Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 22, issue 2, 2012, pp. 185-191

MORPHOGENESIS IN THE CULINARY, APICAL AND FOLIAR MINICUTTINGS, OF SEDUM TELEPHIUM SSP. MAXIMUM L. IN VITRO ON MURASHIGE - SKOOG (1962) MEDIUM CULTURE WITH DIFFERENT GROWTH REGULATORS ADDED

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Summary

Sedum telephium ssp. *maximum* L. is a species crassulacee possessing a variety of active ingredients, such as flavonoids, tannins, saponins, volatile oils, alkaloids, mucilage`s, resins, chlorophyll, vitamins (A, E, C, B complex), minerals.*Sedum telephium* ssp. *maximum* it has been described since antiquity by Dioscoride, Galenos, Hippokrates, Bauhin, is a perennial plant that grows on unfavorable ground where water supplies fluctuates seasonally present napiform rhizomes, fine roots, stems, high 20-50 (70) cm, glabrous, dark green, fleshy leaves, 5-13 cm long and 2-5 cm wide, sessile or short petiolated, at base with the rosette leaves, flowers are hermaphroditic, yellow-green to the spontaneous or those growing roses, arranged in a dense terminal corymb, the fruits consisting from five follicles, 1-2 mm long seeds are shiny and brown .

This ornamental species is cultivated for its evergreen, good looks, *Sedum telephium* ssp. *maximum*, known in our country as fat grass, very less came to the attention of our specialists.

The present experiment aimed to study the regenerative capacity and organogenesis fragments *Sedum telephium* ssp. *maximum* L. up, used as inoculums *in vitro*.

Regeneration of strains of type mini cuttings *nodal* explants taken from the seedlings of *Sedum telephium* ssp. *maximum*, obtained by micro propagation techniques, based on MS medium with the addition of *auxinic* or *citokinine*, have maximum values.

Introduction

Vegetable in vitro culture part of modern biotechnology industry that focus on various areas, including a special interest in plant biotechnology presents, that *in vitro* cultivation of physiotherapeutic interest.

In general, many herbs are micro propagate *in vitro*, were used as starting material in the popular culture media that are filled bioreactors and the biomass collected from a number of days in vitro culture pass extraction and conditioning the compounds of pharmaceutical interest (*Cachiţă* et al., 2004).

To populate bioreactors with herbal plant material of interest, generating secondary metabolic products, or to obtain planting material, plant cloning is done *in vitro*, micro propagation is the only effective way extremely fast propagation of plant species.

In terms of *in vitro* cultivation of species of *Sedum*, in order to obtain material that could be exploited as a source of active principles, the literature lacks articles on this profile.

Materials and methods

The present experiment aimed to study the regenerative capacity and organogenesis fragments *Sedum telephium* ssp. *maximum*, I used inoculate *in vitro* culture.

The culinary inoculums consisted of mini cuttings, apical and foliar harvested in vitro plantlets of

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Sedum telephium ssp. *maximum* elevated for up to eight weeks in vitro culture system of the standard culture medium, *Murashige - Skoog*, to which were added different regulators growth (axing and cytokine) inoculums of length 1 cm. Experimental variants were set:

V_0 - (control group) basic mineral medium MB - MS without growth regulators;

V_1 - base mineral medium MB - MS supplemented with the addition of 1 mg / l benzyl adenine (BA);

V_2 - basic mineral medium MB - MS supplemented with the addition of 1 mg / l indolil butyric acid (AIB);

V_3 - basic mineral medium MB - MS supplemented with 1 mg / l indocile butyric acid (AIB) mixed with 1 mg / l benzyladenine (BA);

V_4 - basic mineral medium MB - MS supplemented with 1 mg / l alpha-naphthyl acetic acid (NAA) mixed with 1 mg / l kinetin (KIN).

Inoculation took place inside the clean room, the distribution of one minicuttings phytoinoculs per container culture. Positioning minicuttings was plagiotrope (mini cuttings location on the surface of the culture medium, along with it).

