Technologies for mass propagation of elite forest trees: 30 years activities at the CRA W.

R. GRUSELLE, J.P. MISSON, J.M. TERZI, O. AREZKI and Ph. DRUART

CRA W Department Biotechnology, chaussée de Charleroi, 234, 5030 Gembloux (Belgium) *druart@cra.wallonie.be*

Cloning elite trees get economical advantage on seed regeneration due to the previous evaluation of the plant development that takes place at the adult stage. However, a technology of mass propagation is required at the lowest cost.

The "Plant propagation Unit" of the CRA W Department Biotechnology is involved in the development of mass propagation technologies of forest trees since 1978 when the first wild cherry clones were established *in vitro* from meristem tips. From that time, several species have been investigated with success, like alder, maple, ash, oak, cork-oak, whitebeam, poplar, elm, walnut, sorb tree, horse chestnut, *Eucalyptus*, *Acacia*, *Terminalia*, Cypress and *Abies nordmanniana*

Several propagation models have been developped according to the species. Starting from meristem culture followed by axillary branching, shoot elongation and *in vitro* rooting successively, adaptations were required about the preparation of the initial explant, the regulation of further steps (Gruselle et al., 1995, Dolcet-Sanjuan et al., 2004).

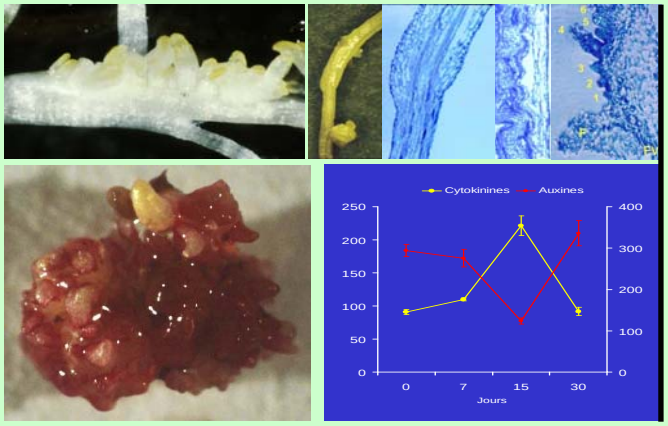
The preparation of initial explants from forest elites

selected at the adult stage is a major step when meristem culture is not workable : shoot flushing from twigs (1), shoot regeneration from forced roots (2) or branches fragments (3), heated graft (4), …are prerequisite before node culture

Meristematic aggregates of *Eucalyptus camaldulensis*

(Arezki *et al*., 2000, 2001)

Established *in vitro* from nodes of 2 years old plants, *Eucalyptus camaldulensis* Dehn., form single structures so called **"Meristematic Agglomerates"** when cultivated in the dark in Petri dishes in presence of auxin and activated charcoal. Those dense axillary shoot clusters (5) mostly limited to meristematic domes of reddish colour (6) appeared from the third subculture after internodes shortening, leaf shape reduction and stem tissue swelling (7). Vascular system changed from straight to sinuous and compacted line. Starting bud proliferation is associated to dramatic increases of the endogenous **cytokinin/auxin ratio**(8) and significant **ethylene** accumulation in the confined atmosphere of the Petri dishes. Bud proliferation enhanced while bud size reduced. Such *de novo* phenotype maintained stable high proliferation rates for more than 2 years and may depend upon an interrelationship between ethylene biosynthesis and **DNA methylation** (unpublished results). A reversion to normal growth occurred after supplying the culture medium with aminoethoxyvinylgnycine (AVG) as anti-ethylene agent.



Great reduction in propagation cost and simplification of handling.

Somatic embryogenesis of elite Christmas tree: *Abies nordmanniana* from mature zygotic embryos to plants (Misson *et al*., 2006)

"Polyclonal variety" of elite wild cherry: vitroplants and softwood cuttings from miniaturized vitroplants

80 elite clones were included in a multiclonal variety of wild cherry. Each year, 13 to 26 clones were established *in vitro* to maintain genetic variability in yearly propagation based on a minimum of 30 clones. Oldest trees issued from *in vitro* axillary branching (among the 50 000 thousands trees) grow in the forest since 1985. Mini stock vitroplants for cuttings have been trained in greenhouse. Softwood cuttings are harvested every 6 (± 1) weeks after GA3 (200mg/l) spray. Darkness, bottom heating and IBA_{KOH} (2g/l) together maintained high rooting rates (around 80%) during 7 years (5) during spring and summer seasons. Plants of 1.8 to 2.0 m height can be produced after one year growth in nursery. The ability to propagate *in vitro* and ex vitro as well is clone dependent.

