

Response of weedy and non-weedy broomrapes to synthetic strigolactone analogue GR24

Citlivosť burinných a divorastúcich záraz k syntetickému analógu strigolaktónov GR24

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Abstract

Parasitic plants of genera *Orobanche* and *Phelipanche* germinate after exposition to chemical signals exuded by roots of the host plants. The most studied germination stimulants belong to strigolactones (SLs), the newly discovered plant hormones which are stimulating hyphal branching of arbuscular mycorrhizal fungi and are involved in regulation of shoot and root architecture of plants. However, little is known about the effect of strigolactones on germination of non-weedy broomrapes. The objective of our study was to investigate the sensitivity of seeds of non-weedy broomrapes to synthetic analogue of SLs, GR24. The seeds of non-weedy broomrapes *Orobanche alba*, *O. alsatica*, *O. caryophyllacea*, *O. elatior*, *O. flava*, *O. lutea*, *O. pallidiflora*, *O. reticulata*, *Phelipanche arenaria*, *P. purpurea* and weedy species *P. ramosa* were collected in natural and cropland plant communities in Slovakia. Seeds of *P. ramosa* and *P. purpurea* were highly sensitive to GR24. On the other hand, effectivity of GR24 in inducing germination of several wild species, *O. alba*, *O. caryophyllacea* and *P. arenaria* was low, while the stimulant shown to be completely not effective on other non-weedy species *O. alsatica*, *O. elatior*, *O. flava*, *O. lutea*, *O. pallidiflora*, and *O. reticulata*. The results point out there are differences in the requirement for germination signals that possibly depend on the host.

Keywords: GR24, germination stimulant, *Orobanche*, *Phelipanche*, strigolactone

Abstrakt

Parazitické rastliny rodov *Orobanche* a *Phelipanche* klíčia len po expozícii chemických signálnych molekúl, ktoré sú vylučované koreňmi hostiteľských rastlín. Najviac študované stimulatory klíčenia sú strigolaktóny (SLs), ktoré boli nedávno charakterizované a zaradené medzi rastlinné hormóny. Strigolaktóny stimulujú vetvenie arbuskulárnych mykoríznych húb a podieľajú sa aj na architektúre koreňov a

výhonkov. Je však málo informácií o vplyve strigolaktónov na klíčenie divorastúcich zázraz. Cieľom našej práce bolo charakterizovať citlivosť divorastúcich zázraz k syntetickému analógu strigolaktónov, GR24. Semená divorastúcich zázraz *Orobancha alba*, *O. alsatica*, *O. caryophyllacea*, *O. elatior*, *O. flava*, *O. lutea*, *O. pallidiflora*, *O. reticulata*, *Phelipanche arenaria*, *P. purpurea* a burinného zázrazovca *P. ramosa* pochádzali z poľných kultúr a prirodzených spoločenstiev Slovenska. Semená *P. ramosa* a prekvapujúco aj semená *P. purpurea* boli vysoko citlivé ku GR24. Na druhej strane, GR24 bol menej efektívny pri indukcii klíčenia niektorých divorastúcich druhov *O. alba*, *O. caryophyllacea*, *P. arenaria*, a úplne neefektívny pri divorastúcich druhoch *O. alsatica*, *O. elatior*, *O. flava*, *O. lutea*, *O. pallidiflora* a *O. reticulata*. Výsledky naznačujú, že pre adaptáciu a špecifický výber hostiteľskej rastliny môže byť dôležitým parametrom aj životný cyklus hostiteľov. Výsledky poukazujú na rozdiely v požiadavkách na stimulátory klíčenia, ktoré môžu vyplývať zo zloženia stimulačných látok vylučovaných hostiteľmi.

Kľúčové slová: GR24, *Orobancha*, *Phelipanche*, stimulátor klíčenia, strigolaktóny

Introduction

Parasitic plants belonging to Orobanchaceae are considered as dangerous pests causing substantial losses of crop production in many countries of the world.

