Electronic Journal of Biotechnology ISSN: 0717-3458 Vol.12 No.4, Issue of October 15, 2009
© 2009 by Pontificia Universidad Católica de Valparaíso -- Chile
Received February 19, 2009 / Accepted July 23, 2009
DOI: 10.2225/vol12-issue4-fulltext-7 RESEARCH ARTICLE

Screening for propagation suitability in vitro of different *Cyclamen* species

Martina Seyring*
Department Plant Propagation
Leibniz-Institute of Vegetable and Ornamental Crops Groβbeeren/Erfurt
Kuehnhaeuser Str. 101, D-99189
Erfurt-Kuehnhausen, Germany
E-mail: seyring@erfurt.igzev.de

Aloma Ewald*
Department Plant Propagation
Leibniz-Institute of Vegetable and Ornamental Crops Groβbeeren/Erfurt
Kuehnhaeuser Str. 101, D-99189
Erfurt-Kuehnhausen, Germany
E-mail: ewald@erfurt.igzev.de

Anke Mueller
University of Applied Sciences Erfurt, Horticulture
Erfurt, Germany

Klaus-Thomas Haensch
Department Plant Propagation
Leibniz-Institute of Vegetable and Ornamental Crops Groβbeeren/Erfurt
Erfurt-Kuehnhausen, Germany

Financial support: Ministries of Agriculture of the Federal Republic of Germany and the States of Brandenburg and Thueringen.

Keywords: propagation, shoot regeneration, somatic embryogenesis, species.

Abbreviations: 2,4-D: 2,4 dichlorophenoxyacetic acid
2iP: 6-(γγ- dimethylallylamino) purine
BAP: 6 benzylaminopurine
IES: Indolyle-3 acedic acid
NES: 1-naphtylacedic acid
SELS: somatic embryo-like structures

In the present study we examined the possibility of propagating different *Cyclamen* species (*C. africanum* Boiss. and Reut., *C. cilicium* Boiss. and Heldr., *C. coum* Mill., *C. hederifolium* Ait., *C. persicum* Mill., *C. purpurascens* Mill.) including some of their subspecies and cultivars in vitro using explants of adult plants. For this purpose two protocols have been applied to eleven genotypes combined with mostly four explant types (placentas with ovules, leaves, petioles and peduncles). The use of these protocols has given rise to either somatic embryo-like structures and/or adventitious shoots in all genotypes. This way it was possible to propagate each of the examined genotypes in vitro using explants of adult plants in a time less than one year. These results may be used in breeding and propagation of *Cyclamen* as an ornamental plant and as a medicinal plant.

The genus *Cyclamen* L. (Myrsinaceae) consists of more than 20 species, *i.e.* 22 species according to Grey-Wilson (2002) or 21 species according to Compton et al. (2004); and Yesson and Culham (2006). Propagation is exclusively performed by seeds. Only cultured varieties of *Cyclamen persicum* Mill. have so far had any commercial importance as an ornamental plant. Other species are largely neglected, due to their poor propagation qualities and other problems in cultivating them. Many species have nevertheless certain properties such as cold hardiness and high levels of resistance to various plant diseases that are potentially of great horticultural interest and value. The most cold hardy species are *C. hederifolium* Ait., *C. coum* Mill. and *C. purpurascens* Mill. Our own research has indicated that some wild species under investigation have higher levels of tolerance against *Cyclamen* wilt than the different *C. persicum* cultivars.

Other species of *Cyclamen* might be of interest for medical purposes (Çaliş et al. 1997a; Çaliş et al. 1997b; Speroni et al. 2007; Foubert et al. 2008; Quave et al. 2008).

