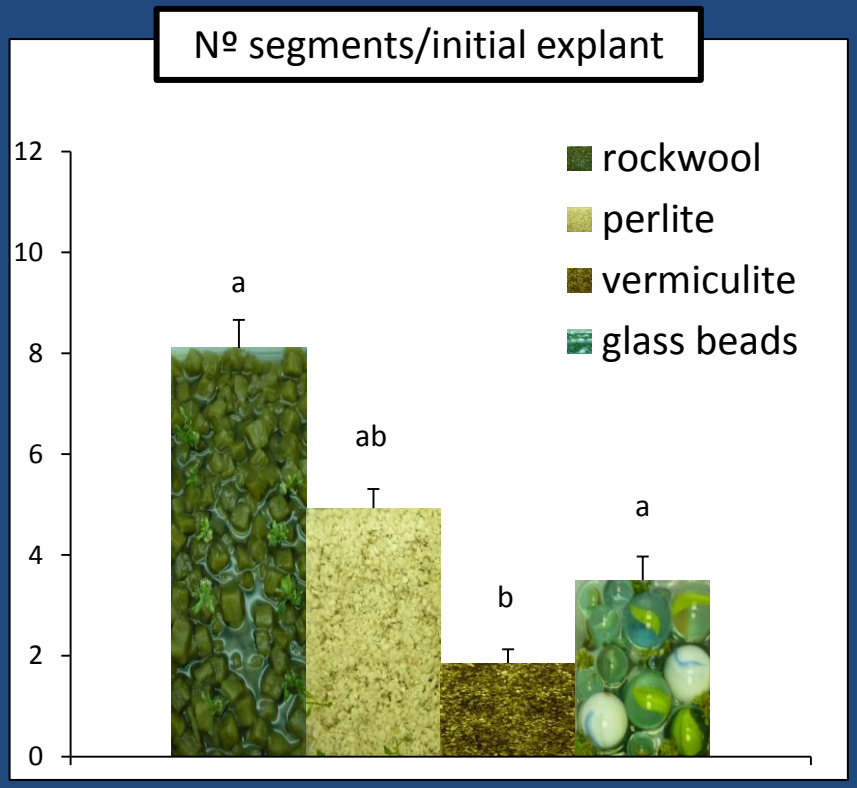
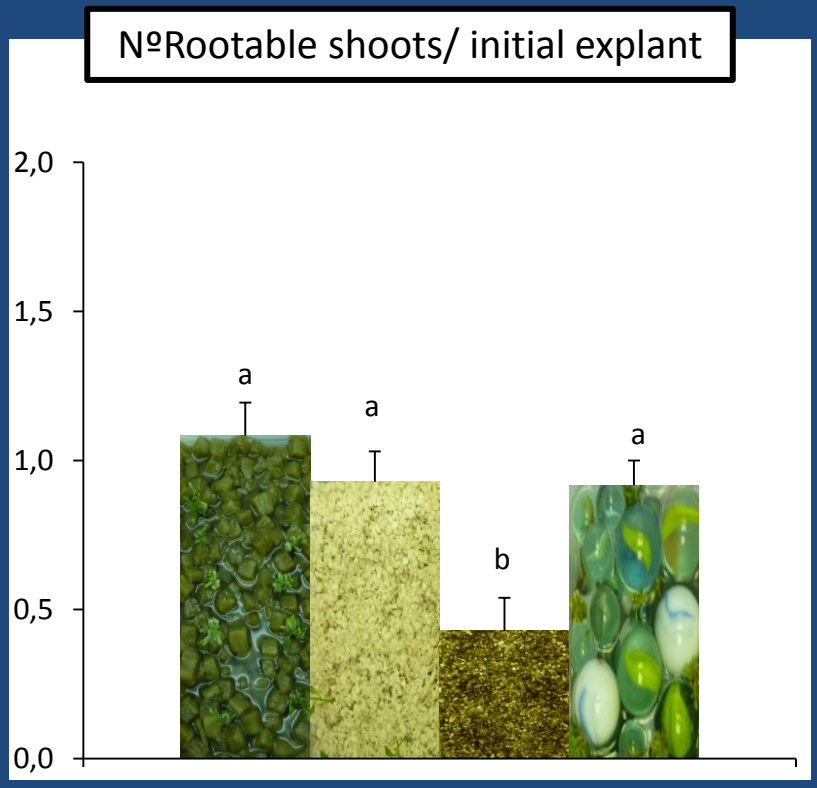
Effect of supporting material: clone 90025

