Biodiv. Res. Conserv. 5-8: 87-94, 2007

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Comparison of regenerative potential of petals, stamens and pistils of five *Sedum* species *in vitro*

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Abstract: The regeneration potential of different explants from flower buds (petals, stamen and pistils) was studied in *Sedum acre*, *S. aizoon*, *S. floriferum*, *S. gracile* and S. *spectabile*. The effects of MS medium, supplemented with nine PGR combinations of 6-benzylaminopurine (BAP 1.0-3.0 mg l^{-1}) and indole-3-butyric acid (IBA 0.1-1.5 mg l^{-1}) on regeneration, were compared. Regenerated plants were obtained in cultures of petal, stamen and pistil in all studies species.
Three-way analysis of variance allowed to assess the effect of medium composition, explant type and genotype on regeneration potential, expressed as the percentage of regenerating explants and the average number of shoots produced from the explants. All analysed factors significantly affected the regeneration process. PGR composition had the most significant effect on the average number of produced shoots. MS medium containing 3.0 mg/l BAP and 1.0 mg/l IBA proved to be optimal for plant regeneration in *Sedum*. Explant type had the most significant effect on the percentage of regenerating explants. For petals the percentage of regenerating explants (40%) was nearly twice as high as in other explant types. Genotype had the most significant effect on the average number of regenerated shoots. The highest number of regenerated shoots per explant (3.25) was recorded in *S. floriferum*.

Key words: flower organ cultures, organogenesis, tissue culture, *Sedum*

1. Introduction

In vitro plant regeneration *via* organogenesis or somatic embryogenesis is important for a wide range of applications from clonal propagation and germplasm conservations to genetic manipulation (Lowe *et al*. 1996). So far, extensive research has been carried out on *in vitro* regeneration of plants and the most commonly used explant types have been fragments of leaves, stems, shoot apices, parts of seedlings, inflorescences, and flower buds. Moreover, some isolated flower parts (petals, stamens and pistils) were applied as explants for micropropagation.

To date, the largest number of studies on the possibility of plant regeneration *in vitro* from petals concerned carnations *Dianthus caryophyllus* (Kakehi 1979; Gimelli *et al*. 1984; Fisher *et al*. 1993; Miller *et. al*. 1991; Nugent *et al*. 1991; Simard *et al*. 1992) and *Chrysanthemum* species: *C. morifolium* (Bush *et al*. 1976; Mandal & Datta 2005) and *C. coccineum* (Fujii & Shimizu 1990). To a large extent this is due to their ornamental qualities and the continuously large commercial demand for those plants. Besides, it has been proved that plant regeneration from petals is possible in *Pelargonium* cv. Klainer Liebling (Bennici 1974), *Hemerocallis* cv. Chipper Cherry (Heuser & Apps 1976), *Saintpaulia ionantha* (Vazques & Short 1978), *Rosa hybrida* cv. Arizona (Noriega & Sondahl 1991; Murali *et al*. 1996), *Araujia sericifera* (Torne *et al*. 1996, 1997), *Cyclamen persicum* (Karam & Al-Matahoub 2000) and *Rhododendron simisii* (Schepper *et al*. 2004).

The aim of this study was to regenerate plants from petals, stamens and pistils and to compare their regenerative potential in five *Sedum* species. Most of *Sedum* species belong to ornamental plants which tolerate dry conditions, what makes them suitable for planting in rock gardens and flower borders (Yoon *et al*. 2002). So far, *in vitro* plant regeneration in *Sedum* genera has been reported from a shoot tip of *S. sieboldii* (Uhring 1983) and leaves of *S. telephium* (Brandao & Salema 1977) and *S. erytrostichum* (Yoon *et al*. 2002). The present report for the first time describes the *Sedum* plant regeneration from petals, stamens and pistils.

2. Materials and methods

2.1. Plant material

Petals, stamens and pistils of *S. acre*, *S. aizoon*, *S. gracile*, *S. floriferum* and *S. spectabile* were used in this study. Flower buds, just before they opened, were taken from plants growing in the Botanical Garden of the Adam Mickiewicz University in Poznań. They were surface-sterilized in a 70% aqueous solution of ethanol for 0.5 min., rinsed in sterile water, and dipped in a sodium hypochlorite solution (0.15% available chlorine) for 8 min. Individual explants were removed from the buds and placed horizontally on the medium. Additionally, in stamen cultures the stamen and the stamen filaments were put on the medium.