Whereas studies have been conducted to elucidate the reproductive mechanisms in generative and vegetative

*Corresponding author

Figure 1. Regeneration of *C. coum* magenta using peduncles applying protocol 1. (a) Induction of callus after eight weeks on medium with 9.05µmol l^{-1} 2,4-dichlorophenoxyacetic acid (2.4-D) und 3.94 µmol l^{-1} 6-(γγ- dimethylallylamino) purine (2iP); (b) Differentiation of somatic embryo-like structures (SELS) on growth regulator-free medium; (c) Development of SELS; (d) Regenerated plants. Bar. 10 mm (a-d).

propagation of cultivated varieties of *C. persicum* Mill. and interspecific *Cyclamen* hybrids (Kiviharju et al. 1992; Ewald and Schwenkel, 1997; Schwenkel and Winkelmann, 1998; Ewald et al. 2000a; Ewald et al. 2000b; Karam and Al-Majathoub, 2000; Ishizaka, 2003; Rensing et al. 2005; Winkelmann and Serek, 2005; Reinhardt et al. 2008), little is known about the reproduction processes of most other species in this genus (Affre et al. 1995; Affre and Thomson, 1997a; Affre and Thomson, 1997b; Affre and Thomson, 1999). An efficient vegetative propagation of *Cyclamen* is possible only by using in vitro culture. Reports about this method are restricted to the wild species C. *persicum* (Karam and Al-Majathoub, 2000) and its cultivars (Hohe et al. 2000). This knowledge has been applied by Prange et al. (2008) for establishing the in vitro propagation of four other wild species of *Cyclamen* starting from juvenile explants. However one prerequisite for the propagation of quality tested plants is that explants from adult plants can be used. The aim of the present study was therefore to perform a screening for the possibility of propagating different wild species and subspecies of the genus *Cyclamen* and their cultivars in vitro using explants from adult plants.

MATERIALS AND METHODS

The study included eleven genotypes of the genus *Cyclamen* (different species, subspecies and cultivars, Table 1).

Explants were extracted from young leaves, petioles, flower buds and peduncles of adult plants from the greenhouse.

The leaves with petioles were disinfected for three minutes in a 0.8% silver nitrate solution and were rinsed three times for 5 min each time in sterile distilled water.

The peduncles and flower buds were immersed for 30 sec in alcohol (70%) before being disinfected for 20 min in a sodium hypochloride solution (3% free chlorine). Following this, the plant material was rinsed three times for 5 min each time in autoclaved tap water.

Since the leaves had a maximum leaf blade width of 1 cm, only a single explant could be extracted per leaf. Explants were extracted with a cork borer (6 mm) right beside the mid vein. Four segments (with a size of 5 mm each) were extracted from each petiole and peduncle. The flower buds, two to four days before start of anthesis, were exposed down to the ovary, and the central placenta with its ovules was vertically quartered. Due to the different growth patters of the original plants, however, not all types of explants from all genotypes could be subjected to exactly the same experiments at the same time. Between 4 and 36 explants per type of explant were analyzed. Each Petri dish contained four explants.

Propagation using protocol 1

Following Schwenkel and Winkelmann (1998), the primary culture (eight weeks) and the first sub-culture (four weeks) were applied on modified MS medium (Murashige and Skoog, 1962) at 9.05 µmol l^{-1} 2,4 dichlorophenoxyacetic acid (2,4-D) and 3.94 µmol l^{-1} 6-(γγ- dimethylallylamino) purine (2iP) and kept at 25ºC in permanent darkness to induce and propagate calluses. In order to allow the differentiation and germination of somatic embryos, the callus was transferred to growth regulator-free MS medium and cultivated for 16 weeks at 23ºC in permanent darkness. The further cultivation of the germinated somatic embryos into young plants was also performed on growth regulator-free MS medium, but at 18ºC under a 16/8 hrs (light/dark) photoperiod with light supplied by white fluorescence lighting at an intensity of 46 µmol s^{-1} m^{-2}.

