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in Branched Broomrape (*Phelipanche ramosa*)
management in Tomato**

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Chapter 1:

General introduction, research objectives and thesis outline



1.1 Introduction

Parasitic plants are a taxonomically and biogeographically diverse group of organisms that include both angiosperms and gymnosperms. There are approximately 3000–4000 parasitic angiosperm species in total (Press, 1998) that belong to 270 genera and 22 families (Nickrent, 2002) with parasitism having independently evolved approximately 11 times (Barkman et al., 2007). Parasitic plants are distributed- from the arctic to the tropics (Watling and Press, 2001). By far the most economically damaging root parasitic weeds are members of the *Orobanchaceae* mainly species belonging to the genera *Striga*, *Orobanche* and *Phelipanche*. They are widespread in the Mediterranean areas in Asia, Southern and Eastern Europe and North Africa attacking crops (Parker and Riches, 1993).

Orobanche and *Phelipanche* species commonly known as broomrapes are found in temperate regions of the northern hemisphere as well as in the sub-tropics and tropics, especially in arid and semi-arid regions. The Mediterranean region where large areas are heavily infested (Parker and Riches, 1993) is assumed to be the center of origin of broomrapes. The parasites have recently been reported in the USA, Australia and some countries in South America (Rubiales et al., 2009). The broomrape species infect important crops including members of the Solanaceae, Fabaceae, Compositae, Cruciferae, and Umbelliferae families (Parker and Riches, 1993). Annual crop losses from *Orobanche spp.* in the Middle East are cautiously approximated at \$1.3 billion to \$2.6 billion (Aly, 2007). Sauerborn (1991) estimated that over one million ha of faba bean in the Mediterranean region and western Asia are infested or at risk from *O. crenata*. The parasite can cause losses of up to 100% on farmers fields, which they often have to abandon due to non- productivity (Kroschel and Klein, 2004). Yield losses due to *Orobanche spp.* vary between 5 to 100% depending on host susceptibility, level of infestation and environmental conditions (Abang et al., 2007).

1.2 Broomrape status in Sudan

In Sudan, three *Orobanche* and *Phelipanche* species *P. ramosa* (L.) Pomel, *O. cernua* Loef. var *desertorum* (G. Beck) Stapf and *O. minor* SM. were reported as early as 1948 as minor pests on horticultural crops, ornamental plants, and common weeds (Andrews, 1956). *O. crenata* Forsk, was introduced recently as it was not



reported prior to the year 2001 (Babiker et al., 2007). *P. ramosa* and *O. crenata*, the most noxious and pestiferous among the *Orobanchae spp.*, are mainly confined to the fertile alluvial soils of the Nile Valley (Babiker et al., 2007). *P. ramosa* (branched broomrape) was first reported in Wadi Halfa on the southern border of Egypt and in Khartoum in 1948 (Andrews, 1956). Since the 1970s, the parasite has become a major pest on solanaceous crops. It has spread into central Sudan and has become a limiting factor to tomato production in the rich alluvial soils along the Nile banks from El Gaily in Khartoum State to southern borders of the Gezira State. Recently, infestations were reported along the Blue Nile to the South of Roseries (Babiker et al., 2007).

1.3 The life cycle of *Phelipanche*

Phelipanche spp are obligate root holoparasites characterized by a lack of chlorophyll and depend completely on their hosts for sustenance. The tiny *Phelipanche* seeds ($0.35 \times 0.25 \mu\text{m}$, 3 to 6 μg) are limited in energy reserves and after germination they must attach themselves to a host root within days or otherwise they will perish (Parker and Riches 1993). Estimates of seed production per single plant range between 31 000 to 500 000.

Seeds can survive in the soil for more than 20 years, display dormancy and in general require after ripening period in a warm and dry environment. This period is broadly called after-ripening or post-harvest ripening (Matusova et al., 2005). A second requirement for germination is the preconditioning of the seed, which requires about 7 days of a warm (15-21°C), wet environment before seeds respond to germination stimulants. Apparently, during the conditioning period, seeds are released from dormancy through an increase in seed coat permeability and /or changes in the levels of endogenous germination promoters or inhibitors (Press et al., 1990). For germination, preconditioned seeds require chemical stimulants from potential hosts.