2.2. Culture media and culture conditions

In this study, MS medium (Murashige & Skoog 1962) was used, with various levels of growth regulators (PGR): BAP (1.0-3.0 mg l^{-1}) and IBA (0.1-1.5 mg l^{-1}) (Tables 1-3). The applied growth regulators were selected on the basis of author's own preliminary experiments, which showed that the addition of the cytokinin BAP and the auxin IBA is an optimum combination of growth regulators, as it most effectively stimulates the process of micropropagation of *Sedum* spp. The media were supplemented with sucrose (30.0 g l^{-1}) and Difco agar (8.0 g l^{-1}). The pH was adjusted to 5.7 before autoclaving. As a control, explants were placed on MS medium without growth regulators.

All cultures were incubated in a growth chamber under a 16 h photoperiod (35 µmol m^{-2} s^{-1}, cool white fluorescent light) at 24°C and 70-80% relative humidity.

2.3. Analysis of regenerative potential of explants

To assess the regenerative potential of explants and perform a statistic analysis, quantitative observations were made.

After 24 days of culture, for each explant type the percentage of explants regenerating shoots and the average number of shoots per regenerating explant on each medium variant were recorded.

A randomized complete block design was used for the experiments. For each species, 50 explants on average were placed on each medium variant. Results from 3 replications were subjected to a statistical analysis. Effects of factors (medium, genotype, explants and their interactions) on regenerative potential were tested by three-way analysis of variance. For multiple comparisons, Tukey test was used, which allowed to distinguish homogeneous groups at the assumed significance level ($p<0.05$). The analysis was made with the use of Statistica 5.0 software.

2.4. Shoot rooting and transfer to the soil

The shoots regenerated from petals, stamens and pistils were transferred to a rooting medium containing half of macronutrient concentrations of MS (½ MS) without growth regulators, or to MS medium supplemented with naphthaleneacetic acid (NAA) at concentrations 0.1 mgl^{-1} and 0.5 mgl^{-1}. They were incubated in a growth chamber under a 16 h photoperiod (35 µmol m^{-2} s^{-1}, cool white fluorescent light) at 24°C and 70-80% relative humidity.

Rooted plantlets were transferred to plastic pots containing a mixture of leaf mould, clay and sand (8:2:2) and hardened in a greenhouse for succulent plants. After the hardening period of 5 to 8 weeks, plantlets were finally moved to the field conditions.

3. Results

In the *in vitro* culture, both the petals, stamens and pistils of the studied *Sedum* spp. regenerated plants. Addition of growth regulators proved to be necessary to induce the process of regeneration. On MS medium without growth regulators (control), the petals, stamens and pistils did not show the capacity for plant regeneration.

3.1. Morphogenesis in petal cultures

Petals of *Sedum* spp., depending on medium variant and species, were subject to indirect or direct organogenesis (Table 1). Adventitious buds and callus were always formed on the adaxial side of explants.

Direct organogenesis. In petal cultures of *S. aizoon*, *S. gracile* and *S. spectabile* regeneration was induced through direct organogenesis on most of the applied medium variants (Table 1). In petal cultures of *S. aizoon*, primordia of adventitious buds were visible on explants as early as on the day 4 of culture on MS VII and VIII, containing BAP 3.0 mg l^{-1} and IBA 0.5 mg l^{-1} and 1.0 mg l^{-1}, respectively, and the first shoots started to develop already on day 7 (Fig. 1A). In petal cultures of *S. aizoon*, an adventitious bud and shoot formation was observed earlier than in other *Sedum* spp. In the other species, depending on medium variant, adventitious buds at the petal base started to be formed between days 8 and 14 (Table 1) and developed into adventitious shoots in 1 week (Fig. 1B).

Indirect organogenesis. In *S. floriferum* and *S. acre* the addition of PGR used in this study induced indirect organogenesis on most of the used medium variants (Table 1).