Rockwool cubes





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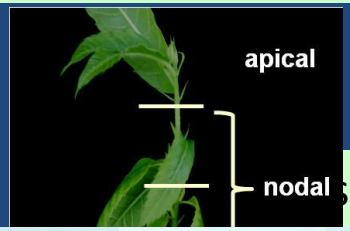

Effect of explant type (apical or basal explants) → higher proliferation rates

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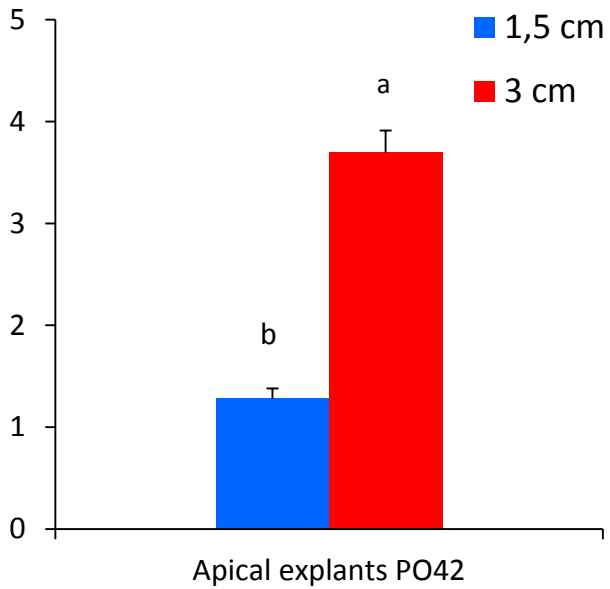
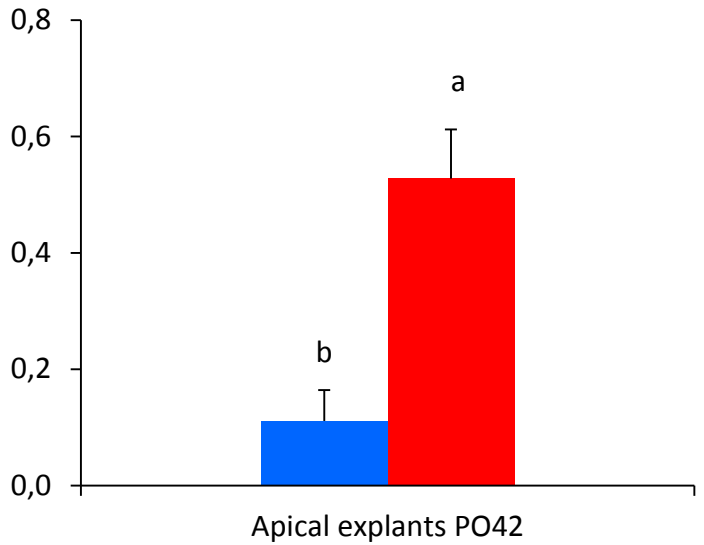
Effect of size of apical explants (1.5 or 3 cm)

Longer explants → higher proliferation rates

Nº segments/initial explant



Nº Rootable shoots/ initial explant



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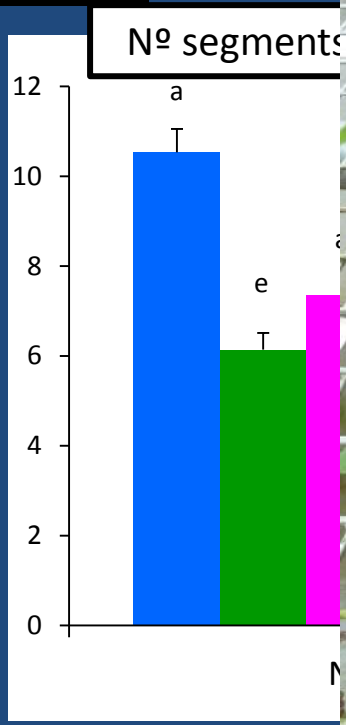
CO63
PO28
CO53
PO42
CO42
90025

Genotypical differences → need to adjust conditions

Problems for acclimatization in some genotypes
↑ Risk of contamination (internal and external)
Loss of more explants



