COOPERATION BETWEEN PUBLIC RESEARCH AND PRIVATE COMPANIES IN PROPAGATION AND CONSERVATION OF PLANTS FROM THE WILD

MARGHERITA BERUTO[1], CRISTINA PORTOGALLO, SIMONA RININO

[1]Istituto Regionale per la Floricoltura (IRF), Via Carducci, 12-18038 Sanremo (IM), Italy; e-mail: beruto@regflor.it

ABSTRACT
In recent years, conserving biodiversity and propagating wild species are becoming subject of debate and research (Rae & Ingram, 1999). Beside aesthetic and cultural reasons, scientific and economic aspects related to the botanical diversity are of great importance. Plants from wild can contribute to the introduction of novelty in the ornamental market through direct exploitation and providing genetic source for interspecific crosses. Application of *in vitro* techniques can greatly contribute to conservation and propagation programs; however, these technologies require the definition of specific protocols and are too expensive for individual small sized companies. On the other hand, introducing new products is a difficult task for public research institutes due to their limited access to markets and to promotion, particularly when the economical potentiality of a product is addressed to amateur public. In this paper, we review the work carried out at the Regional Institute for Floriculture (IRF) of Sanremo with the cooperation of natural parks and growers in order to evaluate the possibility to develop new products to bring to the floriculture market through the exploitation of plants from the Ligurian biodiversity.

KEY WORDS
Axillary bud stimulation; adventitious regeneration; biodiversity; floriculture; *in vitro*; micropropagation

INTRODUCTION

The floriculture industry represents a very important aspect of the European economy; the gross marketable production related to this industry is about 7% of the whole agricultural production. The West side of the Liguria Region is an area where this industry has a tremendous impact with a gross marketable production of 72% of the whole agricultural production of this Region and about 2800 ha devoted to floricultural crops (Sanremo flower market data, 2006). This part of the Liguria Region is the pioneer land for the Italian floriculture; in this area the floriculture industry has began about the end of the nineteenth century with the production of cut flowers from the wild (e.g. stocks and narcissus) and cut foliages. Afterwards, the floriculture could raise towards an industrial application; the favourable environment climatic conditions and the operators' professionality could contribute to the creation of a quality product well known around the world.

Creating new products is a mandatory task for the floriculture industry which is subjected to an increasing competition between breeders and producers in order to meet the demands imposed by the market. Growers sometimes try to use plants from the wild and often breed ornamental plants, purely by picking out chance variants, in other cases by systematically breeding for specific traits of interest. Thus, many ornamentals are "new " introductions , with a short breeding history although different challenges arise from developing an undomesticated plant into an economically viable, cultivated fresh cut flower.

The aromatic plants of Liguria Region provide an interesting floriculture product which started from harvesting species from the wild for the pot market. To date, in this Region the yearly production of pot plants and outside plants is about 100 millions pieces and the half production is represented by aromatic plants (lavender 37%; rosemary 34%; thyme 9%; sage 6%; oreganum 4%; others 10%). In addition, some other species (e.g. *Ranunculus* spp, *Helleborus foetidus*, …) are collected from the wild and seem to represent an interesting alternative for a cut flower niche market which characterizes an important part of exports to the Nord Europe.

The contribution of wild sources to the introduction of novelty in the ornamental market can be managed not only through direct exploitation but also by providing genetic source for interspecific crosses. In this wide context, the application of *in vitro* techniques can greatly contribute to exploitation of the opportunity provided by the wild (Bowes, 1998). *In vitro* collections can show significant potential for the conservation of plant genetic resources and the micropropagation can enhance the number of plants for each genotype for which the *in vivo* selection can be performed in order to shorten the time required to reach the market.

