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SPECIAL FEATURES OF REPRODUCTIVE BIOLOGY IN SOME SPECIES FROM THE GENUS *CAMPANULA* L.

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Introduction

At the present time one of the most important problems is the one of biodiversity preservation. For further development of rare plants preservation methods knowledges of their reproduction processes are needed. Campanulaceae family is numerous and diverse. According to the data of V.N. Golubev 22 species Campanulaceaeies from Campanulaceae family grow in the Crimea including 16 species from the genus *Campanula* L. Thus, A.V. Ena [9] noticed that in the Crimea Campanulaceae family is represented by 17 species including 12 species from the genus *Campanula* L. this genus is problematic enough in its systematics and some species` position is under discussion [7, 8]. As for species we have investigated - *Campanula sibirica* L., *C. taurica* Juz. и *C. talievii* Juz., there is no consensus in the literature sources. Some authers consider them as three separate species [4, 7, 8] others [2, 9] point that *C. taurica* and *C. talievii* are subspecies for *C. sibirica*. Characteristics of reproductive sphere structural elements of these taxons could be useful for defining and claryfying of their systematic position. Besides, representatives of Campanulaceae family are highly decorative and perspective for using in decorative gardening.

Resulting all reported above the aim of our studies was determination of the special features of reproduction, features of similarity and differences in the reproductive system of three *Campanula* L. species (*Campanula sibirica* L., *C. taurica* Juz. и *C. talievii* Juz.) that grow in the Mountain Crimea.

Objects and methods of he research

Species *C. sibirica*, *C. taurica* and *C. talievii* were investigated in there native growth conditions in the Mountain Crimea on three plots: 1) on the north-eastern slope of the Chatyr-Dag mountain, 2) along the road to Baidarskie Vorota (from Yalta – Sevastopol highway) and 3) on the north-eastern slope of the Chelebi mountain. Flowering rythms and terms were studied with the methods by A.N. Ponomarev [18], V.N. Golubev and Yu.S. Volokitin [5, 6]. Seed productivity was performed by E.A. Hodachek [21]. I.V. Vainagii [1] and Yu. A. Zlobin [11]. Temperature indexes were determined using a laboratory alcohol thermometer. For plant material fixation Karnua solution (ethanol 96% - 6 parts: hlorophorm – 3 parts: acetic acid – 1 part) was used. Customary methods of preparation samples for cytoembriological studies were followed. Staining was by methyl-green and pyronin with alcian blue [24, 25].

Evaluation of ornamental efficiency was made according to the method of State Plant Varieties Testing for ornamental plants [14].

Photoes were taken with the camera Canon A 3100 IS. Seed germination was tested in laboratory conditions on distilled water after a year of storage in paper bags under the room air temperature [17].

Results and discussion

In Campanulaceae family various life-forms are presented including herbaceous plants, climbing plants, treelike forms and treelike climbings. Our studied species are mostly herbs. *C. sibirica* is biennial plant up to 70 sm height. The stem is single and erect. Flowers

are numerous (fig. 1A). *C. taurica* is a perennial plant up to 50 sm height with many stems, the central one is erect (fig. 1 B). *C. talievii* according to N.G. Dremluga and S.N. Ziman [8] and our observations is perennial semishrub up to 25 sm height, sometimes up to 50 sm (fig. 1 C). So as in *C. taurica* its stems are numerous with erect central one, persist after winter. Flowers are numerous. An.A. Fedorov [20] and V.N. Golubev [4] considered that *C. talievii* is endemic Crimean species.

Flowering of studied *Campanula* species is long enough in the Crimea – since May to August. Its initiation in *C. taurica* and *C. talievii* starts 1.5-2 weeks earlier than in *C. sibirica*. Single flower lives for 7-10 days in all three species. Then it gradually wilts, cup and corolla don`t fall down but dry. Flowering of a single plant can last from May to August so in August flower buds, flowers and fruits could be noticed on the same plant. The stage of loose flower bud comes on the 4^{th} day and the flower opens on the 7^{th} day. In the period of flowering 2013, in June, temperature indexes on the Chatyr-Dag Mountain were: on the soil surface +23 ^{o}C, of the air - +21 ^{o}C, in July – +26 ^{o}C and +24 ^{o}C, in August – +28 ^{o}C and +25 ^{o}C, correspondantly. On the mountain Cheleby in 2013 the same temperature indexes in the flowering period were: in June– +22 ^{o}C and +20 ^{o}C, in July – +26 ^{o}C and +25 ^{o}C, in August – +29 ^{o}C and +27 ^{o}C, correspondantly. So in the period of flowering the air temperature varies from +20 ^{o}C to +30 ^{o}C.

