STUDY OF *IN VITRO* REGENERATION AND MULTIPLICATION CAPACITY OF *ASTER ALPINUS* L , WITH THE PURPOSE OF *EX SITU* CONSERVATION OF THE BIOLOGICAL AND ORNAMENTAL VALUE OF THE SPECIE

Agud Eliza*, Laslo Vasile*, Zăpârţan Maria, Oneţ Aurelia

*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru, St. 410048 Oradea; Romania; eliza_agud@yahoo.com; laslovasile@yahoo.com

Abstract

The research has followed the in vitro regeneration and multiplication capacity of the apical tissue and of the bud detached from Aster alpinus L, a specie encountered here in the areas of the Apuseni Mountains Natural Park (PNMA). The mother-plants from which there were detached the explants come from Poşaga, Cheile Turzii (Cluj county) and Piatra-Struţu (Bihor Mountains) areas. There were experimented advantageous mediums with a small concentration of phytohormones, with a basal medium according to Murashige-Skoog (MB), on the variants: Mt.=MS; V_1=Mt+0.2mg/lAIA+0.4mg/lBAP; V_2= Mt+0.2mg/lAIA+0.4mg/lK; V_3=Mt+ 0.2mg/lAIA+0.4mg/l2iP. After 45-50 days of in vitro culture there was followed the regeneration and multiplication percentage of the explants, the average of the number of neo-plantlets/explant and of their length; the average of the number of roots/neo-plantlet and of their length, and also aspects of ex vitro acclimatization of the neo-plantlets. We can assert that the specie manifests a good and very good multiplication and regenerative reaction on the mediums with small concentrations of phytohormones (0.4mg/l BA, K, 2iP and 0.2mg/lAIA), with differences depending on the type of cytokinin and of explant

Key words: *Aster alpinus* L., *apex* (apical tissue), *bud*, *ex situ conservation*, regeneration, *in vitro* micro propagation, acclimatization, Murashige-Skoog.

INTRODUCTION

Aster variety encompasses over 650 species of annual, biannual or perennial plants. The name of the variety comes from the Greek word aster = star, after the radial extended flowers of the plant, the central flowers of the disc are tubular and bisexual disposed on grassy stems, glabrous, hairy or glandulous, with simple leaves, disposed on both sides of the stem, unbroken or with toothed edges and a fruit, flattened achene (Flora României, vol. IX, 1964). The simple stem, high of 50 cm has in the soil an oblique, nodulose, rhizome (which ensures the vegetative multiplication of the specie) and which gives out at the level of the soil a bow of simple leaves (Ciocârlan, 1988). The flowers are multicoloured, from purplish-blue, rarely reddish (see Fig. 1 and 2), met in sunny cliffy places, rocky pastures, in the mountains and subalpine regions, especially on calcareous substratum (Pârvu, 2003).

The origin of the majority of species from Aster variety is mainly in North America but also in Central and Southern Europe and in South Asia. Named also Alps Aster, *Aster alpinus* L specie (photo. 1) has its origin in

the mountainous regions of Europe (BOTANICA - Encyclopédie de botanique et d`horitculture, Ed. Könemann, 1997). Through its rusticity and characteristics (it has flowers of different sizes and colours, from white to violet, with a yellow middle, etc.) the specie flourishes in the second part of the summer (photo. 2) and the beginning of autumn, ensuring the ornamentation of the rocks, parks, and gardens where it was acclimatized (Sonea et all., 1987).

In Romania it is met in a great variety of mountain ranges (Maramureş, Ţibleş, Rodna, Retezat, Giurgeu, etc.) in touristic areas of smaller extension but which are important and which are studied from a botanical point of view: as for example Scăriţa Mountains (Poşaga-Turda), Cheile-Turzii, and also in Bihor Mountains at Piatra Struțu, where it can be sporadically found in a smaller number and with a certain degree of vulnerability due to the ornamental value ensured by the form of the bushes and by the colouring of the flowers, preferred by the collectors of spontaneous flora (Flora României, vol IX, 1964).

