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Recent advances in Broomrapes research

ABSTRACT

Orobanchaceae (broomrapes) is a morphologically diverse family of predominantly herbaceous, parasitic plants. The majority of species are facultative or obligate root parasites that subsist on broad-leaf plants, thereby depleting them of nutrients, minerals and water. The taxonomy status of the family *Orobanchaceae* among other flowering plants is often subject of debate. They possess only a few morphological features suitable for taxonomy purposes and yet even they are quite changeable. The variability within the species is too high and hampers the attempts to create proper determination keys. During last two decades several molecular markers were used for reevaluate taxonomy, biodiversity and phylogenetic relationships within the family. Recent investigations supported by molecular taxonomy analyses have resulted in re-definition of *Orobanchaceae* family. According to this classification *Orobanchaceae* consists of 89 genera, containing 2061 species. On the Balkans the family *Orobanchaceae* is represented by 3 genera: *Orobanche* includes 25 species; *Phelipanche* comprises of 9 species and some putative hybrids; *Diphelypaea* occurs with single species, *Diphelypaea boissieri*, in Macedonia and Greece. Only a few recent studies based on modern methods took place during last decade. Their findings confirmed differences between *Phelipanche* and *Orobanche* genera, but raised new question about their internal structure. Several broomrape species parasitize important crops. They are widely spread in Bulgaria, Southern Europe, Russia, Middle East and Northern Africa. They cause losses in crop productivity estimated at hundreds of millions of dollars annually than affect the livelihoods of 100 million farmers. A wide variety of approaches have been explored to control broomrapes, but none have been found to be sufficiently effective and affordable. The new findings about their life cycle and the recent genomic project focused on sequences of *Ph. aegyptiaca* genome open new perspectives for management of the harmful broomrape species and for understanding of their biology and evolution as well.

Key words: Broomrapes, ecosystem management, germination stimulants, haustorium, molecular taxonomy, *Orobanchaceae*

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Extend of parasitism among flowering plants

Non-photosynthetic flowering species that have lost their autotrophic properties of plants in favor of a parasitic lifestyle have evolved in at least 11 independent angiosperm lineages and account more than 1% of all angiosperm species (Cronquist, 1988). These species lack capacity to assimilate sufficient CO_2 amounts to sustain their growth or/and cannot

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absorb nutrients and water from the rhizosphere in sufficient quantities to reproduce successfully. These plants rely on other "host plants" to provide them with the materials they cannot acquire from their abiotic environment (Kuijt, 1969; Fernández-Aparicio et al., 2011). Recent investigation determined at least 4500 plant species within 270 genera in over 22 families predominantly angiosperms (Fernández-Aparicio et al., 2011) rely on a parasitic association with a host plant for their mineral nutrition, water uptake, and/or carbon supply. Only a single parasitic gymnosperm species, *Parasitaxus usta*, has been identified, deriving water and nutrients from its host's xylem, but carbon by mycoheterotrophy (Feild & Brodribb, 2005; Sinclair et al., 2002).

They inhabit ecosystems ranging from the high Arctic to the tropics. This group of plants includes such notable species as the mistletoes (*Arceuthobium*, *Phorodendron*, and related genera), dodder (*Cuscuta* spp.), sandalwoods (*Santalaceae*), broomrapes (*Orobanche* spp.), and witchweeds (*Striga* spp.). Among them broomrapes demonstrate a higher level of adaptation because they are chlorophyll-lacking obligate root holoparasites that depend entirely on their hosts thereby depleting them of nutrients, minerals and water (Young et al., 1999; Wolfe et al., 2005). The family has a worldwide distribution, but the main centers of distribution are the Mediterranean, Northern Africa, and western North America (Musselman, 1980, 1986; Young et al., 1999; Wolfe et al., 2005).

Taxonomy and biodiversity of *Orobanchaceae* family

The phylogenic origin of these plants and their taxonomy is often subject of debates. They possess only a few morphological features suitable for taxonomy purposes and yet even they are quite changeable. The variability within the species is too high and hampers the attempts to create proper determination keys.

The taxonomy status of the family *Orobanchaceae* Vent among other flowering plants is uncertain. Most of the regional "floras" accept that *Orobanchaceae* is independent family (Tzvelev, 1981; Delipavlov, 1995; Zazvorka, 2000; Foley, 2001). Some authors however propose that *Orobanchaceae* is part of *Scrophulariaceae* Juss (Chater & Webb, 1972; Teryokhin, 1997), while other (Olmstead et al., 2001) argue that substantial part of *Scrophulariaceae* belong actually to *Orobanchaceae*.

The recent investigations supported by molecular phylogenetic analyses have resulted in re-definition of *Scrophulariaceae* and related families in the order *Lamiales* (Olmstead & Reeves, 1995; Olmstead et al., 2001; Bremer et al., 2002; Albach et al., 2005). Hemiparasitic species, formerly placed in *Scrophulariaceae* subfamily *Rhinanthoideae*, and *Orobanchaceae* were shown to comprise a monophyletic group. Parasitism is believed to have evolved once in the group, followed by multiple independent origins of holoparasitism from hemiparasitic ancestors (Young et al., 1999). *Lindenbergia philippensis* from a genus of 12 nonparasitic species from northeast Africa and Asia, was resolved as sister to the parasitic species (Nickrent et al., 1998; Young et al., 1999; Young & de Pamphilis, 2000; Olmstead et al., 2001) and included in a clade definition of *Orobanchaceae* (Young et al., 1999).