Callus formation at the petal base was observed as early as on the day 6 in *S. acre* on MS V and IX, with the highest auxin concentration (Table 1), and in *S. floriferum* on media: MS VI, VII, VIII and IX, all with

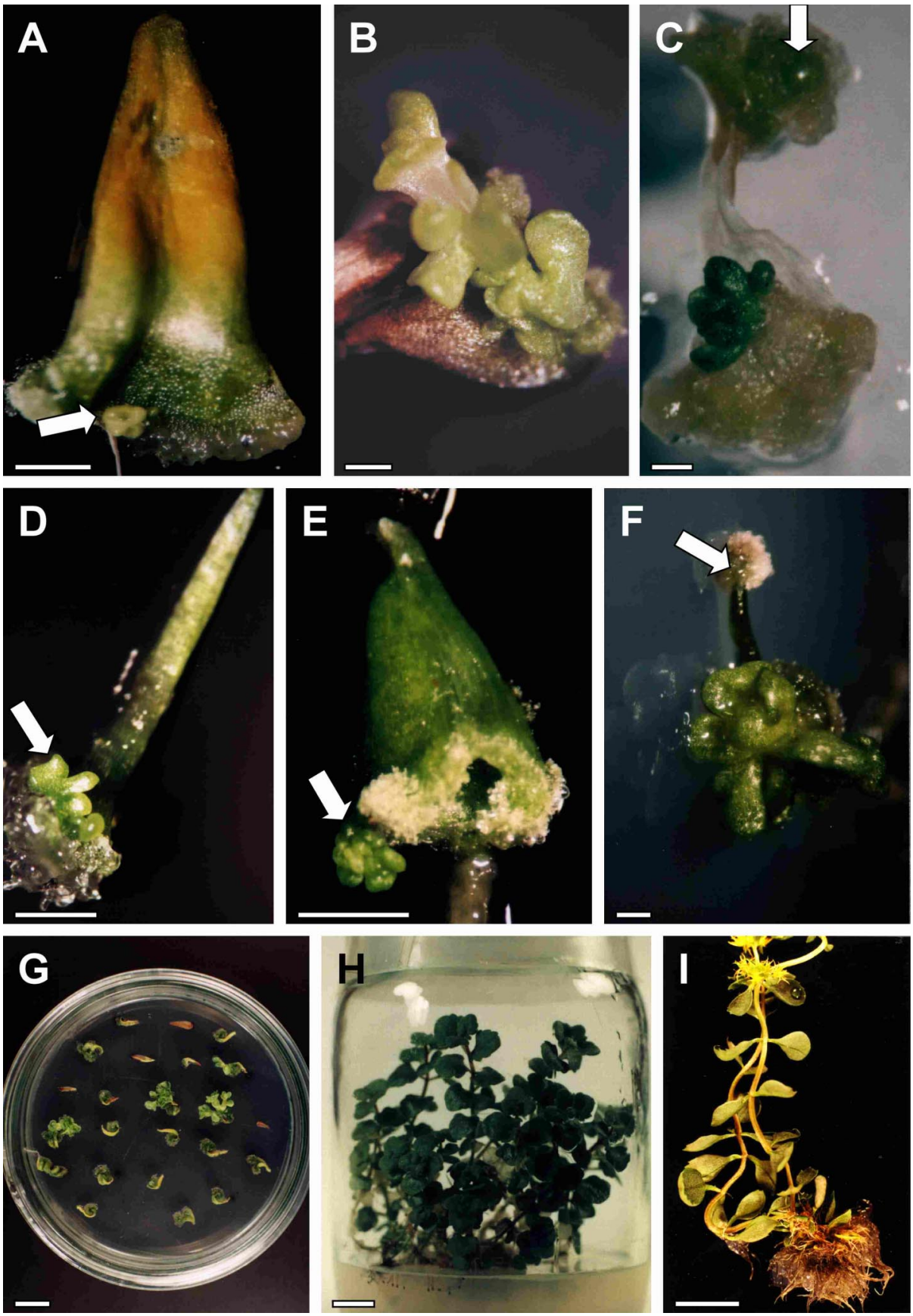

Fig. 1. Plant regeneration from petal, stamen and pistil explants of *Sedum* spp.
Explanations: A, B. Direct organogenesis in petal cultures; A. Adventitious bud formation (arrow) at the base of *S. aizoon* explant, 4 days (MS VIII). Bar 1 mm; B. Shoot elongation at the base of *S. spectabile* explant, 13 days (MS VI). Bar 1 mm; C. Indirect organogenesis in petal cultures – differentiation of adventitious buds from callus at the basal and the apical part (arrow) of *S. acre* explant, 11 days (MS VI). Bar 0.5 mm; D, E. Indirect adventitious buds (arrow) formation (D) in stamen culture of *S. spectabile*, 8 days (MS IX). Bar 1 mm (E) in pistil culture of *S. gracile*, 10 days (MS VIII). Bar 1 mm; F. Callus proliferation at the apical part (arrow) of *S. floriferum* stamen and adventitious buds developed in callus at the base of explants, 14 days (MS VII) Bar 1 mm; G. Petal culture of *S. floriferum* showing the high frequency of explant regenerating shoots, 14 days (MS VII). Bar 1 mm; H. Adventitious shoots of *S. floriferum* subcultured on rooting medium with ½ MS macro nutrients. Bar 1 mm; I. *S. floriferum* flowering plant regenerated *in vitro* from pistil explant with well developed roots before transferring to a pot. Bar 20 mm

Table 1. Effects of growth regulators on measured traits of induction of direct (D) or indirect (I) organogenesis in petal cultures of *Sedum* spp. on nine variants of MS medium

