

Evaluation of Species from Cyprus Flora for Sustainable Use in Commercial Floriculture

D. Chimonidou¹, J.C.Vlahos², E. Odysseos³, K. Georgiou⁴ and A. Della¹

¹ Agricultural Research Institute, P. O. Box 22016, 1516 Nicosia, Cyprus

² School of Agricultural Technology, TEI of Crete, Heraklion 71004, Greece

³ Odysseos Nurseries, 2450 Nicosia, Cyprus

⁴ Athens University, Biology Department, 15784 Athens

Keywords: endemic Cyprus species, in vitro culture, new flower crops, pot plants, propagation, seed germination.

Abstract

Cyprus, despite its small geographic scale, is characterized by a large topographic diversification concerning climate and soil morphology. As a result of these conditions, Cyprus has a large and unique flora of 1910 taxa, with a high (7.3%) percentage of endemism. This work is at a preliminary stage and is a part of a joint project between Cyprus and Greece funded by the Cyprus Research Promotion Foundations. The aim is to study the potential of six endemic species of the Cyprus flora (*Arabis purpurea*, *Centaurea akamantis*, *Onosma fruticosa*, *Origanum cordifolium*, *Ptilostemon chamaepeuce* and *Euphorbia veneris*), for use in sustainable commercial floriculture and at the same time, the project aims at the conservation of these species since all of them are endemic and two of them *Centaurea akamantis* and *Origanum cordifolium* are strictly protected by the Bern Convention.

Tests on seed germination of these species at different temperatures, showed that 81% of the seeds of *Arabis purpurea*, germinated at 20°C between the 32nd and the 40th day and 72 - 76% of *Ptilostemon chamaepeuce* var. *cypria*, between 10 and 16 days. A high percentage of *Origanum cordifolium* (82%), germinated at the temperature of 10°C and 79% at 15°C, in both cases after 6 days. For *Centaurea akamantis*, the percentage of seed germination was lower and reached 70% in 14 days at 15°C and 55% in 25 days at 20°C. Best results for *Euphorbia veneris* propagated by tissue culture, were observed when stem nodes without leaves were used as explants. It is shown that 0,5 mg.l⁻¹ BAP in *Euphorbia veneris* led to satisfactory bud differentiation and shoot proliferation.

INTRODUCTION

Cyprus, is the third largest island in the Mediterranean sea. It is situated between 34°34' - 35°42' N and 32°16' - 34°34' E, covering an area of 9251 km². Its geographical location places it between three continents, Europe, Asia and Africa. It has an extremely irregular outline, great variation in temperature and rainfall and never had a Glacial epoch. So a rich native, naturalized and cultivated flora has developed. Extensive plains, mountain masses, wetlands, coasts, sand dunes, gorges and cliffs provide a home for many indigenous and endemic species. The vegetation of Cyprus consists of typical Mediterranean types, coniferous forest, maquis, garigue and batha vegetation (Meikle, 1977), while more localized communities occur around salt marshes, sand dunes, stone walls, and mountain streams (Della, 1987, 1995). On arable land, the cultivated species predominate, many of which have been naturalized; others, however, have not been naturalized but can be utilized for aesthetic purposes. On the other hand, hundreds of ornamental species, introduced at varying times, have been planted in the inhabited areas; many of these have been naturalized and occur as adventives (Tsintides, 1995; Tsintides et al., 2002).

The introduction of new plants in floriculture is a new trend in countries where horticultural research is well developed. For this reason, native plants are sought and selected for their desirable characteristics (Maloupa et al., 1999; Shillo, 1999). Genetic

improvement and hybridization of native plants produces attractive, vigorous and disease resistant plants, which are then introduced into commercial floriculture (Littlejohn et al., 1999).

The introduction of new plants in the floricultural market has been practiced for some time now in Germany, Denmark, the Netherlands and the United States, where consumers are attracted by new products, thus increasing the market trade. In Greece, recently research has been conducted in Research Centers and Universities where native and endemic species have been tested (Vlahos, 1996, Maloupa et al., 1999, Papaphotiou et al., 2000).

