

IN VITRO PROPAGATION AND CONSERVATION OF RED CHICORY GERMLASM

Previati A.¹, Benelli C.², Da Re F.¹, De Carlo A.³, Vettori C.³ and Lambardi M.²

¹ Centro Sperimentale Ortofloricolo “Po di Tramontana”, Veneto Agricoltura,
45010 Rosolina (Rovigo), Italy, alberto.previati@venetoagricoltura.org

² IVALSIA/Istituto per la Valorizzazione del Legno e delle Specie Arboree, CNR,
50019 Sesto Fiorentino (Fi), Italy

³ IGV/Istituto di Genetica Vegetale, Sezione di Firenze, CNR, 50019 Sesto Fiorentino (Fi), Italy

Summary

Effective procedures for the slow growth storage and the cryopreservation of red chicory (*Cichorium intybus* L.) selected lines are described. Best storage condition (95% of shoot survival after 9 months) was obtained with the maintenance of ‘Treviso precoce’ at 4°C in darkness. As for cryopreservation, shoot tips survived satisfactorily when they were loaded in PVS2, prior to direct plunging into liquid nitrogen (“vitrification/one-step freezing”).

Keywords

Cichorium intybus, cryopreservation, *in vitro* conservation, micropropagation, RAPD

Introduction

Red chicory (*Cichorium intybus* L.) is one of the most economically important vegetables of the Veneto region, North-East Italy, with over 9,000 ha cultivated annually. Introduced into Italy in the XV^o Century, in recent years red chicory has undergone intense work of selection and breeding, which has produced over time several improved typologies (such as ‘Rosso di Treviso’ and ‘Rosso di Chioggia’), highly appreciated for their quality and productivity. An important breeding programme is currently in progress at the experimental farm “Po di Tramontana” in Rosolina (Rovigo, Italy), which belongs to the “Veneto Agricoltura” State Institution, with the aim of selecting high-performance lines. After in-field evaluation, the most valuable lines are introduced *in vitro*, reproduced by micropropagation, and used yearly as a source of plants which are grown in screenhouses to obtain quality seeds. The seeds are then available to farmers for the production of high-quality red chicory. As micropropagation of red chicory requires a regular 3-week subculturing at 21°C and 12h photoperiod, the possibility of achieving long-term storage or, at least, a significant extension of the interval between subcultures is crucial. Hence, a study for the medium-term (by slow growth storage, SGS) and the long-term (by cryopreservation) conservation of selected lines was initiated in the 2002 with a collaboration between “Veneto Agricoltura” and the CNR/IVALSIA of Italy.

Material and Methods

The study is in progress with a total of 4 selected lines of red chicory (‘Treviso precoce’, ‘Chioggia’, ‘Treviso tardivo’ and ‘Castelfranco’). After the introduction *in vitro*, the lines are kept in standard proliferative conditions (SPC), i.e., in MS medium, containing 0.5 µM BA, at 21°C and 12h photoperiod, and subcultured every 3 weeks. This method allows a few years of shoot culturing, after which the lines are replaced at the first signs of culture decline.

Preparation of shoots and conservation by SGS

Shoots of ‘Treviso precoce’ and ‘Chioggia’ were prepared by transferring them into 80-cc sterile plastic cylinders, closed with a gas-tight cap, containing 10 ml of medium (one shoot per cylinder). Two different approaches to SGS were tested: (i) the storage of cylinders at 4°C, in darkness or under a 8h photoperiod (15 µmol m⁻² sec⁻¹ of light intensity), and (ii) the addition of osmotically active compounds (OACs) to the medium, i.e., mannitol (40 g/l) or

NaCl (4.48 g/l), then storing the shoots in SPC. In a second trial, the conservation at 4°C and in darkness was applied to compare 3 different *in vitro* containers, i.e., (i) gas-tight 80-cc plastic cylinders (one shoot per cylinder), (ii) gas-tight 500-cc glass jars wrapped with plastic film (10 shoots per jar), and (iii) gas-permeable “StarPac”™ bags (one shoot per cell).