References

- R. Gruselle, C. Nicaise and P. Boxus, 1995. *Bull. Rech. Agrom. Gembloux* 30(1-2) : 47-53.
- R. Dolcet-Sanjuan, E. Claveria, R. Gruselle, A. Meier-Dinkel, C. Jay-Allemand and T. Gaspar. 2004. *Journal of American Society for Horticultural Science* 129 (2): 198-203.
- O. Arezki, Ph Boxus, C. Kevers and T. Gaspar. 2000. *In Vito Cell. Dev. Biol*. 36:398-401.
- O. Arezki, Ph. Boxus, C. Kevers, and T. Gaspar, 2001. Plant growth Regulation,33:215-219.
- J.P. Misson,P. Druart,B. Panis and B. Watillon (2006). *Propagation of ornamental plants*. 6, (1), 17-23.

Department Biotechnology
http:// www.cra.wallonie.be

IUFRO » T ree biotechnology » , Açores (Portugal), June 2007

Walloon Agricultural Research Centre

Technologies for mass propagation of elite forest trees: 30 years activities at the CRA W.

R. GRUSELLE, J.P. MISSON, J.M. TERZI, O. AREZKI and Ph. DRUART

CRA W Department Biotechnology, chaussée de Charleroi, 234, 5030 Gembloux (Belgium) *druart@cra.wallonie.be*

Cloning elite trees get economical advantage on seed regeneration due to the previous evaluation of the plant development that takes place at the adult stage. However, a technology of mass propagation is required at the lowest cost.

The "Plant propagation Unit" of the CRA W Department Biotechnology is involved in the development of mass propagation technologies of forest trees since 1978 when the first wild cherry clones were established *in vitro* from meristem tips. From that time, several species have been investigated with success, like alder, maple, ash, oak, cork-oak, whitebeam, poplar, elm, walnut, sorb tree, horse chestnut, *Eucalyptus*, *Acacia*, *Terminalia*, Cypress and *Abies nordmanniana*

Several propagation models have been developped according to the species. Starting from meristem culture followed by axillary branching, shoot elongation and *in vitro* rooting successively, adaptations were required about the preparation of the initial explant, the regulation of further steps (Gruselle et al., 1995, Dolcet-Sanjuan et al., 2004).

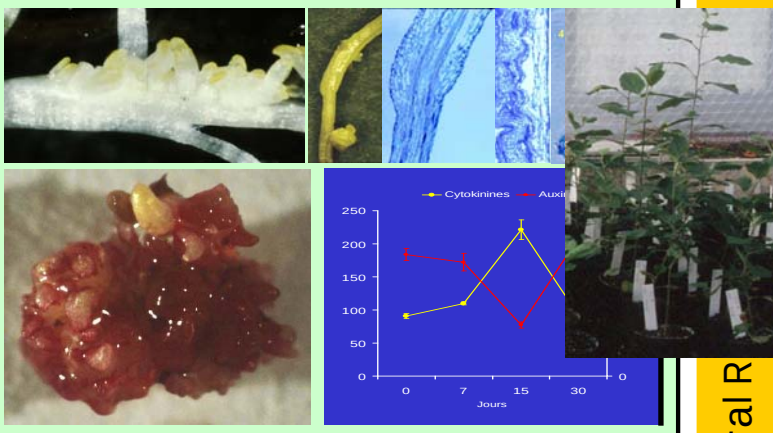
The preparation of initial explants from forest elites

selected at the adult stage is a major step when meristem culture is not workable : shoot flushing from twigs (1), shoot regeneration from forced roots (2) or branches fragments (3), heated graft (4), …are prerequisite before node culture

Meristematic aggregates of *Eucalyptus camaldulensis*

(Arezki *et al*., 2000, 2001)

Established *in vitro* from nodes of 2 years old plants, *Eucalyptus camaldulensis* Dehn., form single structures so called **"Meristematic Agglomerates"** when cultivated in the dark in Petri dishes in presence of auxin and activated charcoal. Those dense axillary shoot clusters (5) mostly limited to meristematic domes of reddish colour (6) appeared from the third subculture after internodes shortening, leaf shape reduction and stem tissue swelling (7). Vascular system changed from straight to sinuous and compacted line. Starting bud proliferation is associated to dramatic increases of the endogenous **cytokinin/auxin ratio**(8) and significant **ethylene** accumulation in the confined atmosphere of the Petri dishes. Bud proliferation enhanced while bud size reduced. Such *de novo* phenotype maintained stable high proliferation rates for more than 2 years and may depend upon an interrelationship between ethylene biosynthesis and **DNA methylation** (unpublished results). A reversion to normal growth occurred after supplying the culture medium with aminoethoxyvinylgnycine (AVG) as anti-ethylene agent.



