On the embryology of *Brassica jordanoffii* (*Brassicaceae*) – an endemic species in the Bulgarian flora*

Elina Yankova-Tsvetkova, Petya Yurukova-Grancharova & Vladimir Vladimirov

Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113 Sofia, Bulgaria; e-mail: e_jankova@abv.bg (corresponding author)

Received: December 03, 2015 ▹ Accepted: June 07, 2016

Abstract. An embryological study of *Brassica jordanoffii* was carried out. The anther is tetrasporangiate and its wall of Monocotyledonous-type development consists of: epidermis, endothecium, one middle layer and glandular tapetum. Predominantly tetrahedral microspore tetrads form after simultaneous microsporogenesis. The mature pollen grains are three-celled. The mature ovule is ana-amphytropous, crassinucellate, bitegmic with unicellular archesporium that functions as a megaspore mother cell after cutting off one or two parietal cells. Development of the embryo sac is of the *Polygonum* type. The embryo and endosperm develop after Onagrad-type embryogenesis. A relatively high pollen and embryo viability was estimated.

Key words: embryo and endosperm formation, glacial relict, male and female gametophyte

Introduction

Brassica jordanoffii O.E. Schulz *(Brassicaceae)* is a Bulgarian endemic species – a glacial relict with status of high conservation concern represented by a single population (with a few small sub-populations) in the Pirin Mts. The species is a herbaceous perennial, insect pollinated, reproducing by seeds. *Brassica jordanoffii* is under the protection of the National Biodiversity Act, Biodiversity Conservation Law and European ecological network Natura 2000 in Bulgaria, and is rated as Vulnerable in the Red List of Bulgarian Vascular Plants (Anchev & Goranova 2009) and Red Data Book of the Republic of Bulgaria, vol. 1. Plants and Fungi (Anchev & Goranova 2015).

Production and level of dissemination of plant seeds are of principal significance for the survival of individuals and conservation of the population size of plant species, especially for plants with limited distribution or endangered as the above-mentioned species. For the sake of conservation, it is very important to investigate the reproductive processes of *B. jordanoffii,* because one of the major causes for the species rarity and small size of its populations might be its inefficient reproductive capacity.

The aim of the present study is to reveal the mode of reproduction and peculiarities of embryological structures and processes of *B. jordanoffii,* as well as to evaluate the pollen and seed viability, so as to clarify the possibilities for fulfillment of its reproductive capacity. The results of this study will also provide valuable information regarding the *ex situ* cultivation of this endemic species.

Material and methods

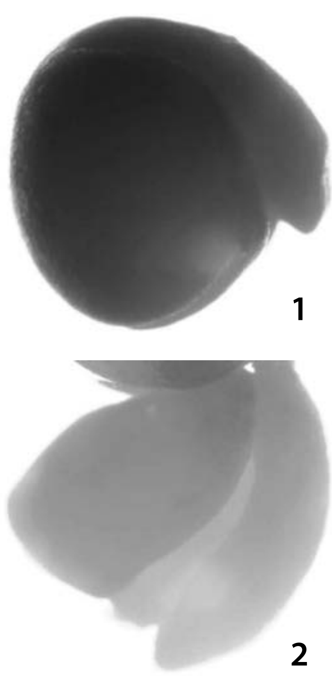
The material for embryological study (flower buds and flowers at different developmental stages) was collected from the single natural Bulgarian population of *B. jordanoffii* in the Pirin Mts (Northern) and fixed in

* The report was presented at the International scientific conference „Plant diversity towards society", Sofia, Bulgaria, 2015.

FAA mixture (formalin: glacial acetic acid: 70% ethanol in correlation 5:5:90 parts), embedded in paraffin, cut into 8–12 μm sections with a rotary microtome and then treated according to the classical paraffin methods (Sundara 2000). The sections were stained with Heidenhain's haematoxylin and included in Enthelan.

To estimate pollen viability, a common method was applied of staining with acetocarmine the mature pollen grains and subsequently counting directly (Heslop-Harrison 1992) the viable and nonviable. For that purpose, anthers from open flowers were suspended in a solution containing 1% acetocarmine for staining (Singh 2003). Then, the solution with pollen grains was dispersed on the slide and the number of stained (viable) and unstained (nonviable) pollen was counted. Mature pollen grains were counted up in 30 anthers (visual field – at an augmentation 100×), using a light microscope.