Cryopreservation procedures

Shoot tips (1-2 mm long) were excised from microshoots, adventitiously induced from portions of red chicory leaves, and used in cryopreservation trials. Two “vitrification/one-step freezing” procedures were developed, i.e., (i) “loading the shoot tips with a vitrification solution”, and (ii) the “encapsulation-vitrification”, consisting in the encapsulation of explants in calcium-alginate beads before vitrification treatment [1]. In both the procedures, the used vitrification solution was the PVS2 [2]. The explants (naked or encapsulated, contained in 2-ml cryovials) were rapidly ultra-frozen to -196°C by direct immersion in liquid nitrogen (LN). For recovering, they were quickly rewarmed by plunging the cryovials into a 40°C waterbath, unloaded from the PVS2 solution, and finally moved to SPC.

Assessment of genome stability of explants from cryopreservation

A preliminary evaluation of genome stability of the cryopreserved material was performed by RAPD markers. About 0.1 g DNA from leaves of control and cryopreserved ‘Chioggia’ shoots was extracted and purified, using a DNeasy Plant Mini Kit (Quiagen). Ten random primers were used. RAPD reaction was performed as described by Vettori *et al.* [3].

Results

With the 'Treviso precoce' line, all the storage conditions were equally effective in reducing shoot growth during 9 months of conservation, as the "relative growth rates" (RGR) were always below 1, i.e., over six-fold inferior to the value recorded from shoot cultures in SPC (RGR=6.0). Also in terms of shoot survival, the conservation up to 6 months was always satisfactory. However, on prolonging the storage time in OACs to 9 months, a marked decrease in shoot survival was observed (70% in mannitol, 35% in NaCl). Differently, 95% of the shoots stored at 4°C were still in perfect condition after 9 months (with no difference between the darkness and the 8h-photoperiod conditions) and they recovered their full proliferation activity when moved again to SPC. A general lower adaptability to SGS was evidenced by the 'Chioggia' line, as 100% shoot survival was recorded after 6 months of conservation at 4°C in darkness, but this value dramatically dropped to 45% after 9 months. The use of OACs allowed only a 3-month conservation, mannitol being more efficacious than NaCl (75% and 50% of shoot survival, respectively).

All the containers allowed a satisfactory conservation of shoots at 4°C and in the dark for 9 months, as 100% shoot survival was achieved. However, a different trend was recorded as regards the RGR values. Indeed, for each conservation period, a higher growth rate was always recorded for the shoot cultures stored in the 'StarPac' gas-permeable plastic bags, and they required a longer time to recover full proliferation activity when moved again to SPC.

All the 4 red chicory lines could be successfully cryopreserved by loading shoot tips in PVS2 for 60 or 90 min, prior to be one-step frozen in LN. Among the lines, maximum survival ranged between 65% and 76%. After the recovering of shoot tips for 3 months in SPC, the plantlets could be potted and acclimatized *in vivo*. The “encapsulation-vitrification” procedure was less effective for red chicory cryopreservation. With the tested primers, RAPD analysis didn't show any genetic alteration between donor and cryopreserved plants.

REFERENCE LIST

- [1] De Carlo A., Benelli C., Lambardi M (2000). Development of a shoot-tip vitrification protocol and comparison with encapsulation-based procedures for plum cryopreservation. *CryoLetters* 21: 215-222.
- [2] Sakai A., Kobayashi S., Oiyama I. (1990). Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis*) by vitrification. *Plant Cell Rep.* 9: 30-33.
- [3] Vettori C., Paffetti D., Pietramellara G., Stotzky G., Gallori E. (1996). Amplification of bacterial DNA bound on clay minerals by the random amplified polymorphic DNA (RAPD) technique. *F.E.M.S. Microbiol. Ecol.* 20: 251-260.