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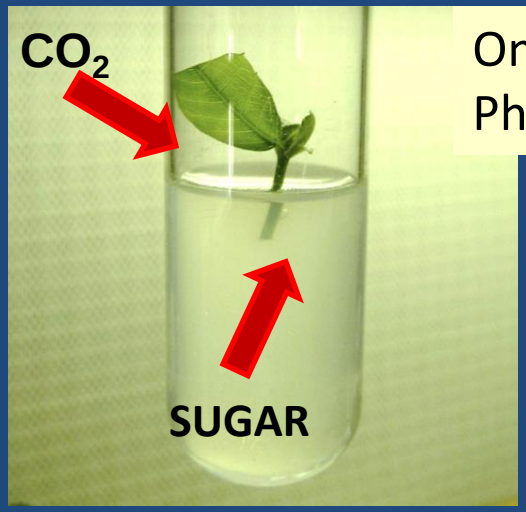
Photomixotrophic conditions

Low light	PPF ~ 50-70 µmol m^{-2} s^{-1}
High sugar content	30 g/liter
No CO_2 added	



Only partially by Photosynthesis

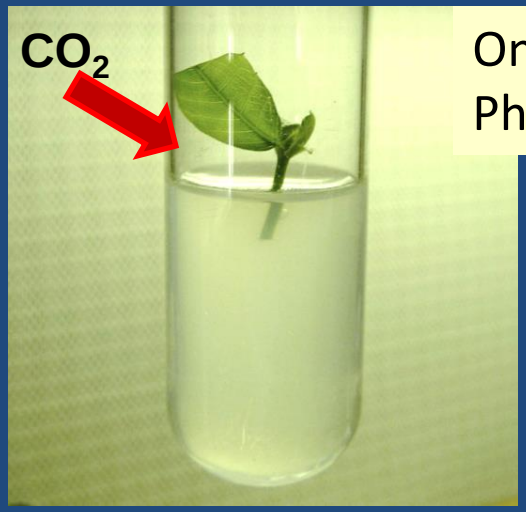
Use of a Continuous Immersion System (CIS) for Micropropagation of Chestnut in Photoautotrophic and Photomixotrophic conditions

Photoautotrophic conditions

High light PPF ~ 100-150 μmol m^{-2} s^{-1}

No sugar added

CO_2 added 1000-1500 μmol mol^{-1}



Only Photosynthesis

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PhotoAutotrophic Micropropagation (PAM)

Kozai T, Afreen F, Zobayed SMA . 2005. Photoautotrophic (sugar-free medium) micropropagation as a new propagation and transplant production system. Springer.

Xiao Y, Niu G, Kozai T (2011) Development and application of photoautotrophic micropropagation plant system. Plant Cell Tiss Organ Cult 105:149–158.

PROPOSED ADVANTAGES:

Elimination of morphological and physiological disorders

High survival percentage/smooth transition to ex vitro environment

Little loss of plantlets due to microbial contamination

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PhotoAutotrophic Micropropagation (PAM) of chestnut shoots