For estimation of the seed (embryo) viability, the tetrazolium test was used. The mature seeds were incubated in 1% solution of 2, 3, 5-tripheniltetrazolium chloride, according to the methods elaborated by AOSA (Association of Official Seed Analysts – Peters 2000). Initially, the tetrazolium solution is colorless, but later on it changes to red as a result of the action of hydrogenic ions derived from the respiration process of seeds. Embryos that show physiological activity (active respiration) turn red and are considered viable (Figs 1-2). The darker the red color, the greater the respiratory activity of the seeds.



Figs 1-2. Estimation of seed viability of *Brassica jordanoffii*: **1.** Dark-red colored viable embryo; **2.** Light-pink colored viable embryo.

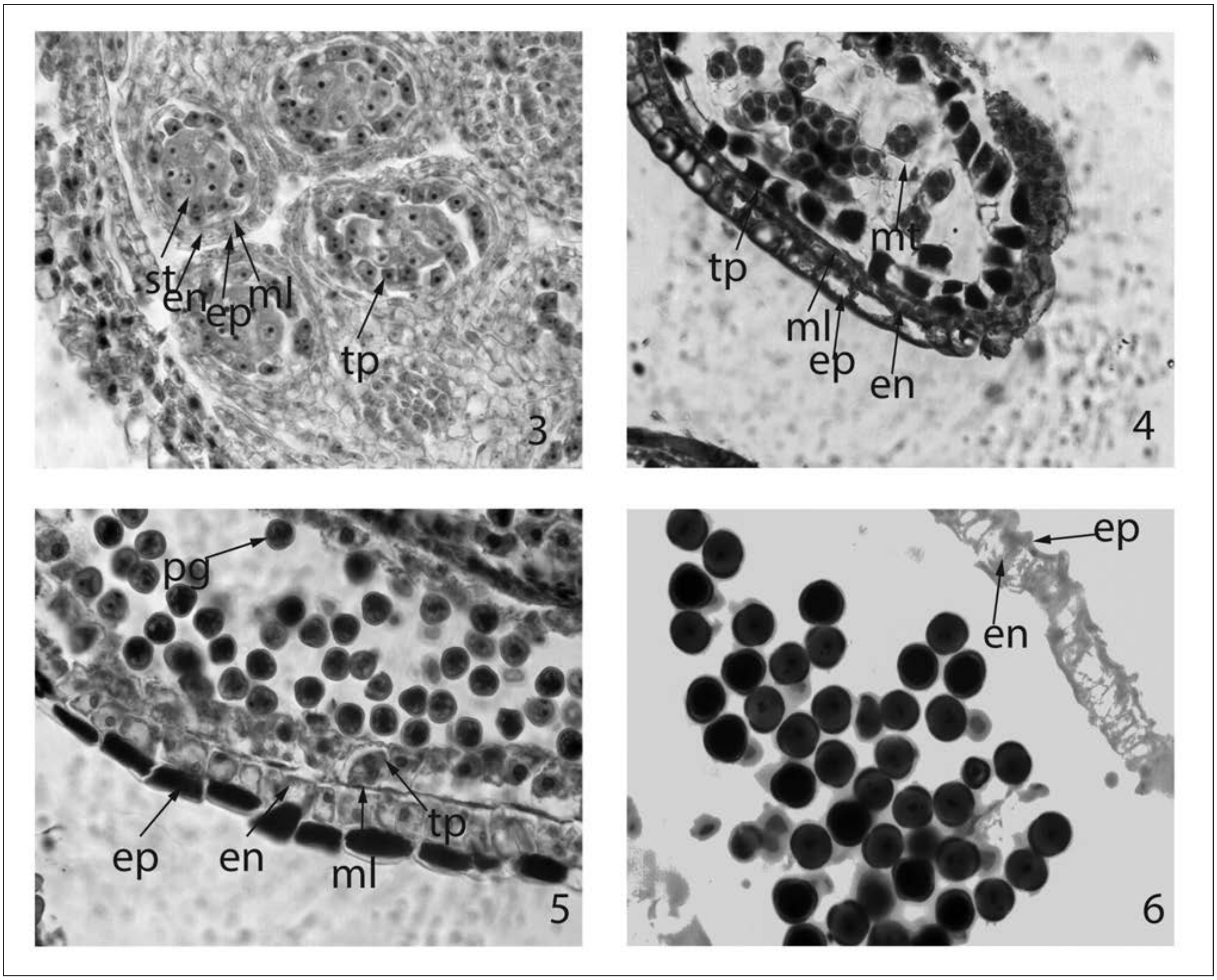
Results and discussion

Anther and development of the male gametophyte. The anthers are tetrasporangiate. The anther wall development is of the Monocotyledonous type, according to Davis classification (Davis 1966; Watson & Dallwitz 1992). It consists of four layers: epidermis, endothecium, one middle layer and tapetum that at the beginning of anther ontogenesis are almost similar in shape but later on begin to distinguish – especially the tapetum, even at the stage of sporogenous tissue (Fig. 3), while the other three layers do it after the formation of microspore tetrads (Fig. 4). The epidermis comprises one row of almost rectangular one-nucleate cells that vastly enlarge during the anther ontogenesis. The