Somatic embryogenesis of elite Christmas tree: *Abies nordmanniana* from mature zygotic embryos to plants (Misson *et al*., 2006)

"Polyclonal variety" of elite wild cherry: vitroplants and softwood cuttings from miniaturized vitroplants

80 elite clones were included in a multiclonal variety of wild cherry. Each year, 13 to 26 clones were established *in vitro* to maintain genetic variability in yearly propagation based on a minimum of 30 clones. Oldest trees issued from *in vitro* axillary branching (among the 50 000 thousands trees) grow in the forest since 1985. Mini stock vitroplants for cuttings have been trained in greenhouse. Softwood cuttings are harvested every 6 (± 1) weeks after GA3 (200mg/l) spray. Darkness, bottom heating and IBA_{KOH} (2g/l) together maintained high rooting rates (around 80%) during 7 years (5) during spring and summer seasons. Plants of 1.8 to 2.0 m height can be produced after one year growth in nursery. The ability to propagate *in vitro* and ex vitro as well is clone dependent.


Production from one Petri dish

Great reduction in propagation cost and simplification of handling.

References

- R. Gruselle, C. Nicaise and P. Boxus, 1995. *Bull. Rech. Agrom. Gembloux* 30(1-2) : 47-53.
- R. Dolcet-Sanjuan, E. Claveria, R. Gruselle, A. Meier-Dinkel, C. Jay-Allemand and T. Gaspar. 2004. *Journal of American Society for Horticultural Science* 129 (2): 198-203.
- O. Arezki, Ph Boxus, C. Kevers and T. Gaspar. 2000. *In Vito Cell. Dev. Biol.* 36:398-401.
- O. Arezki, Ph. Boxus, C. Kevers, and T. Gaspar, 2001. Plant growth Regulation, 33: 159-163.
- J.P. Misson,P. Druart,B. Panis and B. Watillon (2006). *Propagation of ornamental plants*, 6, (1), 17-25.

Druart, Ph. 1999. Somatic embryogenesis in *Prunus* species. In "Somatic Embryogenesis in Woody Plants, edited by S. M. Jain, P.K. Gupta, R.J. Newton, Volume 5: 215-235.

Druart, Ph 2003. Micropropagation of apples (*Malus sp*.) In : « Micropropagtion of Woody Trees and Fruits. Ed. S.M. Jain and K. Ishii. Kluwer Acad. Publish., The Netherlands. 433-463.

Kondakova V., Druart Ph., 2001. True-to-type protoclones regeneration from mesophyll protoplasts of "Inmil" cherry rootstock *(P. incisa x serrula)*. Acta Hort., 521-526.

De Bondt, A., K. Eggermont, Ph. Druart, M. De Vil, I. Goderis, J. Vanderleyden and W. Broekaert. 1994. *Agrobacterium*-mediated transformation of apple (*Malus x domestica* Borkh.): an assessment of factors affecting gene transfer efficiency during early transformation steps. Plant Cell Reports 13: 587-593.

Druart, Ph, Delporte, F., Brazda, M., Ugarte-Ballon, C., da Câmara Machado, A., Laimer da Câmara Machado, Jacquemin, J. and Watillon, B. 1998. Genetic transformation of cherry trees. Proc. Third Int. Cherry Sym. Ed. Jonas Ostaas. Acta Hort 468:71-76.

Da Camara Machado A., Druart Ph., Brazda M., Purhinger H., Watillon B., Kattinger H., Laimer Da Camara Machado M. 1998. Routine transformation via secondary embryogenesis in cherry rootstocks. In : IXth Int. IAPTC Congress, Jerusalem (Israël), p. 158.

Department Biotechnology

http:// www.cra.wallonie.be

IUFRO « Tree biotechnology », Açores (Portugal),
June 2007