LEDs lights for increasing PPF
Forced ventilation system with CO_2 enrichment

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Preliminary experiments

150 PPF

- **Sucrose 3%**
- **Sucrose 1%**
- **Sucrose 1% + CO_2**

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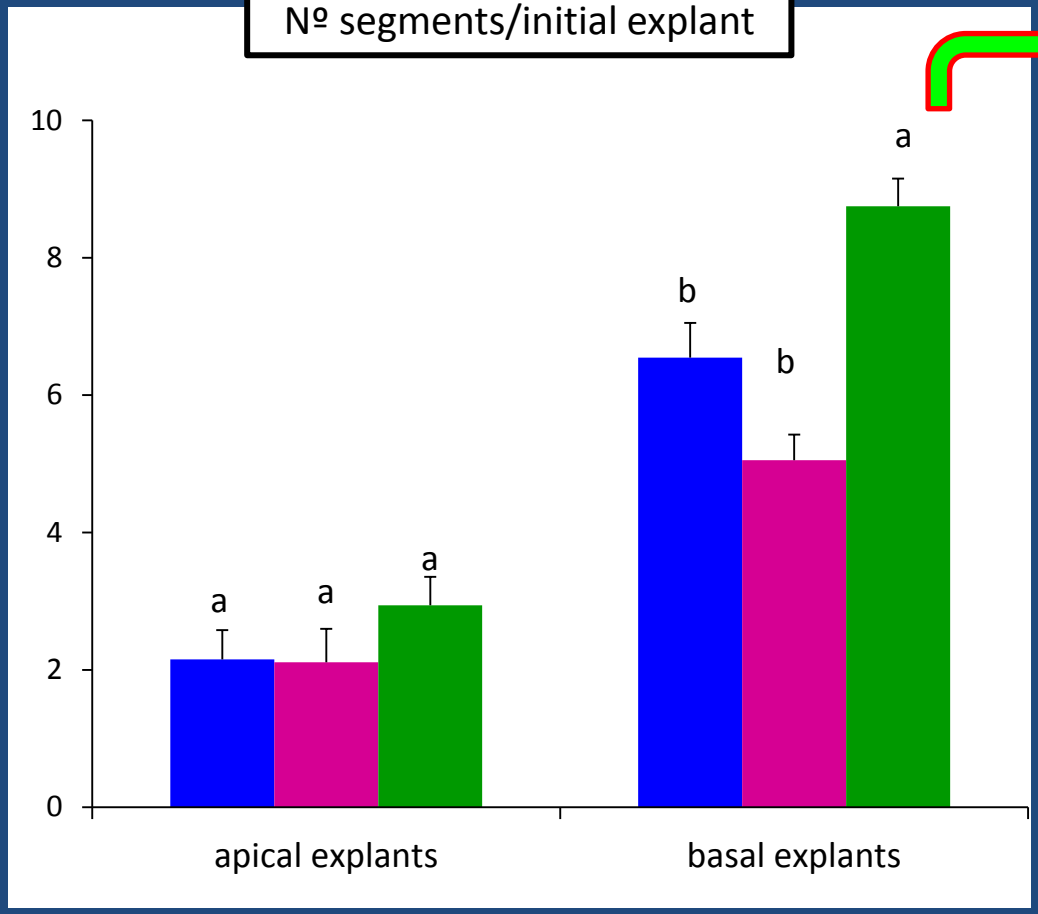
Proliferation parameters

Nº segments/initial explant



Sucrose 3%

Sucrose 1%

Sucrose 1% + CO_2

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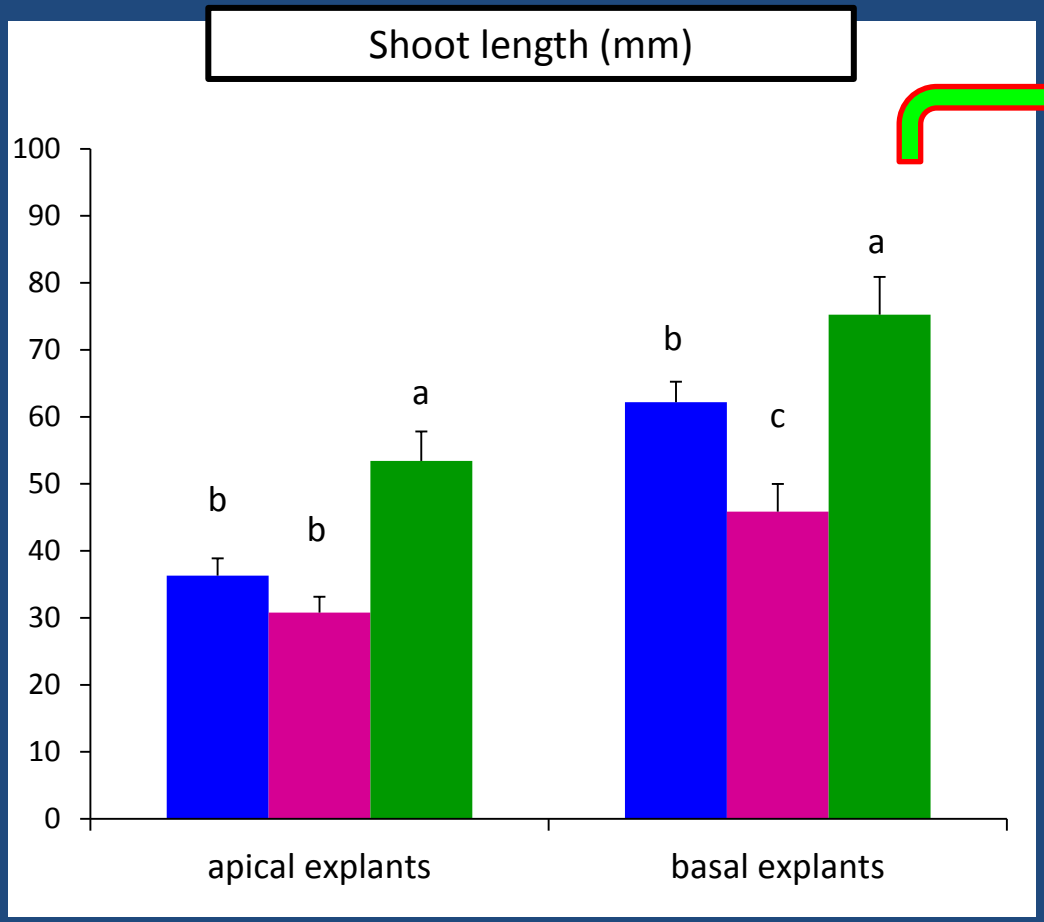
Shoot quality parameters