middle layer is ephemeral and completely degenerates by the end of meiosis in the microspore mother cells (MMCs). At the beginning of anther ontogenesis, the endothecium cells are quite similar in size and shape to the epidermal ones, but subsequently they lengthen radially (Fig. 5). The one-layered tapetum is glandular during the entire anther ontogenesis. Initially, its consisting cells are one-nucleate, but even at the stage of MMCs they become two-nucleate (Fig. 5) as result of mitotic division. At the stage of mature pollen, the anther wall consists of one-row endothecium and partially preserved epidermis (Fig. 6).

The sporogenous tissue is multilayered (Fig. 3) as in most representatives of the family *Brassicaceae* (Poddubnaya-Arnoldi 1982). Initially, the sporogenous cells are polygonal and fit closely each other. Lather on, they elongate, round up and differentiate into MMCs. The meiosis in MMCs passes with insignificant deviations. After simultaneous microsporogenesis, predominantly tetrahedral microspore tetrads form (Fig. 4). At the time of shedding, the mature pollen grains are three-celled and usually morphologically uniform (Fig. 6).

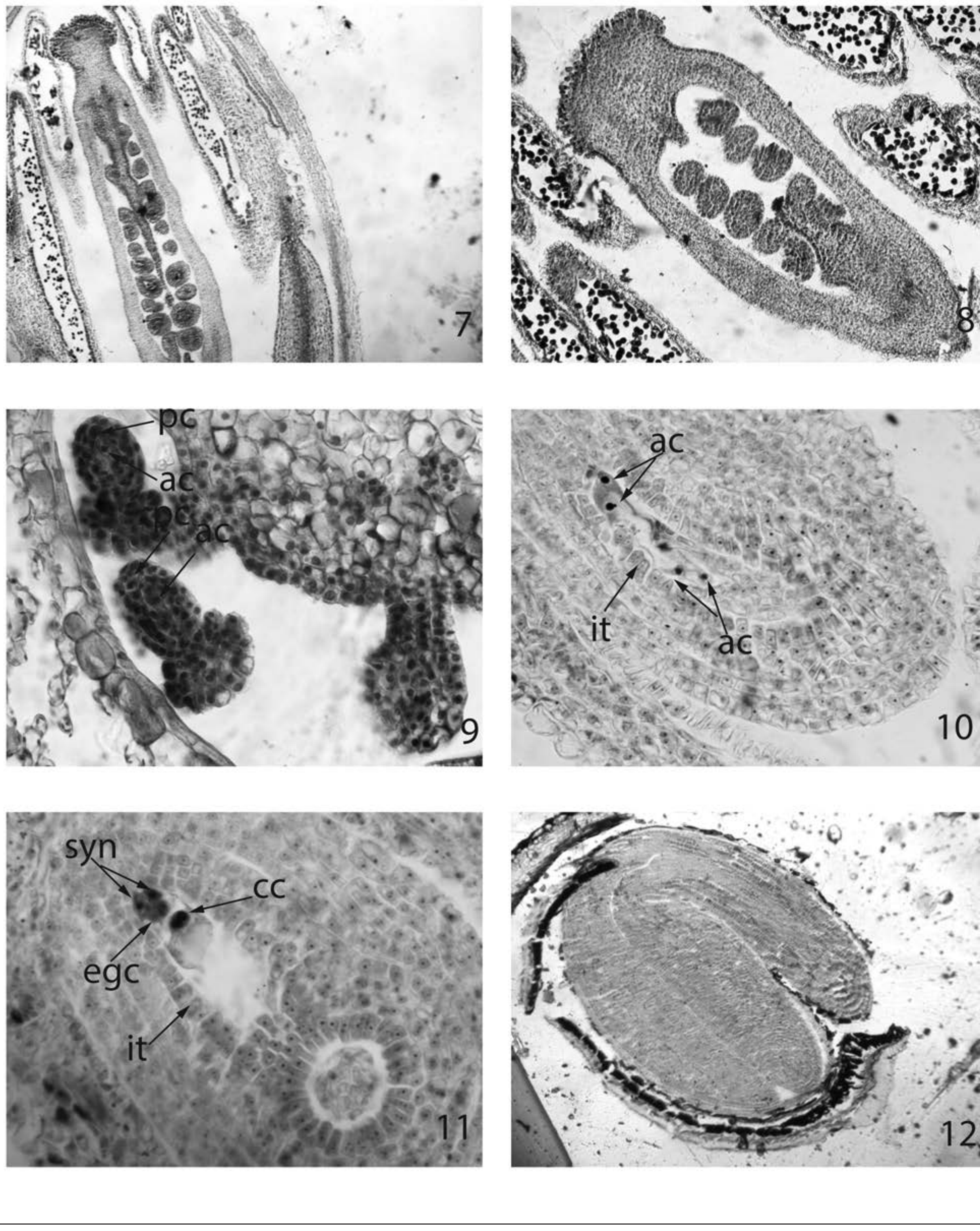
Ovule and development of the female gametophyte. The ovary is bilocular, with many ana-amphytropous, crassinucellate, bitegmic ovules with parietal disposition in each locule (Fig. 7). Like in most Angiosperms, the innermost layer of the single integument differentiates into integumentary tapetum (endothelium).