After inoculation, in all phases of experimental phytoinoculs containers were transferred to incubate and grow in culture room provided with shelves for supporting the bottles and their exposure to appropriate growth regime. Containers with explants (blocked with polyethylene, fixed at the mouth bottles with elastic) were on the shelves at a temperature ranged between 22 – 25^{0}C and a photoperiod of 16 hours light/24h, the light intensity of approx. 1500 - 1700 lux, white light emitted by fluorescent tubes.

Their lighting was every 8 hours light and 16 hours of night.

The experiment was followed for *30 days* after inoculation of plant material, during which they performed biometric respectively direct observations and photographing the most important aspects.

Results and discussion

Observations made within 30 days of starting the experiment initiated in caulinar and apical vitro plantules mini cuttings are:

The average length of strains reached a maximum of 1 cm to inoculums raised on medium supplemented version with the addition of 1 mg / l NAA in combination with 1 mg / l KIN (V_4) marking an increase of 300% (fig.1B) V0 values exceeding the control group (0.25 cm).

Regarding the formation of young leaves, most were generated V_3 variant (basic medium MB - MS supplemented with 1 mg / l AIB mixed with 1 mg / l BA), *the average was 3 leaflet* / inoculums marking an increase of 200% (fig. 1C).

The largest width of young leaf was 1.45 cm with year increase of 314.28% (Fig. 1D) the environment variable V_3 (basal medium MB - MS supplemented with 1 mg / l AIB mixed with 1 mg / l BA), followed by inoculums variant V_4 (basic medium MB - MS supplemented with 1 mg / l NAA in combination with 1 mg / l KIN) Which reached a width of 1.15 cm marking year increase of 228.57% (fig.1D).

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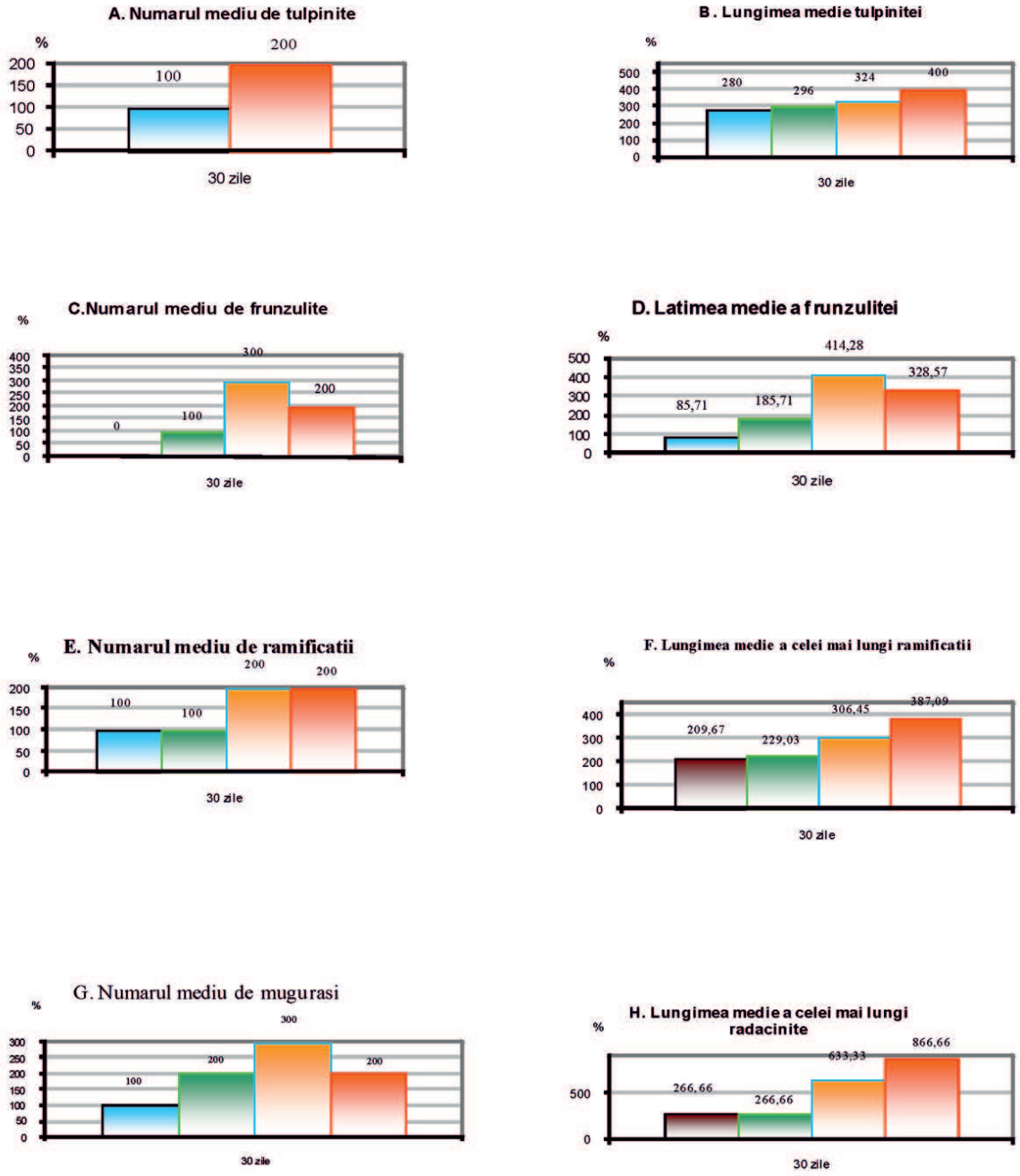