Propagation using protocol 2

The explants were established on modified N69 -medium (Nitsch and Nitsch, 1969) to which 6.66 µmol l^{-1} 6 benzylaminopurine (BAP), 5.71 µmol l^{-1} Indolyle-3 acedic acid (IES) and 888.24 µmol l^{-1} of adenin were added, initially for six weeks at 20ºC in permanent darkness, then for two weeks under a 16/8 hrs (light/dark) photoperiod with light supplied by white fluorescence lamps at an intensity of 46 µmol s^{-1} m^{-2}. Following this, the explants were transferred for eight weeks on to N 69 with 4.44 µmol

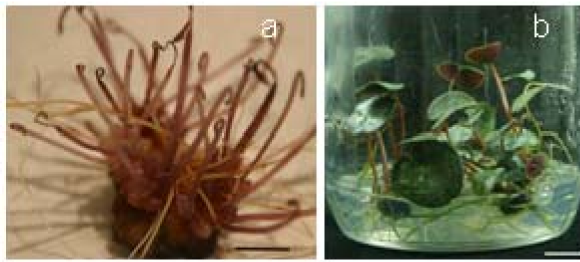

Figure 2. Regeneration of *C. purpurascens* on medium with 9.05 µmol l^{-1} 2.4-D und 3.94 µmol l^{-1} 2iP using protocol 1. (a) Formation of shoots on petioles; (b) Young plants in vitro on growth regulator-free medium. Bar. 10 mm (a, b).

l^{-1} BAP and 5.71 µmol l^{-1} IES. The N69 medium contained 10.74 µmol l^{-1} 1-naphtylacedic acid (NES) to induce root formation.

RESULTS

Regeneration using protocol 1

In a space of eight to 12 weeks, callus had developed in all types of explants of all genotypes. The proportion of explants that had developed callus often exceeded 80%. Only the petioles of *C. hederifolium* Ait. ssp. *confusum* (50%) and the leaf explants of *C. cilicium* (20%) fell short of this mark. Callus development was delayed in the leaf explants. *C. coum* magenta and *C. purpurascens* showed the most pronounced callus development in all explants, the explants being nearly totally covered in calluses.

After a period of three to seven months, structures began to differentiate depending on the genotype and type of explant (Table 1). Some structures appeared as shoots, whereas others resembled somatic embryos. Because no histological examination has been done the latter structures are designated as somatic embryo-like structures (SELS). The proportion of explants producing SELS hovered between 0 and 50% with only *C. persicum* pink developing only callus. After a period of seven months, different levels of developing SELS were observed (Table 1). Petiole explants of *C. cilicium* first grew large numbers of SELS, some of which began to develop while the SELS on the peduncle explants of *C. africanum* failed to develop further and eventually died.

SELS could be successfully induced in all types of explants of *C. coum* magenta (Figure 1). Leaf explants had the lowest proportion of explants with SELS (10%) while placenta explants had the highest (30%). With all genotypes with the exception of *C. africanum* SELS developed into plantlets.

Young plants had already developed after 10 months after beginning the culture except *C. cilicium* in which this process lasted longer.

C. coum magenta - with a total of 462 developing SELS in 11 explants after seven-months - produced the best result (Table 1). Out of 307 developing plantlets, which were transferred again to a growth regulator-free medium and cultivated in 16/8 hrs (light/dark) photoperiod, 89.6% grew into fully transplantable young plants. 135 plants were adapted to greenhouse conditions. Only 3% expressed an aberrant phenotype (autotetraploid).

The regenerants from protocol 1 of *C. africanum* petioles and all other regenerants of *C. purpurascens* with the exception of the placenta explants (Figure 2a) were classified as shoots (Table 1). The shoots were partly vitrified and initially showed signs of an abnormal growth. Further cultivation on growth regulator-free medium, however, produced rooted young plants (Figure 2b). These were phenotypically similar to the plants that had developed from SELS (Figure 1d).

