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BRIEF COMMUNICATION

Sucrose effects on *in vitro* fruiting and seed production of *Centaurium pulchellum*

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Abstract

The effect of sucrose on fruiting, seed production, and seed germination of lesser centaury [*Centaurium pulchellum* (Sw.) Druce] was examined using explants of flowers and flower buds. Sucrose concentrations in the culture medium ranged from 0.003 to 0.3 M. It has been shown that the number of auxiliary buds, capsules dimension, number of viable seeds per capsule and seed dimensions increased with the increase of sucrose concentrations. The highest values were recorded at sucrose concentrations higher than 0.03 M, except for seeds size, which were larger at sucrose concentration ranging from 0.003 to 0.1 M. The germination of *in vitro* produced seeds was affected by previous culture history: a higher germination percentage was obtained in seeds that were raised from explants originally grown on medium with sucrose concentrations higher than 0.003 M.

Additional key words: explants, germination, gibberellic acid, lesser centaury.

Lesser centaury [*Centaurium pulchellum* (Sw.) Druce] is an annual herb that is self-fertilized forming dichasial inflorescence. It's widely spread in Europe, but rather rare in Serbia and Montenegro where its natural habitat is restricted to a few regions in Vojvodina (the northern part of Serbia). Therefore, *in vitro* growth, flowering, fruiting and production of viable seeds of lesser centaury are significant for a biotechnological approach to propagation and preservation of this species in Serbia and Montenegro. It is known that most species from the genus *Centaurium* are medicinal plants. It has been reported that *C. pulchellum* is a rich source of secoiridoides and xanthones (Janković *et al*. 2002, Krstić *et al*. 2003). Our laboratory results showed that lesser centaury cultured *in vitro* retained the capability of producing medicinally important secondary metabolites, sometimes in greater amounts than plants growing in the wild (Krstić *et al*. 2003). Intact plants of lesser centaury were capable of *in vitro* flowering and self-fertilization if grown on hormone-free culture media, which resulted in the production of viable seeds. Another advantage of this system is that time-consuming/laborious steps needed for the transfer of regenerated plants to the soil, acclimatization to ambient atmosphere, rising to maturity and seed production are avoided. The *in vitro* flower induction and production of viable seeds from regenerated spinach shoots was reported (Al-Khayri *et al*. 1991). Fruit production under *in vitro* conditions was obtained from cultured shoot tips of *Capsicum fruitescens* L. (Tisserat and Galletta 1995).

There are data that sucrose is not only a source of carbon and energy for plant growth and development, but it also has a signalling function and modulates expression of genes that encode enzymes, transporters and storage proteins (Lunn and MacRae 2003). In addition, sucrose controls growth (Ket *et al*. 2004), flowering (Bernier

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Abbreviations: F - flowers; FB - flower buds; GA_3 - gibberellic acid.
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et al. 1991, Perilleux and Bernier 1997, Roldan *et al*. 1999, Ohto *et al*. 2001), fruiting (Micallef *et al*. 1995, D'Aoust *et al.* 1999), seed development (Miller and Chourey 1992, Weber *et al*. 1997, Focks and Benning 1998, Weber *et al*. 1998, Cheng and Chourey 1999) and seed germination (Perata *et al*. 1997, Gazzarrini and McCourt 2001, Loreti *et al*. 2001, Shiau *et al*. 2005).

In this paper the results of sucrose effects on *in vitro* fruiting, seed production and germination requirements of lesser centaury's seeds are presented.

Seeds of *Centaurium pulchellum* (Sw.) Druce were collected in the area of Novi Sad (Vojvodina). They were surface sterilized for 5 min with 30 % solution of commercial bleach and then rinsed three times with sterile distilled water. Sterilized seeds were germinated in distilled water, under white light conditions and at 25 ± 2 °C. A 14-d-old seedlings were planted on agar-medium containing MS salts and vitamins (Murashige and Skoog 1962), 3 % sucrose and 0.7 % agar. Prior to autoclaving (25 min at 114 °C) media pH was adjusted to 5.8. All cultures were grown in a growth room under 16-h photoperiod, at 25 ± 2 °C and a 60 - 70 % relative humidity. White fluorescent tubes (Tesla, Pančevo, Serbia and Montenegro) provided 32.5 µmol m^{-2} s^{-1} irradiation.

