IVCHB 2011

Biotechnological advances in *in vitro* horticultural breeding

September 18-22, 2011
Ghent, Belgium

PROGRAM
BOOK OF ABSTRACTS
PARTICIPANTS

11:15 – 11:30 a.m.	**Carmen Martin** Influence of regeneration medium composition in survival and genetic stability of mint (*Mentha x piperita*) cryopreserved apices
11:30 – 11:35 a.m.	**Carla Benelli** *In vitro* conservation of ornamental plants by slow growth storage
11:35 – 11:40 a.m.	**Filomena Gomes** *Arbutus unedo* L. selected clones conservation *in vitro* conditions
11:40 – 11:45 a.m.	**Anna Nukari** Optimising modified droplet vitrification cryopreservation method for lowbush blueberries
11:45 – 11:50 a.m.	**Mailson Monteiro Do Rêgo** *In vitro* conservation of castor bean (*Ricinus communis* L.) embryonic axis by capsulation-dehidratation technical
11:50 – 11:55 a.m.	**Romano Roncasaglia** Effect of the carbohydrate composition in the medium during the *in vitro* slow growth storage of the cherry rootstock '*Gi.Sel.A®5*'
11:55 – 12:00 p.m.	**Teresa Hazubska-Przybyl** The use of the vitrification method for cryostorage of selected embryogenic lines of *Picea abies*
12:00 – 12:30 P.M.	**POSTERS**
12:30 – 2:00 p.m.	Lunch
2:00 P.M. – 5.10 P.M.	**SESSION 8 (NTC): NEW DEVELOPMENTS IN TISSUE CULTURE TECHNOLOGY** **CHAIR: MARIE-CHRISTINE VAN LABEKE** **CO-CHAIR: KEVIN FOLTA**
2:00 – 2:40 p.m.	**Kevin Folta** Increasing regeneration efficiency of diploid strawberry (*Fragaria vesca*)- A powerful translational system
2:40 – 3:00 p.m.	**Barbara Ruffoni** Natural compounds for the control of contaminants during micropropagation
3:00 – 3:15 p.m.	**B.N. Sathyanarayana** Micropropagation to revolutionize plant production scenario in India - Role of universities towards innovations in making the technology a low cost sector and thus empower unemployed youth of the country
3:15 – 3:30 p.m.	**Adriano Sofo** Biochemical and morphological changes in micropropagated shoots of *GiSeLa6®* (*Prunus* spp.) rootstock inoculated with *Trichoderma harzianum* strain T-22

is that the injury produced by the cryopreservation process (action of cryoprotectants and/or oxidative stress) may cause the formation of free radicals, which could potentially lead to genetic alterations *(Benson and Bremner, 2004)*. These stresses are somehow added to those imposed by the employment of *in vitro* techniques.

Therefore, cryopreservation success has to deal with a balance between high survival rate and genetic stability. In order to evaluate these important factors, a study of survival and genetic stability (using molecular markers) after cryopreservation has been carried out in mint apices cryopreserved with the vitrification-droplet method, and regenerated on three different media.

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Benson EE, Bremner DH, 2004. In: Fuller BJ, Lane N, Benson EE, editors. Life in the frozen state. CRC Press
Harding K, 2004. CryoLetters 25: 3–22
Martín C, González-Benito ME, 2005; Cryobiology 51: 281–289
Martín C, Cervera MT, González-Benito ME, 2011. J. of Plant Physiology 168, 158-166

IN VITRO CONSERVATION OF ORNAMENTAL PLANTS BY SLOW GROWTH STORAGE

Benelli, C.[1], Dradi, G.[2], Lambardi, M.[1] and Ozudogru, E.A.[3]

Poster CR1

ARBUTUS UNEDO L. SELECTED CLONES CONSERVATION *IN VITRO* CONDITIONS

Gomes, F.[1], Simões, A.[1], Lopes, L.[2] and Canhoto, J.[2]

Poster CR4

OPTIMISING MODIFIED DROPLET VITRIFICATION CRYOPRESERVATION METHOD FOR LOWBUSH BLUEBERRIES

Nukari, A., Pantsu, H, and Uosukainen, M.