References to the Cyprus flora, and in particular to plants of economic importance, go back as far as Homer (Holmboe, 1914). Plants from Cyprus were mentioned in the work of ancient authors such as Theophrastus, Dioscorides, Plinius and others. At the end of the 18th century scientific work was published by many European botanists as a result of scientific explorations of the island. The most complete recent study is the "Flora of Cyprus", by R.D.Meikle of the Herbarium, Royal Botanic Gardens, Kew, U.K. The study was undertaken by the Kew Herbarium with the assistance and support of the Cyprus Government and in particular the Ministry of Agriculture and Natural Resources. The "Flora" was published in two volumes (Meikle, 1977; 1985). However, research on the evaluation of endemic species for the potential use in commercial floriculture is in very early stages.

The aim of this project (2003-2005), is to study the six selected species under three environments: Greenhouse, Screen house and in the open field and by application of different irrigation regimes, fertilization, application of growth regulators, cultivation techniques (i.e. pruning, pinching etc) according to the species, to transform them into flowering pot plants, or cut flowers and use them in commercial Floriculture.

MATERIAL AND METHODS

All of the selected species are endemic of Cyprus and two of them (*Centaurea akamantis* and *Origanum cordifolium*) are strictly protected by Bern Convention.

Description of the Species

Arabis purpurea (Cruciferae) - Purple Rock-Cress, is a sub shrub or perennial herb up to 30cm high with stems and leaves with dense stellate hairs. Leaves are alternate, simple with colour silvery to glaucous-green. Flowers are hermaphrodite and inflorescences at first densely corymbose, lengthening gradually into a raceme. Petals are pink, purple or rarely white. Flowering from March to April. Locally very common endemic, occurs in crevices of igneous and calcareous rocks at Troodos mountain range between 400-1800 m altitude.

Centaurea akamantis (Compositae) - Akamas Centaury, is a sub shrub with hanging or suberect shoots up to 60 cm long, densely white tomentose. Leaves are alternate, simple, variable, lobed, pinnatisect or bipinnatisect; lobes are linear with 1-5 mm wide, grayish-green, densely tomentose. Flowers (florets) are small, arranged in solitary, terminal capitula (florets purple-mauve, ray-florets ligulate, disk-florets tubular). A very rare endemic, confined to the Akamas peninsula (50-100 m alt.), flowering from May to November

Onosma fruticosa (Boraginaceae) - Cyprus Golden-Drop, is a much-branched, evergreen shrub, 0.2-0.8 m high, with hairy shoots, which become rather spinose when dried. Leaves are alternate, simple, entire, oblong, 7-20 x 2-4 mm, grey-green, with a mixed indumentum of short hairs and long bristles and with revolute margins. Flowers are solitary or 2-3 together, nodding downwards (calyx campanulate, hairy, corolla cylindrical), golden yellow turning into brown-orange with age. Found in areas with garigue vegetation (0-900 m alt.) and flowering from March to May.

Origanum cordifolium (Labiatae) - Cordate-leaved Origan, is an aromatic, much-branched sub shrub or shrub up to 60 cm high. Young shoots are tetragonal, often purple. Leaves are simple, opposite, sessile, rather regularly spaced; Spikes on branched

inflorescences. Flowers are hermaphrodite, arranged in an oblong, pendulous spike (40-70 x 13-25 mm). Bracts are subordicular, glabrous, purple or rosy, sometimes greenish. Flowers appear in June to August. Growing on moist, rocky places along stream sides, on igneous formations (250-900 m alt.).

Ptilostemon chamaepeuce (Compositae) var. *cypria*, is an evergreen shrub up to 1.3 m high with young branches densely white-tomentose and old branches greyish. Leaves are alternate, simple, crowded towards the base of stems and more remote towards apices, entire, linear (3-19 x 0.1-0.3 cm). Flowers (florets) are small, arranged in large capitula, 12-20 mm long (capitula usually solitary, terminal or in loose; florets purple, all tubular). Flowering from May to June. Occurs on rocky slopes on calcareous and igneous formations, very common in the Akamas peninsula (50-1600 m alt.).