For studying sucrose effect on *in vitro* fruiting and seed production, single flowers and/or flower buds, with one node bearing two quiescent auxiliary buds in the bracts axils were used as explants. Flowers and flower buds of *in vitro* raised 10-week-old plants were cut off and transferred on MS-media supplemented with different sucrose concentrations (0.003 to 0.3 M). They were grown in a growth room under the same conditions as intact plants. For each sucrose concentration, in all experiments, 30 explants of flowers and flower buds were used. All experiments were repeated for 3 times.

Development of auxiliary buds was monitored for nine weeks. During experimental period, capsules that developed and matured were collected every third week and dried in growth room at 25 ± 2 °C. Capsules length and width were measured under dissecting microscope (*Leica, WILD, MPS 28/32, M3Z*, Wetzlar, Germany), while seed width and length were measured under *Leica, LETZ DMRB* microscope. Image analysis was performed using *QWIN* software.

In seed germination experiments, spherical and filled seeds, matured *in vitro* on media containing different sucrose concentrations (0.003 to 0.3 M), were used. The mature seeds, collected from greenhouse grown plants, were used as control group. Replicates of ≈ 50 seeds, from explants grown at the same sucrose concentration, were placed in a 6 cm glass Petri dishes (without filter paper) containing either 2 cm^3 of distilled water, or 2 cm^3 aqueous solution of 0.1 M gibberellic acid (GA_3). Seeds were germinated at 25 ºC, in darkness or 16-h photo-period (white fluorescent tubes, irradiance of 32.5 µmol $m^{-2}s^{-1}$). Germination was scored on day 14 after the onset of imbibition. Emergence of radicle was taken as the criterion for germination. Each experiment was repeated twice, employing 4 replicates. Results are presented as percentage of seed germination.

Data statistical analyses were done by *STATGRAPHICS* software, version *4.2* (*STSC, Inc*. and *Statistical Graphics Corporation*, Orem, Utah, USA). Means were compared using LSD's multiple range test at $P < 0.05$.

In culture, both flower and flower bud explants always developed floral, not vegetative buds. In addition, auxiliary buds production was correlated with an increase of sucrose concentration in the culture media. However,

Table 1. The effect of different sucrose concentrations on *in vitro* auxiliary bud production, fruiting and seed production on flower (F) and flower buds (FB) explants. Data for greenhouse grown plants are also shown. Means ± SE, $n = 90$.

Sucrose [M]	Explants	Number of axillary buds [explant^{-1}]	Length of capsules [mm]	Width of capsules [mm]	Capsules with viable seeds [%]	Number of viable seeds [capsule^{-1}]	Length of seeds [µm]	Width of seeds [µm]	Number of germ. seeds [explant^{-1}]
0.003	F	0.86±0.05	5.23±0.16	0.51±0.07	18.75±0.95	4.03±0.12	379.00±4.83	335.60± 4.40	0.83±0.06
	FB	0.00±0.00	4.05±0.18	0.51±0.03	22.58±1.05	0.44±0.05	356.91±7.81	309.56±11.80	0.32±0.05
0.01	F	1.00±0.05	5.04±0.16	0.63±0.04	33.33±1.26	5.06±0.25	389.17±8.12	349.45± 7.27	13.33±0.55
	FB	0.00±0.00	4.09±0.18	0.62±0.04	29.11±1.26	1.94±0.06	370.86±5.47	321.40± 6.51	1.75±0.12
0.03	F	1.84±0.14	5.37±0.14	0.71±0.04	45.45±0.87	10.05±0.17	404.97±6.84	343.03± 7.42	31.10±0.80
	FB	2.50±0.01	4.37±0.10	0.79±0.03	37.50±0.95	5.26±0.10	394.69±6.01	327.87± 4.64	34.70±1.00
0.10	F	4.70±0.70	5.51±0.11	0.88±0.03	46.23±1.56	19.44±0.10	382.33±5.42	334.69± 7.55	59.75±1.25
	FB	5.05±1.19	5.47±0.14	0.82±0.04	32.64±1.44	15.81±0.15	396.97±6.92	331.86± 6.29	48.05±1.20
0.30	F	4.26±0.54	5.43±0.14	0.76±0.03	13.13±0.75	8.93±0.18	375.70±7.48	291.54± 5.78	21.23±0.85
	FB	4.30±0.93	5.55±0.12	0.81±0.03	21.97±0.93	7.93±0.16	368.86±9.69	305.84± 6.45	14.15±0.80
Ex vitro			5.98±0.15	0.77±0.04	78.00±1.25	28.00±0.50	314.10±7.35	254.70± 7.25	