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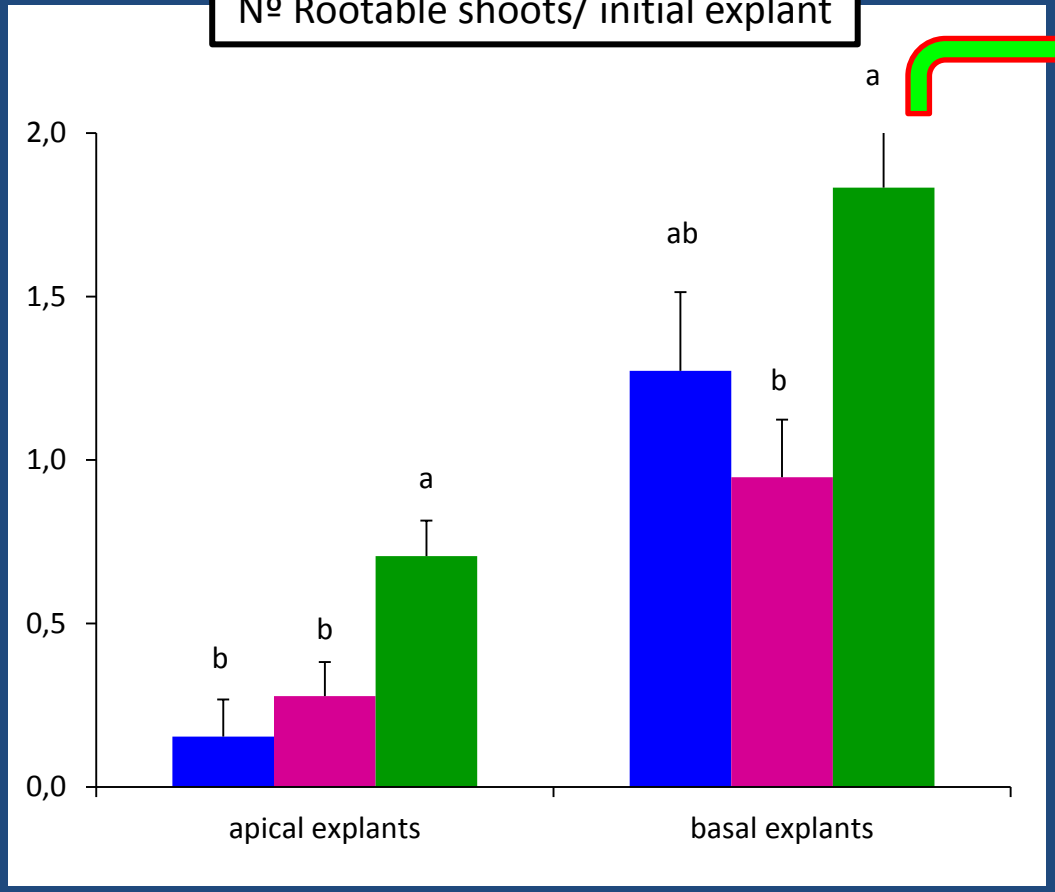
Shoot quality parameters