Fig. 1 - Graphical representation of mean values corresponding to the parameters biometric vitro cultures of Sedum ssp. telephium maximum L. up to 30 days, initiates in mini cuttings with 3 nodes, on average variants: V0 - (control group) basic mineral medium MB - MS without growth regulators, V1 - base mineral medium MB - MS supplemented with the addition of 1 mg / l benzyl adenine (BA), V2 - basic mineral medium MB - MS supplemented with the addition of 1 mg / l indolil butyric acid (AIB) V3 - basic mineral medium MB - MS supplemented with 1 mg / l indolil butyric acid (AIB) mixed with 1 mg / l benzyl adenine (BA), V4 - basic mineral medium MB - MS supplemented with 1 mg / l alpha-naphthalene acetic acid (ANA) mixed with 1 mg / l kinetin (KIN), data expressed as percentage values (where: a - average number of strains, B - average length of strains, C - average number of leaflet; D - average width of young leaf; E - average number of branches, F - average length of the longest branches; G – a bud average number of H - the longest average length of roots).

The highest average values for the *number branches* were found to mini cuttings grown on medium variant V_3 (basal medium MB - MS supplemented with 1 mg / l AIB mixed with 1 mg / l BA) and V_4 (basic medium MB - MS supplemented with 1 mg / l NAA in combination with 1 mg / l KIN) where we won by two branches / inoculums with year increase of 200% (fig.1) Reaching a *maximum length of branches* 1.1 cm of and average year of 287.09% increase (fig.1F), the medium variant V_4 (basic medium MB - MS supplemented with 1 mg / l NAA in combination with 1 mg / l KIN).

Most buds were present environmental inoculums V_3 variant (basic medium MB - MS supplemented with 1 mg / l AIB mixed with 1 mg / l BA) where inoculums generated by 3 leaflet / inoculums marking an increase of 200 % (fig. 1 G).

Regarding the *number of roots* generated strains of *Sedum telephium ssp.maximum* up to *30 days* of vitro culture were obtained for all variants tested and the *average length of the longest roots* reached a length of 1.3 cm and an increase of 766.66% (fig.1H) the environment variable V_4 (basic medium MB - MS supplemented with 1 mg / l NAA in combination with 1 mg / l KIN).

