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Orobanche menace in crop plants: Host resistance as a potential tool to control

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Abstract
Orobanche, **'**the total root parasite' known for more than a century, causes large losses in the agriculture production system in most countries of Asian, African and European continents. A large number of crops such as tomato, sunflower, tobacco, hemp, many legumes and crucifers are parasitized by *Orobanche* spp. The cultural practices and application of herbicide were the measures being adopted for control of this weed which is usually involves high cost. Control through resistance breeding is the most cost effective approach to eradicate the weed. To breed any cultivar against *Orobanche*, it is first important to understand the host and weed complex completely. Here in this review attempts have been made to review the life cycle of parasite, host weed interaction, resistance breeding strategies in few crops and thus coming out with future research perspective for developing host plants resistant to *Orobanche*.

Keywords: *Orobanche*, resistance breeding, strigolactones, host-parasite interaction.

Introduction
Orobanche species, commonly known as broomrapes, is a well-known devastating agricultural weed affecting the quality and yield of many crops such as sunflower (*Helianthus annuus*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), rapeseed (*Brassica napus*) and legumes. Depending on the intensity of infestation, *Orobanche* parasitism leads to yield loss in crops ranging from 20 to 100% [1, 2]. The parasite express greater phenotypic plasticity, wide environmental tolerance, prefer permanently disturbed habitats, and are part of a plant guild associated with colonizing or crop complex species [3]. *Orobanche* species spread, widely, in Bulgaria, Southern Europe, Russia, Middle East and Northern Africa. Approximately 16 million hectares of arable land are threatened due to *Orobanche* around the Mediterranean Sea and in West Asia and represent as a major economical constraint [4].
Losses due to *Orobanche* infestation in various field crops are minimised, in general, through agronomic means and application of herbicides. Complete alleviation of losses is not achieved through cultural practices and application of herbicides found to be harmful to host plant and soil health in addition to involving higher cost. Biological control through breeding host resistance crops is the only means to eradicate this problematic weed. Understanding *Orobanche* life cycle and host parasite relationship can guide in following various strategies to breed for tolerance varieties to this parasite. Hence, an attempt made to review the parasite life cycle, host parasite relationships and the existing resistance breeding strategies for *Orobanche* tolerance and suggestions made on various approaches for making host plant resistance.

Orobanche origin and evolution
Orobanche, a latin name is derived from the greek word *Orobos* (pea) and *anchcin* (strangle). It is commonly called as broomrape after *Orobanche rapum-genistae* which parasïtses broom (*Cytisus scoparius*). *Orobanche* is a holoparasitic under the family Orobanchaceae which parasitize on plants belonging to *Cucurbitaceae, Compositae, Cruciferae, Fabaceae, Solanacae* and *Umbelliferae* families [5]. *Orobanche* is considered to be a native of Northern hemisphere. Based on the recent research supported by molecular taxonomy, the Orobanchaceae family consists of 89 genera with 2061 species [6]. About 20 species are considered as harmful parasitic weeds, among the different broomrape members in important crops, including *O. cumana* Wallr. on sunflower, *O. crenata* Forsk. and *O. foetida* Poir. on legumes, *P. ramosa* L. Pomel on oilseed rape, and *P. aegyptiaca* Pers. on tomato and tobacco. As an obligate root holoparasites, broomrapes demonstrate a higher level of adaptation and depends entirely on their hosts thereby depleting them of nutrients, minerals and water [7].
The parasite had found to arise from Laurasia in the northern Tethys Ocean (probably in East Asia) with a unique origin and during the Tertiary period [7]. There are different studies

quoting evolution of *Orobanche*. Earlier the reports support that the parasitism in flowering plants was evolved at least eleven times [8]. Phylogenetic studies based on several molecular markers distributed the parasites in six major clades in which the transition from hemiparasitism to holoparasitism would have occurred three times in an independent manner [9]. During evolution process few of these species became achlorophyllous and began to grow in tropical and subtropical conditions. Hence the family Orobanchaceae, consist of all major nutritional types of parasites such as holoparasitic (nonphotosynthetic), hemiparasitic (partially photosynthetic), and facultative parasite [5].