Species	Trait	Medium variants																	
		I		II		III		IV		V		VI		VII		VIII		IX	
		1.0 BAP 0.1 IBA		1.0 BAP 0.5 IBA		2.0 BAP 0.1 IBA		2.0 BAP 0.5 IBA		2.0 BAP 1. 0 IBA		3.0 BAP 0.1 IBA		3.0 BAP 0.5 IBA		3.0 BAP 1.0 IBA		3.0 BAP 1.5 IBA	
		D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I	D	I
S. acre	%RE	0	0	21.6	0	24.3	0	0	29.0	0	36.4	0	45.2	0	53.2	0	48.6	0	49.2
	S/RE	0	0	1.4	0	2.2	0	0	2.5	0	2.5	0	3.4	0	3.0	0	2.7	0	2.8
S. aizoon	%RE	13.1	0	27.0	0	45.9	0	53.6	0	0	59.4	43.1	0	62.1	0	56.1	0	0	45.7
	S/RE	1.7	0	1.5	0	2.9	0	4.4	0	0	5.6	3.7	0	4.5	0	7.3	0	0	3.7
S. floriferum	%RE	0	0	0	60.0	27.9	0	0	49.5	0	50.0	0	39.7	0	61.3	0	71.8	0	66.7
	S/RE	0	0	0	3.8	4.2	0	0	4.7	0	5.5	0	5.9	0	4.9	0	7.2	0	5.0
S. gracile	%RE	0	0	0	0	28.6	0	32.7	0	26.0	0	29,4	0	36.0	0	31.4	0	0	28.4
	S/RE	0	0	0	0	2.6	0	3.8	0	4.9	0	3.3	0	3.9	0	6.3	0	0	2.8
S. spectabile	%RE	26.7	0	34.0	0	50.4	0	48.2	0	54.7	0	55.0	0	58.8	0	70.2	0	0	78.1
	S/RE	2.3	0	3.0	0	3.5	0	3.8	0	4.4	0	4.8	0	3.9	0	5.8	0	0	4.1

Explanations: %RE – percentage of regenerating explants; S/RE – average number of shoots per regenerating explant; BAP and IBA – concentrations expressed in mg l^{-1}

the highest cytokinin concentrations (Table 1). Formation of adventitious buds in both species was recorded simultaneously, on the day 8 of culture. Additionally, in *S. acre* callus and adventitious bud formation was observed in the apical part of the petal (Fig. 1C). Callus formation and adventitious bud differentiation in callus took place initially at the petal base and later in the apical part of the petal.

