Proceedings of the meeting “Broomrape: biology and resistance”

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1. Knowledge of seedbank size, germination ecology and emergence dynamics as tool to improve *Orobanche* control strategy

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The possibility to link agronomic and ecological studies could be have in the future a crucial role for *Orobanche* rational control in the agro-ecosystem. Previous works (Benvenuti et al., 2001; Bari meeting) evidenced the possibility to decreasing the seedbank size by using (directly in field) germination stimulant solutions for trigger the “suicide” germination. Indeed parasitic seedlings, in the absence of the host crop, have no any survival chance. To optimise the stimulant efficacy is opportune to know both ecophysiological and agronomic dependence of the stimulant action.

In this background ecological and agronomic experiments are focused on the follows investigation points: 1) seedbank evaluation; 2) buried seed germination-ecology; 3) emergence dynamics. I schematise the importance of the several investigation topics.

a) Seedbank analysis: 1) preventive evaluation of the risk for the cropping of new areas (high seed longevity-> permanent seedbank); 2) preventive “convenience-analysis” for the heavy-infested fields (introduction of the concept of threshold limit of parasitic seedbank level); 3) optimisation of soil tillage operations (seedbank distribution in the several arable soil layers).

b) Buried seed germination-ecology: 1) time of primary dormancy-breaking and possibility to increase it (for an eventual use of germination stimulant after the annual seed production and before the relative burial); 2) maximum soil depth for germination (oxygen involvement as limiting factor?); 3) effect of soil microenvironment (texture, compaction?); 4) maximum soil depth for “suicide” germination stimulant action? Agronomic possibility to increase it?

c) Emergence dynamics: 1) prevision of the emergence periods: by using cumulative thermal degree and/or crop phenological stage; 2) emergence-seed ripening time (seed viability dynamics) for a timely destruction of the annual weed seed production; 3) soil management: no soil inversion (chisel, no tillage) for long-term soil surface seed concentration (of annual production) and a consequent high activity of the germination stimulant (and/or catch crop).

Only by achieving precision in seed dynamics simulation will it become possible to make parasitic weed control optimally effective overall, as in this case, there are no available efficient herbicides. The studied topics could be crucial to explore new agronomic strategy for crop protection.
2. Inheritance of resistance to *Orobanche cumana* in sunflower

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Virulent populations of broomrape (*Orobanche cumana* Wallr.) belonging to races F and G in Spain and Turkey have overcome all known resistance genes *Or1* to *Or5* in cultivated sunflower (*Helianthus annuus* L) and are spreading rapidly. This presentation deals with recent results on the development of resistance to race F in Spain and with preliminary results on inheritance of resistance to this race, not reported in previous meeting of the COST action.

Results of evaluation of sunflower germplasm for resistance to populations belonging to race F have shown that wild *Helianthus* species constitute the major source of resistance genes conferring resistance to new virulent races but valuable sources of resistance can also be found in cultivated germplasm. Four germplasm populations, BR1 to BR4, resistant to race F have been developed through interspecific hybridization of cultivated susceptible material and the perennial wild resistant *Helianthus* species *H. divaricatus*, *H. maximiliani* and *H. grossesserratus*. Moreover, through selection and selfing within cultivated germplasm of different origin, were obtained five lines P-96, K-96, K-96, L-86 and W-14 that bred true for resistance to race F. All these lines were also resistant to races B and E but segregated for resistance when tested against a more virulent population probably belonging to race G. Resistance to race F of broomrape has been also reported by several seed companies. Studies have been carried out to characterize the inheritance of three of the lines derived from cultivated material and one line derived from wild species. The *F*$_1$ obtained crossing the resistant lines derived from cultivated material and a susceptible line showed susceptible reaction indicating that resistance in this material is recessive. Evaluation for resistance of segregating generations *F*$_2$ and BC$_1$F$_1$ to the resistant parent, in the crosses with the resistant sources derived from cultivated material, approached resistant to susceptible ratios of 1:15 and 1:3 respectively, in most of the crosses suggesting a double dominant epistasis but segregations 3:13 and 1:3 for *F*$_2$ and BC$_1$F$_1$, respectively, indicating dominant-recessive epistasis were also found. Conversely, results of crosses using one resistant line derived from the wild perennial species *H. divaricatus* and *H. grossesserratus* indicated dominance reaction of resistance genes. The evaluation of *F*$_2$ and BC$_1$F$_1$ to both parents confirmed the dominance reaction of the *F*$_1$ and indicated that the resistance is under the control of a single dominant gene. Preliminary results on allelic studies and on mapping resistance to race F indicate that resistance lines derived from cultivated material differ at least in one gene and QTL’s associated with resistance to race F.