Nº Rootable shoots/ initial explant



- Sucrose 3%
- Sucrose 1%
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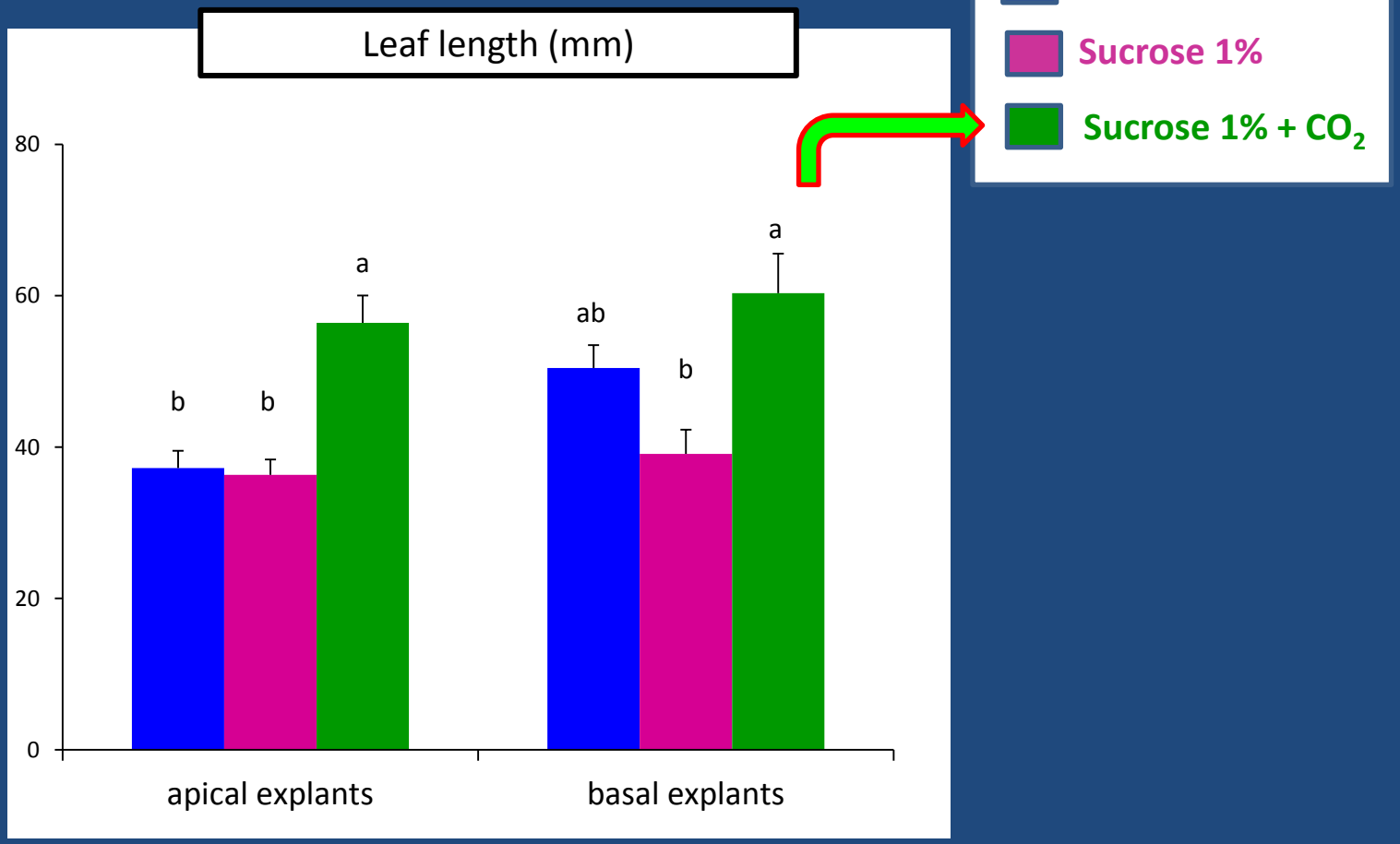
Leaf parameters