Once germinated, *Phelipanche* can survive in the free-living state only a few days due to the small seed reserves. Thus, in nature germination must be synchronized with the presence of a plant root within close proximity of the seed.



After germination, the radicle grows towards the host root and this process is thought to be directed by the concentration gradients of germination stimulants (Dubé and Olivier, 2001). Contact between the tip of the radicle and the host root starts an attachment process that leads to the formation of a haustorium-like structure. The haustorium is a multifunctional organ acting as a conduit for the flow of water and nutrients from host to parasite (Hood et al., 1998). The establishment of this organ requires other host-derived chemical signals to initiate and guide this developmental transition. Among these chemicals are the quinones 2, 6-dimethoxy-1, 4-benzoquinone (DMBQ), a degradation product of host root lignin (Chang and Lynn, 1986) and 5, 7-dihydroxynaphthoquinone (Takeuchi et al., 1995) and the flavonoid peonidin (Albrecht et al., 1999).

Following the establishment of the connection with the host, the parasite develops underground structures the so-called tubercles that assist to accumulate nutrients. The tubercle is a juvenile parasite. At a certain stage, it matures and forms a flowering shoot (plate 1.1) that emerges above the soil surface and produces flowers and seeds. The development of both the juvenile and the mature parasites (plate 1.2) is coordinated with that of the host (Joel, 2000).



Plate 1 1 *Phelipanche ramosa* infestation of tomatoes in the field in Khartoum, Sudan (Photos by Tilal Sayed).

