Post-seminal structure and development of the hemiparasitic plant *Escobedia grandiflora* (Orobanchaceae)

Edison Cardona Medina¹, Marisa Santos² and Rubens Onofre Nodari*¹

¹Laboratório de Fisiologia do Desenvolvimento e Genética Vegetal, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Rodovia Admar Gonzaga, 1546, 88034-000, Florianópolis, Santa Catarina, Brazil. ²Laboratório de Anatomia Vegetal, Departamento de Botânica, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil. *Author for correspondence. E-mail: rubens.nodari@ufsc.br

ABSTRACT. *Escobedia grandiflora* (L.f.) Kuntze is a wild hemiparasitic plant with orange roots. Little is known about the development of initial parasitism with the host, despite the significant value of roots for Central and South American communities. Therefore, this study aimed to characterize post-seminal structure and development of *E. grandiflora* in *Pennisetum purpureum* host. To analyze the structure and development of *E. grandiflora*, seedlings, stems and roots samples were processed and examined under light, confocal and scanning electron microscopy. *Escobedia grandiflora* seeds are composed of seed coat, perisperm, and embryo. Emergence of the radicle began eleven days after imbibition. Seedlings showed a root hair collar encircling the axis at the root-hypocotyl junction with elongation of internal cortical cells. Seedlings formed haustoria and successfully reached the host roots 22 days following root emergence. In the root many starch grains were observed, albeit more scarce in the hypocotyl. After 43 days of root emergence, the seedling stage was finished with the formation of the definitive leaves, and star of the plant stage. After 64 days, root ramification, amount of starch, and orange pigmentation increased with formation of haustoria. The developmental pattern of *E. grandiflora* plants was slow, but the roots grew faster than the stem. *Escobedia grandiflora* seeds were not endospermic and have limited nutritional value. After root emergence, the young seedling must develop roots and starch storage towards to haustorium formation and attachment to host roots.

Keywords: parasitism; seed; seedling survival; orange roots; haustorium.

Received on February 19, 2019. Accepted on July 17, 2019.

Introduction

Parasitic plants comprise an intriguing group characterized by the invasion of host tissues with an organ called the haustorium, which draws water and both inorganic and organic elements from the host (Heide-Jørgensen, 2008; Pielach, Leroux, Domozych, Knox, & Popper, 2014). A large body of knowledge supports the importance of parasitic plants of the Orobanchaceae, a family which includes the largest number of angiosperm root parasitic plant species (Bennett & Mathews, 2006). Some Orobanchaceae genera, such as *Striga* and *Alectra* have a negative impact on crops, like maize, cowpea, and sorghum (Kokla & Melnyk, 2018). Other species are important in natural communities, while a few species are used in medicine and food processing (Ren, Guan, Li, Hu, & Zhang, 2010; Muriel, Cardona, Arias, & Gómez, 2015). The Chibcha civilization was the first culture to recognize the use of hemiparasitic *Escobedia grandiflora* (L.f.) Kuntze (Pennell, 1931), which has orange-colored roots. The roots of this species have been used as a food coloring and as a treatment for liver disorders (Pennell, 1931; Muriel et al., 2015).

Evidence suggests that seedlings represent the most susceptible stage in the parasitic life cycle (Press, 1995; Phoenix & Press, 2005), because those seeds have a low amount of resources, limiting seedling growth; endangering the haustoria formation, and penetration in host tissue, and thus putting seedling survival at risk (Heide-Jørgensen, 2008; Joel & Bar, 2013). For their establishment into hosts, *Alectra vogelii* and *Striga gesneroides*, these plants must concentrate all resources toward growth of the radical system, which is necessary in order to establish a rapid contact with a host tissue for removal of water and nutrients (Okonkwo & Raghavan, 1982). Other studies reporting on parasitic plants belonging to different genera of Orobanchaceae, such as *Alectra, Striga,* and *Orobanche*, reported that those seeds require germination stimulants (Cardoso, Ruyter-Spira, & Bouwmeester, 2011; Joel, Chaudhuri, Plakhine, Ziadna, & Steffens, 2011).
Plant-parasitic researches are focused on parasitic weeds owing to impact on agriculture; yet, the studies into other areas of this issue are few. Cardona-Medina and Muriel (2015) investigated aspects of seed germination, seedlings and development of *E. grandiflora* plants, stating that plants need a host for survival. However, the investigation founded a low survival rate, even in the presence of the host, which points to gaps in our knowledge about the initial development of *E. grandiflora*.