A B C

Fig. 1 Plants of *C. sibirica* (A), *C. taurica* (B) and *C. talievii* (C)

Number of flowers per one generative shoot in *C. taurica* is up to 17, *C. sibirica* – up to 13 flowers, *C. talievii* – up to 30 and number of flowers per plant could be up to 45 in *C. taurica*, up to 35 in *C. sibirica* and up to 70 – in *C. talievii*.

Flower is actinimorphic, full and bisexual. Such flowers are characteristic for Campanulaceae family [3]. Flower stem is 8-10 mm. This feature is important for stuied species as in mature fruit it fuctions as an additional tool for dissemination [16]. Flower bed is convex. Sepals are curved out. Cup is toothed, wilted, nonfallen with curved appendages. Petals are toothed. Corolla is bell-shaped, tomentous (fig. 2).

Fig 2 *C. taurica* flower with tomentous corolla and pollinator

Bud becomes coloured when it`s 10-15 mm length. Its colour varies from light lilac to dark violet (see fig. 1). Flowers of *C. talievii* have the most intencive dark violet colour and in *C. taurica* and *C. sibirica* they are significantly lighter.

In all three studied species androecium is represented with 5 stamens that are direct and equal, attached to the base of nectar disc (fig. 3). Bases of filaments are broad; they fuse and form a dome with an aperture on its top.

Gynoecium is syncarpous with one pistil. Nectaries are inner in the form of a disk on the ovary. Style is central, strait, wilted, nonfallen, covered with lots of one cell hairs of epidermal origin (see fig. 3). In studied species stigma is apical, divided, threelobed, turned out, in open flower it`s partly beyond it. Ovary is trilocular, pubescent and low, with great number of ovules.

Anther has 4 microsporangia and 2 thecas. It also has placentoid – radial growth of connectivum tissue that just inside each microsporangium. Microsporangium wall development is centrifugal [13, 23]. Formed microsporangium wall consists of epidermis, endothecium, one middle layer and secretory tapetum. Microsporangium wall in mature anther consists of flattened epidermal cells and endothecium with fibrous bands.