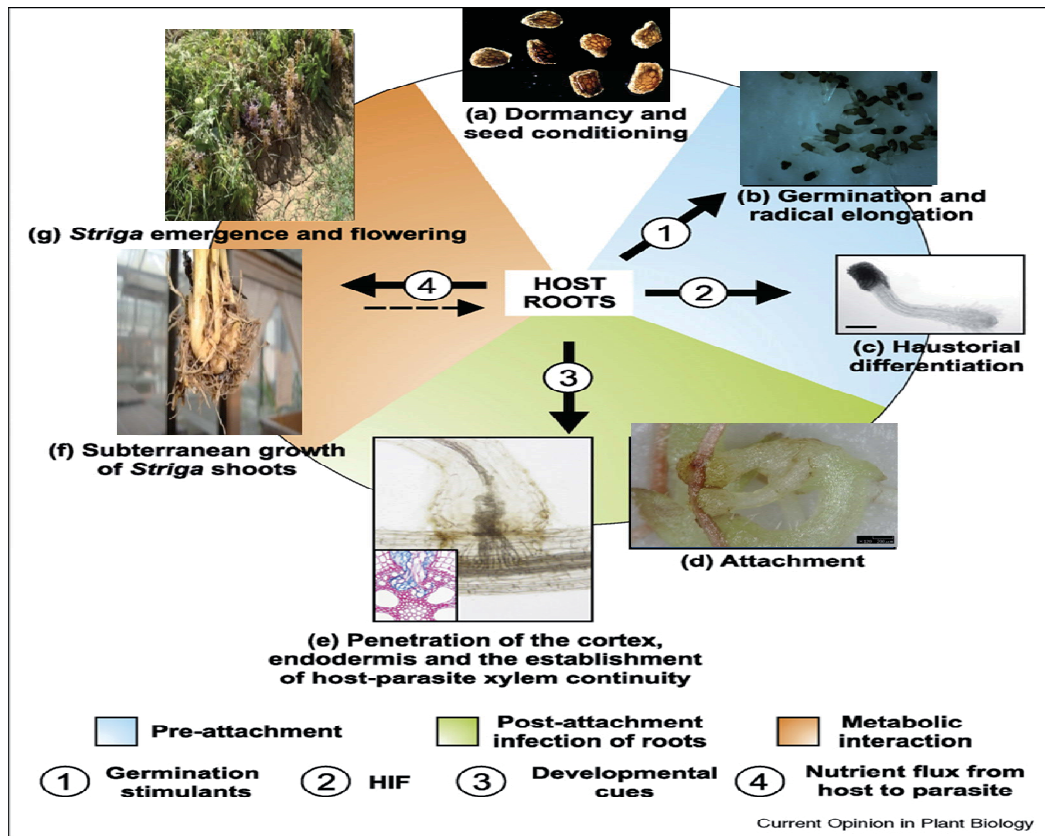


Plate1. 2 *Striga* and *Phelipanche* life cycle (modified from Scholes and Press, 2008)



1.4 Natural and other germination stimulants

The germination stimulants play an important role in the fine-tuning of the lifecycle of the parasites to that of their hosts (Matusova et al., 2005). The first naturally occurring germination stimulant for *Striga* was isolated in 1966 from root exudates of cotton, a non-host for *Striga* or *Orobanchae* (Cook et al., 1966). The rough structure of strigol was elucidated in 1972, and its absolute stereochemistry was established by x-ray diffraction analysis in 1985 (Cook et al., 1972; Brooks et al., 1985). Later on sorgolactone, a compound with a structure similar to strigol was isolated from sorghum (*Sorghum bicolor* (L.) Moench), a genuine host for *Striga* (Hauck et al., 1992). Afterwards, the same authors reported the isolation of a germination stimulant, alectrol from the root exudates of cowpea, which is a host for *S. gesnerioides* (Müller et al., 1992). The collective name 'Strigolactones' was proposed for this class of molecules (Butler, 1994). Another strigolactone, orobanchol, was isolated from red clover, *Trifolium pratense* L., an *Orobanchae* host (Yokota T. et al., 1998). Moreover, Xie and co-workers found two interesting compounds having a 2'-epi stereochemistry and a benzene ring namely: solanacol. 2'-Epi-orobanchol, respectively (Xie et al., 2007). However, more recently, Xie et al. (2008) have shown that alectrol is most probably orobanchyl acetate (Xie et al., 2008). In most cases, the compounds were recently ascribed to be apocarotenoids instead of sesquiterpene lactones (Matusova et al., 2005).

Several studies confirmed that germination of *Striga*, *Orobanchae* and *Phelipanche* seeds is induced by other natural compounds including Sesquiterpene lactones (which are not strigolactones), cytokinins, auxins, gibberellins, cotylenins, fusicoccins and jasmonates (Fischer et al., 1989; Babiker et al., 1992; Logan and Stewart, 1995; Yoneyama, al., 1998a,b ; Xie et al., 2010).

Ethylene has been found to efficiently stimulate witchweed (*Striga spp*) seed germination (Eplee, 1975). In fact, it has been the major component of an integrated witchweed management program in the US from 1956 to 1996 (Eplee, 1975). However, ethylene gas is pressurized, flammable and requires specialized storage and application equipment for soil injection and it is costly. This makes its direct use very hazardous and generally unsuitable for small-scale African farmers. A role of ethylene in *Phelipanche* seed germination has been proposed, but has never been



clearly demonstrated. However, recently, Zehhar et al. (2002) strongly suggested that ethylene synthesis is also required for the induction of *P. ramosa* seed germination. Further research on the role of ethylene as germination stimulant on broomrape species is needed.

There are also several synthetic compounds that induce germination of parasitic plants (Reizelman and Zwanenburg 2002). Among them are the strigolactones GR24 and Nijmegen-1. GR24 is a very potent synthetic stimulant which induces germination of many *Orobanche* and *Striga spp.* and is widely used as a positive control in most laboratory experiments (Reizelman and Zwanenburg, 2002). Nijmegen-1, was developed for possible commercial release by Zwanenburg and Thuring (1997). Later on, Benvenuti and co workers (2002) reported that application of Nijmegen 1 to tobacco field leads to 75% reduction of *P. ramosa* seed bank at 0-10 cm depth and dropped to about 35% at 20 cm depth.