Further research is needed on allelic studies using different sources of resistance, inheritance studies of all the resistant sources and different susceptible lines and molecular markers studies to associate QTL’s with genes of resistance. Research is also needed on testing methodology such as incubation procedures (amount of inoculum, temperature, moisture etc) and correlation between artificial tests and evaluations under field conditions.
3. Seed ecology and crop resistance to *Orobanche*

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Through a simulation of life cycle of *Orobanche* spp., opportunities and control options for each stage of the life cycle may be identified. Using a simulation model it is then possible to simulate the potential impact and benefit of interventions on long-term integrated control. A major component of the life cycle simulation of root parasites such as *Orobanche* – as for many weed species – is the seed cycle. This cycle commences with seed production but the fate of seeds in the soil seedbank is crucial. The impact of the environment on seed depletion and germination and on the attachment of seedlings to the host root must be considered.

Models developed at Reading assist in understanding some of these aspects of seed ecology.

For example, imbibed seeds awaiting stimulation by host root exudates are undergoing three internal physiological processes (Figure 1). These are

♦ Conditioning (development of sensitivity to root exudates)
♦ secondary (wet) dormancy (observed as a loss of sensitivity to root exudates), and
♦ loss of viability

It is also possible to simulate population dynamics of the parasite in different contexts. A good question is how resistant does the crop need to be to stabilise the seed bank in the soil?

Using a simulation model, it is predicted that successful attachments must be reduced by over 99.5% to stabilise the seed bank. Even if other control methods are introduced, the resistance would need to be effective in a very large proportion of cases (Figure 2).

The simulations are necessarily based on simplifying assumptions. It is, therefore, important that all predictions are validated in the field. In addition, the robustness of any simulated control strategy should be tested in a range of environments before being used as an aid to decision-making by farmers.

![Fig. 1 Processes in Orobanche seeds during conditioning at 10°C](image)

![Fig. 2 Annual change in the Orobanche soil seed bank at different rates of unsuccessful attachment to the host. Initial soil seed bank assumed to be 13000 m$^{-2}$. The simulation assumes either no other control method (solid line) or that 80% of mature plants are prevented from seeding. Adapted from Kebreab & Murdoch (2001). *Expl Agric.* 37: 37-51.](image)
4. Crop protection against parasites/pathogens through expression of sarcotoxin-like peptide

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A novel approach for the control of parasitic plants, the most destructive and intractable weed in many economically crops throughout the world had been suggested. The unique technology is based on a cecropin-type antibacterial peptide which enhances parasitic weed resistance in transgenic crops.

The nature of parasitic weeds makes control extremely difficult, costly or hazardous to the environment. While the potentially simplest and most effective approach to parasitic weed control – host resistance – remains an unrealized goal for agriculture, we have developed a simple genetic engineering strategy for conferring parasites resistance on a host plant. Therefore this technique is superior to other methods in that it is: completely effective, low cost of implementation for producers and safe for the environment. The primary component of the system is an antimicrobial polypeptide, sarcotoxin IA. The gene coding for this protein is linked to a promoter that is induced in response to parasitization such that high quantities of the toxic protein are generated specifically at the point of parasite entry. The result is a crop plant with absolute resistance to parasitic weed.

Initial works have made a certain progress towards the control of parasitic weeds. We were able to enhance resistance and to increase yields in transgenic crops such as tobacco, potato and tomato expressing sarcotoxin peptide under the control of a root-specific promoter. We have already linked the sarcotoxin gene to a strong inducible promoter that will target the protein to the point of parasite attachment, where it would be most beneficial in protecting the crop. The use of inducible promoter will ensure that activation of the transgene will occur only in the infested tissue on the desired time. This is a novel approach potentially applicable to many crop species where parasitic weeds or plant pathogens are a problem.
In the interaction between parasitic plants such as *Orobanche* and *Striga* spp. and their hosts, secondary metabolites play an important role in several stages of the parasite’s lifecycle, e.g. for the induction of germination, the direction of radicle growth, haustorium formation and sometimes in determining compatibility. We focus on the first step of this lifecycle, germination, which requires secondary metabolite signalling molecules exuded from the roots of the host plant, known as germination stimulants. The adaptation of the parasitic weeds to these germination stimulants is of great evolutionary importance because their tiny seeds contain only small amounts of reserves and can not survive for more than a few days after germination if a host is not present. However, the germination stimulants may also be a valuable target for mankind to solve or alleviate the problems caused by the parasitic weeds. As these germination stimulants regulate such an important step in the lifecycle of the parasitic plants, we are studying the regulation of their formation in host plant roots using an array of multidisciplinary techniques, such as:

**Pathway research.** Several authors have elucidated the structure of the germination stimulants of the *Striga* hosts maize, sorghum and cowpea, and the false host cotton, and the *Orobanche* host red clover. Considering the fact that these compounds are active for parasitic plants from two distinct genera and are derived from host plants from a range of different plant families, the compounds so far identified are strikingly similar. All of them were categorised to be sesquiterpene lactones. The ubiquitous precursor of all sesquiterpenes is farnesyl diphosphate, which is supposed to be derived from the cytosolic mevalonate pathway. In order to demonstrate the involvement of sesquiterpene biosynthesis, we made enzyme extracts of the roots of high germination stimulant producing sorghum and maize varieties. Feeding of radio-labelled farnesyl diphosphate did not yield any sesquiterpene product, as demonstrated by radio-gas chromatography and GC-MS analysis. To get a better idea of the biosynthetic origin we have designed an in vitro system to grow maize and sorghum, so that we can more easily apply some specific inhibitors of isoprenoid biosynthesis. The first preliminary results suggest that in maize and sorghum the germination stimulants are indeed not sesquiterpenoid in origin, but are derived from the plastidic pathway. For the *Orobanche* hosts, arabidopsis and tobacco, the germination stimulants have not yet been identified, but experiments with specific inhibitors of particular parts of the isoprenoid biosynthetic pathways indicate that in these species the germination stimulants are also more likely to be plastidic than cytosolic in origin, and hence can not be sesquiterpene lactones either. All this suggests that – although many authors have characterised the germination stimulants as sesquiterpene lactones – they seem to actually originate from a different pathway.

**Metabolomics.** Analytical techniques are being developed to enable high throughput analysis of germination stimulants (in collaboration with Prof. Mike Beale of LARS, UK).

**Proteomics.** Attempts are being made to identify the receptor of the germination stimulants (in collaboration with Prof. Binne Zwanenburg of Nijmegen University, The Netherlands)

**Molecular approach.** *Arabidopsis* is also used as a molecular tool to pick up GS-biosynthesis related genes. At Plant Research International we have a collection of several thousands of activation-tagged arabidopsis mutants. From this collection we are screening thousands of tagged lines for changes in the induction of *Orobanche* seed germination. The lines with interesting phenotype are analysed at the molecular level, and so far six genes were found that may be related to parasitic weed seed germination. In addition, we collaborate with Profs Mike Beale and Keith Edwards of Long Ashton Research Station/University of Bristol, UK, in screening a maize transposon knock-out collection for altered germination-phenotypes. In future collaboration we will use arabidopsis genes also to look for maize homologues.
Our Research Groups are currently investigating molecular aspects of the plant-parasitic angiosperm interaction, by using sunflower-\emph{O. cernua} as one of the experimental system models. Our interest is directed to evaluate the specific role played by sunflower secondary metabolites, concretely specific phenolic and terpenoids.

In our past COST meeting at Bari, we presented data indicating that the sunflower 7-hydroxilated coumarins (scopoletin, scopolin and ayapin) can avoid broomrape parasitism by preventing successful germination, penetration and/or connection to the host vascular system. These data have been recently published (Serghini et al., 2001. J. Exp. Bot. 52: 1-8).

We have tested several sesquiterpene lactones as possible inductors of \emph{O. cernua} seed germination, by using an \textit{in vitro} assay on paper. These compounds bear features common to any sesquiterpene lactones previously isolated from sunflower. In general, all the tested compounds induced germination, with some of them being more actives than GR-24. They seem to be quite specific as did not induce germination of other parasitic species, concretely \emph{O. ramosa}, \emph{O. crenata}, and \emph{O. aegyptiaca}. Presented results have already been published (Pérez de Luque \textit{et al.}, 2000. Phytochemistry 53: 45-50; Galindo \textit{et al.}, 2002. J. Agric. Food Chem. 50: 1911-1917).

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6. On the search of \textit{Orobanche cernua} (sunflower broomrape) germination stimulants

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Phytoalexins are low molecular weight compounds from the plant metabolism, which are only synthesized upon the attack by pathogens, and which enable the plant to defend itself against the attacking organism. Generally phytoalexins have been discussed in the context with plant resistance to fungi, bacteria and (a little strange) to viruses. Phytoalexins generally are toxic to the invader and therefore can protect the plant. Indispensable condition is that the phytoalexin synthesis occurs fast enough and can build up sufficient local concentrations at the site of infection; this is often forgotten by people, who deny the usefulness of phytoalexins. The reason is that the pathogen may detoxify the phytoalexin in its own metabolism, and hence overcome the “resistance”.