Based on these molecular analyses Young et al. (1999) proposed that *Orobanchaceae* is a morphologically diverse family of predominantly herbaceous, parasitic plants. The majority of species are facultative or obligate root parasites, which may be photosynthetic (hemiparasites) or totally dependent on the host plant (holoparasites). According to this classification *Orobanchaceae* consists of 89 genera, containing ca. 2061 species (Nickrent, 2008).

The largest among them is genus *Orobanche*, comprised of approximately 170 species that inhabit mainly in the northern hemisphere (Beck-Mannagetta, 1890; Uhlich et al., 1995). The genus was initially divided into four sections: *Gymnocaulis*, *Myzorrhiza*, *Trionychon* and *Orobanche* (Beck-Mannagetta, 1930). In the most recent taxonomic treatments, these sections were recognized as separate genera: *Aphyllon*, *Myzorrhiza*, *Phelipanche* and *Orobanche* (Holub, 1977, 1990; Teryokhin et al., 1993). Teryokhin (1993, 1997) classified *Orobanche* together with *Phelypaea* (syn. *Diphelypaea*) and *Cistanche*, in subtribe *Orobanchinae*, while *Phelipanche* constitutes a separate subtribe *Phelipanchinae*, implying that *Orobanche* in its broad circumscription is not monophyletic. This view is supported by results from nuclear ITS, karyological and genome size data, which indicate that *Phelypaea* is closely related to *Orobanche* sect. *Orobanche* (both share a chromosome base number of x=19), while the remaining sections (all with chromosome base number x=12) constitute a separate lineage (Schneeweiss et al., 2004a, 2004b; Weiss-Schneeweiss et al., 2006). The recognition of *Phelipanche* (syn. *O*. sect. *Trionychon*) as separate genus is prompted also in recent papers of Park et al. (2007a, 2007b). Another viewpoint is the

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separation of *Phelipanche* as subgenus of *Orobanche* (Tzvelev, 1981; Pujadas, 2007).

Unlike many other parts of the world, the current knowledge about broomrapes diversity and distribution on the Balkan Peninsula is based mainly on floristic records. According to them on the Balkans the family *Orobanchaceae* is represented by 3 genera: *Orobanche* includes 25 species, two of which are endemic for the region: *O. serbica* and *O. esulae*. Other species, like *Orobanche pancicii*, do occur also in Central Europe, but originate from the Balkan Peninsula. *Phelipanche* comprises of 9 species and putatively hybrids between *Ph. ramosa* and *Ph. lavandulacea* reported in Bulgaria. The third genus, *Diphelypaea*, has its centre of diversity in Southwest Asia and occurs with single species, *Diphelypaea boissieri*, in Macedonia and Greece (Chater & Webb, 1972). The *Orobanchaceae* populations on the Balkans probably include many intraspecific taxa. However, only a few recent studies based on modern methods have included materials from Balkan countries - Greece and Croatia (Schneeweiss et al., 2004a, 2004b; Weiss-Schneeweiss, 2006; Park et al., 2007a, 2007b).

Recently microsatellite markers were used for evaluation of the biodiversity and phylogenetic relationships between Bulgarian representatives of the family *Orobanchaceae* (Hristova et al., 2011; Stoyanov & Denev, 2011; Stoyanov et al., 2012). This is a relatively new molecular marker technique called inter simple sequence repeat (ISSR) (Zietkiewicz et al., 1994). ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite. The primers are 16-18 bp long composed of a repeated sequence and could be flanked at the 3' or 5' end by 2-4 arbitrary nucleotides – anchored primers (Zietkiewicz et al., 1994). Such amplification does not require genome sequence information and leads to multilocus and highly polymorphous patterns (Zietkiewicz et al., 1994; Nagaoka & Ogihara, 1997). Each band corresponds to a DNA sequence delimited by two inverted microsatellites. The applicability of the ISSR for taxonomic studies of *Orobanchaceae* was demonstrated by Benharrat et al. (2002). The authors studied *Orobanche hederae, O. amethystea, O. cernua* and *O. cumana* by five different ISSR primers and obtained taxonomically significant results. ISSR technique was proven suitable for distinguishing between closely related broomrape species (Benharrat et al., 2002) and even between different populations of *O. crenata* (Román et al., 2002) and *Ph. ramosa* (Buschmann et al., 2005). *Orobanche minor* was recently subjected to extensive study in the UK. Using ISSR and sequence-characterized amplified region (SCAR) markers, Thorogood et al., (2008, 2009a,b) demonstrated that in Britain, *O. minor* comprises of genetically divergent populations associated with different hosts.

Recently one hundred ISSR primers (University of British Columbia Nucleic Acid-Protein Service Unit, UBC Primer Set #9) were tested on specimens from five different species (*Phelipanche ramosa, P. mutelii, P. purpurea, Orobanche alba* and *O. minor*). The plants were collected from different locations in Bulgaria. Thirteen ISSR primers were found to produce polymorphic bands suitable to distinguish the known sections and genera. Other 3 primers could distinguish the genera and probably higher taxonomy ranks (Hristova et al., 2011). These findings were used to study Balkan representatives of *Orobancheceae*.