Life cycle of *Orobanche*

The annuals weedy *Orobanche* spp. reproduced by means of tiny size seeds [10]. Up to 200,000 seeds are produced from each flowering shoot of the parasite which can remain dormant in the soil for several years, even to 20 years [2]. The small seeds of *Orobanche* may easily spread from infested areas to other fields through agricultural implements, cultural practices and irrigation thereby affecting susceptible crops [11]. *Orobanche* seeds are very small in size, dark brown and ovoid in shape and their weight ranges from 0.1g to 3 g per 1000 seeds [2]. Their seed coat is has a honeycomb pattern caused due to the dehydration of a maternal cell layer. These patterns can be used for species identification as they are specific to each species. Lipids, with oleic and linoleic acids are the main storage material found in these seeds [12]. The seed has a well-defined endosperm composing of three to four cell layers and several oil bodies and starch grains and a reduced embryo. The endosperm surrounds the reduced embryo, usually considered as an undifferentiated body which composes a spherical body without any plumule /radicle /cotyledons. Study of Joel *et al*. [13] indicated the presence of two perisperm cells located between the embryo and the micropyle regions of P. *aegyptiaca*, which is assumed to contain the putative receptors of germination stimulants (GSs) [14].

The life cycle of *Orobanche* starts with the germination of the seeds, radicle growth to the host root, haustorium formation and attachment to the host root, the successful establishment of a xylem connection for compatible interaction, and finally production of seeds (Figure 2).

Figure I. Life cycle of *Orobanche* (Source: Delavault, P. 2015. Knowing the Parasite: Biology and Genetics of *Orobanche*. HELIA 2015; 38(62): 15–29) [4]