Phytoalexin synthesis is induced by elicitors. These elicitors can be cell wall constituents of the invader. In many cases, however, they appear as decomposition products from the plant cell wall after a chemical attack. Elicitors can also be specific polypeptides from fungi or bacteria.

The chemical nature of phytoalexins is extremely manifold. There are compounds known from almost all classes of metabolites. Of course a phytoalexin does not necessarily act against all pathogens, in each case the toxicity to a certain pathogen has to be shown.

Phytoalexins can protect plants against Orobanche attack, and probably also against the other root parasites like Striga and Alectra. This requires the additional condition that the phytoalexin is available in the root. This is also sometimes forgotten.

From the breeders’ view phytoalexins are simply and mostly dominantly inherited and hence easy to handle in breeding programs.

We have formerly shown that phytoalexins are involved in Orobanche resistance in sunflower. While scopoletin is synthesized in the sunflower root, unfortunately its relative ayapin is not. Scopoletin is toxic to Orobanche, but also metabolized and thus detoxified. May be that more aggressive Orobanche pathotypes (which are well known from various countries) have a higher potential for metabolic detoxification of scopoletin! Ayapin would not be metabolized and therefore probably would not loose its activity, but it is only synthesized in the sunflower leaf. Jesus Jorrin has presented valuable results with 7-hydroxycoumarines in sunflower. Holger Buschmann also is involved in this field.

We also have seen phytoalexins involved in chickpea. Chickpea lines resistant to Orobanche crenata produce in their roots maackiain and medicarpin.

There is evidence that phytoalexins are also involved in cowpea resistance to Striga and Alectra. It would be very useful to study this system, because the resistances are simply inherited (the lines are available at Kano, Nigeria), but not identical for Striga and Alectra resistance.

There are many crops, in which simple resistance is unknown, Vicia faba, tobacco, sorghum as examples. The phytoalexin trait in such crops may be introduced by alien gene transfer. This requires the knowledge of the metabolic pathways and the mode how the biosynthesis is expressed.

The manifold pathways leading to phytoalexins can not be presented in a short time. In some cases at least the key enzymes are known and found more active under infection conditions, e.g. PAL (phenylalanine ammonia lyase) in the biosynthesis of polyphenols and phenylpropanoids, or stilbene synthase in the biosynthesis of resveratrol and other stilbene-phytoalexins.

Another phenomenon should be included in integrated Orobanche control. With certain fungicides, but also herbicides, the fortification of phytoalexin synthesis as a side effect has been reported. These compounds do not induce phytoalexin synthesis per se. But after a fungal attack they produced up to 80 times as much phytoalexin compared with untreated controls. A careful choice of fungicides or herbicides would contribute to the self-defence of the plants. This
phenomenon has not yet been studied in the context with *Orobanche* control, but should it not be?

Integrated control!

Systemic acquired resistance (SAR) is a further step in this direction. Benzothiadiazol (known as Bion) treatment of crop plants induce fungal resistance. Holger Buschmann currently studies the action of this compound on the *Orobanche* resistance in sunflower.

### Suggestions

**for further exploration of phytoalexins in *Orobanche* control**

- Known phytoalexins should be tested for their toxicity to *Orobanche*. This can be carried out with submersed *Orobanche* tissue cultures.

- Identification of phytoalexins as resistance factors in *Orobanche* resistant crop plant cultivars. Because of their simple inheritance the phytoalexin concept is most favourable in breeding programs.

- Gene banks shall be screened for genes of the key enzymes in biogenetic pathways of phytoalexins in order to win a basis for genetic modification of crops towards *Orobanche* resistance. As phytoalexins against *Orobanche* must be produced in the root, also the search for favourable root-specific promoters is necessary.

- In the programs for integrated *Orobanche* control phytoalexin fortification and cross protection (e.g. by harmless soil microorganisms) should be considered.
8. Hypersensitive reaction and necrosis of *Orobanche crenata* tubercles in legumes: histological studies

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In this work we have studied some histological aspects related to the resistance of legumes to *Orobanche crenata* (broomrape). We re-examined two resistant responses in plant-parasitic plant interaction, previously referred to as (1) hypersensitive reaction (HR) of the host and (2) necrosis of *Orobanche* tubercles. Some host plants were reported to develop HR that block haustorial penetration of various parasitic plants. But detailed histological studies of the relevant mechanisms have hardly been undertaken and there is no evidence so far that HR really occurs in all these host plants in a manner similar to that described for fungal attack including the necrosis of host cells. Another reported phenomenon, is the death of *Orobanche* tubercles after penetration into resistant host tissues. In this latter case, the parasite succeed in establishing connection with host tissues, but the tubercles become dark and die in an early developmental stage.