Eight microsatellite markers were used for preliminary evaluation of the biodiversity and phylogenetic relationships between Bulgarian representatives of *Orobanchaceae.* The plants were collected from various locations in the country and their flowering stems were used to isolate genomic DNA. The ISSR products were separated on agarose gel and visualized by UV-light. The molecular masses the products were determined and used to fill Boolean matrices that were subjected to cluster analysis. Representatives from genus *Lathraea* (*Scrophulariaceae*) were used for external controls. The consequent cladograms, based on the average Euclidean distances, displayed clear grouping by species. The representatives of subsect. *Minores* were grouped in a separate cluster, as well as *O. gracilis* and *O. cumana*. *Orobanche cumana* showed host-dependent genetic variability . The molecular markers confirm the existence of *O. caryophyllacea* var. *macrolepis* T. Georg. and *O. gracilis* var. *sprunerii* (F. Schultz) Beck. Genus *Lathraea* was clearly separated from the representatives of *Orobanchaceae* (Stoyanov & Denev, 2011).

Particularly problematic are the species within the genus *Orobanche* subsection *Minores* (Beck-Mannagetta) Teryokhin (1997). This group is characterized by small-flower species with an exceptionally large range of angiosperm hosts from at least 16 orders (Schneeweiss, 2007) and probably includes many cryptic taxa (Foley, 2001).

According to Stoyanov (2009) subsect. *Minores* is represented by six species in the Bulgarian flora. Because of their high morphological similarity, five of the them (*O. minor* Sm, *O. loricata* Rchb., *O. amethystea* Thuill., *O. esulae* Pančić, *O. pubescens* d'Urv.) were grouped in the

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aggregate *O. minor* (Chater & Webb, 1972; Gilli, 1982; Delipavlov, 1995). According to Musselman (1986, 1994) this aggregate consists of only one species, which displays wide morphological variability caused by the host plant and is poorly resolved even by broad-scale molecular phylogenetic analyses (Manen et al., 2004; Schneeweiss, 2007; Park et al., 2008).

Among them the Balkan endemic species *O. esulae* was described by Pancic (1884) for the region of Pirot. It is represented in Bulgaria by a variety – *O. esulae* var. *bulgarica* T. Georgiev (1937), which has a high morphological similarity to *O. minor*. The taxonomic position of *O. loricata* is problematic as well – according to some authors it is one species: *O. loricata* (Beck-Managetta, 1890; Andreev, 1992; Benharrat et al., 2002), while others described it as *O. picridis-hieracioides* Scultz (Hayek, 1929), *O. picridis* Schultz (Gilli, 1982; Andreev, 1992; Bornet & Branhard, 2001), *O. artemisiae-campestris* Vauch. (Zazvorka, 2000; Foley, 2001; Piwowarczyk, 2012).

On the other hand, *O. crenata* Forssk. was assigned as a member of a separate subsection according to the classic scheme of Beck Mannagetta (1890). The phylogenic investigations based on ITS sequences (Schneeweiss et al., 2004a,b) resulted in a revision that assigned *O. crenata* to subsect. *Minores*. Recently *O. serbica* Beck & Petrovic was incorporated in the *Minores* as a result of the taxonomical revision proposed by Carlón et al. (2008).

Samples of the six species (*O. minor*, *O. loricata*, *O. amethystea*, *O. esulae*, *O. pubescens* and *O. crenata*) were collected from different Bulgarian regions and used for ISSR-based study. The suitability of 16 ISSR primers for distinguishing each of the studied species was discussed (Stoyanov et al., 2012).

The analyses of the distribution of the polymorphic ISSR products suggested that *O. esulae*, in spite of the morphological similarity, is closer to *O. pubescens* and *O. loricata* than to *O. minor* (Figure 1). On the other hand *O. loricata* and *O. amethystea* showed closer relationships with *O. esulae*.