Poster CR11

MP13 PROGRESSES IN TISSUE CULTURE OF *ARBUTUS UNEDO* L. (STRAWBERRY TREE, ERICACEAE)

Canhoto, J. M.[1], Gomes, F.[2] and Lopes M.L.[1]

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Arbutus unedo is a small tree characteristic of Mediterranean countries. The plant produces edible fruits that can be consumed fresh or used to make marmalades, jams and jellies. However, the major part of fruit production is used to obtain an aromatic alcoholic distillate very appreciated in several regions around the Mediterranean basin and in Portugal. Fruits are usually collected from spontaneously growing trees and the farmers interested in the culture of this species find many difficulties to achieve selected plant material that may guarantee interesting incomes. To overcome this drawback we have selected about 30 trees in several places of Portugal based on fruit production and their genetic profiles were compared through SSRs and RAPDs. Techniques of in vitro propagation have been applied for large-scale propagation of these trees and the results so far obtained have shown that axillary shoot proliferation from adult material established from epicormic shoots and somatic embryogenesis from leaves of adult derived micropropagated shoots are effective methods of propagation. Organogenesis was also observed in some culture media but this type or regeneration has yet to be optimized. The results indicated that the genotype and culture conditions are the main factors controlling the morphogenic responses. In particular, we have found that in the conditions tested some clones display a high embryogenic potential whereas in others attempts to induce somatic embryogenesis were unsuccessful. Somatic embryos are quite similar to their zygotic counterparts and converted well into plantlets following development in a plant growth regulator free medium and maturation on a medium containing charcoal. Histological and histochemical studies showed that the embryos are formed from the proliferation of mesophyll cells near the vascular bundles or the epidermis. Somatic embryogenesis only occurs in brownish leaf segments when the total phenolic content reached its maximum. Similar patterns of storage reserve (lipids and proteins) accumulation were detected in somatic and zygotic embryos suggesting a similar pattern of development and maturation. Based on the protocol for axillary shoot proliferation and after treatments of nodal segments with colchicine, tetraploid shoots have been obtained. Attempts to optimize this protocol are being carried out.

MP14 THE EFFECT OF MYCORRHIZATION WITH *PISOLITHUS TINCTORIUS* ON FIELD GROWTH OF MICROPROPAGATED PLANTS OF *ARBUTUS UNEDO* L.

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Arbutus unedo L. (strawberry tree) grows spontaneously in Mediterranean ecosystems. Fruit production represents the major income for farmers interested in this species. From an ecological perspective, it contributes to the biodiversity, helps to stabilize soils and it is well adapted to harsh environments. Mycorrhizae can improve plant adaptation and tolerance to several types of biotic and abiotic stresses. Species of *Arbutus* (Ericaceae) form arbutoid mycorrhizae with several fungi which are also able to form ectomycorrhizae in association with other plant hosts. One of these fungi is *Pisolithus tinctorius. In vitro* cloned plants of *A. unedo* through shoot proliferation were inoculated in the nursery with *P. tinctorius* and two inoculation treatments were tested and compared to control plants: 1) vegetative inoculum produced in liquid medium from sporocarps of a *Quercus suber* stand and 2) dry sporocarps from a *Eucalyptus globulus* stand. In a field trial, the nursery inoculation treatments were compared to fertilized plants (seedlings and selected clones). Plant height was evaluated 4 and 20 months later, in nursery and in the field trial, respectively. After 4 months best results were achieved when dry sporocarps water mixed were tested ($P<5\%$). In the field trial, 20 months later, both mycorrhizae inocula treatments tested in nursery improved plant growth compared to control plants and seedlings ($P>5\%$). Mycorrhization may help to reduce fertilizers and biocides application thus contributing to more friendly environmental agricultural practices and to a decrease of productivity costs. Further studies are needed to ensure fungal persistence, as well as, fungal strains selection for their aggressiveness under nursery conditions, which is a requirement for a successful implementation of these methods. Mycorrhizal synthesis with edible fungi can improve not only the success of ex-vitro plant growth, but can also account for another source of income, for farmers.