This genetic resource has significant value, yet, little is known about its initial development. Therefore, it is necessary to advance in basic scientific understanding of this critical initial development stage of parasitic plant. Therefore, based on analyses with light, confocal and electron microscopy, the present study aimed to characterize the post-seminal structure and development of the hemiparasitic *E. grandiflora*, during the establishment of parasitism on the *Pennisetum purpureum* roots; in order to identify aspects that might influence the survival of initial development for parasitic seedlings and plants.

**Material and methods**

Plant material: *Escobedia grandiflora* fruits were collected in two natural populations in Campos Novos (27° 18.414’ S, 051° 11.728’ W) and Água Doce (26° 36.944’ S, 051° 29.847’ W), both located in Santa Catarina State, Brazil. Naturally grown rootstocks of *Pennisetum purpureum* Schumach. (Poaceae) were collected in the Centro de Ciências Agrárias, Florianópolis, and used as a host based on its rapid growth and strong attachment of *E. grandiflora* haustoria (Cardona-Medina & Muriel, 2015).

**Assays:** We sowed *E. grandiflora* seeds on to moistened absorbent paper at a temperature of 25°C and 12 hours light-1 photoperiod within a growth chamber (Cardona-Medina & Muriel, 2015). To characterize general aspects of seed and penetration of the radicle through the surrounding seed tissues (Assay 1), *E. grandiflora* seeds were collected at six, ten, eleven and fifteen days after imbibition, according to germination sensu stricto, as detailed by Bewley, Bradford, Hilhorst, and Nonogaki (2013). To determinate seed structures, sections were compared with the structures of other seeds in Orobanchaceae, reported by Joel and Bar (2013) and Joel et al. (2012). To describe *E. grandiflora* post-seminal structure and ultrastructure with *P. purpureum* host (Assay 2), the rhizome of host plants was grown over a period of 30 days in 338 cm³ (8×6.5×6.5 cm) pots before sowing parasite seeds in a greenhouse. The pots were filled with a mixture of vermiculite and commercial substrate (Tropusstrato HA-Hortalícias®, Mogi Mirim, Brazil) (1:1). Four seeds, previously imbibed in water for 5 days, were individually sown in pots containing a host plant in each pot. For characterization of stems, leaves, and roots samples were collected at 15, 22 and 64 days after the emergence of the seedling root.

**Histology:** Samples were fixed using a solution containing 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer. After fixation, samples were dehydrated in an ethanolic series according to Ruzin (1999). Samples were infiltrated and then embedded with Historesin® (Leica, Heidelberg, Germany), following a protocol suggested by Gerrits and Smid (1985). Cross and longitudinal 5 μm sections were cut with an RM 2125 RT rotating microtome (Leica®, Nussloch, Germany) and stained with toluidine blue (O’Brien, Feder, & McCully, 1964) for characterization and with lugol in order to observe the presence of starch grains (Johansen, 1940). Sections were examined with a BX-40 microscope (Olympus, Tokyo, Japan), and images were taken with a DP71 digital camera (Olympus, Tokyo, Japan).

**Scanning electron microscopy (SEM):** Samples were fixed and dehydrated, as described above, followed by critical point drying with CO₂ in an EM CPD050 (Leica®, Heidelberg, Germany), according to Horridge and Tamm (1969). Dried samples were fastened to aluminum supports using double-sided carbon tape and covered with 30 nm gold-palladium film in an high vacuum sputter coater EMSCD500 (Leica®, Vienna, Austria). Evaluations were carried out with the JEOL XL 50 scanning electron microscope (JEOL, Tokyo, Japan).

**Confocal laser scanning microscopy:** Five days after radicle emergence, seedlings were fixed using a solution containing 2.5% paraformaldehyde in 0.1 M sodium phosphate buffer and then evaluated using a DMI600B TCS SP-5 confocal laser scanning microscope (Leica®, Germany). Excitation and emission wavelengths were set to 488 and 412 to 501 nm, respectively. Image processing was performed with LAS-AF Lite software (Leica®, Mannheim, Germany).