Euphorbia veneris (Euphorbiaceae) - Euphorbia of Aphrodite, is a perennial, with several simple stems up to 35 cm long arising from woody stock. Cauline leaves are elliptic, 1-2.5 cm long, 4-9mm wide, shortly acuminate, acute or sub acute and mucronate, entire, coriaceous. Ray-leaves are elliptic-oblong to obovate 0.5-1.4 cm long, 0.4-1.6 cm wide. Occurs on rocky mountain slopes at Troodos, up to the snowline, in garigue or *Pinus nigra* woodland; 600-1700 m alt. Flowering in February-June.

Propagation and Cultivation of the Species

Since the six selected species were endemic of Cyprus and new, the first aim was to study their propagation in order to have the expected population needed for experimentation. The Athens University (Biology Department) and the Technological Educational Institute (TEI) of Crete, studied the selected species by seeds and tissue culture.

Seeds from the four species under study were selected in July 2002, cleaned from their calyx and weight. A number of 1250 seeds of *Arabis purpurea* and 300 seeds from *Ptilostemon chamaepeuce* var. *cypria* were selected from Kakopetria area. From Akamas peninsula, 100 seeds of *Centaurea akamantis* were selected and from the area of Kafitsides 2600 seeds of *Origanum cordifolium*. All species were tested for germination at 15 and 20°C in Petri dishes of 20-25 seeds for *Arabis purpurea* and *Ptilostemon chamaepeuce* var. *cypria* and 10 seeds for *Centaurea akamantis* and *Origanum cordifolium*. *Origanum cordifolium* was tested also at 10°C and *Ptilostemon chamaepeuce* var. *cypria* tested twice for 20°C. The collection of seeds for the two species *Euphorbia veneris* and *Onosma fruticosum*, was not possible at that time.

The method of micropropagation was used for plant proliferation using different explant material; leaves and nodes (for the four species) and seeds (for *Onosma fruticosum*) in test tubes and controlled environmental and nutritional conditions. Five out of the six species were tested by the method of tissue culture (*Euphorbia veneris*, *Arabis purpurea*, *Ptilostemon chamaepeuce* var. *cypria*, *Origanum cordifolium* and *Onosma fruticosum*). Different concentrations of cytokinines (0.1 mg l⁻¹ and 0.5 mg l⁻¹ Benzylaminopurine-BAP) were applied. In each treatment (control, 0.1 mg l⁻¹ BAP, 0.5 mg l⁻¹ BAP), there were 13 replications (test tubes for each type of the explant material, for each of the species tested.). The test tubes (contained 10-15 ml of substrate according to the protocol of Murashige and Scoog, 1962), were placed in a controlled environment chamber under 16 h light (2800 lux) and 8 h dark at 18 °C.

At the Agricultural Research Institute in Nicosia, the selected species were transferred under three environments: Greenhouse, screen house and in the open field in order to study their reaction and the treatments/interventions necessary to be applied.

The selected species were propagated by seeds and cuttings and then transferred to the different environments and preliminary measurements on the shape, growth rate, number of lateral shoots, stem length of the shoots, number of leaves on selected shoots, flower bud appearance, number of inflorescences per plant, inflorescence length) were observed for the three of them *Ptilostemon chamaepeuce* var. *cypria* (Compositae), *Centaurea akamantis* (Compositae) and *Euphorbia veneris* (Euphorbiaceae). Plants in the greenhouse were under temperatures of 35-37°C and relative humidity of around 60%. At

the screen house the shading (green net) reduced light by 60%. Due to very high temperatures during summer (39-40°C), no plants were placed at the first trials in the open field. The experimental design in the screen house and the greenhouse, was a Complete Randomized Design with 6 replicates (6 plants in each replication) per species. The substrate used was peatmoss 60% and perlite 40% in pots of 10lit capacity. The irrigation was provided by drippers of 2 l/h and all plants in the screen house received 3 min of irrigation per day. Greenhouse treatments used different quantity of water and irrigation intervals were applied for the species *Ptilostemon chamaepeuce* var. *cypria* (Compositae), *Centaurea akamantis* (Compositae) and *Euphorbia veneris* (Euphorbiaceae), one or two min/day and irrigation day or day and night. Fertilizers (20N-20P₂O₅-20K₂O) 2 g/lit and Fe, 4 g/lit were applied with the irrigation water (with the injector Dosatron), once a week.