CR3 PLANT BIODIVERSITY AT HIGH ALTITUDE: *IN VITRO* PRESERVATION

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Knowledge of the biological diversity of a certain area is an important target not only scientifically but also economically, especially for plants of particular officinal, liquor and ecological interest. The recent climatic changes affect mostly vulnerable habitats, such as those located at 2000 m altitude, seriously threatening their survival. Flora protection is necessary to preserve these habitats as well as the species residing therein. This global scenery of environmental protection offers a wide application field for *in vitro* propagation. In our lab we study strategies to preserve plants endangered because of their indiscriminate collection for ornamental purposes or liquor production, also using the *in vitro* propagation technique. In particular we focused on *Artemisia umbelliformis* subsp. *eriantha* (Apennine wormwood), *Leontopodium nivale* (Apennine edelweiss) and *Androsace mathildae*. *Artemisia* and *Leontopodium* (*Asteraceae*) have been regenerated *in vitro* and they are currently grown in experimental fields at high altitude, accomplished for example at the "Alpine Botanical Garden of Campo Imperatore". The garden, located in the "Gran Sasso e Monti della Laga National Park" at an altitude of 2100 m, is ideal to assess the adaptive responses of the *in vitro* obtained clones, subjected to extreme climatic conditions. Between June 2007 and September 2010 over 200 clones were introduced in experimental fields at the Botanical Garden. Both *Artemisia* and *Leontopodium* post-transplant mortality were 0%, with an excellent adaptation ability of the plants, already evident three months after planting of the clones. For *A. umbelliformis*, in particular, there was a decrease in the size of the new leaves, from 4 to 1.5 cm, and a rise of tomentum covering them, almost absent at the beginning. The bloom, which occurred in the year following the transplant, has been extremely copious, with about 16 flower-heads per rosette. *Androsace mathildae* (*Primulaceae*) is an interesting plant species residing only in Central Italy in restricted high-altitude sites (2100-2700 m a.s.l.). We performed numerous studies on *A. mathildae* ecological and phyto-sociological issues and S.E.M. analyses to assess the presence of endophytes. Finally, we successfully generated clones by *in vitro* propagation, to be able to perform applied studies in the next months.

CR4 *ARBUTUS UNEDO* L. SELECTED CLONES CONSERVATION *IN VITRO* CONDITIONS

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Arbutus unedo L. (Ericaceae) is a Mediterranean species. The edible fruits are used to make a spirit which represents the main income for farmers. This species clearly fits on the concept of neglected or underutilized crops and has been included in the list of underutilized plants by the Global Facilitation Unit for Underutilized Species. Several organizations, FAO included, have developed many efforts to arouse the interest of these species especially in developing countries and in countries where an overexploitation of certain crops occurs, as in the European Union. A few years ago we have started a long-term breeding program. During the last years, in collaboration with farmers, we have selected plants based on fruit production/quality. Biotechnology approaches have been applied to clonal propagation, as well as to the selected clones' *in vitro* conservation. As medium culture, De Fossard (1974) was used, added of BA (8.9µM). Cultures (360 tubes, from the same genotype) were storage from 3 to 10 months. Two conditions were tested growth culture chamber (GCC, 25/20ºC, 18/6h) *vs* cold storage (CS, 4ºC). After the 1st subculture the multiplication rate was dependent of the conservation storage period and culture conditions. The best multiplication rate (3.2) was achieved after 3 months cultures storage in a GCC. The multiplication rate observed after 3 to 5 months was about 2 and significantly higher than the multiplication rate after 6 to 10 months culture storage. When cultures were storage at CS conditions, shoots did not show elongation or axillary shoot proliferation and consequently achieved to an inferior multiplication rate. After the 2nd subculture, no differences were found for the multiplication rate. Cultures had recovered their ability to be multiplied. Thus, the multiple regression of the multiplication rate showed coefficients of -0,41 e de -0,13 for the 1st and 2nd subcultures respectively ($P<5\%$). These results suggest that the storage period should not be longer than 5 months and after the 2nd subculture the plant material may be kept for a new conservation cycle. Studies using artificial seeds and cryconservation should be implemented to reduce manual labor and to ensuring the long-term cultures conservation.