3.2. Morphogenesis in stamen and pistil cultures

Stamens and pistils, irrespective of medium variant and plant species, regenerated plants only through indirect organogenesis (Tables 2 and 3). Callus at the base of stamens of *S. aizoon* and pistils of *S. acre* was observed on days 4 and 5, respectively, in media with the highest cytokinin concentration (Tables 2 and 3). In stamen and pistil cultures, depending on medium variant and *Sedum* species, primordia of adventitious buds were differentiating in callus at the base of explants between days 8 and 17 (Fig. 1D, 1E). Although, callus was induced both at the base and in the apical part of the stamen filament and on the stigma and style, the adventitious buds developed in callus only at the explant base (Fig.1F).

3.3. Effect of PGR composition, explant type and donor plant genotype on regenerative potential in *Sedum* spp.

An analysis of variance revealed that medium variants, explant types, genotype, and their interactions, affected significantly the regenerative potential, expressed as the percentage of regenerating explants and the average number of shoots *per* regenerating explant (Table 4).

The average number of shoots *per* regenerating explant and percentage of regenerating explants in the cultures of petals, stamens and pistils in individual medium variants and species are presented in Tables 1-3.

Explant type had the most significant effect on the percentage of regenerating explants (Fig. 2). The sta-

Table 2. Effects of growth regulators on measured traits of induction of indirect organogenesis in stamen cultures of *Sedum* spp. on nine variants of MS medium

Species	Trait	Medium variants								
		I	II	III	IV	V	VI	VII	VIII	IX
		1.0 BAP 0.1 IBA	1.0 BAP 0.5 IBA	2.0 BAP 0.1 IBA	2.0 BAP 0.5 IBA	2.0 BAP 1. 0 IBA	3.0 BAP 0.1 IBA	3.0 BAP 0.5 IBA	3.0 BAP 1.0 IBA	3.0 BAP 1.5 IBA
S. acre	%RE	0	0	10.1	14.0	14.1	28.1	33.3	35.0	25.9
	S/RE	0	0	1.1	1.3	1.5	2.3	3.2	3.6	2.5
S. aizoon	%RE	0	0	2.1	14.0	10.6	43.1	21.5	20.9	20.0
	S/RE	0	0	1.3	1.7	2.5	2.8	3.1	2.5	3.9
S. floriferum	%RE	0	0	4.6	10.0	13.2	13.9	19.2	27.9	20.9
	S/RE	0	0	1.5	1.7	2.5	4.2	6.9	5.4	4.9
S. gracile	%RE	0	10.2	18.4	24.0	19.1	29.4	28.8	23.2	24.0
	S/RE	0	1.2	1.2	1.6	2.3	2.6	2.9	2.3	3.5
S. spectabile	%RE	0	0	5.1	12.5	16.4	25.0	22.5	30.0	33.3
	S/RE	0	0	1.2	1.4	2.0	3.3	5.4	4.2	3.9

Explanations: %RE – percentage of regenerating explants; S/RE – average number of shoots per regenerating explant; BAP and IBA – concentrations expressed in mg l^{-1}

Table 3. Effects of growth regulators on measured traits of induction of indirect organogenesis in pistil cultures of *Sedum* spp. on nine variants of MS medium

Species	Trait	Medium variants								
		I	II	III	IV	V	VI	VII	VIII	IX
		1.0 BAP 0.1 IBA	1.0 BAP 0.5 IBA	2.0 BAP 0.1 IBA	2.0 BAP 0.5 IBA	2.0 BAP 1. 0 IBA	3.0 BAP 0.1 IBA	3.0 BAP 0.5 IBA	3.0 BAP 1.0 IBA	3.0 BAP 1.5 IBA
S. acre	%RE	0	0	20.9	21.3	22.2	29.8	31.7	28.8	0
	S/RE	0	0	1.2	1.9	2.0	2.5	3.0	2.3	0
S. aizoon	%RE	0	9.6	24.5	17.8	20.4	47.7	29.3	36.8	25.5
	S/RE	0	1.2	2.2	2.4	2.5	4.2	4.3	2.9	2.6
S. floriferum	%RE	0	0	6.1	6.7	8.3	12.2	11.1	12.5	16.9
	S/RE	0	0	1.9	3.0	2.3	3.2	3.0	4.6	2.5
S. gracile	%RE	0	6.9	11.1	24.2	29.4	27.5	41.9	54.0	48.1
	S/RE	0	1.4	1.3	1.3	1.6	2.0	1.8	2.1	2.9
S. spectabile	%RE	0	2.2	8.5	14.7	17.3	22.2	27.5	29.5	22.0
	S/RE	0	1.1	1.6	2.4	1.9	2.7	2.5	3.9	2.1