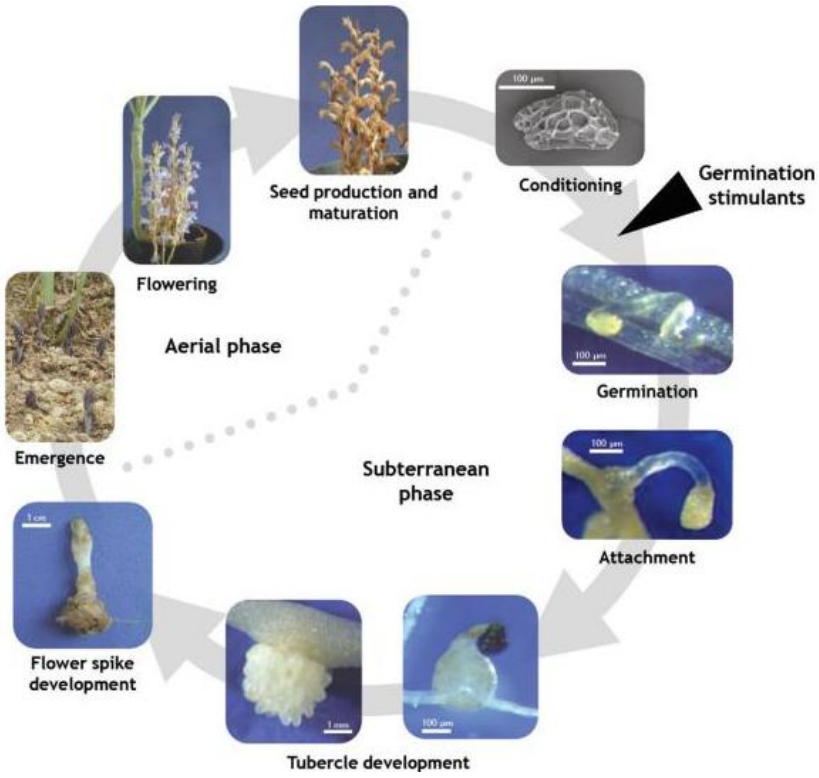

Fig 1: Life cycle of *Orobanche*

The seeds germinate only after receiving chemical signals secreted from nearby host root[2]. It also requires a conducive temperature of around 15 -20°C and the soil has to be moist to favour seed germination. Once the seeds are germinated the radicle has to reach a host root and establish a connection very soon as they can survive for a few days due to its limited food reserve in the seed. The radicle of parasite has no root cap and no procambium or conductive tissues are developed [15]. The parasite seedling radicle after growing for a few millimetres forms a connection with the host by a unique structure, the haustorium, a multi-cellular organ that invades host tissues and serves as a physical and physiological bridge between host and parasite. Invasive cells of the haustorium perforate host tissues and reach the conductive system of the host. The middle lamella of the plants that holds the cells together is digested by the secretion of lytic enzymes, such as pectin methylesterase (PME) and polygalacturonase [15, 16]. This secretion makes the host cell walls weaker and more vulnerable to the attack by changing the composition of the middle lamellae. Once vascular connections are formed, the parasite starts extracting water, nutrients, and photosynthates from the host vascular tissue. The *Orobanche* radicle outside of the host root swells and develops into a globular mass of tissue called tubercle. Finally, the tubercle initiates a floral meristem that develops into a floral spike. Only the flower shoots appear above the ground, and when there is no

blossom no shoot is found above the soil surface. The flower shoots are packed with densely arranged terminal spike of between ten and twenty flowers with a wide range of colour variation. The leaves are modified as triangular scales.

The destruction of these weeds seeds by reducing the seed bank in the soil can be done through cultural operations or through chemical spraying. Once the seed germinates the growth of the weeds cannot be controlled unless the host is made unavailable to the parasite. The biological control is also possible but again all the above control measures are dependent on environmental factors. So for a complete eradication of this parasitic weed the most efficient approach is to develop resistant plant through breeding techniques. Before starting up any breeding programmes it is important to have a clear knowledge on the host plant interaction, the physiological changes and stages of parasite where an intervention can be made.

Physiology of host-parasite interaction

Strigolactones - Germination stimulant produced by the host

It is a well-known fact that the seeds of boomrape germinate in repose to a signalling compound secreted by the host plant roots. A group of natural strigol-related stimulants inducing root parasitic plant seed germination have been identified and the compounds are termed as strigolactones (SL) [17]. During early 20th century there were several attempts to identify the chemicals inducing germination of root parasitic plants. Brown *et al.* [18] reported assay techniques, germination stimulation activity of root exudates from various plant species, and partial purified of *O. minor* germination stimulants in the early 1950s. Strigol and strigyl acetate, were isolated from root exudates of cotton, a false host of Striga are the first described SLs [19]. During 2006, Goldwasser *et al.* [20] conducted a study to extract, purify and analyse the root exudates from *Arabidopsis thaliana* using a specially designed hydroponics culture system where the exudates were collected on the activated charcoal placed under the medium. The exudates were then extracted from the activated charcoal with ethyl acetate and analysed using high performance liquid chromatography. They reported that orobanchol was the major seed germination stimulant produced by *A. thaliana* to induce *Orobanche* seed germination. Similarly exudates from the roots of red clover were containing alectrol, orobanchol and a third unidentified compound which stimulated *O. minor* germination [21]. Two germination stimulants structurally related to strigol, sorgolactone and alectrol [22], were isolated from root exudates of sorghum and cowpea, respectively. Isolation of orobanchol [21], the first *Orobanche* germination stimulant, clearly demonstrated that *Orobanche* species utilize SLs as germination signals. To date, about 14 SLs have been detected in root exudates of various plant species [23].

Structure of Strigolactones

The natural occurring SLs are four-ringed molecules (A, B, C and D rings) [24] containing a tricyclic ring system (ABC part) connected to butenolide (D ring) via an enol ether bridge (Figure 3) [23]. Extensive studies have been conducted on the structure–activity relationships of SLs in germination stimulation of parasitic plant seeds. Research results revealed that the C–D ring functional group is the essential structure for exhibiting germination stimulation activity whereas A-B ring is responsible for the stability of the molecule in the soil [25]. The most active germination stimulants are three mono hydroxy-SLs, 2′-epiorobanchol, orobanchol and sorgomol. Strigol and solanacol are slightly less active than these three monohydroxy-SLs. Sorgolactone and 5-deoxystrigol are lipophilic, no oxygen-containing substituents are found on the A/B-ring group and thus are less active germination stimulants on *O. minor* seeds [26]. GR24 is synthetic analogue of SL which gives >60% germination at 100 nM and 100-fold less active than the natural SLs [23]. So far more than 20 naturally occurring SL derivatives have been described[27] fulfilling a number of roles in plant growth and development [28].