Out of approximately 170 Orobanchaceae species (Weiss-Schneeweiss, et al., 2006), 11 species are serious threat to food security. From genus *Striga*, three species *S. hermonthica*, *S. aspera*, and *S. forbesii* parasitize warm-climate cereal crops across Africa, including maize, sorghum, upland rice and *S. gesnerioides* parasitises dicotyledonous (broad leaved) hosts. *Orobancha cumana*, *O. crenata*, *Phelipanche ramosa* and *P. aegyptiaca* are important parasitic weeds damaging many agricultural crops in Europe, North Africa, Middle East and Asia (Parker, 2012). *P. ramosa*, the only weedy *Phelipanche* species in Slovakia, decreased tomato yield in Slovakia by 43-53% (Cagáň and Tóth, 2003), in Greece by 25% (Parker, 2009), tobacco yield in Turkey by 33% (Emiroglu, et al., 1987) and rapeseed yield in France by 85-90% (Gibot-Leclerc, et al., 2001). Non-weedy broomrapes are growing in wild plant communities and range of their hosts is limited, they parasitize one or few specific hosts only (Teryokhin, 1997).

Orobancha and *Phelipanche* spp. are obligate holoparasites. They are lacking chlorophyll and therefore they must attach to suitable host from which developing seedling acquire all nutrients and water to complete life cycle. Small seeds have very limited reserves for developing seedling. Therefore, they have to attach to the host root within a few days after germination. To assure, that seeds will germinate in the close vicinity of the host root, parasitic plants developed specific recognition mechanism. The seeds must be exposed to specific chemical signal exuded from the roots of host plants to be able to germinate. Several chemical compounds were identified as a germination inducing compounds. They include dihydrosorgoleone, the strigolactones, the sesquiterpene lactones (Bouwmeester, et al., 2003), dehydrocostus lactone (Joel, et al., 2011), peagol, peagoldione (Evidente, et al., 2009) and glucosinolate-breakdown products (Auger, et al. 2012). The most studied compounds are host-derived strigolactones (SLs). Strigolactones are apocarotenoids (Matusova, et al., 2005) and they were recently characterized as new plant hormones (Gomez-Roldan, et al., 2008; Umehara, et al., 2008). SLs induce germination of

parasitic plants, the strigolactones have been shown beneficial for establishment of plant-arbuscular mycorrhizal fungi (AMF) symbiosis by induction of hyphal branching (Akiyama et al. 2005), SLs are involved in regulation of shoot branching and root morphology (Gomez-Roldan, et al., 2008; Umehara, et al., 2008; Ruyter-Spira, et al., 2011; Kapulnik, et al., 2011) and are involved in symbiotic interaction of plants with nitrogen-fixing bacteria (Foo and Davies, 2011). Until now, 19 naturally occurring SLs were characterized (de Saint Germain, et al., 2013). Currently, biosynthesis and perception mechanism of strigolactones in plants is extensively studied by many research groups. Strigolactones are biosynthesized from the carotenoid pathway (Matusova, et al., 2005), but complete biosynthetic pathway is not discovered yet. A model for the interaction of the germination stimulants with their receptor has been proposed by Zwanenburg and co-workers ((Wigchert and Zwanenburg, 1999). Strigolactone-biosynthetic and perception pathway is studied mainly by analyses of mutants with SL-deficient and SL-insensitive mutants of *Arabidopsis*, rice and pea. Recent knowledge on SLs biosynthesis and perception was reviewed by Seto and Yamaguchi (2014). The characterization of *Arabidopsis* (*max2*) (Stirnberg, et al., 2002), rice (*d3*) (Ishikawa, et al. 2005), and pea (*rms4*) (Johnson, et al., 2006) mutants revealed involvement of the F-box protein in perception of SLs. This protein is a subunit of SCF ubiquitin E3 ligase for proteasome-mediated proteolysis. D53 protein (in rice) was identified as a repressor of strigolactone downstream responses (Jiang, et al., 2013; Zhou, et al., 2013). Jiang, et al. (2013) and Zhou, et al. (2013) proposed model, in which strigolactone binding to D14 protein (in rice) induce conformational changes of D14 and subsequent polyubiquitination of D53 for degradation.