We observed a darkening in the site of contact with the root. However such darkening does not really include necrosis of host cell tissues but is the result of a different resistance mechanism which colour the tissues around the penetration site: Lignification of the endodermis cells in contact with the parasite intrusive cells seems to be the responsible for the unsuccessful broomrape seedling penetration. Then the parasite releases a higher amount of enzymes in order to overcome this barrier, and they give a dark aspect under low magnification resembling necrosis. If the parasite succeed avoiding this defensive mechanism and establishes vascular connections with the host, the excess of enzymes can reach the host xylem following the transpiration stream, blocking the vessels or activating a further defensive process related to wilt diseases. As a consequence the nutrient flux between host and parasite is interrupted and the *O. crenata* tubercles become necrotic and die in an early developmental stage.

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The resistance mechanism of *Vicia atropurpurea* against *Orobanche aegyptiaca* is based on thickening and lignification of root cell walls. This is effective in all temperature conditions tested (27-12°C). The effect of temperature is indirect, only through influence on the growth of the host and the parasite. In contrast, the resistance in the sunflower variety ‘Ambar’ to *Orobanche cumana* is effective only at temperatures above 25°C. Temperature dependence was also found in resistant pepper variety ‘Ma’or’ against *Orobanche aegyptiaca*. Pot experiments in soil heavily contaminated with broomrape seeds were conducted in different seasons. In the summer only 1 inflorescence per plant emerged above the soil and a total of 2 parasites were found on the roots of ‘Ma’or’ variety plants, while 23 parasites were found on the roots of susceptible pepper varieties. At the autumn, severe damage was induced by broomrape to the plants of ‘Ma’or’ variety, that were tolerant in the summer. Few parasites emerged above the soil, but more then twenty were found on the roots of both resistant and susceptible pepper plants. To elucidate this phenomena polyethylene bag experiment was conducted under three temperature regimes: 27-22°C; 22-17°C; 17-12°C. Under the highest temperature conditions, broomrape infection was very low on the resistant pepper roots, while under intermediate temperature regime, pepper resistance was totally lost, (8-14 parasites per plant). The pepper plants developed poorly, reaching height, that was two folds less then the plants grown at the highest temperature regime. All pepper varieties germinated *Orobanche* seeds at the same rate (50-60%) at the high and intermediate temperature regimes, but under low temperature regime, seed germination started a month later and achieved only 23%. Under those conditions both resistant and susceptible pepper plants were three folds smaller and the germination rate and the virulence of the parasite significantly decreased, therefore the rate of infection dropped. Low temperatures are not suitable for growth of both the parasite and the host. We assume that the resistant mechanism of ‘Maor’ plants was not activated under unsuitable growth conditions (intermediate temperature), but these regime still were favorable for *Orobanche* development. These results indicate that the temperature effect should always be considered in host-*Orobanche* interactions.
10. Chemical mutagenesis of tobacco for broomrape resistance

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Tobacco is one of the most important crops, which are attacked by *Orobanche* spp. in Bulgaria. There are no natural sources of resistance to broomrape identified in genera *Nicotianae*. Tobacco seeds from cultivar Nevrokop 1146 and Burley 21 were treated with EMS and NEU and plants as well as their M₁ to M₄ progenies were screened for resistance to broomrape by growing in artificially infested soil with *Orobanche* spp. seeds under greenhouse conditions. Increasing the number of no infected tobacco plants was observed in M₃ generation. In M₄ progeny number of plants that escaped infestation with broomrape declined. Only five plants of cultivar Nevrokop 1146 and no one plant from Burley 21 were broomrape-free at the end of the growing season. The investigation of the resistance in the next progeny of the non-infested tobacco plants is under test this year. Treatment of the tobacco seeds by NEU and EMS can drop the susceptibility of tobacco to broomrape and tobacco forms can be selected, eventually resistant to the parasite, but the selection process is suggested to continue at least five generations.
11. Resistance mechanisms in sunflower