1.5 *Orobanche* and *Phelipanche* control

A multitude of control options against *Phelipanche* and *Orobanche* have been studied including cultural measures like hand weeding and tillage (Parker and Riches 1993), soil solarization (Sahile et al., 2005), delayed sowing (Grenz et al., 2005), crop rotation (Babiker et al., 2007), intercropping (Fernández-Aparicio et al., 2010), suicidal germination (Eplee 1975), chemical control (Qasem 1998), biological control (Klein and Kroschel, 2002), and host plant resistance (Echevarría-Zomeño et al., 2006; Fernández-Aparicio et al., 2010). Despite the high potential of some of those solutions so far no single option has shown to be both sufficiently effective and durable as well as economically and practically applicable for low-input farming systems (Joel, 2000).

Because the plant parasites exert much of the damage to host crops during the early phases of attachment, control approaches should target the initial steps in the host–parasite interaction, of which germination is the first (López-Ráez et al., 2009). As germination stimulants play an essential role in the life cycle of parasitic plants this might be an important target for the development of new control strategies for agriculturally important parasitic weeds. Bouwmeester et al. (2003) suggested that the infection of the parasitic plant can be reduced by lowering strigolactone production in hosts. Some consequences of management approaches using knowledge of germination stimulants are discussed below.

1.5. 1 Control through enhanced germination

1.5.1.1 Suicidal germination

Suicidal germination is regarded as the induction of germination in the absence or away from the hosts root. Suicidal germination could be achieved by introducing either natural or synthetic germination stimulants into the soil in the absence of a suitable host leading to both seed bank depletion and death of weed seedlings because of complete dependence on the host for their sustenance (Parker and Riches, 1993).

Evidente et al, (2006) tested about 25 natural analogues and derivatives of fusicoccin and cotylenol for their stimulatory effects on in vitro seed germination of *P. ramosa*.



Among these compounds, 8, 9-isopropylidene of the corresponding FC aglycone and the dideacetyl derivative were considered as the most active FC derivatives (Andolfi et al., 2004; Evidente et al., 2006). Later on, Algit Super an extract of *Ascophyllum nodosum* was found to be an effective stimulant for the germination of *P. ramosa* seeds (Economou et al., 2007). Other synthetic stimulants include Strigol analogues and ethylene. However, most of these chemicals are labile and have a very short persistence (Babiker and Hamdoun, 1983). Lack of stability precludes leaching of the chemical to desired soil depths (Vurro et al., 2012). Another limitation of this approach is that the synthetic stimulants should be easy to handle and affordable to peasant farmers particularly in the African continent, where the problem exists.

1.5.1.2 Trap and catch Crops

Trap cropping relies on plants which offer the advantage of stimulating germination of the root parasites without themselves being parasitized (Parker, 1991; Fernández-Aparicio et al., 2009). Most recently, Fernández-Aparicio et al., (2011) suggested that pea could be a promising candidate as trap crop for *O. foetida* and *P. aegyptiaca*. Catch-cropping is another means of depleting *Striga* and *Orobanche* seed reserves in soil. Contrary to trap cropping, which relies on false hosts, catch cropping employs true hosts of the parasite which are allowed to be parasitized, however, the crop has to be destroyed before the parasite can reproduce (Babiker et al., 2007). The efficacy of catch and trap crops could possibly be increased if overproduction of germination stimulants can be achieved through selection and breeding.