Explanations: %RE – percentage of regenerating explants; S/RE – average number of shoots per regenerating explant; BAP and IBA – concentrations expressed in mg l^{-1}

tistical analysis showed that the regenerative potential of petals was the highest (on average c. 40%). In comparison to other explant types, petals had nearly twice as high values of both the average percentage of regenerating explants and the average number of shoots *per* regenerating explant (Fig. 1G).

Average values for stamens and pistils also diverge from each other (Fig. 2) and the statistical analysis did not allow distinguishing them as one homogenous group. Each of the studied explant types constituted one separate homogeneous group.

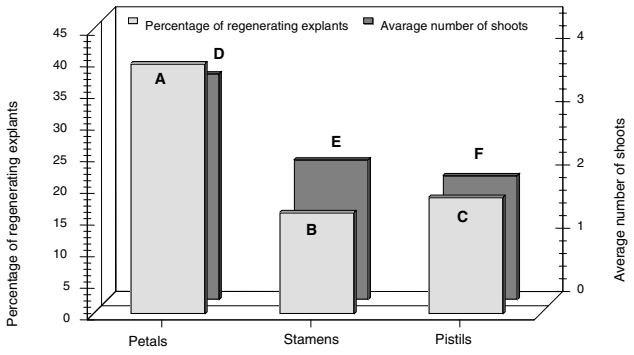

Fig. 2. The effect of a type of explant on the mean values of percentage of regenerating explants and an average number of shoots (n=90 observations)

Explanations: Homogeneous groups (significant at $p< 0.05$). Trait: percentage of regenerating explants A, B, C: 1-element groups: explants: petals, stamens, pistils. Trait: average number of shoots per regenerating explant D, E, F: 1-element groups: explants: petals, stamens, pistils

PGR concentrations in medium had the most significant effect on the average number of shoots *per* regenerating explant. With regard to the applied medium variants, the statistical analysis revealed that the increase in BAP concentration from 1.0 mg l^{-1} to 3.0 mg l^{-1} caused a significant increase both in the average number of shoots per regenerating explant and in the average percentage of regenerating explants (i.e. the growing

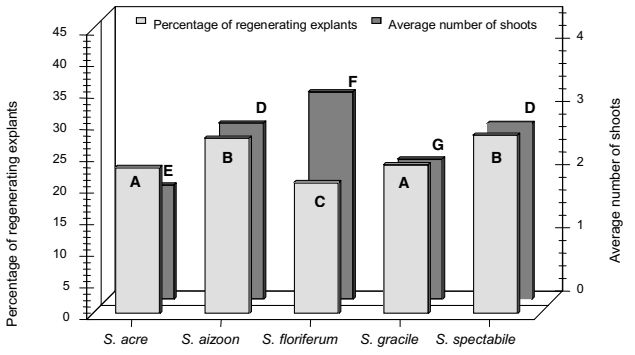

Fig. 3. The effect of genotype on the mean values of percentage of regenerating explants and an average number of shoots (n=54 observations)

Explanations: Homogeneous groups (significant at $p< 0.05$).Trait: percentage of regenerating explants. A. 2-element group: *S. acre* + *S. gracile*; B. 2-element group: *S. aizoon* + *S. spectabile*; C. 1-element group: *S. floriferum*. Trait: average number of shoots per regenerating explant; D. 2-element group: *S. aizoon* + *S. spectabile*; E, F, G. 1-element groups: *S. acre*; *S. gracile*; *S. floriferum*

Genotype had the most significant effect on the average number of shoots *per* regenerating explant. The highest value (3.25 shoots per explant) was recorded in *S. floriferum* (Fig. 3). The statistical analysis allowed to distinguish homogeneous groups, particularly *S. floriferum* alone and two groups composed of pairs of similar species: *S. aizoon* + *S. spectabile* and *S. acre* + *S. gracile* (Fig. 3).