A rustic specie, *Aster alpinus* L experimented by us comes from Poşaga parish and from Cheile-Turzii (Apuseni Mountains) and it was multiplied in vitro with the purpose of introducing it within the culture of different spaces with a scientific value (botanical gardens, dendrological parks) or with an ornamental value (parks, etc.). The research literature recognises the value of the ornamental semi-bushes as they ensure a characteristic ornamental-paysagist space. The bushes can be conserved in other spaces too, besides the ones of origin (Iliescu, 2008). Ex situ conservation of the aster variety demands a classical technology in steps, depending of the preferences of the three groups of Aster met. Hence, group 1 prefers a rich, fresh soil, sunny or semi-shaded; group 2 prefers a rich enough soil, well worked, sewed and in full sun (a group from which *Aster alpinus* L is also a part of); group 3 prefers the most rich soil, fresh and semi-shaded (Encyclopédie universelle des 15.000 de plantes, Christopher Brickell editor, 1999).

Normally the specie multiplies form seeds, and in the regions where it does not tie seeds it multiplies vegetatively through the detachment from the bush and from the cuttings (Enciclopedia ilustrată a florilor şi florei sălbatice, 2008, Editura Aquila'93). The species of the variety have different uses added to their botanical value, being used in compact groups, isolated or in unique exemplaries. *Aster alpinus* L is ideal for the delimitation and formation of some kerbstones, as rock plants, etc. (Warton, 1995). It was proved that for the stimulation of the vegetative multiplication and for the adjustment of the growth of the semi-bushed species it is important the use of the growth stimulators (Milică, 1983). They are administered to the cuttings or to other vegetative parts from the plants under the form of

solutions, stimulating the formation of the radicular system (Neamțu, and Irimia, 1992), and their use is signalized since the middle of the last century (Gergen, et. all., 1988).

It is known that vegetal biotechnologies represent a technique with a powerful impact and a direct involvement in the ex situ conservation of the species (Agud, 2014a), for the rescue of some species which are endangered with extinction in some areas of Romania and for the repopulation of these areas through efforts for their ecological reconstruction (Zăpârțan, 2001). Unconventional methods of multiplication were successfully applied to a great number of species from the spontaneous flora and to the ex vitro acclimatization of the neo-plantlets obtained (Zăpârțan, et all. 2014). In vitro multiplication of the botanical elements proved its efficiency at some species with a certain degree of vulnerability, specified in the red book and in the red lists of the species which are endangered with extinction (Laslo, et all., 2011; Laslo et all., 2011a). The objective of conserving the botanical species with a certain sozological degree, through the in vitro multiplication method is encompassed into the research programmes elaborated by the State research institutions and by the higher education ones (Agud, 2014).

Photo. 1 Aster alpinus

Photo. 2. Aster alpinus L., var. glabratus (Herb.)

MATERIAL AND METHOD

The vegetal material for the in vitro culture of *ASTER alpinus* L specie was made of a young (uncoloured) *bud* and *apical tissue* (apex) harvested before the flowering. The plant had a great porosity and a harsh tissue, so we betook to the application of the following stages of sterilization: the maintenance of the springs for about 15 minutes under tap water jet; the immersion for about 4 minutes in sterile water + detergent; the maintenance for 10 minutes in alcohol of 80% with continuous shaking; the placement of the vegetal material for about 10 minutes into an "Ace" solution of 89%; repeated rinses into sterile water (about 3 rinses); afterwards we passed to the sectioning of the desired explants (apex, bud). The inoculation was made at the end of summer 2016 and the beginning of autumn 2016, in the laboratory of Biotechnology of the Faculty of Environment protection in Oradea, basing on the classical technology of culture of the semi-bushed species (full vegetation and the moment before the flowering), when the growing and development processes of the specie favour in vitro culture

(Cachiţă et. all. 2008). There were conceived simple medium variants with very small doses of phytohormones, 0.2 – 0.4 mg/l, added in the basal medium according to MS (Murashige-Skoog, 1962) in which there were also included the vitamins (Thiamine, Pyridoxine and Nicotinic Acid, according to MS). There was conceived a witness variant (Mt.) and other three with the same basal medium and with a different hormonal balance, with phytohormones in the same concentration, but of a different nature (Table 1).