Figure II. Structure of natural strigolactones and the synthetic analog GR24 (Reproduced from: K. Yoneyama, *et al*. Strigolactones as Germination Stimulants for Root Parasitic Plants. Plant Cell Physiol. 2010; 51(7): 1095–1103) [23]

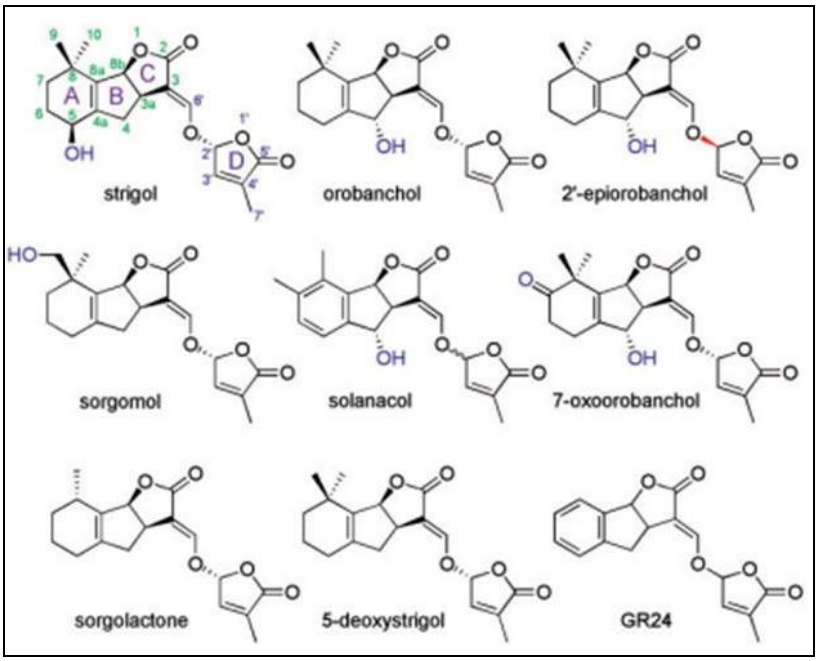

Fig 2: Structure of natural strigolactones and the synthetic analog GR24

Synthesis of Strigolactones

SLs originate in the plastids of higher plants which are carotenoid derived phytohormones [29]. DwaRF27 (D27) [30], ccD7 and ccD8 [31] are three plastid localised proteins involved in the stepwise conversion of all-trans-β-carotene into carlactone in the plastids which is further translocated to the cytoplasm [32]. In the cytoplasm the MAX1-type monooxygenases transform CL into carlactonic acid that is later converted into 5-deoxystrigol ororobanchol, two main precursors of other SLs [33]. Earlier studies carried out by Matusova [29] supported that the biosynthetic pathway of SLs starts from plastid. Fluridone is known to specifically inhibit phytoene desaturase enzyme that is involved in carotenoid biosynthesis [34]. When the maize seeds are treated with fluridone which is a carotenoid inhibitor to treat the seeds of maize, the plants produced very low germination stimulant which supressed the germination of parasitic seeds [29].

Role of Strigolactones

Apart from inducing germination of parasite seeds the strigolactones have been suggested to play a key role in formation of arbuscular mycorrhizal symbiosis [35]. The role of SLs were studied vastly in the last decade, it has been identified as a crutial regulator for plant branching [36, 37], root development [38], leaf senescence [39], responses to nutrient stress[38] and potential role during biotic stresses [40]. Under optimal growth conditions, strigolactones promote root hair elongation but suppress lateral root formation [41]. A work by Brewer *et al.* [32] reported that under ideal condition at a basal level of SL production in wild plants, reduced lateral shoots and roots, enhanced plant height, secondary growth, senescence, and root hairs are evident. This was proved in mutant plants which displayed more lateral branches and lateral roots, and less secondary growth and arbuscular mycorrhizal (aM) fungi symbiosis at reduced levels of SLs. SL production is triggered by reduced phosphate which leads to greater branch repression [32].