BAP concentration was connected with growing regenerative potential). A rise in IBA concentration to 1.0 mg l^{-1} also improved the regenerative potential, but a further increase in its concentration led to a slight reduction of the potential (Fig. 4). MS medium containing

Table 4. Results of analysis of variance (values of Snedecor *F* variable) for factors: genotype of donor plant (species), explant type, medium, and their interactions

Traits	Main effect			Interactions			
	Medium (M)	Explant (E)	Species (S)	M × E	M × S	E × S	M × E × S
	f_1=8,f_2=135	f_1=2,f_2=135	f_1=4,f_2=135	f_1=16,f_2=135	f_1=32,f_2=135	f_1=8,f_2=135	f_1=64,f_2=135
%RE	473.63**	1505.75**	56.17**	21.88**	8.22**	137.44**	11.94**
S/RE	881.74**	1176.43**	306.71**	61.27**	16.28**	39.98**	20.73**

Explanations: ** – significant at p<0.01; %RE – percentage of regenerating explants; f_1, f_2 – degrees of freedom for factors; S/RE – average number of shoots per regenerating explant

3.0 mg l^{-1} BAP and 1.0 mg l^{-1} IBA (MS VIII) proved to be optimal for plant regeneration in *Sedum* (Fig. 4).

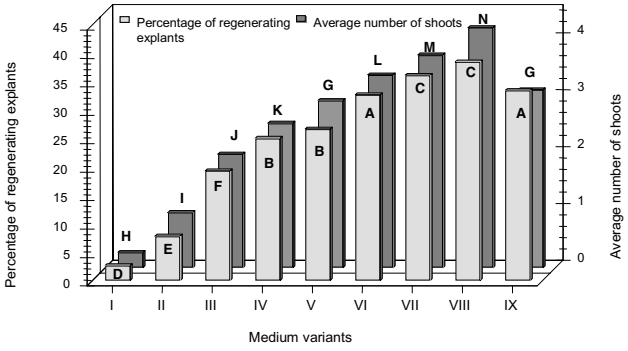

Fig. 4. The effect of PGR composition on the mean values of percentage of regenerating explants and average number of shoots (n=30 observations)

Explanations: Homogeneous groups (significant at p< 0.05). Trait: percentage of regenerating explants; A. 2-element group: medium variants VI + IX; B. 2-element group: medium variants IV + V; C. 2-element group: medium variants VII + VIII; D, E, F. 1-element groups: medium variants I; II; III. Trait: average number of shoots per regenerating explant; G. 2-element group: medium variants V + IX; H, I, J, K, L, M, N. 1-element groups: medium variants I; II; III; IV; VI; VII; VIII

3.4. Shoot rooting and *ex vitro* culture

Adventitious shoots of the studied species, irrespective of explant type from which they were isolated, took roots between week 3 and 4 of the culture (Fig. 1H) Root systems were well-established on 1/2 MS medium without growth regulators (Fig. 1I)

As a result of the experiments, rooted plants of the studied *Sedum* spp. were obtained. In the field, all *in vitro* regenerated plants flowered and set seeds. A plant habit, size and stem firmness as well as leaf size and shape in specimens derived from *in vitro* culture did not differ from those of the donor plants. The plants regenerated from petals were green and their petal colour was the same as in the donor plants.

4. Discussion

In this study the regeneration potential of isolated petals, stamens and pistils was compared *in vitro* in *Sedum acre*, *S. aizoon*, *S. floriferum, S. gracile* and *S. spectabile*, in the presence of growth regulators: the cytokinin BAP and the auxin IBA. As a result of these experiments, fully developed plants were regenerated from all kinds of explants in five studied *Sedum* spp. In contrast, among three applied explant types in *Theobroma cacao* (Almanno *et al.* 1996) – petals, stamens, and ovules – only stamen filaments showed a morphogenetic potential.