Observations made within *30 days* of starting the experiment initiated in *mini cuttings* vitroplantules of *vitro young leaves with or without stems,* pointed out the following:

The average length of the longest stems peaked at 0.95 cm inoculums grown on medium supplemented version with the addition of 1 mg / l NAA in combination with 1 mg / l KIN (V_4) scoring 375% (Fig. 2A) V_0 values exceeding the control group (0.20 cm).

Regarding the formation of leaflet, most were generated by variant V_4 (basic medium MB - MS supplemented with 1 mg / l NAA in combination with 1 mg / l KIN), *the average* was 4 *leaflet* / inoculums marking 300% (fig. 2 B), and *average width young leaf the largest* was 1 cm with an increase of 280% (fig. 2 C).

The highest average values for the *number branches* were found to mini cuttings raised the V_4 variant (basic medium MB - MS supplemented with 1 mg / l NAA in combination with 1 mg / l KIN) where we obtained 2.65 branch / inoculum with an increase of 381.81% (fig. 2D), reaching a *maximum length of branches* average of 1.25 cm and an increase of 331.03% (fig. 2E). *The average size of the largest callus* showed the highest values only the addition of 1 mg / l NAA in combination with 1 mg / l KIN in the medium (variant V_4), that it was 1.65 cm (Fig. 2G).