In this paper, we review the work carried out at Istituto Regionale per la Floricoltura (IRF, Sanremo) over 7-8 years in order to improve the availability of new products to the market by the use of plants from the wild. The different programs were set up with the cooperation of other public institutions (namely University of Turin and Genoa, INRA- France), natural parks (namely National Park Maritime Alps-Valdieri and Park National du Mercantour-France) and growers. This latter aspect has been carefully taken into consideration because the policy of our institution is based upon the consideration that a mutual cooperation between public institution and private company can led both to overcome the challenges of the *in vitro* technologies requiring specific and expensive professionality and to increase the potentiality to penetrate the market with a new product.

AREAS OF STUDY

A part of our programs considered the possibility of exploitation, under an ornamental point of view, for different species distributed in the area from Ventimiglia and Savona. Terrestrial orchids and different wild species (i.e. *Astrantia major, Lilium sp, Helleborus sp, Rosa sp.*.) were gathered in the four areas of the Maritimes and Ligurian Alpes showed in Fig. 1. Application of *in vitro* culture for conservation of the endangered species *Campanula sabatia* was considered too.

Further research program considered to set up a collection of wild species of *Ranunculus* genus; for that, species with particular aesthetic traits were collected from botanical gardens, collectors, nurseries and seeds' producers all over the world. The plant material was collected in the form of *in vivo* plants and *in vitro* clones.

MATERIALS AND METHODS

In vitro germination of seeds

The *in vitro* techniques for germination of seeds were applied for terrestrial orchids, the different species of *Ranunculus* and *Campanula sabatia*. The choice of this *in vitro* system was related to different reasons according to the different plant material.

In the case of terrestrial orchids, the asymbiotic growth is generally considered in order to overcome the germination problems arising from the reduction of endosperm in the seeds and the required symbiotic relationship with mycorrhizal fungi which led to a low germination rate. In our study, twelve different species of terrestrial orchids were considered: *Anacamptis pyramidalis, Barlia robertiana, Dactylorhiza maculata, Dactylorhiza sambucina, Epypactis helleborine, Gymnadenia conopsea, Ophrys fusca, Ophrys sphegodes, Orchis coriophora, Orchis mascula, Platanthera* sp and *Serapias* sp. The entire capsules, few days after collection from plants in the wild (late Spring- Summer 2000 and 2001), were sterilized with a

NaOCl solution (1% available chlorine; 15 min) and rinsed with sterile double distilled water. Then they were opened under aseptic conditions and seeds inoculated on Orchimax (cd O 0262, Duchefa) gelled medium (0.7% w/v agar) supplemented with sucrose (20 g L^{-1}) and the cytokinins BA (0.5 mg L^{-1}) and kinetin (1 mg L^{-1}). The pH of the medium was checked in the sol state (75 ± 1°C) and adjusted to 5.85 before sterilization (120°C, 15 min; flasks filled with 250 mL of medium). After autoclaving, medium (20 mL) was poured into Petri dishes (60 mm diameter). Attention was taken that the seeds could cover all the surface of the medium and that conglomerate of plant material was avoided. The incubation conditions considered a temperature of 20 ±1°C photoperiod 16h/day and PAR 50 μmol $m^{-2}s^{-1}$ (fluorescent tubes: TLD 36W/33 Philips). The germination rate was scored after five-six months of culture and the subsequent development of seedling was followed. For the species *Dactylorhiza maculata* two additionals observations were considered concerning the influence of the maturity state of the capsule on the further germination rate and the multiplication potentiality of different seedlings developed from the *in vitro* sown. For that, capsules collected from the same stem (25-30 cm length) were numbered progressively (up-down versus) and the subsequent development of the corresponding seeds was followed. The multiplication efficiency was evaluated by culturing 10-20 genotypes (seedlings) over a period of a year of culture under the same conditions previously described. Gradual acclimatization of *Dactylorhiza maculata* plantlets to *in vivo* conditions was performed by transferring the *in vitro* plants to glass vessels (500 mL) filled with a sterilized substrate composed of peat and perlite (1:1), maintained under the same conditions at culture rooms, for two months. Subsequently, the plantlets were transferred to greenhouse conditions (unheated greenhouse) and were delivered to the growers for a first field evaluation.