Identification and isolation of natural strigolactones is very difficult due to very low level in plants and rhizosphere. Therefore, synthetic strigolactone analog GR24 (Fig. 1) is used in prevalent studies dealing with dormancy and germination of weedy parasitic plants belonging to *Orobanche*, *Phelipanche* and *Striga* spp. The objective of our study was to investigate the sensitivity of seeds of non-weedy broomrapes (*Orobanche alba*, *O. alsatica*, *O. caryophyllacea*, *O. elatior*, *O. flava*, *O. lutea*, *O. pallidiflora*, *O. reticulata*, *Phelipanche arenaria* and *P. purpurea*) and weedy *P. ramosa* to synthetic analogue of SLs, GR24.

Materials and Methods

Plant material

Seeds of *Phelipanche ramosa* (L.) Pomel, *P. arenaria* (Borkh.) Pomel, *P. purpurea* (Jacq.) Soják, *Orobanche reticulata* Wallr., *O. alba* Stephan ex Willd., *O. caryophyllacea* Sm., *O. lutea* Baumg., *O. elatior* Sutton, *O. flava* Mart. Ex F.W. Schultz and *O. alsatica* Kirschl. were collected from dry inflorescences in natural communities and fields in Slovakia (Tab. 1). All seed samples were collected, treated and kept under lab conditions in the same manner.

Table 1.

The broomrapes taxa used, their origin and host plants

Species	Location	Location co-ordinates	Host plant
Weedy broomrapes			
<i>Phelipanche ramosa</i>	Branovo	48°00'N 18°17'E	<i>Solanum lycopersicum</i>
<i>Phelipanche ramosa</i>	Domadice	48°10'N 18°46'E	<i>Nicotiana tabacum</i>
Wild broomrapes			
<i>Phelipanche purpurea</i>	Domica 1	48°28'N 20°28'E	<i>Achillea</i> spp.
<i>Phelipanche purpurea</i>	Domica 2	48°28'N 20°32'E	<i>Achillea</i> spp.
<i>Phelipanche arenaria</i>	Nitrica	48°41'N 18°25'E	<i>Artemisia campestris</i>
<i>Orobanche alba</i>	Nitrica	48°41'N 18°25'E	<i>Thymus serpyllum</i>
<i>Orobanche caryophyllacea</i>	Hačava	48°39'N 20°51'E	<i>Galium mollugo</i>
<i>Orobanche alsatica</i>	Turňa nad Bodvou	48°36'N 20°51'E	<i>Peucedanum cervaria</i>
<i>Orobanche elatior</i>	Penhýbel	48°32'N 18°32'E	<i>Centaurea scabiosa</i>
<i>Orobanche flava</i>	Blatnická dolina	48°54'N 18°57'E	<i>Petasites albus</i>
<i>Orobanche flava</i>	Oravská Polhora	49°33'N 19°24'E	<i>Petasites albus</i>
<i>Orobanche flava</i>	Východná	49°03'N 19°53'E	<i>Petasites albus</i>
<i>Orobanche lutea</i>	Gemer	48°27'N 20°18'E	<i>Medicago falcata</i>
<i>Orobanche pallidiflora</i>	Tisovec	48°42'N 19°52'E	<i>Cirsium</i> spp.
<i>Orobanche reticulata</i>	Pusté	48°59'N 19°36'E	<i>Carduus glaucinus</i>

Sterilization and conditioning

Seeds of parasitic plants *Phelipanche* and *Orobanche* spp. require period of conditioning to break dormancy and to be able to respond to germination stimulant. Sterilization and conditioning of seeds were performed according to Matusova et al. (2004). Briefly, seeds were sterilized in 2% commercial bleach containing 0.02% (v/v) Tween 20 for 5 min and washed several times in sterile demineralized water. Approximately 120-150 seeds were spread on each 8 mm (diameter) glass fibre filter paper (GFFP) disc. 12 discs were placed into 9 cm Petri dish containing 2 layers of Whatmann filter paper wetted with 2.7 ml of sterilized demineralized water. Petri dishes were sealed with parafilm and incubated in darkness at 21 °C for 14 days to break dormancy (Matusova et al. 2004).