RESULTS AND DISCUSSION

Results showed that 81% of the seeds of *Arabis purpurea* (with mean weight of 0,09 mg), were germinated at 20°C after 32 days. A high percentage (97%) germinated after 40 days at 20°C. The seed germination of *Arabis purpurea*, in the dark at 15 and 20°C, remained in very low levels for a long period (18 days). The transfer of the seeds to the room conditions, resulted in high levels of seed germination (Fig. 1). This is consistent with the results obtained with previous work on *Arabis cypria* and *Arabis purpurea* (Paraskeva, 1996).

For the species *Ptilostemon chamaepeuce* var. *cypria*, a percentage of 72% of the seeds (with mean weight of 10,0 mg), germinated after 10 days at 20°C and 76% after 16 days. A percentage of 80% germinated after 22 days in both cases treated at 20°C. On the contrary at 15°C, only 53% of the seeds germinated after 10 days and remained constant up to 45 days (Fig. 2).

The germination *Origanum cordifolium* was tested at 10, 15 and 20°C. A high percentage of 82% of the seeds (with mean weight of 0,22 mg), germinated at 10°C and 79% at 15°C, in both cases after 6 days. The highest percentage of germination was 88% in both cases in 9-10 days. At 20°C the germination was 82% after 16 days and 88% after 21 days (Fig. 3). For *Centaurea akamantis*, the percentage of seed germination was lower and reached 70% in 14 days at 15°C and 55% in 25 days at 20°C. The mean weight of the seeds of *Centaurea akamantis* was 2,64 mg (Fig. 4). These results on the germination of *Origanum cordifolium* and *Centaurea akamantis*, were confirmed by previous work on the same species (Kadis, 1995).

Propagation by tissue culture, for *Euphorbia veneris*, best results were observed when stem nodes without leaves were used as explants, in spite the fact that shoots were lactiferous. It is shown that 0,5 mg.l⁻¹ BAP in *Euphorbia veneris* led to satisfactory bud differentiation and shoot proliferation (Fig. 5). In *Arabis purpurea*, explants had a lot of infections in spite of the high chlorine concentration used (0.75% of NaOCl for 10min). This can be due to the fact that its leaves are very thick with many short hairs that make disinfections difficult (10,2% successful disinfections). The shoots of the plant were not used in vitro, as they were not easy to use as explants (too short internodes). *Ptilostemon chamaepeuce* explants from the nodes, had also high infection rates (only 5% successful disinfections in 0.75% of NaOCl for 10 min), because of the thick white hair covering its shoots. The leaves of the plants were not used in vitro as they were not easy to handle. The disinfection for *Origanum cordifolium* was quite successful both with leaves as well as with stem segments. Successful disinfection with leaves and with node and leaf, reached 60 and 55% respectively, both in 0.25% NaOCl for 10min. Results with the different concentrations of cytokinine, showed that 0.1 mg.l⁻¹ BAP in *Origanum cordifolium* led to shoot development from a leaf axil. The seeds of *Onosma fruticosum* had a low (15.4%) infection rate, which shows that seeds can be disinfected satisfactorily.

Ptilostemon chamaepeuce var. *cypria* (Compositae) was propagated by seeds at Solomou Nurseries in 3 weeks at 20°C (95% success) and transferred in the greenhouse. In the period of six months, the 6 replications (6 plants in each replication) had flowering

shoots (inflorescence in one stem). The plants from cuttings transferred in the screen house had more stems (branches) and the time required to reach flowering stage was longer. The aim was to produce flowering pot plants using different irrigation intervals or quantity of irrigation/fertilization.

Centaurea akamantis (Compositae) in nature is a shrub with hanging shoots and few flowers. The aim is to transform this species into a flowering pot plant. Plants of *Centaurea akamantis* propagated by seeds and by cuttings (with 25% success), were transferred in the greenhouse and screen house. After 6 months, all plants under greenhouse conditions with frequent irrigation/fertilization were in full bloom (Fig. 6).