The choice of *in vitro* germination for *Campanula sabatia* is related to the fact that this is an endangered species exclusive of western Liguria for which an in vitro conservation and propagation program of different genotypes can be envisaged. The *in vitro* collection of *Ranunculus* was mainly based upon *in vitro* sown due to the easier availability of seeds from the different botanical gardens and private companies contacted during our work. Seeds were treated with a 10% calcium hypochlorite solution for 10 min, rinsed with sterile double distilled water. *Campanula sabatia* seeds were inoculated on gelled (0.7% w/v) medium supplemented with the Quoirin & Le Poivre (1977) macroelements, Murashige and Skoog (1962) microelements and vitamins, sucrose (30 g L^{-1}), NAA (0.01 mg L^{-1}), BA (0.5 mg L^{-1}) and Kinetin (1 mg L^{-1}) (25 x 150 mm test tubes, containing 10 mL of medium) and kept at the same conditions described for the terrestrial orchids. The developed seedlings were subsequently cultured on the same medium; after *in vitro* rooting, plantlets were transferred to *in vivo* conditions and the subsequent growth to flowering was followed.

The *in vitro* collection for *Ranunculus* genus was undertaken for 33 species (Costa et al., 2007) which once sterilized were cultured on modified Murashige and Skoog (1962) medium (MS) containing half strength salts, sucrose 30 g L^{-1}, agar 8 g L^{-1} and different hormonal combinations (mg L^{-1}) (NAA 0.02+BA 0.5+Kin1; NAA 0.02+BA1+Kin2; control= no hormones). *Ranunculus* seeds were cultured at 10°C for three months and subsequently were transferred at 15°C for additional three months; half of cultures were maintained under dark, the other part under light (50-60 μmol $m^{-2}s^{-1}$ provided by fluorescent tubes TLD 36W/33 Philips- 12h- photoperiod). Germination rate was scored at the end of the two culture periods (three

and six months) and the obtained plantlets were acclimatized under greenhouse conditions at standard cultural practices regarding nutrition, irrigation and pest control.

In vitro axillary bud stimulation

Most micropropagation is achieved by using axillary buds as explant source. In fact, shoot explants are relatively easy to manipulate, they establish and proliferate quite rapidly and a low percentage of variation is expected for the plantlets issued from the micropropagation process. This kind of *in vitro* growth was chosen to face the production of species with are overcollected from the wild in order to satisfy a niche market (e.g. *Helleborus* sp. and *Paeonia* sp.), to be used as rootstock (e.g. *Rosa* sp.) or to provide a novelty to the market (e.g. *Astrantia* sp.). The explants were sterilized by a solution of NaOCl (0.5-1% available chlorine; 10-15 min depending on the initial tissue) followed by a rinse with sterile double distilled water. For each species a proper medium and culture conditions were selected; the culture was carried out in culture tubes (25x 150mm tubes; 10 mL of medium) or glass jars (320 mL filled with 100 mL of medium). The classical scheme for micropropagation was followed concerning of three different stages (Stage 1= initiation of culture; Stage 2= multiplication; Stage 3= *in vitro* rooting) and plantlets were acclimatized under *in vivo* conditions and followed in the field growth.

In vitro adventitious bud stimulation

The techniques used to regenerate adventitious shoots represent an alternative way to build propagation and conservation programs although genetic changes can occur through these growth systems. To minimize somaclonal variation, the direct adventitious regeneration is desirable. In our trials we selected the adventitious regeneration from bulb scale of three *Lilium* species (*L. bulbiferum*; *L. martagon* and *L. pomponium*). Tissues were sterilized with a NaOCl solution (1% available chlorine; 15 min) and rinsed with sterile double distilled water; each bulb scale was divided in three parts (upper, medium and basal one) and cultured on gelled (0.8% w/v agar) MS medium supplemented with sucrose (30 g L^{-1}), NAA (0.1 mg L^{-1}) and BA (10 mg L^{-1}). The regeneration efficiency was evaluated after five months of culture; plantlets were acclimatized under *in vivo* conditions and transferred to the growers for a field evaluation.