Germination bioassay

GFFP discs with conditioned seeds were shortly dried in a flow cabinet to remove surplus moisture and transferred into new Petri dishes containing ring of wet filter paper according to Matusova et al. (2004). The synthetic strigolactone analogue GR24 at the concentrations of 100, 10, 1, 0.1, 0.01, 0.001, 0.0001, 0.00001 and 0.000001 mg·l⁻¹ was added to each of three replicate disks (40 µl/ disk). Demineralized water was used as a negative control. Petri dishes were sealed with parafilm and incubated in darkness at 26 °C for 7 days. The germinated and nongerminated seeds were counted using Zeiss stereomicroscope DV4.

Calculation of logistic dose–response curves

Calculation of dose-response curve was used to quantify the response of the seeds to the germination stimulant GR24. Logistic dose-response curves with non-linear regression were calculated using GraphPad Prism, Version 6.05 (GraphPad Software, Inc.).

Results and Discussion

The sensitivity of *Orobanche* spp. and *Phelipanche* spp. seeds to the germination stimulant GR24 at broad range of GR24 concentrations (from 0.000001 mg·l⁻¹ up to 100 mg·l⁻¹) was tested. Germination was concentration dependent for all responsive species and germination assays showed different sensitivity of weedy and non-weedy broomrape seeds to the applied GR24 (Fig. 1, Tab. 2).

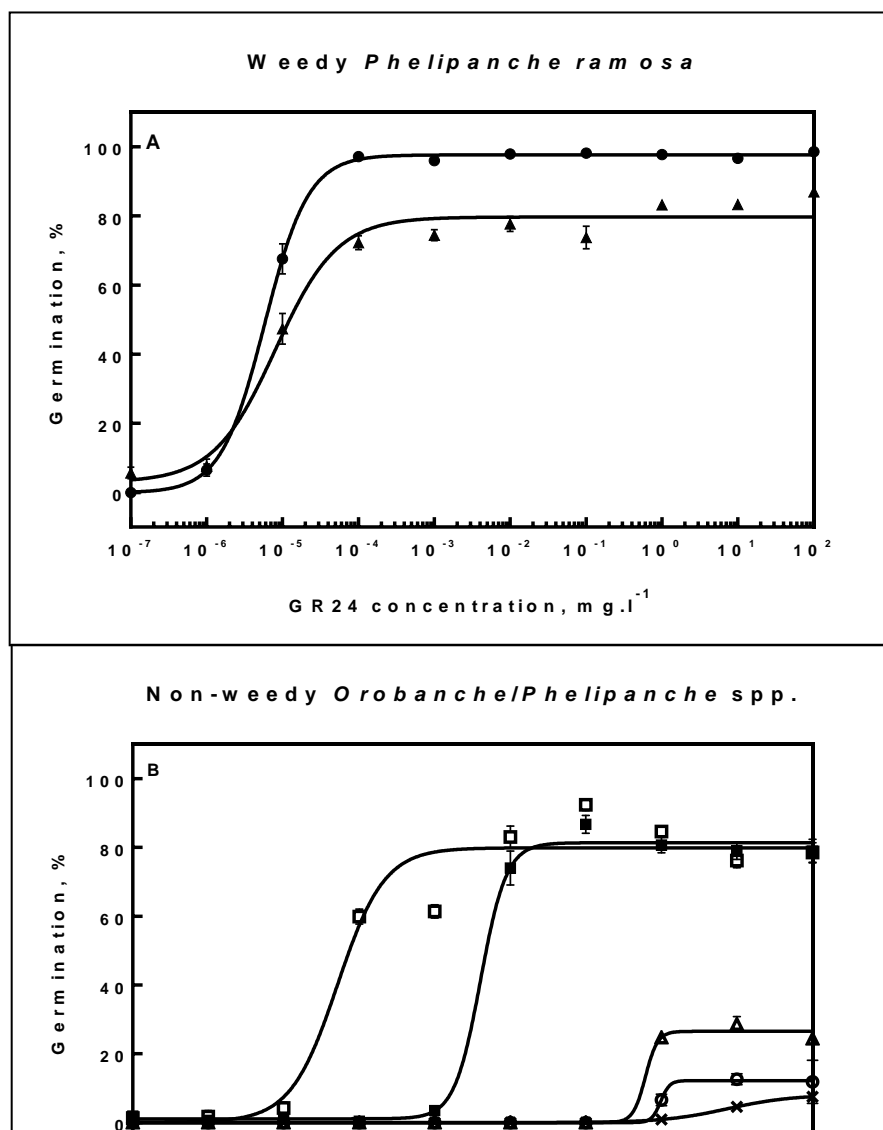


Figure 1.