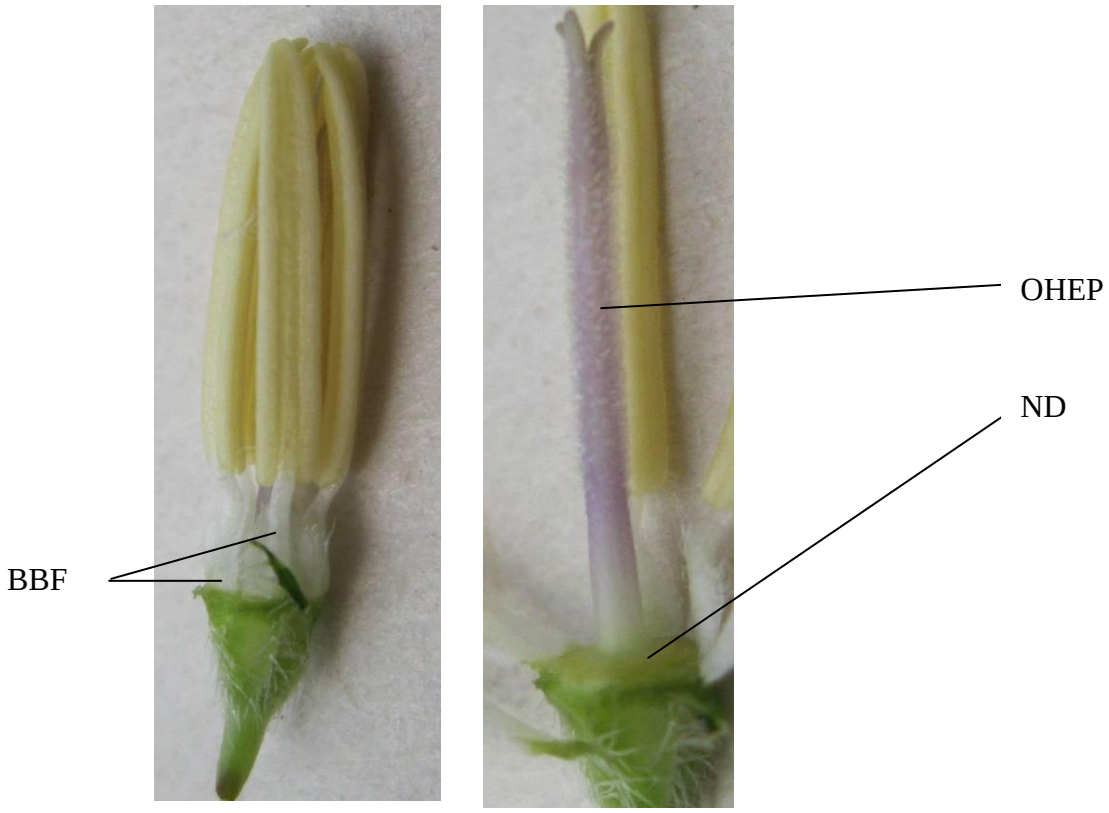

Fig. 3 Parts of C. taurica flower (BBF – broad bases of filaments; ND – nectar disc; OHEP – one cell hairs of epidermal origin on the pistil)

Endothecium could consist of two rows and fibrous bands present both on the cell walls of the outer side of microsporangium and on the cell walls at the side of connectivum. As the result each microsporangium is covered with fibrous layer. Mature pollen grains have three cells, 3 furrows and 3 pores. Together with normal pollen grains defective ones could be noticed in microsporangiums (fig. 4).

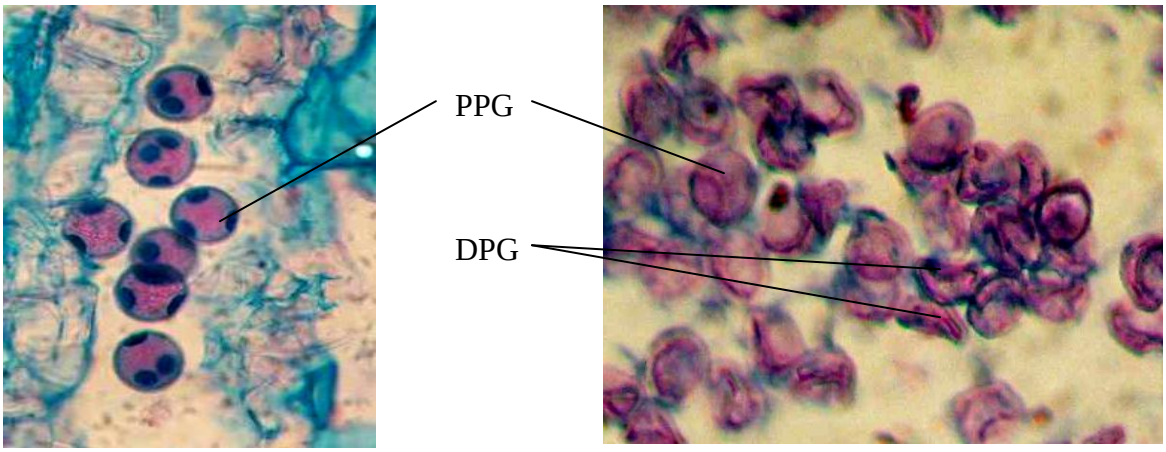

Fig. 4 C. taurica pollen grains (PPG – proper pollen grains, DPG – defective pollen grains

Ovule is anatropous, medianucellate, unitegmal. Integument is of epidermal origin, consists of 6-8 cell rows. Micropyle is simple, narrow, strait. Funiculus is short, funicular obturator presents. Raphe is formed. Integumental tapetum is strongly developed; it reaches the egg apparatus and is presented with flattened round cells with nuclei and nucleoli.