RESULTS AND DISCUSSION

In vitro germination of seeds

The *in vitro* germination of non-epiphytic, terrestrial orchids is reported to be difficult because of the great dependence on mycorrhizal fungus and the several types of seeds dormancy (Sgarbi, 2001). In our experiment, evidence of germination was considered when embryos were swollen, the testa integument cracked and the rhizoids longs. The germination rate, scored after about six months of culture, was depending upon the different genera and species. The highest germinating ones were the genera *Dactylorhiza*, *Platanthera* and *Serapias* (about 80%, 60% and 100% respectively) and the species *Gymnadenia conopsea* (about 80%) and *Orchis coriophora* (about 60%). Intermediate germination rate (20-50%) were found for the species *Barlia robertiana*, *Epypactis helleborine*, *Ophris fusca* and *sphegodes* (Tab. 1). These findings are consistent with the available literature (Debergh & Van Waes 1986; Read& Szendrak, 2000; Sgarbi et al., 2001). In addition, the experiment carried out for *Dactylorhiza maculata*

evidenced that the seed maturity can influence the further germination efficiency. Seeds collected from capsules in an intermediate position provided the highest germination percentage (Fig. 2). It could be envisaged that, under the experimental conditions, immature seeds (in the upper capsules) were not able to complete the germination process, while seeds at a high state of maturity (in the lower capsules) could not overcome the obstacles posed by the already formed impermeable integuments. Most of the protocorms from the different species turned quite rapidly green and within two addition months of culture the development of young plantlets was observed (Tab. 2). Plantlets continued to develop leaves and roots and for *Dactylorhiza maculata* a multiplication cycle was performed. According to the genotypes, a variable number of plantlets (range from 10 to 80) were obtained after one year of culture and they showed a further growth when transferred to a gradual *in vitro* acclimatization. The *in vivo* survival rate for *Dactylorhiza maculata* was satisfactory (about 70%) but the subsequent growth in the field showed some important constraints (Beruto et al., 2002; Beruto, 2003).

Tab. 1. Germination and further development of protocorms after six and eight months of *in vitro* culture for different species of terrestrial orchids.

Genere/Specie	Germination (%) after 6 months of *in vitro* culture	Protocorm developed towards young plantlets (%) after about 8 months of *in vitro* culture
Anacamptis pyramidalis	6,7 a*	-
Barlia robertiana	27,8 b	29,8 c
Dactylorhiza maculata	76,9 d	65,0 e
Dactylorhiza sambucina	90,9 e	62,5 e
Epypactis helleborine	50,0 c	-
Gymnadenia conopsea	83,3 d	58,6 de
Ophrys fusca	14,3 b	-
Ophrys sphegodes	20,0 b	-
Orchis coriophora	62,5 c	0,0 a
Orchis mascula	0,0 a	
Platanthera sp	62,5 c	13,3 b
Serapias sp.	100,0 e	49,6 d

*Means, in the same column, followed by the same letter are not different according to the Duncan test
($p < 0.05$)

Fig. 2. *In vitro* germination of *Dactylorhiza maculata* according to different degrees of maturity of starting capsules (1-7= up-down versus). The reactivity was evaluated according to a visual scale from 0 (no germination) to 2 (developed plantlets).

Our preliminary results on *in vitro* culture of *Campanula sabatia* showed that, under our experimental conditions, about four-five months of culture were required before that germination (20-30%) was observed. A development to young plantlets was observed in any case and the further multiplication phase carried out over a year, (may 2006-april 2007), with regular subcultures, showed quite satisfactory rate although dependent on the genotype (from 20 to 60 shoots from each plantlet developed from seed). The acclimatization phase carried out in February 2007 provided plants able to flower at the end of July 2007 .