Dose-response curves for germination induced by GR24 in (A) weedy *Phelipanche ramosa* (Br – locality Branovo, Do – locality Domadice) and (B) wild *Orobanche/Phelipanche* spp. Seeds of *P. ramosa* Br (●), *P. ramosa* Do (▲), *P. purpurea*1 (□), *P. purpurea*2 (■), *O. alba* (x), *P. arenaria* (Δ) and *O. caryophyllacea* (○) were preconditioned at 21 °C for 14 days. Each point represents mean of three replicates ± SE. Dose-response curves with non-linear regression were calculated using GraphPad Prism, Version 6.05. GR24 logistic dose-response curve parameters are shown in Tab. 2.

Table 2.

Parameters of GR24 dose-response curves of *Phelipanche* and *Orobanche* spp. in Fig. 1A, B. R_{max} – maximum germination induced by GR24, $[EC]_{50}$ – dose required to induce 50 % of maximum germination, p – Hill-coefficient, negative values and “~”(ambiguous) values are the results of the curve fitting.

Species	R_{min} (%)	R_{max} (%)	$[EC]_{50}$ (mg.l ⁻¹)	p
<i>P. ramosa</i> Br	-0.071	98	0,000006	1,506
<i>P. ramosa</i> Do	2.966	80	0,000008	1,070
<i>P. purpurea</i> 1	0.145	80	0,000051	1,400
<i>P. purpurea</i> 2	1.110	81	0,004025	2,531
<i>O. alba</i>	-0.013	8	7,5480	1,015
<i>P. arenaria</i>	0.000	27	~ 0,6139	~ 5,371
<i>O. caryophyllacea</i>	0.000	12	~ 0,9786	~ 7,334

GR24 was highly effective in inducing germination of weedy *P. ramosa*, what is consistent with previous findings (Fernández-Aparicio, et al., 2009; Thorogood, et al., 2009; Matusova et al., unpublished data). *P. ramosa* germinated up to 99% at exposition to 10⁻⁴ mg*l⁻¹ for population Branovo (Fig. 2A). Seeds collected at locality Domadice were slightly less viable and maximal germination reached 87 %. The sensitivity of both seed populations is about the same (for both population in range of 10⁻⁶ mg*l⁻¹ GR24 to induce half maximal germination, Tab. 2). The different geographic regions and growth conditions of different host plants, are most likely reasons for observed differences in viability between the two *P. ramosa* populations. Seeds of other weedy broomrapes including *O. cumana* (EC_{50} at 10⁻³ mg*l⁻¹, Matusova, et al., 2004), and *O. crenata* (EC_{50} at 10⁻² mg*l⁻¹, Matusova, et al., unpublished data) were also highly sensitive to the GR24.

Compared to weedy *P. ramosa*, we observed less or no stimulation of non-weedy broomrapes seeds germination by GR24. Seeds of *Orobanche alba*, *O. caryophyllacea*, *P. arenaria* and *P. purpurea* responded to germination stimulant

GR24. Germination of *O. alba*, *O. caryophyllacea*, and *P. arenaria* were induced only by concentration of GR24 $1 \text{ mg}\cdot\text{l}^{-1}$ or higher. *P. purpurea* was intermediately sensitive to GR24, half maximal germination was induced by a concentration of $10^{-5} \text{ mg}\cdot\text{l}^{-1}$ for population *P. purpurea*1 and by $10^{-3} \text{ mg}\cdot\text{l}^{-1}$ GR24 for *P. purpurea*2. In this species we observed the decrease in the seed germination upon higher concentrations of GR24 ($1\text{-}100 \text{ mg}\cdot\text{l}^{-1}$, Fig. 2B). The similar effect was found for *Orobanche crinita* and *Orobanche densiflora* (Fernández-Aparicio, et al., 2011), *S. asiatica*, *S. hermonthica*, *S. aspera* and *O. crenata* (Wigchert, et al., 1999). The high concentration of germination stimulant does not necessarily induce maximal germination of seeds and the non-optimal high concentration of GR24 might inhibit germination of parasitic weed seeds. Therefore the optimal conditioning and concentration of germination stimulant should be tested before routine germination tests.