**Results**

Seed and seedling: The mature seeds were composed by three recognizable regions (Figure 1A-D): the seed coat, including seminal tegument, perisperm and the embryo. The embryo occupied the central part of
the seed and was composed of a hypocotyl-radicular axis and two cotyledons. The apex of the radicle was not yet well developed, and densely cytoplasmic cells were observed in the cotyledons (Figure 1B). In the perisperm, we found a simple layer of densely cytoplasmic cells with thick exterior wall (Figure 1C-D). Endosperm traces were verified, but some seed regions were imperceptible (Figure 1C-D). *Escobedia grandiflora* root emergence began eleven days after imbibition (Figure 1E-G), ending the germination phase and giving rise to seedling growth (G-I). In principal root, root apex had slow development (Figure 1G). In the proximal region of root there developed an annular area densely covered by hairs was verified (Figure 1H-I). However, other hairs were present along the root, as well (Figure 1I). In the rootcap, mature xylem elements were noted near the promeristem region (Figure 1I).

**Figure 1.** Seed (A-D) and seedling (G-J) morphoanatomy of *Escobedia grandiflora*. Scanning electron micrograph (D, F, H), longitudinal sections in light microscopy (B, C, G); stereoscopic (A) overview of seed, embryo and suspensor observed for transparency and the seminal tegument involving the entire structure (short arrow); (B) internal embryo structure; (C-D) seminal tegument and perisperm detail, including the presence of endosperm traces (short arrow); (E) seed with root emergence (short arrow) (F) root emergence detail (short arrow), showing the seminal tegument and root hairs; (G) seedling emergence detail; (H-I) seedling with glandular trichomes in the adaxial surface of cotyledons and root hairs in root proximal zone (short arrows); (J) seedling root detail with root hairs (short arrow). Abbreviations: CD = Cotyledons; EM = Embryo; GTR = Glandular trichome; HT = Hypocotyl; RH = Root Hair; PR = Parasite root; PC = Procambium; PER = Perisperm; PM = Promeristem; RC = Rootcap; ST = Seminal tegument; SS = Seed suspensor; XE = Xylem elements. Scale: (A, E, I) = 500; (B, F) = 100; (C) = 20; (D) = 10; and (G, H, J) = 200 μm;
Seedling and initial parasitism: Soon after the emergence of the radicle (Figure 1E, I) the root lacked pigmentation. Fifteen days after the beginning of root emergence, we detected initial root pigmentation (Figure 2A). In a number of cross sections, the hypocotyl tissues were homogeneously distributed, with the vascular cylinder, cortex and epidermis. The vascular cylinder was composed of pericycle, formed by a ring of cells, smaller in size than the cells of the endoderm, and vascular elements arranged in a form similar to that of the root, with two protoxylem strands (diarch) alternating with phloem strands (Figure 2B). In the root proximal zone (Figure 2C), the vascular cylinder showed a structure similar to that described for the hypocotyls. We noted peripheral swelling, a manifestation of the elongated cortex, parenchyma and endoderm cells. In the internal structure, the protoxylem was observed to alternate with phloem, and root branching also occurred (Figure 2A, D).

Figure 2. Escobedia grandiflora morphoanatomy in the presence of host Pennisetum purpureum for 15 (A-D), 22 (E-H), and 64 days (I-M). Cross sections in light microscopy (B-D, H, J-M), stereoscopic microscopy (A, E, G, I), and scanning electron microscopy (F). Escobedia grandiflora seedlings (A) and plants with haustoria attached on roots of the host (short arrows) (E, I), hypocotyl internal structure in the seedling (B); root proximal zone structure of seedling (C); root structure with initial root branching in the seedling, consisting of cells with visible nucleus and dense cytoplasmic content (D); root hairs in root proximal zone (short arrow) (F); E. grandiflora haustoria attached to P. purpureum root (G); vascular connection between haustoria and host root (short arrow) (H); stem structure (J); root structure with starch grains in cortical cells (short arrow) (K); detail of starch grains in cortical stem cells, (L) and in cortical root cells (M), stained with toluidine blue and lugol. Abbreviations: CD = Cotyledon; CT = Cortex; EM = Endoderm; HR = Host root; HT = Hypocotyl; LR = Lateral root; PE = Pericycle; PH = Phloem; PM = Promeristem; PR = Parasite root; RC = Rootcap; VC = Vascular cambium cells; XL = Xylem elements. Scale: (A, E, I) = 2 mm; (B-D) = 50; (F, J-K) = 100; (G) = 200; and (H, L-M) = 20 μm.
At 22 days after root emergence, the seedlings showed secondary haustoria only in principal root, and was not developed primary haustoria (Figure 2E). Some developed haustoria began to attach to *P. purpureum* roots (Figure 2E-G), and haustorium endophyte penetrated within the host’s tissue connecting to host vascular elements and causing fragmentation of the surface (Figure 2H). Therefore, *E. grandiflora* parasitism on *P. purpureum* appeared to begin at 22 days post-root emergence.