Regeneration using protocol 2

After two months of cultivation, shoot formation could be induced in seven of the 11 genotypes (Table 2). The proportion of different types of explants with shoot formation fluctuated between 0 and 75%. In addition to well-developed shoots, there were also some deformities and single leaves (Figure 3a to Figure 3d). Shoot formation was successfully induced in all types of explants of *C. africanum*, while *C. cilicium* and the three types of *C. coum* showed no reaction. In contrast to the results of protocol 1, all explants of *C. coum* magenta eventually died. The highest numbers of normally developed shoots (after seven months) were observed in *C. africanum, C. hederifolium* ssp. *confusum* and *C. purpurascens* with 44, 40 and 20 shoots respectively.

Root formation was observed in shoots that had been transferred to N 69 medium with 10.74 µmol l^{-1} NES. After five weeks, rooting rates in excess of 50% had been established in *C. purpurascens* (93%), *C. persicum* (83.3%), *C. africanum* (61.4%) and *C. hederifolium* cv. 'Rosenteppich' (55.6%) while C. *hederifolium* ssp. *confusum* lagged behind at only 15%. In line with the rooting patterns of the different varieties, between one and six roots were developed per shoot.

Young plants had already developed after eight months after beginning the culture.

DISCUSSION

The previous knowledge concerning the in vitro propagation of *Cyclamen* is restricted to the species C. *persicum* (Karam and Al-Majathoub, 2000) and its cultivars. A comprehensive survey has been supplied for the latter by Winkelmann et al. (2000). Wild species of this genus possess a great potential for the improvement of *Cyclamen* as an ornamental plant and as a medicinal plant. For its use it is necessary to establish in vitro propagation

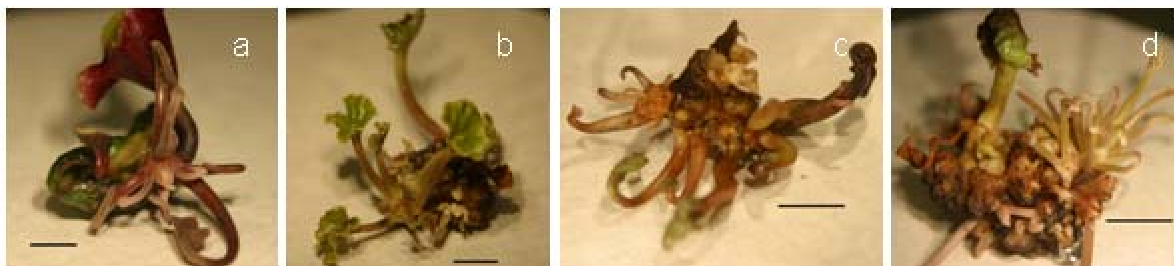

Figure 3. Formation of shoots in different *Cyclamen* species using protocol 2 after 14 weeks (cultivation for six weeks on N 69-medium (N69) with 6.66 µmol l^{-1} 6 Benzylaminopurine (BAP), 5.71 µmol l^{-1} Indolyle-3 acedic acid (IES), 888.24 µmol l^{-1} Adenin followed by eight weeks on N 69 with 4.44 µmol l^{-1} BAP and 5.71 µmol l^{-1} IES using leaves (a-c) and peduncles (d). (a) *C. purpurascens.* (b) *C. africanum.* (c) *C. hederifolium* ssp. *confusum.* (d) *C. persicum.* Bar 10 mm (a-d).