Archesporial cell differentiates in subepidermal layer and after the first division it forms parietal and sporogenous cells, later transforms into megasporocyte. Meiosis results in megaspores tetrad formation and halasal one developes into the embryo sac (fig. 5).

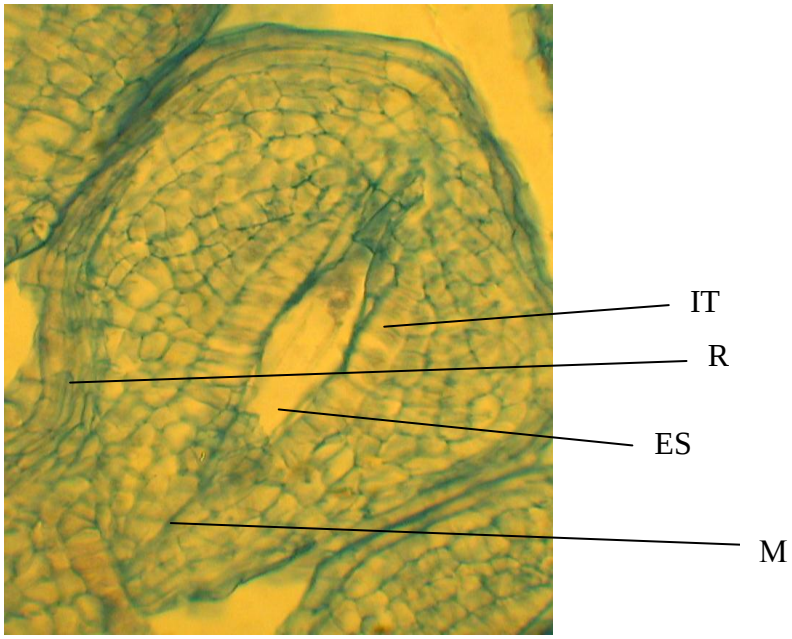

Fig 5 C. taurica ovule (R – raphe, IT – integumental tapetum, M – micropyle, ES – embryo sac)

Embryo sac is 7-celled, monosporic, of Polygonum type. Egg complex is presented by two sinergids with hookshaped outgrowths and paer-shaped egg. Polar nuclei fuse before fertilization. Antipods are placed in T-shape and stay long enough. Between the embryo sac and hypostaze postament-podium forms and its cells have thicker walls than the cells nearby [23].

Pollination process in *Campanula* is very specific. In closed flower bud staments fully cover thestyle – anthers densly surround the style and broad bases of filaments form, as it has been noted above, a kind of dome (see fig 3 A). Anthers open introrse in the closed flower bud and at that time stigma lobes are cerried.

As pistil growth hairs that cover the style hooks the pollen grains and take them out of the anthers and as the result the whole style is covered with pollen grains. In the upper part of the dome there is a whole through which a pollinator reaches a nectar disk with its haustellum. Nectar disk becomes coloured when a flower size is 14-16 mm and it could be from white to bright-lemon.

As an insect moves towards the nectar disk it takes the pollen grains from the style with its legs, abdomen and, sometimes, even wings. When it comes back it touches the style again and takes additional pollen grains (fig. 6)

Fig. 6 Pollinators on *C. Sibirica* flowers

After pollination the staments wilt and curve before the stigma opens so in an open flower we noticed only parts of anthers. When a flower is fully open stigma lobes disclose. For studied species allogamy (geitonogamy and kleistogamy) is typical, sometimes autogamy occurs as it also has been reported by S. Vogel [27] for *C. rotundifolia* L. during the pollination process hairs are pulled in the covers of style so phenomenon of invagination or retraction is observed.