On day 64 after radicle emergence, the parasitic root system grew faster with more ramification compared to previous developmental stages and more haustoria attached to host root. Roots, mainly older, showed strong orange pigmentation (Figure 2I). As shown in Figure 2J-K, we saw evidence of vascular cambium cells, even though no evidence of secondary xylem and phloem elements was found through histological analysis. We observed the accumulation of starch in the stem and root cortex of *E. grandiflora* (Figure 2L-M).

Presence of the primary definitive leaves was verified about 43 days after root emergence (Figure 3). This stage was indicative of the transition from seedling to plant, and it was possible to detect three foliar types; cotyledons, eophylls, and definitive leaves (Figure 3A-B). The three foliar types presented both glandular and non-glandular trichomes (Figure 3A, C-D) on both adaxial and abaxial surface. Glandular trichomes were visible after radicle and cotyledon emergence (Figure 1I-J). Non-glandular trichomes had a sharp apex and were visible 15 days after radicle emergence.

**Figure 3.** Leaf surface of *Escobedia grandiflora*. Stereoscopic microscopy (A); scanning electron microscopy (B-D). *Escobedia grandiflora* plant with evidence of definitive first leaves developed about 43 days after radicle emergence (A); adaxial surface of the three foliar types (B); adaxial surface of the eophyll with glandular and non-glandular trichome details (C); definitive first leaves developed (D). Abbreviations: CD = Cotyledons; EO = Eophyll; GT = Glandular trichome; LE = leaf; NG = Non-glandular trichome. Scale: (A-B) = 500; and (C-D) = 100 μm.
Discussion

The research elucidated the post-seminal development of *E. grandiflora*. We founded a presence of a reserve for nutrients in the perisperm and cotyledons. Perisperm is also an important reserve tissue in other Orobanchaceae seeds, including such genera as *Striga*, *Alectra*, *Phelipanche*, *Orobanche*, and *Aeginetia* (Joel et al., 2012; Joel & Bar, 2013). Seed reserve tissues accumulate carbohydrates, proteins and lipids, during germination these reserves are degraded and mobilized to different parts of the embryo (Sert, Bonato, & Souza, 2009). Perisperm cells are involved in the transfer of reserve nutrients to the embryo during germination (Joel et al., 2012). The cotyledons, the first leaves of the embryonic axis, have a role in photosynthesis, but also function as nutrient reserves (Souza, 2009). For germination *E. grandiflora* seeds only have reserves in the perisperm and cotyledons. However, according to Joel and Bar (2013), the low amount of seed resources limits the parasitic seedling growth. After germination, therefore, the survival of *E. grandiflora* seedlings depends on their own root absorption and host parasitism.

*Eschobia grandiflora* seedling root showed mature xylem elements near the promeristematic region. It can be argued that this feature contributes to the slow growth of the roots, as claimed earlier by Esau (1959). This interpretation is in agreement with other studies. For example, in the principal root of *A. vogelii* and *S. gesnerioides* seedlings, mature xylem elements were far from the promeristem region (Okonkwo & Raghavan, 1982), resulting in relatively fast root growth (Dörr, 1997). Despite the slow growth of *E. grandiflora*, the trend is toward faster root growth, compared to that of stem, since the parasite’s root must develop haustoria for attachment to the host.

In addition, since *E. grandiflora* forms only secondary haustoria after the formation of the main root, it needs more time to reach the parasitism phase in the host, when compared to parasite species that form primary haustoria. Also, because of the need to use its own resources to form the main root first, growth and the seedling survival could be affected. Conversely, after seed emergence, *Striga* seedlings develop primary haustoria. This haustorium type is developed in the root apex, allowing fast parasitism and increased seedling survival (Dörr, 1997; Hood, Condon, Timko, & Riopel, 1998). The faster root growth compared to that of stem is related to the presence of starch grains, which is characterized by a higher proportion in the root and haustoria cortex than stem. Starch storage in the root and haustoria, as founded in roots of *E. grandiflora*, is key to parasite survival because it supports plant growth, flowering, and seed production, even after the death of the host (Joel & Bar, 2013).