During the study, it was established that *B. jordanoffii* is a strongly proterandrous species: when in the anthers forms one-celled pollen, archesporium was observed in the ovule (Fig. 8).



Figs 3-6. Anther and development of the male gametophyte:
3. Sporogenous tissue and four-layered anther wall comprising epidermis, endothecium, one middle layer and tapetum; **4.** Tetrahedral microspore tetrads and anther wall; **5.** One-nucleate pollen and anther wall; **6.** Mature pollen grains and anther wall. Magnification 400×.

Within the still young ovule, unicellular archesporium forms hypodermally (Fig. 9). The formation of unicellular archesporium in the genus *Brassica* is reported for the first time. So far two- or three-celled archesporium was observed in this genus (Belyaeva & Rodionova 1983). The single archesporium cell functions as a megaspore mother cell (MMC) after cutting off one or two parietal cells (Fig. 9). For the genus *Brassica,* formation of one (Sulbcha 1957; Davis 1966) and two (Pearson 1933) parietal cells is reported. Later on, the MMC undergoes meiosis to produce a linear megaspore tetrad. The embryo sac (ES) development runs according to the *Polygonum* (monosporic) type from the chalazal megaspore of the tetrad that functions as an embryo sac mother cell (EMC), like in most representatives of family *Brassicaceae* (Davis 1966; Belyaeva & Rodionova 1983; Watson & Dallwitz 1992). The other three megaspores degenerate progressively during the advancement of ES development and their remains can be seen until the four-nucleate ES (Fig. 10). After three successive mitoses, two-, four- and eight-nucleate ES forms. The mature ES consists of a three-celled egg apparatus (usually a pear-shaped egg cell and two synergids), a central cell (Fig. 11) and a three-celled antipodal apparatus in the chalazal part of the ES. The synergids degenerate after fertilization. The antipodals are ephemeral, which is typical for the family *Brassicaceae* (Davis 1966; Poddubnaya-Arnoldi 1982; Belyaeva & Rodionova 1983) and begin to degenerate before fertilization. In the present study, synergids with a filiform apparatus reported for the *Brassica* representatives (Davis 1966) were not observed.



Figs 7-12. Ovule and development of the female gametophyte:
7, 8. Flower with bilocular ovary and anthers; **9.** One-celled archesporium in the ovule; **10.** Four-celled embryo sac (ES); **11.** Mature embryo sac with egg apparatus and central cell; **12.** Mature embryo.