1.5.2 Control through reduced germination

1.5.2.1 Using Chemicals

Classically, strigolactones have been considered to be sesquiterpene lactones (Akiyama et al., 2005; Akiyama and Hayashi, 2006). However, recently it was shown that strigolactones are derived from the carotenoid biosynthesis pathway (Matusova et al., 2005). Therefore, it has been hypothesized that the strigolactone pathway and ultimately seed germination can be inhibited by carotenoid biosynthesis inhibitors and Jamil et al. (2010) found that the carotenoid inhibitors, fluridone, norflurazon, clomazone and amitrole, applied to rice either through irrigation or through foliar



spray significantly decreased strigolactone production and *S. hermonthica* germination and attachment. However, addition of fluridone and norflurazone during the conditioning period of *S. asiatica* shortened the conditioning period (Kusumoto et al., 2006) and prevented the inhibitory effects of both light and supraoptimal temperature (40°C) on seed germination (Chae et al., 2004). In addition, seed treatment with the same compounds after conditioning in water induced germination in a manner similar to the effects of natural germination stimulants (Chae et al., 2004).

Ibrahim et al (1985) and Babiker et al. (1988) reported that aqueous extracts from several *Euphorbia* spp. including *E. hirta* and *E. aegyptiaca* induced germination and haustorium initiation in *Striga hermonthica*. However, to the best of our knowledge, no similar work has been reported with *Orobanchae* and/or *Phelipanche* spp.

1.5.2.2 Breeding for low germination stimulants

One of the best characterized mechanisms of host resistance to parasitic weeds is reduced exudation of the compounds required for stimulation of the parasite seed germination by host plant roots (Hess et al., 1992). Genotypic variability in production of *Striga* germination stimulants in *Sorghum* has been described (Hausmann et al., 2001; Ejeta 2007). Genes encoding low stimulant production were identified and introduced into high-yielding sorghum cultivars in several African countries. Genetic variation for the induction of *P. aegyptiaca* germination has also been described in tomato (El-Halmouch et al., 2006) making breeding for low germination stimulants (LGS) feasible and attractive. In addition, it has recently been shown that different cultivars of tomato produce/exude largely different amounts of strigolactones (López-Ráez et al., 2008a). The tomato mutant high pigment-2 (hp-2dg), an important mutant line endowed with high levels of carotenoids including lycopene, was found to be less susceptible to *O. aegyptiaca* infection than the corresponding wild-type. The reduced susceptibility to the parasite correlated well with a lower production of strigolactones (López-Ráez et al., 2008b). Several other mutants in tomato and maize, such as notabilis and vp14, respectively, also produce less strigolactones than their corresponding wild types (Matusova et al., 2005; López-Ráez et al., 2008b). Overall, results indicate that selection of *Striga* and *Orobanchae* spp. resistant cultivars and/or lines based on low germination stimulant production is a valid and



promising strategy. However, strigolactones also play a role in the interaction with AMF (see 1.6.2) and reducing them may not only have positive effects.

1.6 Bringing together different components into a systems approach

Many of the factors discussed above may contribute to the control of parasitic weeds. However, many of the possible interactions are not fully understood and need more attention.

1.6.1 *Strigolactones and nutrient status of the plants*

As described above, *Striga*, *Orobanche* and *Phelipanche* tend to be associated with low soil fertility. Several reports showed that fertilizers, mainly phosphorus and nitrogen, lead to significant reductions in infestation of host crops by *Striga*, *Orobanche* and *Phelipanche* and the reduced infestation appears to be linked with alteration in strigolactone production (Yoneyama et al., 2001; Raju et al., 2006). On the other hand, elements such as K, Ca or Mg seem to have no influence on strigolactone production (Yoneyama, et al., 2007a,b).

Recent research showed that production of strigolactones was invariably higher under phosphorus starvation (Yoneyama, et al., 2007b; López-Ráez et al., 2008a). López-Ráez et al., (2008 a,b) reported that tomato plants grown for 4 days in phosphorus deficient nutrient solution induced over three fold higher germination of *P. ramosa* seeds than that induced by the corresponding control with full strength phosphorus. In sorghum, N deficiency and/or P deficiency was reported to promote production and exudation of 5-deoxystrigol (Yoneyama et al., 2007 a,b). In rice, P deficiency (Umehara et al., 2010) as well as N deficiency (Jamil et al., 2011) was shown to enhance strigolactone exudation. The relationship between production of strigolactones and nutrient availability may explain the prevalence of root parasitic weeds on soils of poor fertility especially those low in organic matter or those with high pH where nitrogen and available phosphorus are predominantly low. Therefore, fertilizer rate and composition should be carefully optimized.