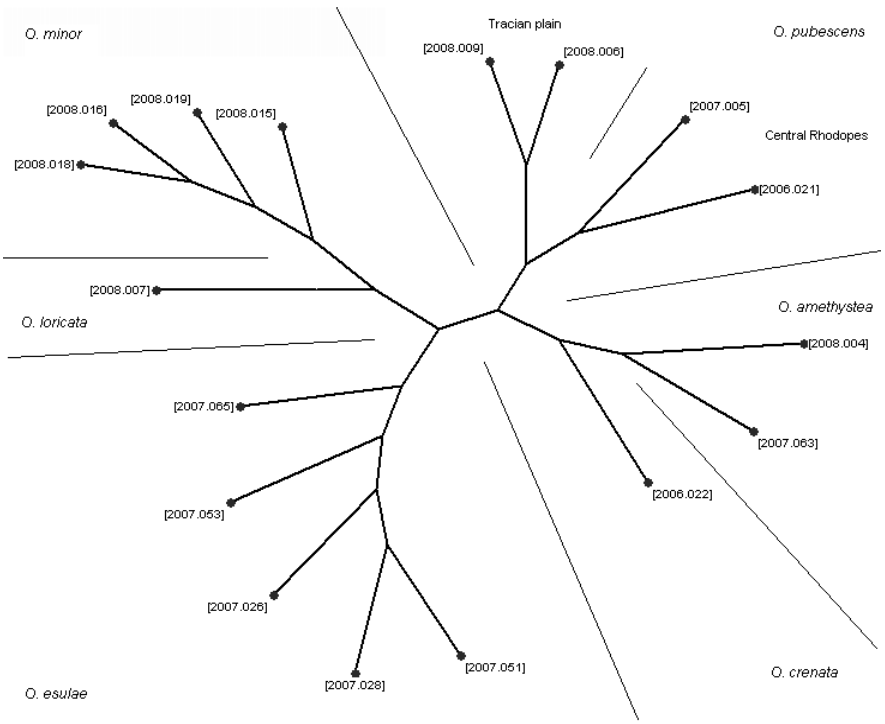

Figure 1. *The consequent cladograme based on genetic distances of the studied species using Unweighted Neighbor Joining method. The dendrogram was plotted by the PhyloDraw software ver. 0.82.*

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The primer that produced the closest results to this presumption was p836, followed by p857, which can also distinguish geographically isolated populations of *O. minor*. A specific marker for *O. esulae* could be p826, while for *O. minor* the best one is p855.

The results demonstrated grouping not only by species but also, in some cases, by geographically isolated population. This was shown for the samples of *O. pubescens*. According to the final cladogram it is obvious that in spite of the high morphological similarity, the members of agg. *O. minor* are individual species. The only case of high similarity was detected between *O. minor* and *O. loricata*. This study confirmed the status of the Balkan endemic species *O. esulae*, because it formed a separate clade from *O. minor*, regardless of the similar morphology (Stoyanov et al., 2012).

Subsection *Glandulosae* (Beck) Teryokhin is represented by four species in Bulgaria: *O. alba* Steph. ex Willd., *O. reticulata* Wallr., *O. serbica* G.Beck et Panč., and *O. pancicii* G.Beck & Petr. Samples of all four Bulgarian representatives were collected and analyzed with ISSR markers. Five ISSR primers were used. The amplified polymorphic bands were scored, processed by cluster analysis and used to build consequent cladogram. This confirmed the grouping of the known species. However the group of *O. alba* showed quite high diversity, which was probably due to the fact that the species comprises of two subspecies and about ten different forms (Stoyanov, 2009; Stoyanov & Denev, 2010).

Biology of *Orobanchaceae*

Years of investigation have uncovered an elegant system of chemical signaling by which root parasitic plants *(Striga* and *Orobanche)* recognize a potential host plant and regulate their development in order to optimize their chances for survival. (Kuijt, 1969; Musselman, 1980; Parker & Riches, 1993). Several mechanisms exist that insure tighter coordination between developmental stages of parasite life cycle and the one of the host plants (Figure 2).

Germination

Mature broomrape plants produce between 50,000 and 500,000 seeds per generation, which are capable of persisting for over a decade in the soil. According to Uematsu et al. (2007), for germination to proceed, the seeds require an after-ripening period and next specific preconditioning, which has been linked with water imbibition, production of gibberellins and cAMP, which seem to be prerequisites for germination.

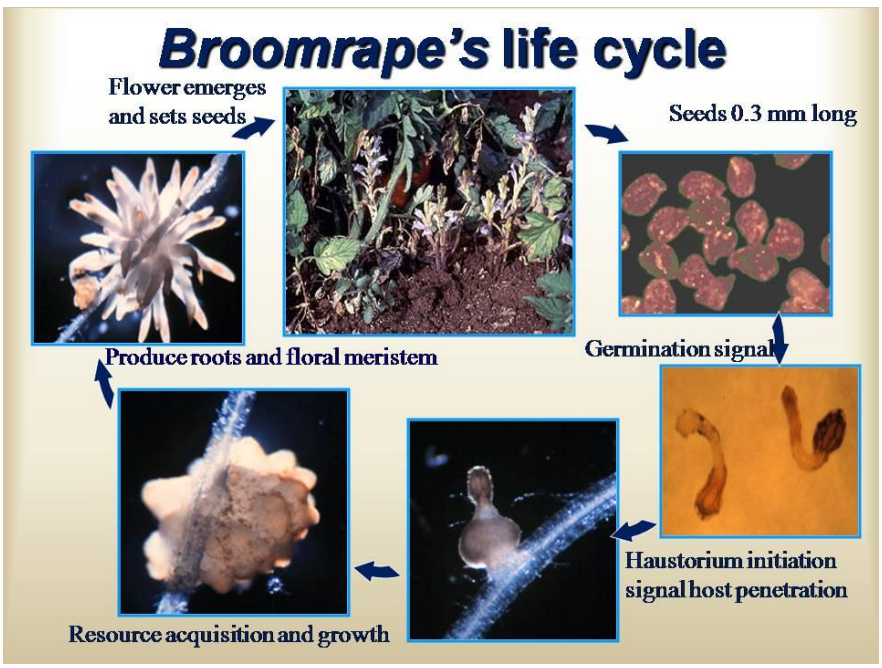

Figure 2. *Life cycle of Orobanche spp.*

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The final requirement for germination is exposure of the "conditioned seed" to an exogenous xenognosins - representing the primary chemical class of germination factors, specifically strigolactones, SXSg, and resorcinol (Butler, 1995; Pierce et al., 2003) usually emitted by in the host-root exudates (Yoder, 2001).