Table 1

Medium variants used at the in vitro multiplication of *ASTER alpinus* L specie

Var.	Basal medium (MS)	AUXIN (mg/l)	CYTOCHININ (mg/l)
Mt.	MS+ vit. MS	-	-
V_1	Mt.	0.2 AIA	0.4 BAP
V_2	Mt.	0.2 AIA	0.4 K
V_3	Mt.	0.2 AIA	0.4 2iP

(MS= basal medium according to Murashige-Skoog. 1962 ; AIA = α naftil acetic acid ; BAP= benzylaminopurine ; K = kinetin ; 2iP= 2 izopentyladenine)

RESULTS AND DISCUSSIONS

The observations were made after about 45-50 days from the in vitro incubation and there were established the medium values of the parameters concerning the number of plants and roots, the average of their length, values encompassed in Table 2.

Table 2

The reaction of the apical explant of *ASTER alipnus* L after 50 days of in vitro culture

Explant	**Var.**	**No. of plants** (average)	**Length of plants** (cm)	**No. of roots** (average)	**Length of roots** (cm)	**Observations**
BUD	Mt.	2	5.0	4	< 5.0cm	Uniform, good evolution
	V_1	**8**	**5.0**	**12**	**< 10.0cm**	Uniform, good and very good evolution
	V_2	2	0.5	1	2.0	Non-uniformly evolution
	V_3	*3*	*2.5*	*7*	*5.0*	Uniform evolution, better than on V_2
APEX	Mt.	1	6.0	7	< 8cm	Uniform evolution, good regeneration
	V_1	**6**	**7.0**	**15**	**< 10-15cm**	Very good evolution, regeneration and multiplication
	V_2	1	0.5 – 1.0	2	2.5cm	Non-uniformly evolution
	V_3	*3*	*7.0 – 8.0*	*8*	*4.5cm*	Uniform evolution, better than on V_2

The average of the number of neo-plantlets obtained from an explant presented in Fig. 1, points out the superiority of the variants with BAP (V_1) in the case of both explants on which there was obtained an average of about

8 neo-plantlets/bud and 6 neo-plantlets/apex. In exchange, on the other variants the number of neo-plantlets reaches only 1-3 neo-plantlets/explant.

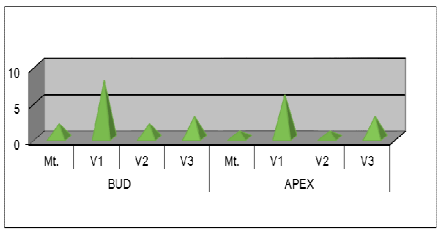

Fig. 1. The average of the number of plants/explant at Aster alpinus L specie (after 45-50 days of in vitro culture)

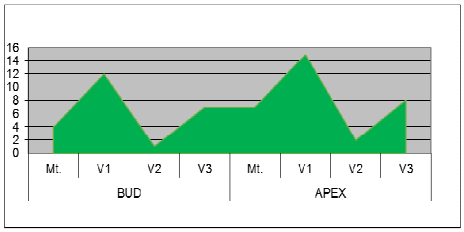

Fig. 2. The average of the number of roots/neo-plantlet at *Aster alpinus* L specie (after 45-50 days of in vitro culture)

The measurements concerning the average of the length of the plantlets (in cm) proved to be different depending on the nature of the phytohormones and very good on the witness sample (Mt.), being known that the basal medium (without a hormonal addition) stimulates the elongation of the internodes (Cachiță, et all. 2004). On the variants with phytohormones the average of the length of the plantlets differentiated from the bud is of 0.5cm (V_2), 2.5 (V_3) and up to 5 cm on V_1, and from the apex the values are somewhat higher reaching up to 7-8 cm on V_1 and V_3 (see Table 2).

There was also followed the average of the number of roots differentiated at the level of a neo-plantlet and we observed that the presence of auxin AIA – 0.2mg/l, had a beneficial effect on the differentiation of the radicular system, especially in association with BAP (V_1), the average of the number of roots being able to reach up to about 15 roots/plantlet, with a length of de 10-15cm (the roots circle the bottle a couple of times). On the other variants the average is comprised between 2-8 roots/neo-plantlet, and the length is of 2.5- 8.0 cm. We remark good values of the radicular system at the neo-plantlets differentiated on Mt. (Fig. 2) being well known that some neo-plantlets form a radicular system in the absence of phytohormones too, depending on the specie, in this case interfering with the contribution of endogenous phytohormones existing in the tissue of the plant (Cachiță et. all. 2008).

Simultaneously there were calculated the perceptual values concerning the regeneration and multiplication capacity of the two types of explants (bud and apex) after about 45-50 days of in vitro culture, values also presented in Figure 3 (regeneration percentage) and in Figure 4 (multiplication percentage).