Plants of *Euphorbia veneris* from cuttings were transferred to the greenhouse and to the screen house. Propagation by seeds was very difficult due to the limited number of seeds selected (the seeds shake off the flower). The percentage of propagation by cuttings was low (20%) due to the lactiferous shoots of *Euphorbia veneris*. The aim is the transformation of *Euphorbia veneris* (Euphorbiaceae), to flowering pot plant using growth regulators or plant growth retardant. There are similar studies for *Euphorbia characias*, a common wild plant of Greece found throughout the Mediterranean, treated with paclobutrazol (Papaphotiou et al., 2000). The elongation of the shoots of eighteen months old pruned plants of *Euphorbia characias*, treated with paclobutrazol applied as a foliar spray (at 50 and 100ppm), or as a soil drench (at 25 and 50 ppm), was restricted to 47% and 58% or 31% and 24% of the control respectively, with the extent of restriction remaining almost stable for four months (Papaphotiou et al., 2000).

CONCLUSIONS

The duration of the project is 3 years and started in November 2002. These results mentioned above, are preliminary results. The project within the 3 years, aims to study the reaction of the six plants in control environments (greenhouse and screen house) and in the open field and find the optimum conditions for massive production in order to specified species to meet the commercial standards for their introduction in local and international markets. Preliminary results showed that the species *Ptilostemon chamaepeuce* var. *cypria* (Compositae), *Centaurea akamantis* (Compositae) and *Euphorbia veneris* (Euphorbiaceae) could become potentially pot plants. By the application of different irrigation regimes, fertilization or application of growth regulation according to the species, the project aims to transform the six selected species in to flowering pot plants. At the same time the project aims at the conservation of these species since all of them are endemic and two of them *Centaurea akamantis* and *Origanum cordifolium* are strictly protected by the Bern Convention.

ACKNOWLEDGEMENTS

This work is funded by the Cyprus Promotion Foundations. Great appreciation is expressed to the people at the Forestry Department of the M.A.N.R.E, Cyprus, for the collection of the seeds of the six plants under investigation as well as to P. Polyviou, S. Christoforou and D. Philipetis working on this project at ARI and to Ch. Karagiannakis and Maria Kralli at the TEI of Crete.

Literature Cited

- Della, A. 1987. The plant world of Cyprus. Cyprus To-day 25: 2-13.
- Della, A. 1995. History and Geography Combine to make Cyprus a Floristic Wonder. DIVERSITY, The Mediterranean 11:92-95.
- Holmboe, J. 1914. Studies on the Vegetation of Cyprus. Bergens Museums: 344p.
- Kadis, K. 1995. The reproductive Biology of the strictly protected species of Cyprus flora. PhD thesis. Athens University, Athens.
- Littlejohn, G.M., Robyn, A., Blomerus, L.M. and Allies, H. 1999. Proteaceae genetic resources, sampling and ex situ conservation. Proc. of the IV International Symposium on New Floricultural Crops. Acta Hort.541:243-245.
- Maloupa, E., Zervaki, D. and Marnasidis, A. 1999. Introduction of the Mediterranean

- native species *Thymus mastichina*, *Lotus cytisoides*, *Lavandula stoechas*, *Centranthus ruber*, *Limonium pectinatum* and *Limonium sinense* into commercial floriculture. Proc. of the IV Internat. Symp. on New Floricultural Crops. Acta Hort. 541:57-65.
- Meikle R.D. 1977. Flora of Cyprus. Published by the Bentham-Moxon Trust Royal Botanic gardens, Kew, U.K., Volume I, 832p.
- Meikle R.D. 1985. Flora of Cyprus. Published by the Bentham-Moxon Trust Royal Botanic gardens, Kew, U.K., Volume II, 1969p.
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio-assay with tobacco tissue cultures. Plant Physiology 15:473-497.
- Paraskeva D. 1996. Ecophysiological study of seed germination of the endemic species of Cyprus flora *Arabis cyprica* Holmboe and *Arabis purpurea* Sibth & Sm. B.Sc. thesis (supervisor K. Georgiou), Athens University, Athens.
- Papaphotiou Maria , Garavelos E., Antonopoulos C. and J. Chronopoulos. 2000. Studies on Growth manipulation of *Euphorbia characias* Acta Hort. 541:201-206.
- Shillo, R. 1999. The importance of new crops for Israeli floriculture. Proc. of the IV Internatinal Symposium on New Floricultural Crops. Acta Hort. 541:221-225.
- Tsintides, T. 1995. The Endemic Plants of Cyprus. Published by Cyprus bank of Cyprus, Nicosia 1995, p.123.
- Tsintides, T., Hadjikyriakou, N. and Christidoulou, Ch. 2002. Trees and Shrubs in Cyprus. Published by Foundat. A.G. Leventis, Cyprus Forest Assos. 2002, p.442.
- Vlahos, J.C. 1996. *Ebenus cretica* L., An Attractive Endemic Plant of Crete with Potential for Floricultural Use. Hort Science 31:769-774.