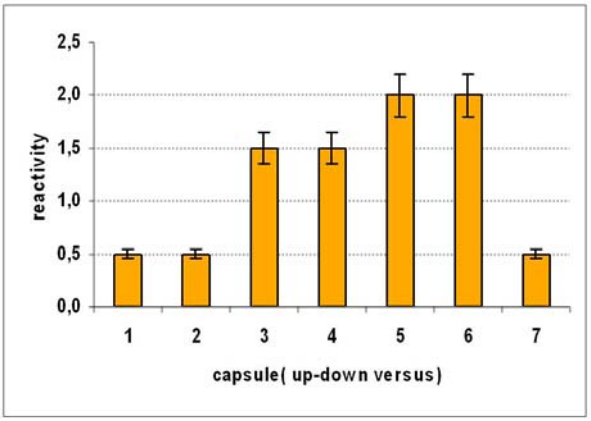
The aptitude for germination for the different *Ranunculus* species was shown to be genotype-dependent. Particularly for 17 species no germination was observed under our experimental conditions. Generally speaking, germination could occur on medium with no hormones; only for *R. glacialis* and *R. montanus* hormone supply was essential to induce germination. Other species (*R. godleyanus*, *R. illyricus*

Tab. 2. Contamination and adventitious regeneration from bulb scale of different wild lilies.

Lilium	**(%)**	
	contamination	**reactivity**
bulbiferum	31.7 b*	73.2 a
pomponium	1.7 a	83.5 ab
martagon	1.0 a	86.9 b

* Means, in the same column, followed by the same letter are not different according to the Duncan test ($p < 0.05$).

and *R. tricophyllus*) enhanced their germination on media supplemented with hormones, although hormonal requirements was not essential to stimulate sprouting. Light conditions were essential to elicitate the germination for *R. aconitifolius*, *R.. acris*, *R. godleyanus* and *R. insignis*, while dark conditions promoted the germination for *R. bulbosus* and *R. glacialis* (Costa et al., 2007). The observation on the further multiplication efficiency of seedlings showed that a different degree of variation among the genotypes could be observed according to the species (Fig. 3). Plantlets were successfully acclimatized under *in vivo* conditions (survival rate after five months from the acclimatization: 60-70%) and they start to form our *in vivo* collection which is still under field evaluation. (Fig. 4).



In vitro axillary and adventitious bud stimulation

The decisive factor for successful *in vitro* work is to obtain aseptic cultures and this aspect is particularly incisive when a vegetative propagation from an *in vivo* mother plant is faced. In our experiments, a variable percentage of contamination, ranging from 25% and 50%, was scored and this allowed to grow explants according to the different stages outlined for a micropropagation scheme (Debergh & Maene, 1981).

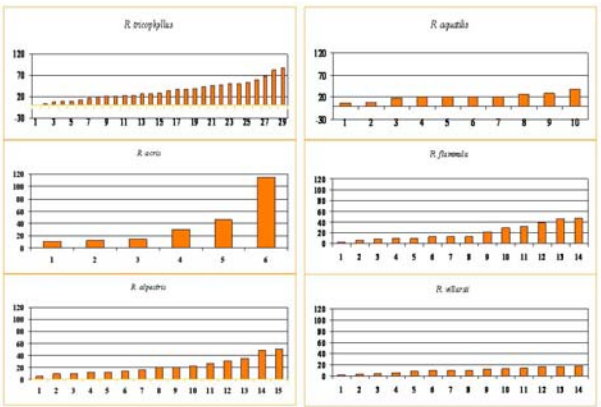


Fig. 3. Moltiplication efficiency of different seedlings of *Ranunculus* wild species cultured over a period of about eight months.

Fig. 4. *In vivo* collection of different *Ranunculus* wild species obtained through *in vitro* technique (see text).