Seeds of *O. alsatica*, *O. elatior*, *O. flava*, *O. lutea*, *O. pallidiflora*, and *O. reticulata* did not germinate after treatment with GR24. A number of other wild broomrape species were discovered not to respond to GR24 likewise (Fernández-Aparicio, et al., 2008; Thorogood, et al., 2009). Thus it seems to be questionable to utilize GR24 as the universal standard for germination tests.

Seeds of all species were conditioned and germination tests performed under the same conditions. Increase of germination stimulant concentration may lead to reduction of radicle length or to short radicle not emerging from the seed coat (Joel and Bar, 2013). Despite of the different sensitivity of seeds to GR24 resulting to different germination percentage, emerging seedlings of all responding *Phelipanche* spp. and *Orobanche* spp. in our experiments have an elongated radicles, which indicate optimal concentrations for all species tested.

Weediness or non-weediness of parasitic plants is based basically on the host plant and the damage they cause to economically important agricultural crops (Rubiales, et al., 2009; Parker, 2013). It is obvious that weedy broomrape species are less specialized in germination requirements; their seeds germinate upon exposition to different plant root exudates (Fernández-Aparicio, et al., 2009), purified strigolactones or GR24 and grow on various annual crops. However, there are a few known exceptions. In contrast with other weedy broomrapes, *O. cumana* evolved as a weedy species more recently (Parker, 2013). *O. cumana* specifically attacks sunflower (*Helianthus annuus* L.) and seeds of *O. cumana* are less responsive to strigolactone analogue GR24 and more responsive to sunflower root exudate (Fernández-Aparicio, et al. 2009). Indeed, seeds of *O. cumana* are highly sensitive to dehydrocostus lactone, which was recently identified as an active germination stimulant for *O. cumana* in sunflower root exudates (Joel, et al., 2011). The importance of other compounds for germination of broomrapes was also demonstrated for weedy *P. ramosa* and its host *Brassica napus*. In the rhizosphere of non-mycotrophic *B. napus* were identified isothiocyanates, which are breakdown products of glucosinolates, to be the main germination stimulants for *P. ramosa* present in *B. napus* (Auger, et al., 2012). The authors did not detect any known strigolactone by ultraperformance liquid chromatography-tandem mass spectrometry in the rhizosphere. The low/no exudation of strigolactones by roots *Arabidopsis thaliana* (Brassicaceae family) was observed by Kohlen, et al. (2011). The authors detected two strigolactones (orobanchol and orobanchyl acetate) in root exudates and also other no strigolactones-like compounds with the germination-inducing activity on *P. ramosa* seeds.

In contrast to weedy broomrapes (except *O. cumana*), the non-weedy species have only one or just a few, usually perennial hosts (Teryokhin, 1997). For instance, *Artemisia campestris* is practically the only host of *P. arenaria* (Piwowarczyk and Przemysky, 2010), *Centaurea scabiosa* is a host of *O. elatior* (Zázvorka 2010) and *Orobancha ballotae* A. Pujadas is strictly monophagous on *Ballota hirsuta* (Pujadas Salva 1997). We detected very low or no germination of non-weedy broomrapes induced by GR24. Wild species have probably more specific germination requirements. Fernández-Aparicio, et al. (2009) tested seeds of nine broomrape species for their sensitivity to root exudates of 41 different plants. Seeds of non-weedy *O. hederiae*, *O. gracilis* and *O. densiflora* highly specifically germinated with one or a few root exudates only. Our results are in agreement with work of Fernández-Aparicio, et al. (2009). In general, GR24 was much less effective for non-weedy broomrape.

The single broomrapes might require unique combination and concentrations of signaling chemicals (Fernández-Aparicio, et al., 2011; Höniges, et al., 2012). In wild ecosystem there are growing many different plants (potential hosts) in the same area, colonized by arbuscular mycorrhizal fungi. They also might produce less strigolactones or strigolactones might be released to the rhizosphere in very low quantity. Therefore, other compounds might be crucial for germination of some non-weedy broomrapes as it was already shown for some weedy species.

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