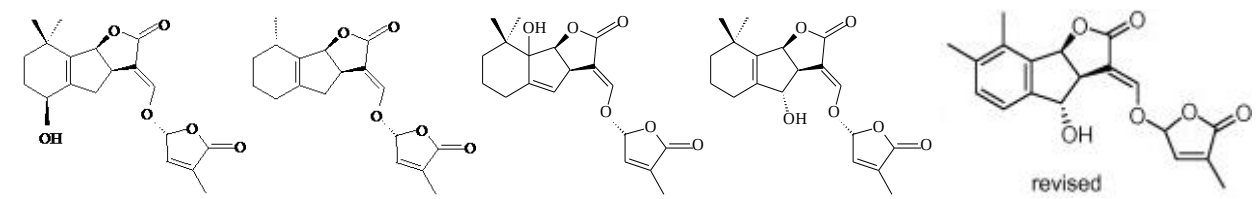
Some recent observations however reported that non-conditioned seeds of both *Orobanche cumana* and *Ph. aegyptiaca* were able to germinate in response to chemical stimulation by GR24 even without prior conditioning (Plakhine et al., 2009). The authors hypothesized that conditioning is not involved in stimulant receptivity but it includes (a) a parasite-specific early phase that allows the imbibed seeds to overcome the stress caused by failing to receive an immediate germination stimulus, and (b) a non-specific later phase that is identical to the pregermination phase between seed imbibition and actual germination that is typical for all higher plants (Plakhine et al., 2009).

Host-parasite interaction at germination step are very specific and depends on chemical recognition. Several natural GS have been isolated from their hosts (Galindo et al., 2004). With one exception (sorgoleone), all GS isolated so far from host plants belong to the so-called strigolactones (Figure 3).

Strigolactones have been identified and isolated as GS from sorghum (Hauck et al., 1992; Siame et al., 1993) red clover (Yokota et al., 1998) and cow pea (Muller et al., 1992). Many non-host also emit cocktails strigolactones like cotton (Cook et al., 1966), *Menispermum dauricum*, and *Stephania sepharantha* (Yasuda et al., 2003). Recently Kohlen et al. (2011) proposed that strigolactones may play role of new class plant hormones that are involved not only in seed germination but also in many other processes like hypocotyl elongation, reproductive development. Therefore the use of strigolactones as GS by parasites might be a result of long coevolution process during which the parasite takes advantage of, and recognizes “chemical signature” exuded by the prospective host plant for other purposes (Akiyama et al., 2005; Akiyama & Hayashi, 2006; Kohlen et al., 2011).

The biosynthetic pathway of GS, enzymes and genes involved in the process and the regulation steps of their synthesis and excretion are still unknown. It has been proposed that in tomato the biogenetic origin of GS lies in the carotenoids pathway (Rani et al., 2008). This assumption however is in contrast with findings in *Arabodopsis* and tobacco were plastids identifies as an origin of the GS blocking of carotenoid biosynthesis did not affected GS production (Denev et al., 2001, 2007). The root exudates of tobacco contained at least five different stimulants (Xie et al., 2007). Four of them were strigolactones: a tetradehydrostrigol isomer named solanacol, a didehydrostrigol isomer, a (+)-orobanchol and its 2′-epimer. The 2′-epiorobanchol and solanacol are the first natural strigolactones having a 2′-epi stereochemistry and a benzene ring, respectively (Xie et al., 2007).

Several works showed the presence of specific inducers of *O. cumana* seeds germination that do not induce a germination response in other *Orobanche* species (Pérez de Luque et al., 2000; Galindo et al., 2002). A comparative structure-activity relationship study has been conducted with several guaianolide sesquiterpene lactones as inducers of the germination of sunflower broomrape (*O. cumana*) seeds. Compounds were selected and synthesized to study the influence of the lactone-enol-γ-lactone moiety on the selectivity of GS toward the stimulation of sunflower broomrape germination.



Strigol (maize, cotton) **Alectrol** (cowpea, red clover) **Sorgolactone** (sorghum) **Orobanchol** (red clover) **Solanacol** (tobacco)

Figure 3. *The chemical structure of some isolated germination stimulants.*

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The results clearly illustrate that this type of GS are recognized only by *O. cumana*, while the introduction of a strigol-like second lactone moiety in the guaianolide backbone results in the loss of specificity and hence the germination of other broomrape species. Macías et al. (2009) named this new class of compounds guaianestrigolactones (GELs). Joel et al. (2011) illucidated the chemical structure of yet onoder GELs - guaianolide sesquiterpene lactone dehydrocostus lactone (DCL). Low DCL concentrations effectively stimulate the germination of *O. cumana* seeds but not of *Phelipanche aegyptiaca* (Joel et al., 2011).