1.6.2 Mycorrhiza and strigolactones

A recent investigation into the effect of AMF on parasitism by *Striga* on sorghum and maize reported a strong correlation between an increased soil mycorrhizal load, and increased resistance of host plants to damage by *Striga* (Lendzemo et al., 2005). The effect was ascribed partly to improved crop performance owing to enhanced nutrient uptake by the successfully colonized roots, and partly to a reduction or delay in *Striga* germination and emergence (Lendzemo et al., 2005, 2007). Accordingly, the use of mycorrhizae for integrated management of parasitic weeds was proposed (Lendzemo et al., 2005).

Besides being natural germination stimulants for the seeds of root parasitic plants (Bouwmeester et al., 2007) strigolactones are also host detection signals for AMF in the rhizosphere (Akiyama et al., 2005). This can explain in part the impact of AMF on parasitic plants. AMF inoculation leads to a reduction of the impact of *Striga*, apparently related to a reduction in strigolactone production (Lendzemo et al., 2007). Most recently, López-Ráez et al. (2011) observed that root extracts from tomatoes colonized by *G. mosseae* induce lower germination of *P. ramosa* seeds compared to the corresponding control non-mycorrhizal plants (López-Ráez et al., 2011). Moreover, a reduced production of strigolactones in a tomato mutant correlated with less susceptibility to *Phelipanche* spp. (López-Ráez et al., 2008 b). Thus, down regulation of strigolactone production may be achieved by enhanced AMF colonization instead of breeding for reduced strigolactone production.

1.6.3 Integration of various components into the a system approach

It is possible to affect tomato root production of germination stimulants by altering host plant growing conditions. Proper soil management practices involving the use of AMF, crop residues as good source of phosphorus, and nitrogen application could contribute to control parasitic weed genera *Phelipanche*. In order to be able formulate an effective systems for control *P. ramosa* in tomato, it is important to know which AMF species present in import Sudanese ecosystems. As *Phelipanche* germination is dependent upon the quantity and quality of strigolactones, we need to know are different tomato genotypes and AMF fungal species influence the stimulation pattern,



and the exact relationship between fertilizer application, mycorrhiza inoculation and *Phelipanche* infestation.

1.6.4 Research objectives and thesis outline

The present study was undertaken with the primary objective of developing simple, environmentally friendly methods for control of *P. ramosa* on tomato focusing on arbuscular mycorrhizal fungi (AMF) and plant extracts of *E. hirta*. A second objective was to determine if and what mycorrhiza fungi are present in important crops in Sudan

The thesis is divided into seven chapters. Chapter 2 describes the taxonomic diversity of Sudanese AMF associated with thirteen agricultural crops at the White Nile state, Sudan. In the subsequent chapter (Chapter 3) the effects of colonization of tomatoes by different *Glomus* species on the effects of tomato root and stem exudates on *Phelipanche* seed germination in vitro are studied. For this, three tomato varieties commonly grown in Sudan were inoculated with three *Glomus* species at two inoculum densities. Root exudates were collected 21 or 42 days after transplanting (DAT) and stem exudates 42 DAT and tested for their effects on germination of *P. ramosa* seeds in vitro. The effects of air-dried powder and an aqueous extract from *E. hirta* on germination and haustorium initiation in *P. ramosa* are studied in Chapter 4. In Chapter 5, results of a glasshouse pot experiment are described that was undertaken to investigate whether AMF could have an influence on *Phelipanche*-tomato interactions in vivo. The effects of three *Glomus* species on Tomato 'Strain B' which is sensitive to *Phelipanche* were tested. In Chapter 6, the combined effect of AMF, organic amendments based on sugar cane filter mud and N fertilization on occurrence of and damage by *P. ramosa* under field conditions in Sudan are described. Finally, Chapter 7 presents the general discussion and possible implications of the study for *Phelipanche* management.