Parasitism takes place after seedlings have formed cotyledons, and leaf formation occurs after development of the many haustoria and its attachment to host root. In the present study, the development of first leaves occurred 45 days after root emergence. According to Souza (2009), leaf development indicates the conclusion of the seedling stage and the beginning of the plant stage. Therefore, this developmental time would be the most critical stage for *E. grandiflora* conforming to the definitions of Press (1995); Phoenix and Press (2005); Těšitel, Lepš, Vráblová, and Cameron (2011).

Trichomes of cotyledons, eophylls and definitive leaves of *E. grandiflora* present morphological features similar to the leaf trichomes of the parasitic plant *Orobanche ramose* L (Orobanchaceae) (Sacchetti, Ballero, Serafini, Muzzoli, & Tosi, 2003). According to the authors, *O. ramose* trichomes showed such chemical compounds as terpene and flavonoids. The trichomes are important for protection against pathogens and herbivorous insects (Glas et al., 2012).

Conclusion

This is the first research to describe the structures of the initial development and successful parasitism of the hemiparasitic plant *E. grandiflora* on the host *P. purpureum*. The main features observed during parasitism were poor seed reserve, fast growth of roots and poor stem development, accumulation of starch grains in the roots and formation of haustoria. The information here in should contribute to better understanding of the development of parasitic plants relative to their establishment in their hosts. In addition, with such basic knowledge, it will be possible to develop strategies of conservation and cultivation of the specie, especially considering the value of this genetic resource.

Acknowledgements

We thank Lido Jose Borsuk for his help with the fieldwork, members of the Central Electron Microscopy Laboratory (LCME) for technical assistance with SEM and Confocal Analyses, and members of the LAVEG.
and LFDGV laboratories for the use of their facilities. We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Proc. 307144/2013-5) for financial resources and for the scholarships to RON and MS. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) - Finance Code 001.

References

Bennett, J. R., & Mathews, S. (2006). Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome. *American Journal of Botany, 93*(7), 1059-1051. doi: 10.3732/ajb.93.7.1059

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). *Seeds.* New York, NY: Springer.

Cardona-Medina, E., & Muriel, S. B. (2015). Seed germination and plant development in *Escobedia grandiflora* (Orobanchaceae): evidence of obligate hemiparasitism? *Acta Biológica Colombiana, 20*(3), 133-140. doi: 10.15446/abc.v20n2.43776

Cardoso, C., Ruyter-Spira, C., & Bouwmeester, H. J. (2011). Strigolactones and root infestation by plant-parasitic *Striga, Orobanche* and *Phelipanche* spp. *Plant Science, 180*(3), 414-420. doi: 10.1016/j.plantsci.2010.11.007

Dörr, I. (1997). How *Striga* parasitizes its host: a TEM and SEM study. *Annals of Botany, 79*(5), 463-472. doi: 10.1006/anbo.1996.0385

Esau, K. (1959). *Plant anatomy.* Barcelona, ES: Omega.

Gerrits, P. O., & Smid, L. (1983). A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *Journal of Microscopy, 132*(1), 81-85. doi: 10.1111/j.1365-2818.1983.tb04711.x

Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., & Kant, M. R. (2012). Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences, 13*(12), 17077-17103. doi: 10.3390/ijms131217077

Heide-Jørgensen, H. S. (2008). *Parasitic flowering plants.* Boston, MA: Brill.

Hood, M. E., Condon, J. M., Timko, M. P., & Riopel, J. L. (1998). Primary haustorial development of *Striga asiatica* on host and non-host species. *Phytopathology, 88*(1), 70-75. doi: 10.1094/PHYTO.1998.88.1.70

Horridge, G. A., & Tamm, S. L. (1969). Critical point drying for scanning electron microscopic study of ciliary motion. *Science, 163*(3869), 817-818. doi: 10.1126/science.163.3869.817

Joel, D. M., & Bar, H. (2013). The seed and the seedling. In D. M. Joel, J. Gressel, & L. J. Musselman (Eds.), *Parasitic Orobanchaceae: parasitic mechanisms and control strategies* (p. 147-163). Heidelberg, DE: Springer.