A different reactivity to the *in vitro* culture was observed for the different bulb part, showing the upper one poor regenerative potentiality (20% regenerated explants versus 60-100% for the medium and basal part). After five months of culture, a satisfactory reactivity for the three tested species was scored, being the *L. martagon* the most reactive species (Table 3; Beruto et al., 2001). The regenerated plantlets were successfully acclimatized and the following evaluation at growers' conditions led to consider that the wild lilies cut flowers can represent an interesting product for a niche market (Beruto, 2003).

The micropropagation of *Rosa* rootstock was considered under an industrial point of view due to the fact that it is normal agronomical practice to graft the commercial varieties sold in the market. The *in vitro* culture can provide health and homogeneous plant material; particular performing genotype can be multiplied by starting from a reduced number of mother plants and the overcollection from the wild can be reduced. Our results on micropropagation of different clones of *Indica major* showed that *ex vitro* plantlets provided an increased number of cuttings compared to the corresponding plant material propagated under *in vivo* conditions and this enables to consider that the *in vitro* techniques could be considered by the different companies involved in the commercial production of roses (Beruto et al., 2001).

Astrantia major is a species of herbaceous plants in the family Apiaceae which was considered for a possible exploitation as cut flower production. Our preliminary results on micropropagation of this species showed that a poor initial contamination percentage (about 13%) and an average multiplication rate of 1: 2/2,5 could be scored. Further investigations will be addressed to evaluate the plants under field conditions (Beruto et al.,2001).

In the past, the peonies were often classified in the family *Ranunculaceae*, alongside hellebores and anemones. The members of this family present different constraints in the definition of a reliable micropropagation protocol. Contamination of the initial explants, low multiplication rate, losses during rooting and acclimatization are still problems to be solved (Wang et al., 2000; Albers et al., 1992; Beruto et al., 2004; Seyring, 2001; Beruto et al., 2007). In our institution, we have start a project dealing with the micropropagtion of different *Helleborus* species and hybrids to be evaluated as cut flower and pot production. *H. foetidus* and *H. viridis* were ascribed among the wild flora of Liguria Region (Nicolini and Moreschi, *Fiori di Liguria*) and are already used as cut flower production addressed to the Nord Europe customers. Our trials on *H. foetidus* pointed out that, under our experimental conditions, a not yet satisfactory multiplication rate could be scored although this agrees with other findings (Dhooghe & Van Labeke, 2007). Once transferred to *in vivo* conditions, plantlets developed in their growth and the first flowering was observed after one year from the acclimatization.

Peonies are herbaceous perennials common to beyond and the central and western Mediterranean which can be found also in Liguria Region; indeed, *Paeonia officinalis* subsp. villosa can be diffusely found in the mountains beyond Ventimiglia and near the French border. Due to the ornamental value of these plants, a commercial exploitation has been already considered. However, the usual *in vivo* propagation methods are time-consuming and expensive beside the fact that un undesirable variation among seedling can be scored. As previously said for the *Helleborus*, in our institution the micropropagation of peony is considered under a wider project addressed to multiply different species and hybrids of this plant. This study is still under consideration and to date only preliminary results on the first steps of *in vitro* culture are

available. From that, an initial contamination rate of about 50% and a different multiplication efficiency depending upon the genotype could be evidenced.

CONCLUSIONS

The researches carried out at Istituto Regionale per la Floricoltura, Sanremo, has been undertaken in order to save, collect and propagate wild genetic resources which can be considered for the exploitation for an ornamental use. Innovation in floriculture field which came from the wild resource can contribute to the renewal imposed by the pressing competion. The possibility to exploit a local wild flora can offer different advantages compared to the exploitation of wild plants coming from foreign countries; plants can be better adapted to the local climatic conditions and the plant source to start the collection and propagation can be easy available. Notwithstanding of it, the direct or indirect (e.g. by breeding) use of wild flowers cultivars for use in commercial floriculture present important challenges and the cooperation between public institutions and private companies could contribute to shorten the time to penetrate the market with novelties.