Figures

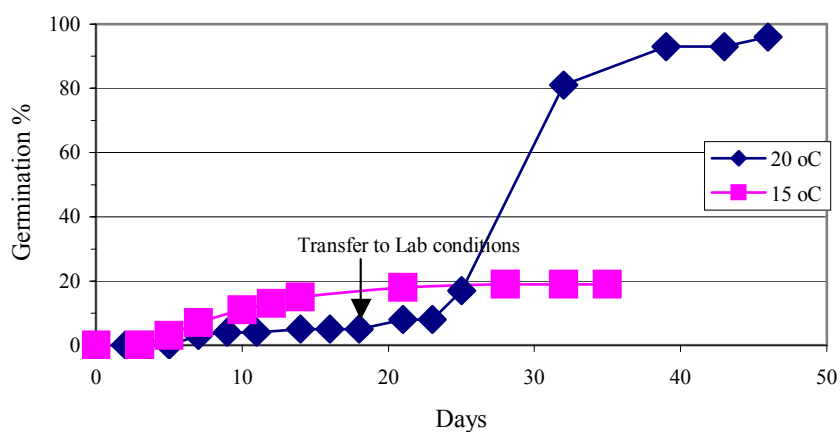


Fig. 1. Percentage (%) of germination from seed of *Arabis purpurea* (Cruciferae) in relation to time (Days) for 15 and 20°C.

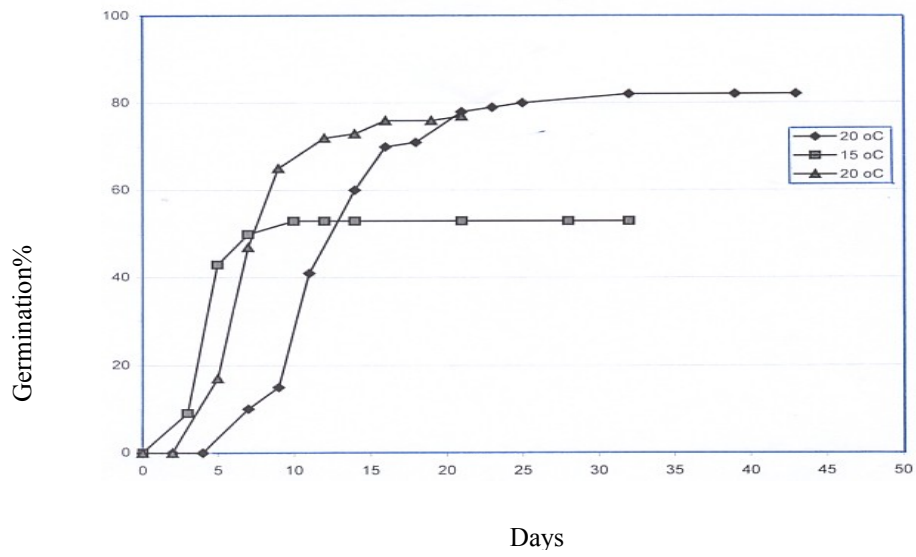


Fig. 2. Percentage (%) of germination from seed of *Ptilostemon chamaepeuce* var. *cypria* (Compositae) in relation to time (Days) for 15 and 20°C (tested twice).