Joel, D. M., Bar, H., Mayer, A. M., Plakhine, D., Ziadna, H., Westwood, J. H., & Welbaum, G. E. (2012). Seed ultrastructure and water absorption pathway of the root-parasitic plant *Phelipanche aegyptiaca* (Orobanchaceae). *Annals of Botany, 109*(1), 181-195. doi: 10.1093/aob/mcr261

Joel, D. M., Chaudhuri, S. K., Plakhine, D., Ziadna, H., & Steffens, J. C. (2011). Dehydrocostus lactone is exuded from sunflower roots and stimulates germination of the root parasite *Orobanche cumana.* *Phytochemistry, 72*(7), 624-634. doi: 10.1016/j.phytochem.2011.01.037

Johansen, D. A. (1940). *Plant microtechnique.* New York, NY: McGraw-Hill.

Kokla, A., & Melnyk, C. W. (2018). Developing a thief: haustoria formation in parasitic plants. *Developmental Biology, 442*(1), 55-59. doi: 10.1016/j.ydbio.2018.06.013

Muriel, S. B. M., Cardona, E. C., Arias, E., & Gómez, A. G. (2015). Indagaciones acerca delazafrán de raiz (*Escobedia grandiflora* (L.F.) Kunze) Antioquia - Colombia: una especie olvidada. *Ethnobiología, 15*(2), 85-93.

O’Brian, T. P., Feder, N., & McCully, M. E. (1964). Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma, 59*(2), 368-373. doi: 10.1007/BF01248568

Okonkwo, S. N. C., & Raghavan, V. (1982). Studies on the germination of seeds of the root parasites, *Alectra vogelii* and *Striga gesnerioides.* I. Anatomical changes in the embryos. *American Journal of Botany, 69*(10), 1636-1645. doi: 10.2307/2449218

Pennell, F. W. (1931). *Escobedia: a Neotropical genus of the Scrophulariaceae.* *Proceedings of the Academy of Natural Sciences of Philadelphia, 83*(1951), 411-426.
Phoenix, G. K., & Press, M. C. (2005). Linking physiological traits to impacts on community structure and function: the role of root hemiparasitic Orobanchaceae (ex-Scrophulariaceae). *Journal of Ecology, 93*(1), 67-78. doi: 10.1111/j.1365-2745.2004.00950.x

Pielach, A., Leroux, O., Domozycz, D. S., Knox, J. P., & Popper, Z. A. (2014). Arabinogalactan protein-rich cell walls, paramural deposits and ergastic globules define the hyaline bodies of rhinanthoid Orobanchaceae haustoria. *Annals of Botany, 114*(6), 1359-1373. doi: 10.1093/aob/mcu121

Press, M. C. (1995). *Parasitic plants*. London, GB: Chapman & Hall.

Ren, Y.-Q., Guan, K.-Y., Li, A.-R., Hu, X.-J., & Zhang, L. (2010). Host dependence and preference of the root hemiparasite, *Pedicularis cephalantha* Franch. (Orobanchaceae). *Folia Geobotanica, 45*(4), 443-455. doi: 10.1007/s12224-010-9081-6

Ruzin, S. E. (1999). *Plant microtechnique and microscopy*. New York, NY: Oxford University press.

Sacchetti, G., Ballero, M., Serafini, M., Muzzoli, M., & Tosi, B. (2003). Morphological and histochemical investigation on glandular trichomes of *Orobanche ramosa* subsp. nana (Orobanchaceae). *Phyton, 43*(1), 207-214.

Sert, M. A., Bonato, C. M., & Souza, L. A. (2009). Germinação da semente. In L. A. Souza (Org.), *Sementes e plântulas: germinação, estrutura e adaptação* (p. 15-88). Ponta Grossa, PR: Todapalavra.

Souza, L. A. (2009). *Morfologia e anatomia vegetal célula, tecidos, órgãos e plântula*. Ponta Grossa, PR: UEPG.

Těšitel, J., Lepš, J., Vráblová, M., & Cameron, D. D. (2011). The role of heterotrophic carbon acquisition by the hemiparasitic plant *Rhinanthus alectorolophus* in seedling establishment in natural communities: a physiological perspective. *New Phytologist, 192*(1), 188-199. doi: 10.1111/j.1469-8137.2011.03777.x