REFERENCES

Albers M.R.J., Kunneman B.P.A.M., 1992. Micropropagation of paeonia. Acta Horticulturae 314: 85-92.

Beruto M., Portogallo C., Atzei E. & Lanteri L., 2002. Impiego delle tecniche in vitro per la valorizzazione di alcune essenze della flora delle Alpi Marittime a scopi ornamentali. Abstract: Workshop – VI Giornate scientifiche SOI, 23-25 April 2002, 57-58

Beruto M., Lanteri L. & Portogallo C., 2004. Micropropagation of three peony (Paeonia suffruticosa). Plant Cell, Tissue and Organ Culture 74: 249-255

Beruto M., Portogallo C. & Curir P., 2007. Effect of chilling and hormonal supplìes on rooting and in vivo establishment of micropropagated plantlets of Helleborus hybr. Abstract: 3rd International Symposium on acclimatization and establishment of micropropagated plants. 12-15 September 2007, Faro – Portugal.

Beruto M., Portogallo C. & Gazzano C., 2001. Micropropagazione di portainnesti di rosa. In : Programma pluriennale di ricerca e sperimentazione in campo floricolo in zone obiettivo 5B, misura 4.1. Art & Stampa, Sanremo: 84-87.

Beruto M., Lello S. & Portogallo C. 2001. L'utilizzo ornamentale di specie autoctone. In: Impiego ornamentale della flora spontanea delle Alpi Marittime e Liguri. Tipografia Ghibaudo, Cuneo: 105-126

Beruto M., 2003. San Romolo: un bosco dal cuore antico. Abstract: 27-28 June 2003, Sanremo.

Bowes B. G., 1998. Plant propagation and conservation (Vol. 1). Grafos SA, Barcelona.

Costa E., Scariot V. & Beruto M., 2007. The development of new products throught the collection of old cultivars and wild species of Ranunculus genus. In press: XXII EUCARPIA Symposium section Ornamentals, Breeding For Beauty, 11-15 Sept, 2006, Sanremo – Italy

Debergh P.C. & Maene L.J. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. Scientia Horticulturae 78: 335-345

Dhooghe E. & Van Labeke M.C. 2007. In vitro propagation of Helleborous species. Plant Cell Tissue Organ Culture, in press.

Murashige T. & Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497

Nicolini G. & Moreschi A., Fiori di Liguria (Vol. 1). Cassa di Risparmio di Genova e Imperia. Edizione e Stampa: SIAG, Genova.

Quorin M., Le Piovre P., 1977. Etudes de milieux adaptes aux cultures in vitro de Prunus. Acta Horticulturae. 78: 437-442

Rae D., Ingram D.S., 1999. The rationale of conservation. In: B.G. Bowes (Ed) Plant Propagation and Conservation, Manson Publishing: 15-24

Seyring M. 2001. In vitro cloning of Helleborus niger. Plant Cell Rep. 20: 895-900.

Sgarbi E. 2001. La germinazione in vitro delle orchidee: un approccio metodologico. Atti Soc. Nat. Mat. Modena 132: 66-74

Sgarbi E., Del Prete C., Ronconi L. & Dallai D. 2001. Asymbiotic micropropagation of wild Italian orchids: from seed to plant in a project for in situ reintroduction. Journal Europaischer Orchideen 33: 395-404.

Szendrak E. & read P. E., 2000. In vitro propagation and anatomical studies of temperate orchid species (Orchidaceae). Acta Horticulturae 520: 75-81.

Van Waes J. & Debergh P. C., 1986. Adaptation of the tetrazolium method for testing the seed viability, and scanning electron microscopy study of some western European orchids. Physiologia. Plantarum 66: 435-442.

Wang H., Van Staden J., 2000. Establishment of in vitro cultures of three peonies. South African Journal of Botany 67: 358-361