Biochemical changes in parasite after infection

The nutrients acquired are metabolized by the haustorial cells and the concentration of carbohydrate in the parasite's xylem sap is much higher than that of the host plant [42]. The major carbohydrate reserve differs from host to that of the parasite. Mannitol accumulation is found to be around 150 mg/g of dry weight in *Orobanche* which accounts for about 75% of total soluble sugars [42]. Much higher concentration of mannitol accumulation reaching upto 20% of dry weight has also been observed [43]. The rate of turnover of manitol is very slow might be the major reason for such higher accumulation of these compounds. The studies on algae and fungi suggest mannitol involvement in various physiological role as listed, even though precise role of mannitol in angiosperms is unclear, viz., 1) reducing power and storage of carbohydrate 2) stabilization and regulation of enzyme systems and 3) osmoregulation [43, 44]. Mannitol accumulation in *Orobanche* also plays an important role in parasite's recruitment of water and nutrients from its host and involvement in *Orobanche* tubercles development [45]. In *Orobanche ramose* a study done by characterization of the molecular components, such as cellwall invertase (CWI), vacuolar invertase (VI), neutral/alkaline invertase (NI) and sucrose synthase (SuSy), showed that cell expansion occurs through vacuolar hexose, SuSy is involved in the sucrose mobilization and for starch accumulation in parenchyma cells whereas CWI activity is constant to a basal level in tubercles and stems [46].

Physiology of infected hosts

Host plants response due to infection varies from intense growth defects to an virtually complete non-appearance of visible symptoms [47]. In general, four factors determine the type and extent of the impact: 1) the size of the parasite, 2) the rate of growth and metabolic activity of the parasite, 3) the degree of dependency on the host for resources, and 4) the stage of development of the host [48]. Now the germinated *Orobanche* compete with the host for water and metabolities. *Orobanche*-induced yield reductions are due to carbohydrate loss to the parasite. It further reduces the water uptake capacity of the host roots. Evidence for nutrient deficiency in hosts is rare, although *Orobanche* certainly acts as a strong sink for inorganic ions. One of the few examples was found in tobacco infected with *O. ramosa*, in which the phosphorus concentration was reduced by more than 50% in roots of infected plants and leaf potassium concentration was reduced by 60% [49]. These changes in the nutrient availability of infected plants were considered to be the principal reason for a 30% reduction in host growth.

Status of development of host plant resistant

As an important strategy to control through host plant resistance, researchers made attempts to develop *Orobanche* tolerant cultivars in Sunflower, *Arabidopsis*, tobacco, Faba Bean, tomato etc. The resistance breeding approaches followed in few of these crops are discussed below.

Resistance breeding in Sunflower

Worldwide Sunflower production is affected by *Orobanche cumana* which parasitizes sunflower roots. Earlier five broomrape races were identified *viz*., A to E with a set of differential lines carrying corresponding monogenic dominant resistance genes *Or1* to *Or5* [50]. Until mid-1990's *Orobanche* was not a major problem since single dominant gene were available for the five different races. The race F appeared in Spain in late 1990's by overcoming the *Or5* gene, later race G and H was reported [51,52]. *Or6* and *Or7* two independent recessive genes were identified from the cultivar KI374 giving resistance towards F race [53]. The resistance genes were derived from various wild relatives of sunflower like *Helianthus divaricatus* and *H. grosseserratus*, *H. divaricatus*, *H. mutelii*, *H. grosseserratus* and *H. Tuberosus* [54].

Mutated lines of *Arabidopsis thaliana*

In *A. thaliana* natural host plant resistance mechanism to the parasite is not found. A study was conducted where 50 different ecotypes of *A. thaliana* were analysed for their ability to encourage *O. aegyptiaca* germination. Inspite of using large ecotypes the "low germination stimulant" phenotype was not identified among them. In *Arabidopsis*, where natural resistance is not available, mutagenesis has established fruitful results in identifying resistant lines. In fact, 94 out of 13 000 *A. thaliana* fast neutron mutated M_2 lines have been detected as low inducers of *O. aegyptiaca* germination [55].