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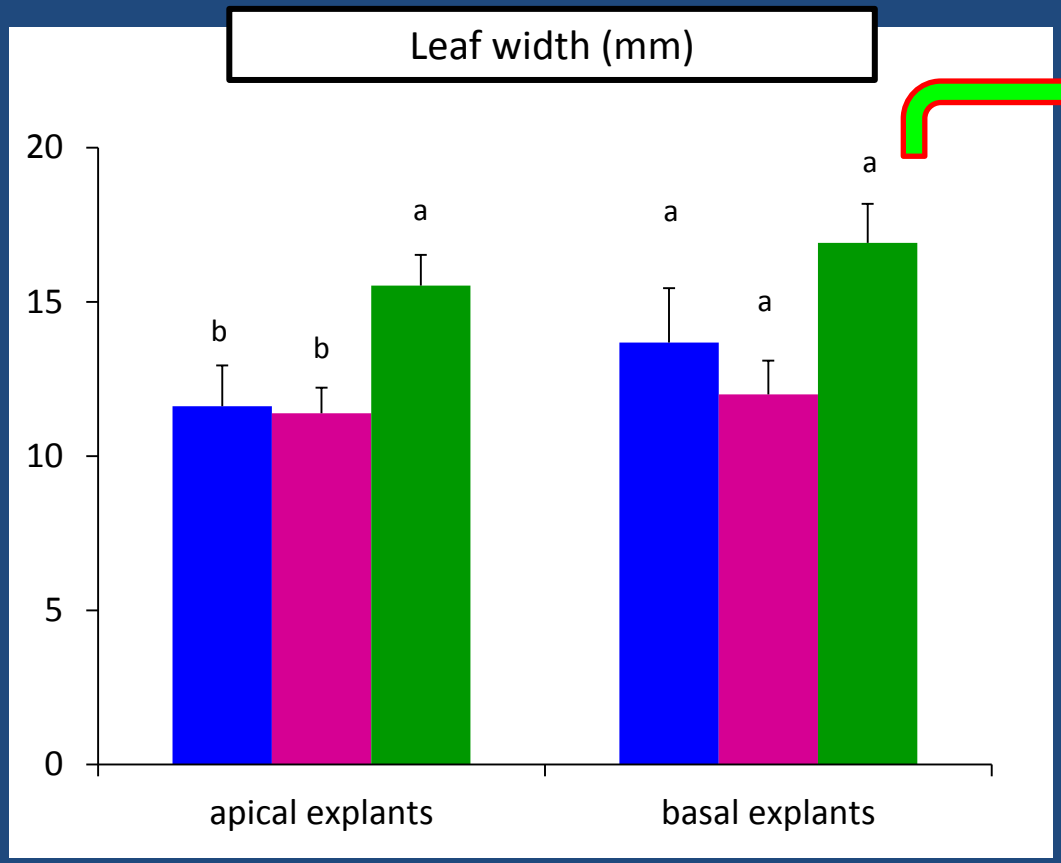
Leaf parameters



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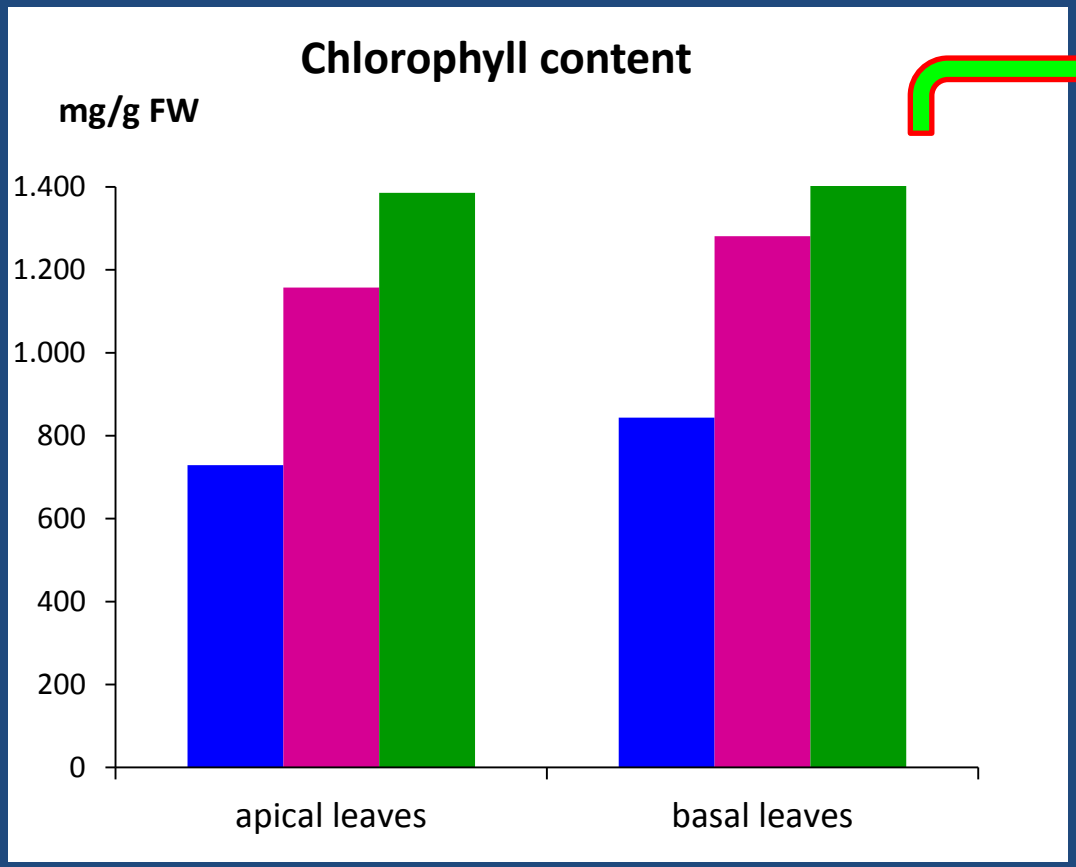
Leaf parameters