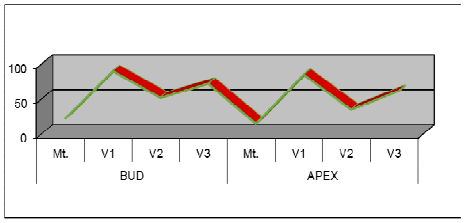

Fig. 3. Percentage of in vitro regeneration of the bud and apex of *Aster alpinus* L (after 45-50 days of in vitro culture)

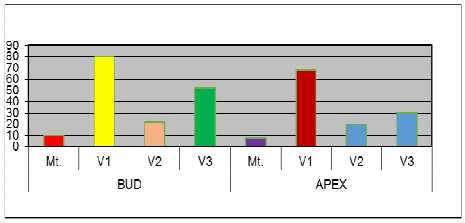

Fig. 4. Percentage of in vitro multiplication of the bud and apex of *Aster alpinus* L (after 45-50 days of in vitro culture)

The percentages of *in vitro* regeneration of the bud of *Aster alpinus* L are situated around 22 up to 90%, depending on the presence of the cytokinins and on their nature; on the witness it reaches the smallest percentage, and in the presence of BAP (V_1) the bud reaches even 90% regeneration. The apex also proves a good regenerative capacity especially on V_1 (with BAP) and on V_3 (with 2iP), but somewhat lower than in the case of the bud (between 35-85% on the variants with phytohormones). The witness in the case of the apex regenerates in s small percentage of 15% (Fig. 3).

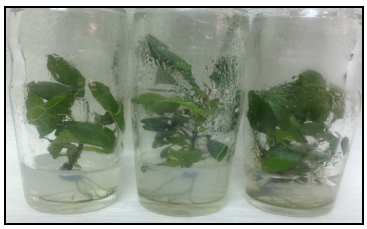

Photo. 3= Mt. Satisfactory, uniform evolution,

Photo. 4 = V_1 Very good, uniform evolution

The multiplication of the types of Aster explants analysed, expressed in percentages reaches superior values at the level of the bud (Fig. 4); if on the witness (Mt.) there is obtained only 10% multiplication on the variant with BAP (V_1), the bud multiplies up to 80% and over 50% in the presence of 2iP (V_3), in exchange the presence of kinetin (V_2) determines a small multiplication percentage, namely about 20-22% (at both explants). The percentage of multiplication is inferior in the case of the apex on all variants, and to this explant also, the best percentage is obtained in the presence of BAP - V_1 too (see Fig. 4).

CONCLUSIONS

1. *Aster alpinus* L specie manifests a regenerative and a multiplication reaction in vitro on mediums with very small doses of cytokinins (0.4mg/l

BA, K, 2iP) and auxins (0.2 mg/lAIA), being recorded differences depending on the nature of the explant and of the cytokinin.

2. Regeneration and multiplication capacities recorded at the level of the apex and of the bud of *Aster alpinus* L are percentually close, the bud recording values greater with 10% up to 20%, depending on the composition of the variant (on the nature of the phytohormones).

3. Among the cytokinins, BAP determines in vitro multiplication in the greatest percentage of 80% (at the bud) and respectively 68% (at the apex); the regeneration capacity in the presence of the same cytokinins (BAP) reaches up to 90% at the bud and up to 85% at the level of the apex;

4. In the presence of 2iP the in vitro reaction of the tissues is good (but inferior to BAP), regeneration reaches up to 73% (from the bud) and up to 62% (from the apex) and multiplication up to 53%, and respectively 30%; in the presence of kinetin (K) in vitro reaction of the tissues is inferior.

5. On the witness sample (Mt.), the percentages of the two parameters are inferior: we observe a regeneration of 22% at the bud and of 15% at the apex, and a multiplication between 10-8%.

6. We recommend a very small concentration of phytohormones (between 0.2-0.4mg/l), for the induction of the in vitro regeneration and multiplication of *Aster alpinus* L specie; and for obtaining a greater percentage, the presence of BAP-0.4mg/l, is absolutely necessary.

7. The acclimatization percentage of the neo-plantlets of *Aster alpinus* L ex vitro is situated between 70-90%, and the percentage grows directly proportionally with the value of the radicular system and with the forcefulness of the plantlets.

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