methods. Therefore the experience with the in vitro propagation of *C. persicum* has been applied by Prange et al. (2008) to four other wild species of *Cyclamen* using explants of juvenile plants, *i.e.* cotyledons, tubers and roots of very young sterile seedlings. However one prerequisite for the use of in vitro propagation for large scale propagation as well as in the breeding process is that it is possible to use explants which are available after the plants have been tested for appearance, health, or technological traits. Until now there are no scientific reports about the regeneration of wild species of *Cyclamen* starting from explants disposable after quality tests. Therefore in the present report we examined the possibility of propagating different wild species and subspecies of the genus *Cyclamen* and their cultivars in vitro using explants of adult plants. For this purpose two protocols, which have been successfully used in the propagation of *C. Persicum,* have been applied to eleven genotypes combined with mostly four explant types. The intention of this study was to establish a comprehensive survey about the regeneration responses in the genus *Cyclamen* using a high number of treatment combinations. This was only possible by tolerating relative small sample sizes in some combinations and by performing the experiment without replications because of biological reasons. This special statistical approach allows to get such a survey (Baetz et al. 1982) but prevents to make differences between single treatments statistically evident. This must be considered when special treatments will be compared.

Protocol 1 has been reported by Schwenkel and Winkelmann (1998) to be suitable for the induction of somatic embryogenesis in isolated ovules of *C. persicum*. The application of this protocol to the genotypes examined in the present study resulted in most of the treatment combinations in the formation of structures which resembled somatic embryos (Table 1), *i.e.* bipolar structures as they have been defined by Haccius (1978) and as they have been examined in *C. persicum* by Wicart et al. (1984). They are designated in this study as SELS because the clarification of their nature needs histological examinations, which has not been the intention of the present study. Furthermore the application of protocol 1 gave rise to shoots in some treatment combinations (Table 1).

Applying protocol 2 resulted only in the formation of shoots but not SELS. Differentiation of shoots has been observed in seven genotypes (Table 2). The genotypes *C. cilicium*, *C. coum*, *C. coum* ssp. coum and *C. coum* magenta did not form shoots.

The period necessary for the in vitro propagation using protocol 1 was in most genotypes 10 months and using protocol 2 about eight months after beginning the culture until to the formation of young plantlets. The use of protocol 1 and / or protocol 2 has given rise to either SELS or shoots in all the genotypes. This way it was possible to propagate each of the examined genotypes in vitro using explants of adult plants in a time less than one year. This is of practical importance because such a method could be integrated into the breeding process of *Cyclamen*. Single plants of the examined genotypes which have been assessed for their quality traits can now be cloned for their further use in programs for cross breeding or inter-specific hybridisation. Seed propagation would always lead to a strong segregation of traits in the descendent plants in these genotypes. Another advantage would be to use such in vitro propagation methods for the large scale propagation of *Cyclamen* as an ornamental plant and as a medicinal plant.

REFERENCES

AFFRE, Laurence; THOMSON, John D. and DEBUSSCHE, Max. The reproductive biology of the mediterranean endemic *Cyclamen balearicum* Willk. (Primulaceae). *Botanical Journal of the Linnean Society*, August 1995, vol. 118, no. 4, p. 309-330.

AFFRE, Laurence and THOMSON, John D. Variation in the population genetic structure of two *Cyclamen* species on the island of Corsica. *Heredity*, February 1997a, vol. 78, no. 2, p. 205-214.

AFFRE, Laurence and THOMSON, John D. Population genetic structure and levels of inbreeding depression in the

Mediterranean island endemic *Cyclamen creticum* (Primulaceae). *Biological Journal of the Linnean Society*, April 1997b, vol. 60, no. 4, p. 527-549.

AFFRE, Laurence and THOMSON, John D. Variation in self-fertility, inbreeding depression and levels of inbreeding in four *Cyclamen* species. *Journal of Evolutionary Biology*, January 1999, vol. 12, no. 1, p. 113-122.

BAETZ, Gerd; DOERFEL, Hannelore; FUCHS, Armin and THOMAS, Erhard. *Einführung in die Methodik des Feldversuchs*. Berlin; VEB Deutscher Landwirtschaftsverlag, 1982. 327p. ISBN 3-33-100125-2.