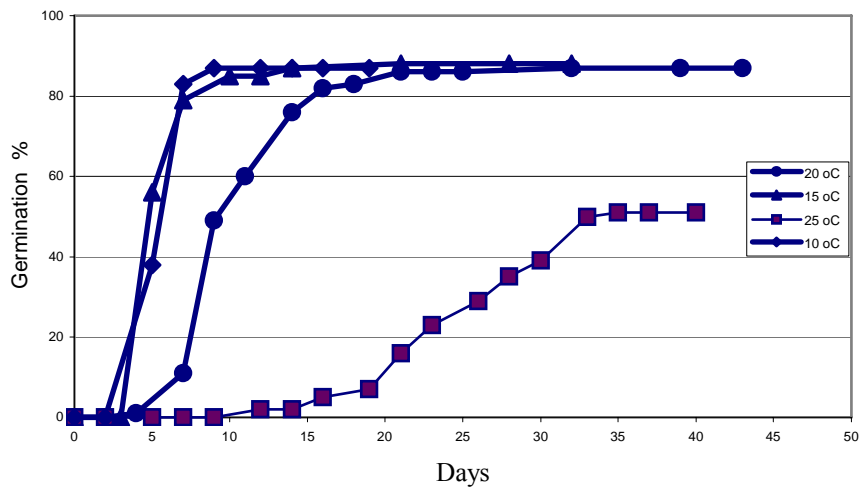


Fig. 3. Percentage (%) of germination from seed of *Origanum cordifolium* (Lambiatae) in relation to time (Days) for 10, 15 and 20°C.

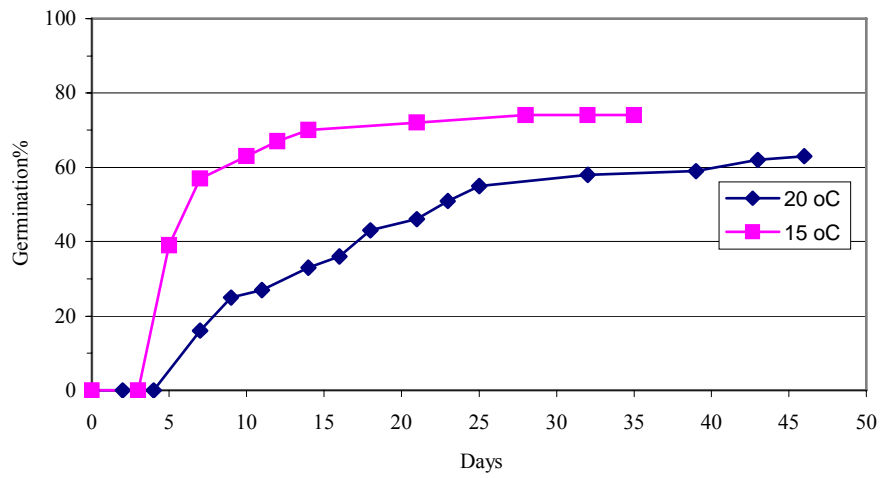


Fig. 4. Percentage (%) of germination from seed of *Centaurea akamantis* (Compositae) in relation to time (Days) for 15 and 20°C.

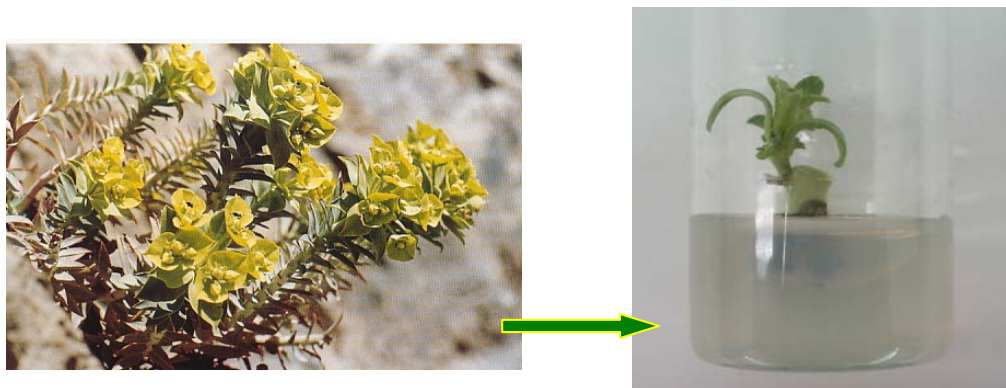


Fig. 5. Left: *Euphorbia veneris* in nature
 Right: Proliferation of adventitious shoots from stem nodes of *Euphorbia veneris* at 0.5 mg.l^{-1} BAP.



Fig. 6. Left: *Centaurea akamantis* (Compositae) in nature with fewer flowers
Right: *Centaurea akamantis* (Compositae) in the greenhouse after 6 months.