The embryo and endosperm develop after porogamous double fertilization accompanied with destruction of one synergid from the pollen tube penetrating into it through the micropyle of the ovule. The first division of the zygote is transversal and usually runs before the beginning of endospermogenesis. Direction of the cell wall setting in young embryo, after a small number of following mitoses indicates that embryogenesis is of the Onagrad type – a typical embryogenesis type for the family *Brassicaceae* (Davis 1966; Poddubnaya-Arnoldi 1982; Belyaeva & Rodionova 1983). At the beginning of endospermogenesis, the endosperm is nucleate but later on, at the stage of globular embryo, it differentiates into cellular.

In the mature seed, the embryo is curved, filling the entire ES cavity (Fig. 12), while the endosperm is completely consumed. Apomixis was not registered.

Pollen and seed viability. After application of the acetocarmine stain technique for estimation of pollen viability, the cytoplasm and nuclei of viable pollen grains were stained in red, while the nonviable, empty and shrunken pollen remains colorless. The results of the study show a relatively high viability of the mature pollen in the studied population of *B. jordanoffii*: 71.97 %.

On the basis of results obtained after tetrazolium testing, the seed (embryo) viability (embryos) was 71.46 %, which is considered relatively high.

Conclusion

As result of the present study, the mode of reproduction and reproductive capacity of *Brassica jordanoffii* were established, in order to define the character and state of its populations. The observed features of the embryological structures and processes, and absence of apomixis characterize *B. jordanoffii* as a sexually reproducing species.

The low plasticity of the male and female generative sphere (balanced processes and stable structures and only sexual reproduction that restricts the adaptive mechanisms) determined during this study is very likely the fundamental cause that defines the restricted distribution of *B. jordanoffii*.

The established relatively high viability of the pollen and seeds of *B. jordanoffii* contributes to successful realization of its reproductive capacity, but the state of this species as a vulnerable Bulgarian endemic suggests that these two studied parameters of the reproductive capacity mainly depend on the conditions of its habitats.

Acknowlegments. The present study was supported by the EEA Grants/Norway Grants within the framework of the project "Conservation of biodiversity in hot-spots of glacial relict plants in Bulgaria" (BG 0034).

References

Anchev, M. & Goranova, V. 2009. – In: **Petrova, A. & Vladimirov, V.** (eds), Red List of Bulgarian Vascular Plants. – Phytol. Balcan., **15**(1): 82.

Anchev, M. & Goranova, V. 2015. *Brassica jordanoffii* O.E. Shulz. – In: **Peev, D**.(ed.), Red Data Book of the Repiblic of Bulgaria, Vol. **1**, Plants and Fungi. Bulgarian Academy of Sciences & Ministry of Environment and Water of Bulgaria. Digital Edition. http://www.e-ecodb.bas.bg/rdb/en

Belyaeva, L.E. & Rodionova, G.B. 1983: Family *Brassicaceae*. – In: **Batygyna, T.B., Yakovlev, M.S.** (eds), Comparative Embryology of Flowering Plants. *Phytolaccaceae – Thymelaeceae*, Vol. **3**, pp. 154-164, Leningrad, Nauka (in Russian).

Davis, G. 1966. Systematic Embryology of the Angiosperms. John Wiley & Sons, New York.

Heslop-Harrison, J.S. 1992. Pollen capture adhesion and hydration. – In: **Cresti, M. & Tizzi, A.** (eds), Sexual Plant Reproduction, pp. 81-88. Berlin, Springer.

Pearson, O.H. 1933. Study of the life history of *Brassica oleraceae*. – Bot. Gaz., **94**(3-4): 534-550.

Peters, J. (ed.) 2000. Tetrazolium Testing Handbook, Contribution № 29 to the Handbook on Seed Testing, revised. The Association of Official Seed Analysts (AOSA).

Poddubnaya-Arnoldi, V.A. 1982. Characteristics of Angiosperms Families according to Cytoembryological Features. Nauka, Moskva (in Russian).

Singh, R.J. 2003. Plant Cytogenetics. 2nd ed. CRC Press, Boca Raton.

Sulbcha, K. 1957. Embryology of *Brassica juncea* Gzern. & Coss. – J. Indian Bot. Soc., **36**(3): 292-301.

Sundara, R.S. 2000. Practical Manual of Plant Anatomy and Embryology. Anmol Publ. PVT LTD, New Delhi.

Watson, L. & Dallwitz, M.J. 1992. *Cruciferae* Juss. – In: The Families of Flowering Plants: Descriptions, Illustrations, Identification and Information Retrieval. Version: 20th May 2010. http://delta-intkey.com.