In the present study, among the used types of *Sedum* explants, the highest regeneration potential was recorded in the case of petals. For stamens and pistils the potential was only half as high as that of petals. These results and reports by some other authors (Miller *et al.* 1991; Nugent *et al.* 1991) show that petals of various plant species are characterized by a high regenerative potential. Petals of one carnation cultivar regenerated plants from 100% of explants (Nugent *et al.* 1991), while fragments of filaments of *Dianthus caryophyllus* (Miller *et al.* 1991) only sporadically formed leaf primordia. In stamen cultures of *Rosa hybrida*, 14% of explants showed plant regeneration (Noriega & Sondahl 1991), whereas in *Brassica campestris* subsp. *napus* var. *pekinensis* only 6.5% (Choi *et al.* 1998). In ovary cultures of *Citrus* spp. (Carimi *et al.* 1999), only 9.3 % of explants displayed the capacity for plant regeneration.

The interesting feature of petal cultures is the rate of adventitious buds development. In *Dianthus caryophyllus* (Simard *et al.* 1992) primary adventitious buds appeared on the day 14 and shoot elongation occurred within 4 weeks. In the studied *Sedum* spp. organogenesis started much earlier and adventitious buds of *S. aizoon* were already visible on the day 4 and shoot were formed on the day 7. Equally, in stamen and pistil cultures adventitious buds were formed on the day 8.

In petal cultures of *Sedum* spp., plants were regenerated through direct and indirect organogenesis. In petal cultures of S. *aizoon*, *S. gracile* and *S. spectabile* direct organogenesis prevailed, like in cultures of *Saintpaulia ionantha* cv. Blue Rhapsody (Vazquez & Short 1978) and *Dianthus caryophyllus* (Nugent *et al.* 1991).

An analysis of the regeneration activity of petals, stamens and pistils of *Sedum* showed that morphogenetic processes were the most intensive in the basal parts of explants. The process of regeneration at the basal parts of petals, stamens and pistils was probably associated

with the still preserved capacity for elongation of those flower parts. When the *Sedum* explants were placed on the medium (the explants were taken just before flower opened), probably, the intercalary meristem was still active, so the parenchyma cells at the petal base, as ontogenically younger, were characterized by a greater sensitivity to growth regulators and were subject to dedifferentiation and next, regeneration.

A similar course of regeneration at the bases of flower parts was also observed in petal cultures of *Dianthus caryophyllus* (Kakehi 1979; Nugent *et al*. 1991; Miller *et al*. 1991; Simard *et al*. 1992) and *Araujia sericifera* (Torne *et al*. 1997), in stamen cultures of *Brassica campestris* subsp. *napus* var. *pekinensis* (Choi *et al*. 1998) and in pistil cultures of *Citrus deliciosa* and *C. tardiva* (Carimi *et al*. 1999).

In *Sedum acre* plant regeneration was additionally observed in the apical part of petals, but the process always started at the petal base, whereas the apical part produced callus and regenerated new plants much later. Such a sequence of regeneration in petals of *S. acre* could be due to a prolonged activity of the apical meristem of petals in this species, which prolonged the process of differentiation. Regeneration from lateral parts of petals was not recorded in any of the studied *Sedum* spp. On the contrary, in the genus *Citrus* (Carimi *et al*. 1999), cultures of thin sections from various parts of pistils showed that stigma tissues, in four out of the six studied species, were subject to morphogenetic induction.

The *Sedum* plants obtained in petal cultures through direct and indirect regeneration did not show any phenotypic differences. In contrast, carnation plants deriving from petal cultures were characterized by a high variation in features like plant height and flower colour (Biautti *et al*. 1986).

The results of the study showed an efficient protocol for rapid plant multiplication of *Sedum* species from petal, stamen and pistil explants. Additionally, petal ability to induce direct organogenesis, seems to be a valuable material for further studies, especially for improvement of ornamental values in *Sedum* species by mutation or *Agrobacterium* transformation.

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