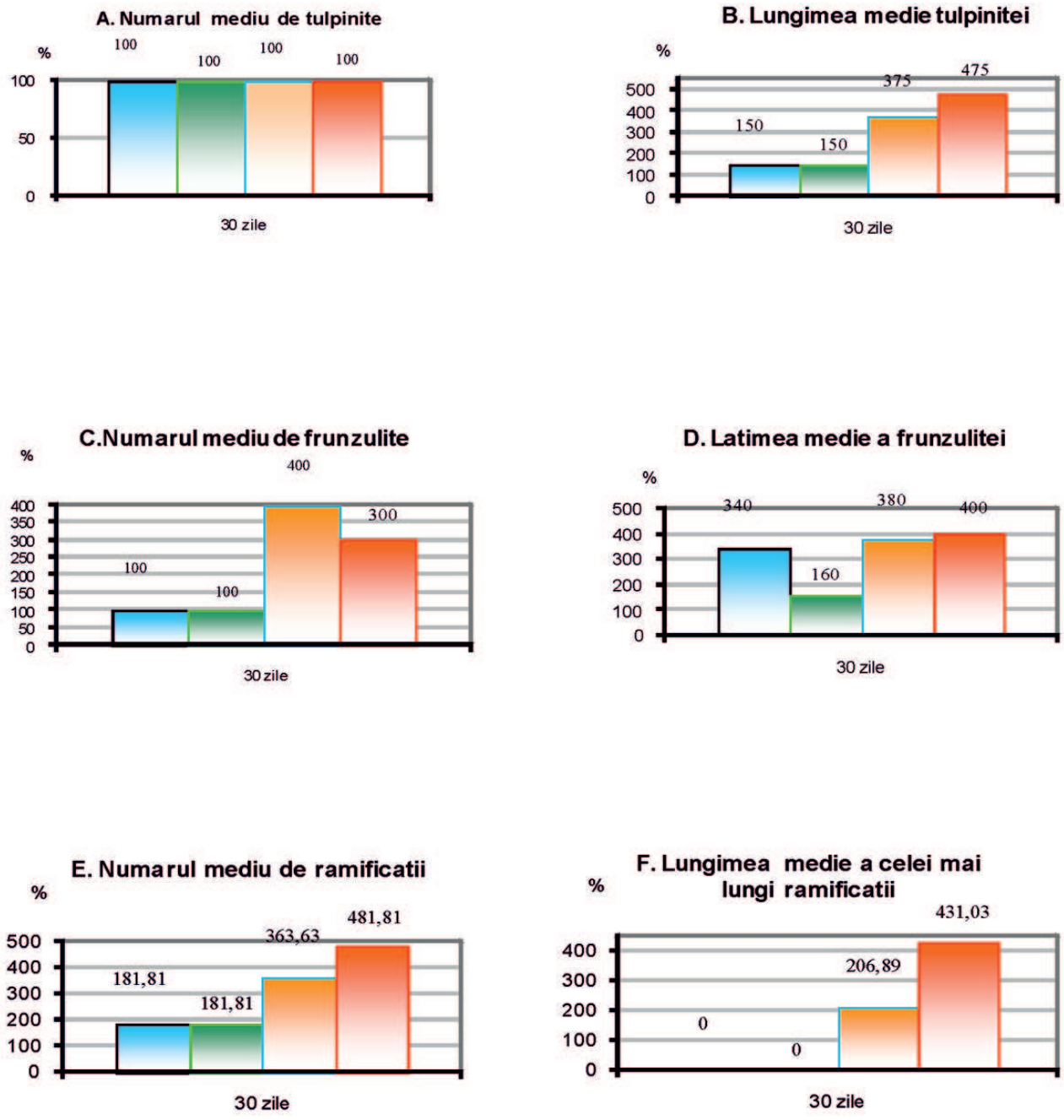

Fig. 2 - Graphical representation of mean values corresponding to the biometric parameters in vitro cultures of Sedum telephium ssp. maximum up to 30 days, initiate mini cuttings type of leaves, on average variants: V0 - (control group) basic mineral medium MB - MS without growth regulators, V1 - base mineral medium MB - MS supplemented with the addition of 1 mg / l benzyl adenine (BA), V2 - basic mineral medium MB - MS supplemented with the addition of 1 mg / l indolil butyric acid (AIB), V3 - basic mineral medium MB - MS supplemented with 1 mg / l indolil butyric acid (AIB) mixed with 1 mg / l benzyl adenine (BA), V4 - basic mineral medium MB - MS supplemented with 1 mg / l alpha-naphthyl acetic acid (ANA) mixed with 1 mg / l kinetin (KIN), data expressed as percentage values (where: a - average number of strains, B - average length of strains, C - average number of leaflet; D - average width young leaf; E - average number of branches, F - average length of the longest branch).

Vitrocultures observations on the species Sedum telephium ssp. maximum revealed that the environment variable V4 - that MB-MS with the addition of NAA and KIN, the concentration of 1 mg / liter, presented the best results in the caulogenesis, branches and callus formation.

In figure 3 are images of Sedum telephium ssp. maximum L. vitroplantules up to 30 days after their inoculation in vitro medium environment variable V4 - that MB-MS with the addition of NAA and KIN.

Conclusions

Regeneration of strains of type mini cuttings nodal explants taken from the seedlings of Sedum telephium ssp. maximum L., obtained by micro propagation techniques, based on MS medium with the addition of auxinic or cytokine, have maximum values.

Thus, addition of 1 mg / l NAA in combination with 1 mg / l KIN favored increasing the number and length of seedlings, formation and elongation of

Fig. 3- Comparison of the process of organogenesis type median minicuttings, culinary or foliar limb type inoculated on basal medium Murashige Skoog (1962) modified by us, with the addition of NAA and KIN, 1 mg / l of each, at 7 days (positions A and B), 30 days (positions B and E) and 60 days of their location in vitro (positions C and F).

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branches, respectively roots elongation.

Regarding the formation of young leaves, basic medium MB - MS supplemented with 1 mg / l AIB mixed with 1 mg / l BA favored their generation and increase their size, these bud formation.

Rootedness manifested on all variants tested, only after 30 days of primary cultivation, most generating on average MB version - MS supplemented with 1 mg / l AIB mixed with 1 mg / l BA.

Regeneration of strains of type mini cuttings leaf explants taken from the Sedum telephium ssp.maximum L. seedlings obtained by micro propagation techniques, both on MS basal medium with the addition of auxinic or cytokine have been some delay in the initiation and training leaflet ramification regenerated from them. But once started on a culture medium which was added a mixture of growth regulators, this process of growth, continued to give satisfactory results.