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In order to find broomrape resistant \textit{Helianthus} genotypes a screening of numerous wild, hybrids, lines and varieties was carried out under glasshouse conditions. A more accurate study of the most interesting genotypes shows that \textit{H. debilis debilis} –215 x \textit{H. annuus} derived genotype (LR1) induces parasite necrosis leading to a decrease in broomrape emergence and flowering. 92B6, an inbred line derived from interspecific gene pool (\textit{H. argophyllus}-92 X \textit{H. annuus}) exhibits broomrape necrosis at a later stage and only seldom flowers of the parasite were observed. Development of a sunflower/broomrape hydroponic co-culture system allowed a study of defence reactions in LR1. The response of this genotype involved cell wall thickening, xylem vessel occlusion and cell division in cortical parenchyma and phloem. All these defence reactions decrease water and nutrient transfer to parasite. Radiolabelled (\textsuperscript{14}C) photoassimilates transfer from the host to \textit{O. cumana} was lower when the parasite was growing on the LR1 genotype than when it was growing on the susceptible sunflower. Resistance study of recombined inbred lines (RIL), derived from a cross of sunflower with LR1, firstly show the existence of lines more resistant than LR1. Secondly, resistance mechanisms (low stimulation of broomrape germination and parasite necrosis) are not linked since low stimulating lines could also induce parasite necrosis and inversely.

This will lead to, both the localisation of gene groups involved in the different resistance mechanisms to \textit{Orobanche cumana} and a more precise understanding of resistance inheritance. A further objective would be to obtain broomrape polygenic resistant sunflower.
12. Resistance to \textit{(Orobanche crenata)} in grain legumes

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Broomrape \textit{(Orobanche crenata)} is a major constraint for grain legumes in the Mediterranean region and West Asia. Breeding for resistance is still the most economic, feasible, and environmentally friendly method of control. However, the lack of a suitable screening method and of an effective selection index and the fact that resistance to broomrape in legumes seems to be scarce and of a complex nature, make breeding for broomrape resistance a difficult task. Host plants might escape broomrape infection by reduced root biomass, root architecture, and phenology, or they might hamper infection by resistance mechanisms acting at different phases of the infection process. Little is known about the nature of available sources of resistance and on its inheritance. Resistance is under quantitative genetic control. Recent molecular studies have shown that resistance in faba beans (Vf136) is controlled by two major QTLs, explaining 58% and 18% of the phenotypic variance for resistance.

Varying levels of resistance against broomrape in faba beans have been identified in field screenings, but no complete resistance has been identified so far. Considerable attention has been given to exploit the faba bean genetic resistance of F402. As a result, resistant cultivars have been released to farmers in Egypt under the commercial names ‘Giza 674’, ‘Giza 429’ and ‘Giza 843’. In Spain the original source of resistance was the line VF1071 (a selection from F402). It was used in breeding to develop the well adapted, high yielding cv. Baraca.

Resistance to broomrape is not available in commercial cultivars of pea and scarce in pea germplasms but available in wild species of \textit{Pisum}, mainly \textit{P. fulvum}. These species have been hybridised successfully with pea and a breeding programme is underway to accumulate this resistance in adapted pea cultivars. Field screenings have yielded identification of levels of resistance also in species of the genera \textit{Lens}, \textit{Cicer}, \textit{Vicia} and \textit{Lathyrus}. 
13. Strategies for the Application of Marker-Assisted Selection

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The question was raised whether Marker-Assisted Selection (MAS) should be considered as 'Most Advanced Selection method' or as a kind of 'Money-Absorbing System'. To give an answer, it is necessary to distinguish between qualitative characters and complex quantitative traits.

Marker-assisted back-crossing of a qualitative trait from a donor genotype into elite lines may consist of foreground selection (i.e., selection of the desired donor genes), background selection (i.e., selection against the undesired genetic background of the donor), or a combination of both. By using a combination of both techniques, the trait transfer can be finalized within 3 - 4 instead of conventional 6 generations. MAS for qualitative traits can therefore be considered as established, advanced selection technique.

When aiming at MAS for quantitative traits, QTL (quantitative trait locus) identification in a mapping population is followed by the transfer of the identified QTL into elite materials via pure MAS or MAS combined with phenotypic selection. Alternatively, "Advanced Backcross QTL Analysis" may be used which is a combination of QTL analysis and development of superior genotypes. For complex, quantitative traits, the efficiency of MAS is not uncontested. There are a number of risks which can result in MAS becoming a „money-absorbing system“:

- Unreliable QTL estimates (i.e., too few QTL with highly overestimated effects); this may be avoided by using a large mapping population, a marker set with good genome coverage, phenotypic values based on multi-environment field trials, and cross validation of the gained data.
- QTL not being expressed in a new genetic background; avoidance by verification of QTL effects using independent population samples or different genetic backgrounds.
- Recombination between marker and QTL; avoidance by close linkage and verification through phenotypic tests all 3-4 generations.
- Bad alleles of other genes being linked to good QTL alleles; avoidance by high marker density around QTL to allow for a marker-based reduction of the linkage drag.
- Marker analyses being too expensive; avoidance by checking the economic parameters and optimizing individual procedures.