Genetically engineered tobacco

Genetic engineering approach is an additional tool which aids the traditional breeding in control of parasitic weed *Orobanche*. Two important elements are required for this statergy *viz*., a parasite-responsive gene promoter and a

parasite-inhibitory gene product. During stress condition when the pathogen infects, the plants starts to respond to the infection by producing a number of signalling molecule which in turn promotes the gene which acts against the pathogen. These defensive molecules are called as photoalexins. The gene *HMG2* in tomato is one among the four genes which encodes for 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), reflected as the rate-limiting step in the isoprenoid biosynthetic pathway [56]. This gene usually expresses only in the infected tissue in response to the wound caused by the pathogen [57]. In flesh fly (*Sarcophaga peregrine*) a gene cluster namely *sarcotoxin IA* secretes Sarcotoxin which is a toxic material [58]. This toxin functions as anti-microbial peptides for the fly, which feeds on decaying flesh. The N-terminal of this mature peptide is rich in positively charged amino acids and is hydrophilic, whereas the C-terminal half is hydrophobic [59]. The N-terminal half of the toxin networks with acidic phospholipids on the bacterial membrane surface, while the C-terminal half enters into the membranes, causing distraction of the membrane potential [60]. In a recent study, in tobacco and tomato plants, expression of sarcotoxin under a root-specific promoter (Tob promoter) improved host resistance to *O. aegyptiaca* and did not appear to have unfavourabe affect the growth of the host plant. In a study conducted by Westwood *et al*. [5] the expression of HGM2 was contained to the region around the site of parasite penetration in transgenic tobacco. He also confirmed that the expression increased during early parasite growth and continued over the course of parasite development, signifying that HMG2 expression does not represent a transient response to host injury. Now cloning of HGM2 gene along with the toxin gene *Sarcotoxin IA* will ultimately act against the *Orobanche* penetration as the HMG2 promoter will determine the expression of the toxin only after parasite attack and the toxin will be accumulated only at the site of attachment where it is will be accessible for uptake by the growing parasite.

Incomplete and quantitative resistance in Faba Bean

Unlike sunflower in faba bean the available resistant seems to be incomplete and quantitative resistance [61]. *Orobanche* resistant breeding in legume crop was started with faba bean in the early 1960s [62]. Untill 1980's only a slight resistance to *O.* crenata was only avalilable[63]. The Egyptian line F402 derived from cross between Rebaya 40 and F216 developed through traditional breeding served has a resistance source to broomrape [63, 64]. This source of resistance has been widely used in Spain, Egypt, Morocco, Tunisia and at the International Centre of Agricultural Research in the Dry Areas (ICARDA) for different breeding programs. Numerous resistant lines and cultivars have been released in the last few decades. According to Perez-de-Luque *et al*., [61] these defensive responses correspond to: (i) callose depositions in the host cell walls from the cortex and in contact with the parasite tissues, and (ii) lignification of host pericycle and endodermal cells. The second response (lignification) occurs after the obstruction of parasite intrusive cells in the host cortex has been overcome, and prevents further penetration into the central cylinder and formation of a haustorium.

Mutated tomato lines conferring resistance towards parasite

In tomato a mutated line of M82 was found to inhibit the growth of *Orobanche* as the strigolactone secretion was found to be low. These lines were further mutated to completely supress the SL secretion. The lines showed a greater degree of resistance towards the parasite but the major disadvantage of these lines were the fruits were of altered quality which was not acceptable. [65]

Future approaches towards resistance breeding

Based on the current understanding of host parasite interactions, various approaches which can be used to breed crops tolerant against *Orobanche* are briefed in the figure 4. When there is availability of resistance source in the crop of interest then gene transfer could be made possible through inter/intra specific crosses. Gene transferred can be also done after identifying the candidate gene through gene expression profiling and marker assisted selection. In the absence of resistance source, alternate methods like creating mutations and silencing of key host genes responsible for pathogenesis can be employed for developing resistant cultivars. If the resistance source is available in related crops, the relevant resistant genes can be identified and transferred to target crop. In case of absence even in the related species then 'model plant approach' can be applied.

A brief note on the possible ways of exploring resistance breeding from the available resources is discussed below.

If true resistance is available in crop of interest it can be transferred from wild species or expression profiling can be employed to develop resistant cultivars.

Transfer of resistance genes from wild species

Wild species acts as a reservoir to the diversity of the crop and contribute directly to food security. In sunflower the resistance against all the races of *Orobanche* were derived from the wild relatives. Tobacco being an allopolyploid crop has a rich diversity in the form of wild species. Parasite tolerant lines have been identified in Tobacco at ICAR-CTRI which will serve the purpose for resistance breeding (Sarala *et al.*, unpublished). These wild species can be exploited for incorporating resistance genes towards the parasite in the host.