Host plants only produce and release tiny amounts of GS into the soil, and seeds of parasites respond to very low concentrations (10^{-7} _ 10^{-15} M) of the stimulants. Concentrations of GS below or above this range block seed germination (Wigcher & Zwanenburg, 1999). Some plant varieties promote lower rates of parasite germination than others, which has been suggested to be a result of reduced production of germination signal molecules. For example, some *Arabidopsis* (Goldwasser & Yoder, 2001) and *Pisum* (Pérez-de-Luque et al., 2005) varieties induce the germination of fewer *Orobanche* seeds under controlled conditions than others. The mechanism by which this is affected is unknown, although it may be due to reduced stimulant production, the production of chemically discrete stimulant isoforms with altered properties, or potentially *de novo* production of germination suppression factors. Recent work has shown that the amino acid methionine was both able to almost completely inhibit the germination of *Phelipanche ramosa* seeds, and lead to severe reductions in the number of tubercles noted on infected tomato roots (Vurro et al., 2006). Since it appears distance from the host to the seed was a major factor, we might postulate that some plants' germination stimulants are less diffusible than others, or that they degrade faster.

Formation of haustoria

The formation of haustorial connection between the parasite and the host vascular tissue allows broomrapes to withdraw water and photosynthates from the host. The haustorium develops when intrusive cells of the parasite penetrate host tissues, eventually reaching the conductive system of the host. The mechanisms of intrusion are only partly understood and probably includes (but are not limited to) mechanical pressure employed by the intrusive cells to force their way through host tissues and secretion of the pectolytic enzymes pectin methyl esterase (PME) and polygalacturonase to insure a smoother penetration (Ben-Hod et al., 1993; Losner-Goshen et al., 1998). Upon contact of the parasite root with that of a host, there is an almost immediate cessation of parasite tip growth. This is soon followed by an isodiametric expansion of cortical cells within the parasite root that result in a noticeable bump at or near the tip meristem within 24 h. There is a concomitant elongation of epidermal cells into long, densely positioned haustorial hairs that are capable of adhering to host tissues (Baird & Riopel, 1985). Cortical swelling and haustorial hair proliferation are visual phenotypes of early haustorium development that occur prior to host contact.

Once the parasite has firmly attached to the host, a penetration peg invades the host epidermis and cortex by a combination of physical and enzymatic processes until it reaches the host stele. Within a few days of host contact, a successful haustorium will have invaded the host and established a functional connection between host and parasite vascular systems.

Development of the primary haustorium occurs by transformation of the radicle meristem of the parasite following its exposure to a suitable haustorial initiation factor (HIF) present in host root exudate (Riopel & Timko, 1995; Yoder, 1999; Keyes et al., 2000; Yoder, 2001). Perception of HIF in *Striga* results in the cessation of radicle elongation, an enlargement of cells in the parasite root protoderm and cortex, and the development of haustorial papillae, formed on epidermal surface (Joel & Losner-Goshen, 1994; Hood et al., 1998). A variety of molecules have been shown to function as HIFs like phenolic acids, flavonoids, and substituted benzoquinones (e.g., 2,6-dimethoxy -p-benzoquinone [2,6-DMBQ]) (Riopel & Timko, 1995; Yoder, 1997; Albrecht et al., 1999).

The first chemical haustorium-inducing factors (HIFs) identified were the flavonoids xenognosin-A and xenognosin-B. These were isolated from a fractionation of gum tragacanth, a commercially available, water-soluble mixture of dried *Astragalus* sap (Steffens et al., 1982). The first and only HIF isolated from host roots is 2,6-dimethoxy-p-benzoquinone (DMBQ) (Chang & Lynn, 1986). Benzoquinones are produced in plants by biosynthesis on the shikimate acid pathway, by oxidative decarboxylation of phenolic acids, and by the enzymatic degradation of cell wall phenols by peroxidases and laccases (Caldwell & Steelink, 1969). Interestingly, DMBQ was identified from sorghum (*Sorghum bicolor*) roots only after they were physically abraded or coincubated with *Striga* cultures (Chang & Lynn,

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1986). HPLC analyses showed that coincubation of root washes with *Striga* results in the generation of DMBQ through peroxidase-mediated oxidation of cell wall components (Lynn & Chang, 1990). Later experiments demonstrated that hydrogen peroxide generated at the *Striga* radical tip activates host plant peroxidases, which convert host cell wall phenols into haustorial-inducing benzoquinones (Kim et al., 1998; Keyes et al., 2007). The active extraction of HIFs from host roots provides a mechanism by which *Striga* ensures proximity to a host root prior to haustorial commitment.

The identification of natural HIF molecules has led to evaluation of other phenolic derivatives for their ability to induce haustoria (Riopel & Timko, 1995; Albrecht et al., 1999). Several active HIFs have been identified, including the simple phenolics syringic acid and vanillic acid; flavonoids, such as xenognosin A and peonidin; and p-benzoquinones, like DMBQ.

Not all HIF molecules are equally active, and different concentrations or times of exposure are needed for optimal haustoria development.