the potential for auxiliary buds production was dependent not only on sucrose concentration in the medium, but also on the type of explants. High sugar concentrations (0.1 and 0.3 M) caused in both type of explants development of a large number of auxiliary buds. However, at lower sucrose concentrations (ranging from 0.003 M to 0.01 M), younger explants (floral buds) failed to develop auxiliary buds (Table 1). Since both type of explants had very low photosynthetic ability, a probable cause for the reduced number of auxiliary buds was shortage of carbon source. Besides, the vigorous growth and higher metabolic turnover of younger explants (floral buds) may be due to their greater sensitivity to lack of sucrose. Fruit and seed development, were also affected by sucrose thus, the higher the sucrose concentration the larger capsules length and width. The effect was similar for both explants tested and maximum width and length of capsules was observed on 0.1 M concentration. In addition, capsules length and width of plantlets grown on 0.1 M sucrose concentration were comparable to those collected from plants grown in the greenhouse (Table 1).

Table 2. Germination of seeds which were formed *in vitro* on flower (F), or flower bud (FB) explants that were grown on different sucrose concentrations. Data for greenhouse grown plants are also shown. The seeds germinated in darkness, or under white fluorescent light, with or without 0.1 M GA_3. Means ± SE, n = 400. No seeds germinated in darkness without GA_3.

Sucrose [M]	Explants	H_2O light [%]	GA_3 light [%]	GA_3 dark [%]
0.003	F	15.38±5.00	8.51±4.10	23.08±6.70
	FB	28.57±7.00	16.67±7.90	50.00±7.10
0.01	F	37.50±4.50	48.50±7.50	47.50±4.50
	FB	43.05±5.00	57.35±2.40	65.32±5.20
0.03	F	52.75±5.20	73.03±4.70	63.33±5.10
	FB	65.56±5.00	94.44±2.40	80.00±5.20
0.1	F	78.18±3.90	92.11±3.10	79.10±3.50
	FB	94.62±2.30	92.13±2.90	78.65±4.30
0.3	F	83.50±3.70	90.29±2.90	74.74±4.50
	FB	97.83±1.50	93.62±3.60	84.00±5.20
Ex vitro		98.00±5.30	95.00±5.00	98.56±6.00

Seed production was assessed on the basis of the percentage of capsules with viable seeds and average number of viable seeds per capsule. By raising sugar concentration in culture medium both the percentage of capsules with viable seeds and the number viable seeds per capsule were increased. Maximum stimulation was obtained on sucrose concentration ranging from 0.01 M to 0.1 M. However, the higher sugar concentration (0.3 M) had opposite effect (Table 1), *i.e.* there was negative correlation between seed dimensions and sucrose concentration in the culture medium. In the case of flower explants, the largest seeds were produced at sucrose concentrations ranging from 0.003 to 0.1 M, however, when the flower bud explants were used, the largest seeds were produced at slightly higher concentrations (0.01 to 0.1 M). The seeds collected from the greenhouse plants were smaller than those matured *in vitro*. However, the average number of viable seeds per capsule, as well as the percent of capsules with viable seeds matured on greenhouse grown plants, was higher than that obtained for *in vitro* raised seeds (Table 1). *In vitro* produced seeds of lesser centaury exhibited similar germination characteristics as those collected from natural habitats (Table 2). The former are positively photoblastic and require light for germination. Light requirement may be replaced by the application of 0.1 M GA_3. The germination of *in vitro* produced seeds was affected by the previous culture history. Namely, a higher percentage of germination was always obtained in seeds that were raised from explants originally grown on medium containing high sucrose concentrations (0.03 to 0.3 M). Even high concentrations of exogenously applied GA_3 (0.1 M) could not stimulate germination of seeds, which were originally raised on low sucrose concentration (0.003 M). No significant differences were found in the seed germination characteristics whether derived from floral and/or floral bud explants (Table 2). As can be seen, the highest number of germinating seeds was found in explants (regardless of their origin) grown on medium containing 0.1 M sucrose. Statistical analysis has revealed a high correlation between seeds dimension and their ability to germinate (correlation coefficients ranged from 0.72 to 0.98).

Based on these results it may be concluded that sucrose, being a source of carbon in the medium, is necessary for all developmental stages of *in vitro* grown lesser centaury. Besides, *in vitro* fruiting and seed production of lesser centaury allowed us to establish the optimal system for studying some developmental processes, particularly the role of certain signaling molecules, in greater details than *in vivo*. Moreover, this model system offers the unique opportunity to deal with genetically modified organisms without leaving laboratory.

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