ÇALIŞ, Ihsan; SATANA, Mesut Ersan; YÜRÜKER, Aysen; KELICAN, Pelin; DEMIRDAMAR, Rümeysa; ALACAM, Ruhi; TANKER, Nevin; RÜEGGER, Heinz and STICHER, Otto. Triterpene saponins from *Cyclamen mirabile* and their biological activities. *Journal of Natural Products*, March 1997a, vol. 60, no. 3, p. 315-318.

ÇALIŞ, Ihsan; YÜRÜKER, Aysen; TANKER, Nevin; WRIGhT, Antonny D. and STICHER, Otto. Triterpene saponins from *Cyclamen coum* var. coum. *Planta Medica*, April 1997b, vol. 63, no. 2, p. 166-170.

COMPTON, James A.; CLENNETT, J. Chris B. and CULHAM, Alastair. Nomenclature in the dock. Overclassification leads to instability: a case study in the horticulturally important genus *Cyclamen* (Myrsinaceae). *Botanical Journal of the Linnean Society*, November 2004, vol. 146, no. 3, p. 339-349.

EWALD, Aloma; ORLICZ-LUTHARDT, Anna; WINKELMANN, Traud and SCHWENKEL, Hans-Georg. Interspecific hybrids of *Cyclamen persicum* Mill. x *C. purpurascens* Mill: propagation, somaclonal variation, resistence to Fusarium wilt and suitability as an outdoor crop. *Acta Horticulturae*, 2000a, vol. 508, p. 309-310.

EWALD, Aloma; SCHWENKEL, Hans-Georg; LEPPER, Lothar and LIPPOLD, Rico. Sexual reproduction of interspecific hybrids between *Cyclamen persicum* Mill. and *Cyclamen purpurascens* Mill. *Gartenbauwissenschaft*, 2000b, vol. 65, no. 4, p. 162-169.

EWALD, Aloma and SCHWENKEL, Hans-Georg. Fertilization and seed formation of *Cyclamen persicum* Mill. Basic and applied aspects of seed biology. In: *Proceedings of the Fifth International Workshop on Seeds, Reading*. Kluwer Academic Publishers, 1997, p. 113-118.

FOUBERT, K.; THEUNIS, M.; APERS, S.; VLIETINCK, A.J. and PIETERS, L. Chemistry, distribution and biological activities of 13, 28-epoxy-oleanane saponins from the plant families myrsinaceae and primulaceae. *Current Organic Chemistry*, May 2008, vol. 12, no. 8, p. 629-642.

GREY-WILSON, Christopher. *Cyclamen* a guide for gardener, horticulturists and botanists. New edition; Batsford, London, 2002. 192 p. ISBN 978 0881923865.

HACCIUS, Barbara. Question of unicellular origin of non - zygotic embryos in callus cultures. *Phytomorphology*, March 1978, vol. 28, no. 1, p. 74-81.

HOHE, Annette; PUESCHEL, Anke-Kathrin and SCHWENKEL, Hans-Georg and WINKELMANN, Traud. Somatic embryogenesis in *Cyclamen persicum* Mill. *Research Trends*, 2000, vol. 2, no. 1, p. 51-62.

ISHIZAKA, H. Cytogenetic studies in *Cyclamen persicum*, *C. graecum* (Primulaceae) and their hybrids. *Plant Systematics and Evolution*, June 2003, vol. 239, no. 1-2, p. 1-14.

KARAM, Nabila S. and AL-MAJATHOUB, Mohannad. In vitro shoot regeneration from mature tissue of wild *Cyclamen persicum* Mill. *Scientia Horticulturae*, December 2000, vol. 86, no. 4, p. 323-333.

KIVIHARJU, Elina; TOUMINEN, Ulla and TÖRMÄLÄ, Timo. The effect of explant material on somatic embryogenesis of *Cyclamen persicum* Mill. *Plant Cell Tissue and Organ Culture*, February 1992, vol. 28, no. 2, p. 187-194.