It should be noticed that a typical feature of studied species is protandry, when development of male reproductive structures is much earlier than of female ones and anthers open in closed bud. After pollen grains landed on the stigma they germinate, pollen tube reaches the embryo sac, comes through one of the sinergids, burst and its content pour out. Fertilization takes place. After double fertilization process embryo and endosperm develop. According to Campanulaceae family characteristics [10, 13] its species are characterized with cellular endosperm. Our observations demonstrate that according to O.P. Kamelina classification it`s tubifloral and characterized with transverse division of the first endosperm nuclei and formation of micropillar and halasal haustorium. Exactly endosperm forms from the cells that lie between hausturias. I.I. Shamrov [22] supposed that this type of endosperm formation could be determined as micropilar-halasal with terminal haustorias as a subtype of cellular type.

Almost all flowers in studied species form fruits but they have different number of seeds [15]. Processes of seed maturing and dissemination are gradual and last from Augest to September. Seeds` scattering is also gradual due to ballochory that is one of the most efficient ways for spreading seeds in a short distance. Particularly ballistoanemohorry is typical for these species, when dry stems and capsules move under the wind blows and seeds fall out from the capsules. The species also characterized with ballistozoohorry when parts of the plant move due to the touches of animals. One more type of seed spreading typical for these species is epizoohorry when dry capsules attach to the animals with their hooks and are spreaded for long distances. Besides, after falling from the capsule small and light seeds are carried for a long distance by the wind blowings, that are typical for mountain Crimea, hence anemohorry occurses. All this ways of seed spreading are favourable for species diffusion and colonization of the new territories.

Seeds of all species are small, about 1 mm length, light-brown. Seed coat consists of two layers; endosperm is represented with large cells. Embryo takes nearly 1/3 part of the seed volume and it doesn`t full the entire embryo cavity which is well noticeable. Viability of seed increase correlative to the terms of their storage, just ripened seeds don`t germinate almost, because of undeveloped embryo. Germination of seeds collected in 2011 after year storage under the room temperature was: in *C. sibirica* more than 50%, in *C. taurica* - 65%,

in *C. talievii* – 35%. Germination of seeds collected in 2012 and germinated in December 2013 was: in *C. sibirica* nearly 90%, in *C. taurica* – более 85%, in *C. talievii* – 65%.

Fruit in studied species is wilted, threecelled, hair-covered capsule with numerous seeds. It is formed by dry flower cup and curved appendages (fig. 7)

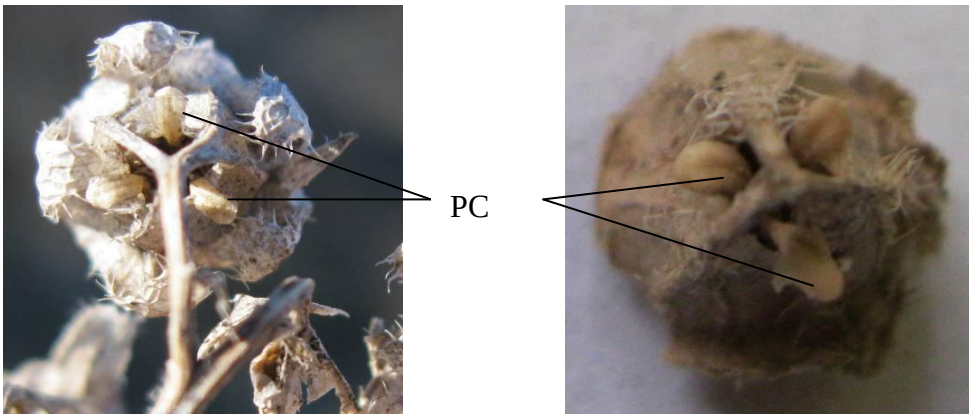

Fig. 7 Dry capsules of *C. talievii* (PC – pore cover)

At the base of the capsule there are three pores formed by axicorns – structure of crescent shape on the centre-axial column of the fruit. Along with the fruit dry axicorn tears a cover between the ridges at the base of the capsule with one of its tips and so pore and its cover are formed (fig. 8).