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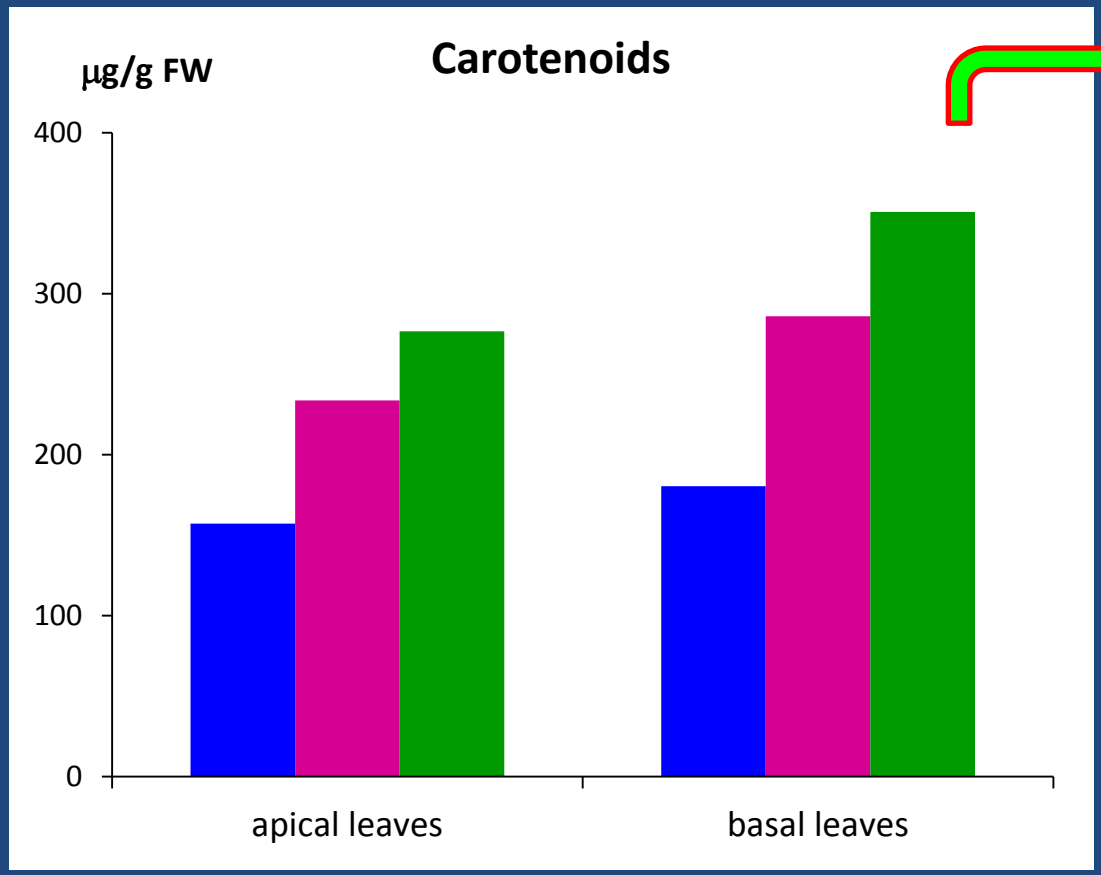

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CURRENT EXPERIMENTS:

Testing more treatments and clones

Optimization of the quantitation of CO_2 levels (IR probe or sampling and GC)

Determination of other photosynthetic parameters (sugar content in leaves, RuBisCO activity…)