In breeding for resistance to parasitic weeds, MAS can be highly advantageous when: parasite appearance is erratic; resistance tests are difficult, expensive and unreliable ($h^2$); resistance can only be determined after flowering; parasite is quarantined; breeding materials are advanced in off-season nurseries where the parasite does not occur; resistance genes are recessive, restricting the utility of classical back-cross schemes; and when resistance is determined by a few „big“ QTL. The latter are easier to identify and can be transferred quickly by the mentioned combination of foreground and background MAS, leading to resistant cultivars in a relatively short period of time.
14. Analysis for susceptibility / resistance to Orobanche using a set of sunflower recombinant inbred lines

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Recombinant inbred (RI) lines are obtained by self pollination at each generation of one plant since the F2 to the F8-F9 generations. Since half of the loci are fixed at each generation after nine generation the loci remaining heterozygous are \(1-(1/2)^8\). They enable both to construct a map and to evaluate resistance / susceptibility of each progeny that leads to assign QTLs.

A set of RI lines was produced between HA89 a susceptible sunflower line and another line LR1 derived from H. debilis 215 that displayed an original mechanism of resistance to O. cumana. Labrousse et al. 2002 have shown that the RI lines displayed a 0% to 100% range of aptitude to stimulate Orobanche seed germination whereas the two parents display 35% and 89% for LR1 and Ha89, respectively. Moreover 28 days after infestation, 100% of Orobanche plants spontaneously died on LR1 whereas only 60% died on Ha89. Moreover, these two different criteria for measuring Orobanche resistance were found unlinked that means different mechanisms and therefore different genes contribute to the expression of Orobanche resistance in sunflower.

The RI lines set will be used for map construction to look for Orobanche QTL analysis.
15. *Orobanche ramosa* control in tomato with herbicides or using transgenic glyphosate-resistant crop.

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In Greece some 30% of tomato area, or 15,000 ha are thought to be infected by *Orobanche ramosa* to a varying and increasing degree with losses averaging 25%. Control if any (on 60% of farms) is by hand-pulling only. In island Crete, however, where the problem is localized within 200 ha, control is achieved by solarization (80%) or methyl bromide (20%).

In order to find a solution to this problem we used several herbicides. Chlorsulfuron, glyphosate and imazaquin were the most effective of them. All three herbicides reduced the dry weight of *Orobanche* shoots and underground attachments more than the number of these organs. This indicates that these herbicides suppress the growth of *Orobanche* rather than kill its underground attachments. However, chlorsulfuron applied at 5.0 g A.I./ha gave 100% control of the number and dry weight of emerged broomrape shoots and underground attachments, showing that under certain conditions this herbicide may completely prevent parasite development. The results of the pot experiments, also indicated that chlorsulfuron applied at 5 g AI/ha was the most effective herbicide for broomrape control and the least toxic to the crop.

Under field conditions chlorsulfuron applied at 10 g AI/ha controlled broomrape emergence by 88%. When the herbicide was applied, twice (5+10 g AI/ha) it gave complete control of broomrape but delayed crop maturity.

Imazaquin applied at 9.25 g AI/ha did not effectively control broomrape in pot experiments. Higher rates of this herbicide increased its effectiveness but reduced crop yield. In field experiments, the same rates and even higher were less effective against broomrape and less toxic to the crop.

Glyphosate applied at 45 g AI/ha did not control broomrape. Its effectiveness was increased at the rates of 90 and 180 g AI/ha, but not consistently. All the rates used of this herbicide were not toxic to the crop grown either in pots or in the field.

The results of this study indicated that chlorsulfuron could effectively control broomrape in tomato, while imazaquin and glyphosate may have some potential uses for this purpose. However, more research is needed to determine the period of herbicide application during which the parasite is most sensitive while the crop is most tolerant.

The other direction of our studies was the use of transgenic crop. Since last years, research has been directed towards the use of transgenic crops against parasites, we decided to investigate whether *Orobanche* could be controlled efficiently in transgenic glyphosate-resistant tomatoes. Greenhouse experiments were conducted using the line 1232 of processing tomatoes, engineered with the plasmid pMON894, which contained a gene from *Escherichia coli*, coding for an altered glyphosate-resistant enzyme: 5-enolpyruvyl-shikimate 3-phosphate synthase. Non-transgenic tomato plants (UC82B) were also used. Monsanto kindly provided seeds of both tomato lines. Glyphosate was applied at 180, 270 and 360 g AI/ha, in three periods (20, 30 and 20+50 days after tomato transplanting).