For example, haustorium initiation with syringic acid requires several more hours of exposure or severalfold higher concentrations than DMBQ. HPLC analyses showed that haustoria activity is dependent on syringic acid being enzymatically oxidized to DMBQ (Lynn & Chang, 1990). An important insight into the mechanism of haustorium signaling resulted from the observation that different haustoriuminducing benzoquinones had similar first-half volt redox potentials (Smith et al., 1996). This led to the hypothesis of a redox model for HIF signaling in which semiquinone intermediates, formed during redox cycling between quinone and hydroquinone states, initiate haustorium development. This model was evaluated with the chemical spin trap cyclopropyl-p-benzoquinone. A single electron reduction of the cyclopropyl ring of cyclopropyl-pbenzoquinone activates a reactive electrophilic center that irreversibly inhibits haustorium development in *Striga* in response to DMBQ (Zeng et al., 1996). The redox model hypothesizes that the first step in HIF recognition is the univalent reduction of benzoquinone to semiquinone.

Quinone redox changes are catalyzed by quinone oxidoreductases (EC 1.6.5), a subfamily of medium-chain dehydrogenase/reductases conserved in plants, primates, yeasts, and eubacteria (Persson et al., 2008). We previously isolated cDNAs from *Triphysaria* roots representing transcripts predicted to encode two classes of quinone oxidoreductases (Matvienko et al., 2001). Transcripts for both QR1 and QR2 are rapidly upregulated in *Triphysaria* roots as a primary response to treatment with DMBQ and other quinones (Matvienko et al., 2001).

Based on sequence homologies, QR1 was classified as a member of the z-crystallin-like quinone oxidoreductases (EC 1.6.5.5) (Thorn et al., 1995; Edwards et al., 1996) and QR2 as a quininereducing flavoprotein (EC 1.6.5.2) (Sparla et al., 1996; Matvienko et al., 2001). Later purifications of the QR2 enzyme showed that it catalyzes NAD(P)H-dependent quinone reduction with substrate and inhibitor specificity consistent with its placement into the *Diaphorase* family (Sparla et al., 1999; Wrobel et al., 2002). Recently Bandaranayake and co-authors (2010) hypothesis that quinolic-inducing factors activate haustorium development via a signal mechanism initiated by redox cycling between quinone and hydroquinone states. Two cDNAs were previously isolated from roots of the parasitic plant *Triphysaria versicolor* that encode distinct quinone oxidoreductases. QR1 encodes a single-electron reducing NADPH quinone oxidoreductase similar to z-crystallin. The QR2 enzyme catalyzes two electron reductions typical of xenobiotic detoxification. QR1 and QR2 transcripts are upregulated in a primary response to chemical-inducing factors, but only QR1 was upregulated in response to host roots. RNA interference technology was used to reduce QR1 and QR2 transcripts in *Triphysaria* roots that were evaluated for their ability to form haustoria. There was a significant decrease in haustorium development in roots silenced for QR1 but not in roots silenced for QR2. The infrequent QR1 transgenic roots that did develop haustoria had levels of QR1 similar to those of nontransgenic roots. These experiments implicate QR1 as one of the earliest genes on the haustorium signal transduction pathway, encoding a quinone oxidoreductase necessary for the redox bioactivation of haustorial inducing factors (Bandaranayake et al., 2010).

Effect of parasitism of the hosts

The broomrapes belong to phloem-feeding parasites that abstract their nutrition predominantly from the phloem of their host plant (Irving & Cameron, 2009). Although phloem-feeding parasites typically retain a xylem connection, they derive the majority of their C and N requirements from the host plants phloem and are often classified as obligate holoparasites. *Orobanche spp*. growing on *T. pratense* sequesters 10–30% of N uptake by the host plant, although this represented 73.6% of N taken up by the individual root(s)

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parasitized (Kawachi et al., 2008). This is a clear demonstration that parasitism has severe effects on the individual roots, while N uptake was negligible in the roots that were not directly parasitized. Interestingly, in Kawachi et al. (2008) the host plants were fed ^{13}N nitrate, which cannot be transported in the phloem, yet, *Orobanche* was apparently still able to sequester significant amounts of the ^{13}N tracer supplied. The reasons for this are unknown, although seem most likely to indicate significant N assimilation during the experimental period. In *Orobanche* parasitized tobacco plants, over 95% of N assimilated by the parasite is phloem derived, and thus amino acids, rather than N taken up directly from the soil (Hibberd et al., 1999). As an achlorophyllous holoparasite *Orobanche* is completely unable to photosynthesize by itself, and is therefore unable to survive and complete its life cycle without a host. Nitrogen assimilation is an energy and carbon dependent process, and without photosynthesis to supply the necessary reducing potential and C-skeletons, utilizing host N assimilation products is an energy efficient way of providing N for growth. Transfer of nutrients and carbon from the host plant is not facilitated by xylem-based mass flow, as in the xylem-feeding hemiparasites, since transpiration rates are very much lower in *Orobanche* than its host, even considering relative mass (Cernusak et al., 2004). *Orobanche* derives approximately 0.2% of its C supply from its host's xylem, while the amount of N measured in the xylem sap of the host could supply only 5% of that accumulated by the parasite (Hibberd et al., 1999).