MURASHIGE, Toshio and SKOOG, Folke. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, July 1962, vol. 15, no. 3, p. 473-497.

NITSCH, J.P. and NITSCH, C. Haploid plants from pollen grains. *Science*, January 1969, vol. 163, no. 3862, p. 85-89.

PRANGE, Anika Nadja Sabine; SEREK, Margrethe and WINKELMANN, Traud. Vegetative propagation of different *Cyclamen* species via adventitious shoot formation from seedling tissue. *Propagation of Ornamental Plants*, December 2008, vol. 8, no. 4, p. 204-209.

QUAVE, Cassandra L.; PLANO, Lisa R.W.; PANTUSO, Traci and BENNETT, Bradley C. Effects of extracts from Italian medicinal plants on planctonic growth, biofilm formation and adherence of methicillin-resistant *Staphyllococcus aureus*. *Journal of Ethnopharmacology*, August 2008, vol. 118, no. 3, p. 418-428.

REINHARDT, Sandra; EWALD, Aloma and HELLWIG, Frank. The correlation between ovule quality parameters and the seed yield at *Cyclamen persicum* Mill. *Journal of Applied Botany and Food Quality*, 2008, vol. 82, no. 1, p. 76-82.

RENSING, Stefan A.; LANG, Daniel; SCHUMANN, Eik; RESKI, Ralf and HOHE, Annette. EST sequencing from embryogenetic *Cyclamen persicum* cell cultures identifies a high proportion of transcripts homologous to plant genes

involved in somatic embryogenesis. *Journal of Plant Growth Regulation*, June 2005, vol. 24, no. 2, p. 102-115.

SCHWENKEL, Hans-Georg and WINKELMANN, Traud. Plant regeneration via somatic embryogenesis from ovules of *Cyclamen persicum* Mill. *Plant Tissue Culture and Biotechnology*, March 1998, vol. 4, no. 1, p. 28-34.

SPERONI, E.; CERVELLATI, R.; COSTA, S.; DALL'ACQUA, S.; GUERRA, M.C.; PANIZZOLO, C.; UTAN, A. and INNOCENTI, G. Analgesic and antiinflammatory activity of *Cyclamen repandum* S. et S. *Phytotherapy Research*, July 2007, vol. 21, no. 7, p. 684-689.

WICART, G.; MOURAS, A. and LUTZ, A. Histological study of organogenesis and embryogenesis in *Cyclamen persicum* Mill. tissue cultures: evidence for a single organogenetic pattern. *Protoplasma*, October 1984, vol. 119, no. 3, p. 159-167.

WINKELMANN, Traud and SEREK, Margrethe. Genotypic differences in callus formation and regeneration of somatic embryos in *Cyclamen persicum* Mill. *Euphytica*, July 2005, vol. 144, no. 1-2, p. 109-117.

YESSON, Chris and CULHAM, Alastair. A phyloclimatic study of *Cyclamen. BMC Evolutionary Biology*, September 2006, vol. 6, p. 72.

Note: Electronic Journal of Biotechnology is not responsible if on-line references cited on manuscripts are not available any more after the date of publication.
Supported by UNESCO / MIRCEN network.

APPENDIX

TABLES

Table 1. Regeneration of eleven *Cyclamen*-genotypes using protocol 1.

Genotype / Source of explants	Number of explants	Number of sterile explants	Start of differentiation after months	Explants with somatic embryo-like structures or shoots*		Number of plantlets after 7 months
				%	total	
C. africanum						
Placenta	8	8	0	0		0
Peduncle	8	4	3	50.0	2	0
Leaf	8	7	0	0		0
Petiole	20	16	7	12.5*	2	2*
C. cilicium						
Leaf	8	4	0	0		0
Petiole	20	20	6	15.0	3	1
C. coum						
Placenta	36	24	3	4.2	1	8
Peduncle	36	20	0	0		0
Leaf	8	6	0	0		0
Petiole	28	19	6	15.8	3	3
C. coum ssp. ***coum***						
Leaf	20	7	0	0		0
Petiole	24	20	6	5.0	1	3
***C. coum* magenta**						
Placenta	20	20	3	30.0	6	249
Peduncle	20	12	3	25.0	2	200
Leaf	20	10	6	20.0	2	10
Petiole	24	20	3	10.0	2	3
C. hederifolium ssp ***confusum***						