Fig. 8 C. taurica fruit part with the axicorn (A – axicorn)

Such wilted capsules with pores in their base were noticed in *C. rotundifolia* L. but contrary to *C. carpatica* L. with erect capsule and pores on its top [26, 27]. One more helpful tool for seed spreading is tough hairs that cover the cup and after they dry became hooks and let seeds to attach to animals.

Processes of seed formation and fruit ripening in studied species is long, dissemination starts in the middle of August and lasts to late September. Seeds through pores fall down under the lightest shoots` movement.

Our observations and literature data demonstrates that in *C. talievii* stems wilt but they don`t fall. In the next year, in spring these wilted plants produce new rosettes of leaf so besides seed propagation in *C. taurica* and *C. talievii* vegetative one is possible while in *C. sibirica* we haven`t observe such trait (fig. 9 and 10).

Fig. 9 Parts of the root system in *C. sibirica* (A), *C. taurica* (B) and *C. talievii* (C) (VS – vegetative shoots, NR – new rosette)

Number of new rosettes in studied populations varies from 2 to 4. We observed *C. taurica* plants both with 2 and 4 vegetative shoots. In *C. talievii* population only plants with 2 vegetative shoots were noticed. Studied populations are not full, left-handed with great part of virgin and generative plants while senile plants haven`t been noticed yet. Such structure of populations demonstrates potential possibilities of selfreproduction and propagation in these species.

Fig. 10 *C. talievii* at the stage of wilted plant (A) in November 2013 and with new generative shoot (B) in May 2014

Studied *Campanula* species, as many other representatives of Campanulaceae family, have decorative appearance. Most of signs according to 5-point scale by the method of State Plant Varieties Testing for ornamental plants [14] have marks 4 and flowering duration was evaluated as 5 points (tabl.).

Table

Decorative features evaluation (points)

Name of the sign	Points		
	C. sibirica	C. taurica	C. talievii
Colour of flower	4	4	5
Odour	4	4	4
Stem (length, firmness)	4	4	4
Inflorescence	4	4	4
Flowering abundance	4	4	5
Flowering duration	5	5	5
Flowers` resistance to unfavourable weather conditions	4	4	4
Decorative of plant vegetative part	4	4	4

Due to their ornamental features, long term flowering and number of formed seeds studied species could be used in park expositions.

Conclusions

1. The main embryological features of the studied species could be considered: centrifugal formation of microsporangium wall, presence of placentoid, pollen grains with three cells, 3 furrows and 3 pores, anatropous ovule, integumental tapetum development, 7-celled, monosporic, Polygonum type embryo sac, tubifloral endosperm micropillar and halasal haustorium, Solanad-type of embryo development, protandry.

2. *C. sibirica*, *C. taurica* and *C. talievii* flowering in native growth conditions lasts from May to August under the average day temperature from +20^{o}C to +30^{o}C. C. sibirica flowering starts 1.5-2 weeks later. Great number of flowers is formed both on the single generative shoot and on the whole plant.

3. Allogamy is typical for studied species but autogamy, when pollen grains are taken out of the anthers by one cell hairs of epidermal origin placed on the style, is possible. Phenomenon of invagination or retraction has been noticed.

4. Fruit in studied species is wilted, threecelled, tough hair-covered capsule with three pores at it base and numerous seeds inside. For their ripenning seeds need the period of biological rest.

5. The main type of propagation is seed propagation but in C. taurica and C. talievii vegetative propagation is also possible.

6. Long flowering period of *C. sibirica*, *C. taurica* and *C. talievii*, great number of fruits and seeds formation, data about seed productivity and germination, different tools for successful dissemination demonstrates potential possibilities of reproduction, propagation and spreading of these species.

7. Due to their ornamental features, long term flowering and number of formed seeds studied species could be recommended for using in decorative gardening.

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The article reveals the research results in formation of male and female generative spheres, flowering, pollination, dissemination, seed formation of three species from the genus Campanula L. (C. sibirica L., C. taurica Juz. and C. talievii Juz.). Also the age structure of their populations, seed and vegetative multiplication as the most important factors of species self-reproduction have been described.

Key words: *Campanula sibirica L., C. taurica Juz. and C. talievii Juz., flowering, pollination, dissemination, seed and vegetative propagation.*