Broomrapes parasitizing crops

Several broomrape species parasitize important crops (Amsellem et al., 2001; Rubiales, 2001; Goldwasser & Kleifeld, 2002; Rubiales et al., 2003a, 2003b, 2006, 2009a, 2009b; Joel, 2007; Satovic et al., 2009; Thorogood et al., 2009a,b). At present, over 73 million hectares of farmland under cultivation in the Middle East, Southern and Eastern Europe, and regions of North Africa are infested with broomrapes (Amsellem et al., 2001; Abang et al., 2007). The yield losses range from 5% to 100% depending on host susceptibility, level of infestation and environmental conditions losses and is estimated at hundreds of millions of dollars annually than affect the livelihoods of 100 million farmers (Amsellem et al., 2001; Abang et al., 2007).

For instance several *Orobanche* and *Phelipanche* species are of major importance in Europe where about 70% of the farming land in infected with seeds of broomrapes. *Orobanche crenata* Forsk. causes considerable damage to legume crops (faba bean, lentil, pea and common vetch) in Southern Europe and the Middle East (Amsellem et al., 2001; Rubeales et al, 2009a,b). *Orobanche cumana* Wallr. threatens sunflower crops in many countries around the world, especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, China (Shindrova et al., 1998). Recent announcements indicated probable existence of F and G races (Pujadas - pers. commmun.). .*O. cernua* attacks solanaceous crops in the Eastern Mediterranean and Southern Asia. *Orobanche minor* is common in Central Europe in clover. Recent investigation demonstrated its presence in UK where *O. minor* parasitizes not only clover but carrots as well (Thorogood et al., 2008). *Phelipanche ramosa* (L.) Pomel (formerly *O. ramosa*) attacks potato, tobacco, tomato and hemp (Slavov et al., 2001; Joel, 2007; Parker, 2009).

In the USA broomrapes are currently under control, *Orobanche* infestations exist in the states of Virginia and Georgia and in both *Orobanche* have been declared Federal Noxious Weed. Despite aggressive eradication measures infestations world-wide still persist and new outbreaks of the parasites have been reported (Joel, 2007; Parker, 2009; Rubiales & Heide-Jørgensen, 2011)

Currently there are no effective, inexpensive control measures for broomrape that can be applied in case of large scale outbreak. A wide variety of approaches, like hand weeding, use of selective herbicides, breeding for resistance, biological control, suicidal germination, and soil treatments by fumigation and solar heating have been explored, but none have been found to be sufficiently effective and affordable (Rubiales et al., 2009b; Westwood et al., 2010; Yoder & Scholes, 2010). Control strategies have largely focused on agronomic practices (Rubiales et al., 2009b), the use of resistant crops (Pérez-de-Luque et al., 2010) and the use of herbicides (Hershenhorn et al., 2009), although success has been marginal. There is, thus, an urgent need to re-evaluate control methods in the light of recent developments in crop breeding and molecular genetics and to place these within a framework that is compatible with current agronomic practices. Novel integrated management programmes should be sympathetic to agricultural intensification and exert minimal harmful effects on the environment. In addition, global environment changes, together with changing land use patterns, mean that some geographical areas and farming systems that do not currently suffer from parasitic weeds in Europe could become affected within the coming decades. It is essential, therefore, to pre-empt the spread of parasitic

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weeds and to consider, for example, how quarantine regulations might achieve this goal.

Future perspectives in Broomrape research

Recently (2009-2010) several new initiatives were launched: An international team of scientists from Albania, Bosnia & Herzegovina, Bulgaria, FYR of Macedonia, Spain, Serbia, and USA are using a combination of classical taxonomic approaches and modern techniques like ISSR markers, massive gene sequensing, and other molecular taxonomy in order to study broomrape's diversity and regional distribution on the Balkans. This study will allow us to achieve the following aims: 1) to make taxonomic revision of *Orobanchaceae* species for the region based on modern molecular techniques; 2) to obtain knowledge about tendencies in phylogeny and evolution of these unique plants; their co-evolution with the hosts, mechanisms of transition on crops/new host; and probably some data about the effects of the climate changes on their recent habitats; 3) to obtained data about habitats of unique and relict broomrape species which have to be preserved; 4) to survey of distribution of (potentially) dangerous parasitic weeds in agro-ecosystems and to create a map (including GIS data) that will allow better predictability of risks upon cultivation of crops and better in planning of control measures, crop rotation and treatments.

Simultaneously a Parasitic Plant Genome Project has started. It aims to sequence transcripts from three parasitic species and a nonparasitic relative in the *Orobanchaceae* with the goal of understanding genetic changes associated with parasitism. Parasitic species used were *Triphysaria versicolor*, *Striga hermonthica* and *Orobanche aegyptiaca*. *Lindenbergia philippensis* represents the closest nonparasite sister group to the parasitic *Orobanchaceae* and was included for comparative purposes. Tissues for transcriptome sequencing from each plant were gathered to identify expressed genes for key life stages from seed conditioning through anthesis. Two of the species studied, *S. hermonthica* and *O. aegyptiaca*, are economically important weeds and the data generated by this project are expected to aid in research and control of these species and their relatives. In addition, the sequences provide important information on target sites for herbicide action or other novel control strategies such as trans-specific gene silencing.

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