The results showed that the two higher rates of glyphosate were the most effective against *Orobanche* (number of nodules/plant) in all periods of application. None of the glyphosate rates reduced shoot dry weight in transgenic tomatoes whereas the higher rates were toxic to the non-transgenic tomatoes. These results clearly indicated that the use of glyphosate-resistant transgenic plants could be a solution to the *Orobanche* problem because it allows the use of higher rates of this non-selective herbicide.
Report on WG3 meeting discussions: Marker analysis for susceptibility / resistance QTLs to parasitic plant species (*Striga* and *Orobanche*).

By B. Haussman

Different methodological points have been discussed:

I Choice of parameters to evaluate resistance:

- From parasite seed germination, attachment, emergence, growth vigor, blossoming, to seed set: more assays (possibly simple, fast and cheap) are needed to enable breeders to evaluate their materials for individual resistance mechanisms and to identify the corresponding QTLs.
- On the other hand, field evaluation remains essential and allows identification of QTLs involved in more complex resistance reactions: attention should be given to selection of trial location and field design.
- Repeated trials across locations, years or parasite strains (in laboratory assays) are essential to estimate the heritability of resistance parameters: only highly heritable traits will lead to the identification of reliable QTLs and therefore of suitable markers for employment in marker-assisted selection.
- Determination of the correlation among resistance parameters can help to reduce the evaluation load (traits that are closely correlated to other traits can be dropped) - multitrait methods for detection of a pleiotropic QTL could be employed.

II Structure of the segregating population (F2, BC1, …, recombinant inbred (RI) lines):

- RI lines are time consuming to produce but they enable repeats and are homogeneous in their responses.
- BC generations can be advantageous if the resistance donor is not adapted to the target area (but a larger population size is needed here to cover the whole donor genome).

III Evaluation of environmental effects, genotype (QTL) * environment interactions and parasitic variability:

- Is possible with RI lines because these allow the breeder to conduct multi-location trials and therefore to check (i) the reaction of host progenies in different environments (locations, years, temperature regimes etc.) and (ii) potential parasite variation in different places (mixed with environmental and parasite × environment interaction effects).
- Possible differential interactions between host genotypes and parasite strains could be checked by QTL mapping using different crosses (different sources of resistance crossed by the same universally susceptible genotype) and different parasite strains.
- Population genetics approach and molecular genetics techniques are recommended for the analysis of parasite populations.

IV Map construction and choice of molecular markers:

- Employ PCR markers (SSR, SNPs)
- Use bulk segregant analysis to saturate the region around a putative QTLs.

V Methods of QTL mapping:

- Analysis of variance for each marker separately: neglects variation at other marker loci or QTL and is therefore no longer recommended.
- Composite interval mapping: considers variation at other marker loci or QTL and has been shown to be more powerful than simple analysis of variance or simple interval mapping.
- Cross validation of QTL results is recommended to obtain an unbiased estimate of the genotypic variance explained by the model. Cross validation is possible, i.e., with the PLABQTL software which can be downloaded from: http://www.uni-hohenheim.de/~ipswwww/soft.html.
VI Managing QTLs in breeding:
- With regard to oligogenic resistance to parasitic weeds, the breeding progress can be significantly enhanced by backcrossing a few "big" QTL using a combination of foreground and background marker-assisted selection.
- For quantitative resistance that is determined by many genes with small effects, identification of reliable, consistent QTL is critical/very difficult.
- Advanced backcross QTL analysis could be appropriate if the aim is to improve a good agronomic genotype for i.e., parasitic plant resistance using a resistance donor with bad agronomic performance. The major advantage is that the method directly combines QTL analysis with the development of superior genotypes.

VII Future perspectives:
- Knowledge of mapped or even isolated resistance genes in other species should be exploited (synteny relationships, association studies).
- Proteomics and reverse genetics may help to identify resistance genes.
- The future will probably lie in the identification of expressed sequence tags (ESTs)/ candidate genes and in direct allele selection.
- The role of regulatory genes may also have to be considered.
- The model species *Arabidopsis* could help to understand host-parasite interaction and (hopefully) to identify resistance genes against Orobanche.

VIII Mutation challenge:
- Mutagenesis is considered as "the last resource for breeders" but is encouraged where no resistance can be found.
- One related question is: are there gene silencing mechanisms causing *Orobanche* resistance?