Placenta	4	4	0	0		0
Peduncle	4	4	0	0		0
Leaf	4	4	6	25.0	1	1
Petiole	16	14	6	14.3	2	8
C. hederifolium cv. **'Perlenteppich'**						
Placenta	20	11	0	0		0
Peduncle	20	8	0	0		0
Leaf	16	7	0	0		0
Petiole	20	16	3	12.5	2	2
C. hederifolium cv. **'Rosenteppich'**						
Placenta	20	10	0	0		0
Peduncle	20	7	7	42.9	3	3
Leaf	16	12	0	0		0
Petiole	20	20	0	0		0
C. persicum						
Placenta	20	16	3	12.5	2	10
Peduncle	20	4	3	50.0	2	8
Leaf	4	4	0	0		0
Petiole	16	16	0	0		0
***C. persicum* pink**						
Leaf	4	4	0	0		0
Petiole	16	15	0	0		0
C. purpurascens						
Placenta	20	15	3	6.7	1	16
Peduncle	20	20	3*	20.0*	4	13*
Leaf	12	12	6*	25.0*	3	50*
Petiole	20	20	3*	25.0*	4	71*

* Regenerants were classified as shoot formation

Table 2. Regeneration of eleven *Cyclamen*-genotypes using protocol 2.

Genotype / Source of explants	Number of explants	Number of sterile explants	Start of shoot regeneration after months	Explants with shoots		Number of shoots after 7 months
				%	total	
C. africanum						
Placenta	20	12	2	8.3	1	0
Peduncle	20	16	2	6.3	1	0
Leaf	4	4	2	75.0	3	28
Petiole	16	16	2	18.8	3	16
C. cilicium						
Leaf	16	14	0	0		0
Petiole	20	20	0	0		0
C. coum						
Placenta	20	8	0	0		0
Peduncle	20	16	0	0		0
C. coum ssp. ***coum***						
Leaf	16	8	0	0		0
Petiole	20	19	0	0		0
C. coum **magenta**						
Placenta	20	12	0	0		0
Peduncle	32	12	0	0		0
Leaf	8	3	0	0		0
Petiole	20	20	0	0		0
C. hederifolium ssp. ***confusum***						
Placenta	28	20	0	0		0
Peduncle	28	16	0	0		0
Leaf	12	5	2	40.0	2	3
Petiole	20	19	2	36.8	7	37
C. hederifolium cv. **'Perlenteppich'**						

Placenta	20	15	0	0		0
Peduncle	20	12	0	0		0
Leaf	8	4	2	25.0	1	2
Petiole	20	20	2	5.0	1	0
C. hederifolium cv. **'Rosenteppich'**						
Placenta	16	16	0	0		0
Peduncle	16	16	2	12.5	2	2
Leaf	12	12	2	8.3	1	3
Petiole	20	16	2	6.3	1	4
C. persicum						
Placenta	20	12	0	0		0
Peduncle	20	16	2	18.8	3	2
Leaf	4	4	2	25.0	1	10
Petiole	16	14	2	7.1	1	0
***C. persicum* pink**						
Placenta	20	8	2	50.0	4	5
Peduncle	20	20	2	45.0	9	9
Leaf	4	4	2	25.0	1	0
Petiole	12	12	0	0		0
C. purpurascens						
Placenta	20	4	0	0		0
Peduncle	20	20	2	20.0	4	3
Leaf	8	8	2	50.0	4	13
Petiole	20	19	2	5.3	1	7