Expression profiling

Understanding the genes that govern response to parasitic attack is helpful in breeding crops with improved tolerance to the broomrape. Expression profiling proves an effective approach in global picture of changes occurring in the transcriptome of the plant under stress conditions as thousands of genes can be investigated in a more comprehensive and holistic way. Candidate genes for broomrape tolerance-related traits associated with adaptive processes can identified by expression analysis of these tolerant lines. Due to high efficiency, comparative transcriptomics is done in many crops and plant species to understand differently expressing genes during the parasite attack. The plant response to the parasitic weed and the molecular basis of the resistance was studied in *Medicago truncatula* using proteomic approach [66]. They found 49 differential peptide spots by peptide mass fingerprinting (PMF) following MALDI-TOF/TOF mass spectrometry. Many of the proteins were belonging to the functional category of defense and stress-related proteins which showed significant differences between genotypes and after parasitic infection. Parasitic Plant Genome Project (PPGP) has started recently and it aims to sequence transcripts from these weeds with the goal of reading the genetic changes associated with

parasitism. Through this project they are trying to identify expressed genes during key life stages from seed conditioning through anthesis.

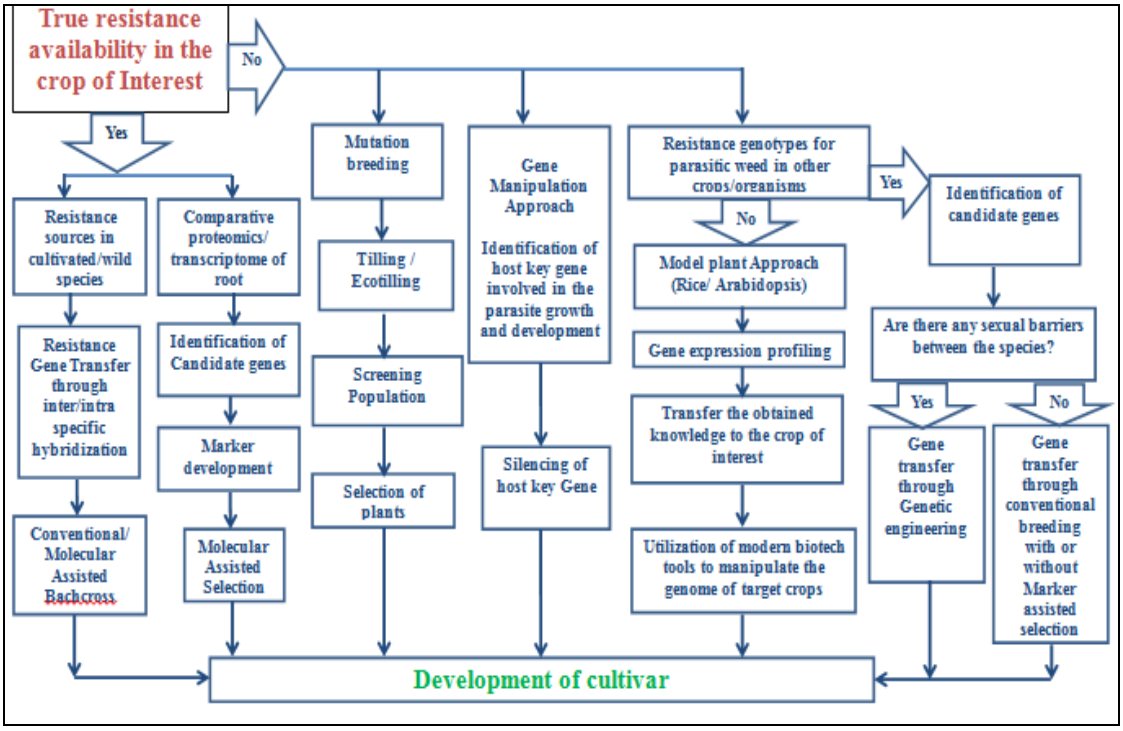

Fig 3: Future perspective in Parasitic weed research

In the absence of resistance source in the crop of interest mutation breeding could be followed to alter few gene of interest and develop resistant cultivars. TILLING and EcoTILLING also can be used to identify unknown and known point mutations from a set of candidate genes. Both methodologies provide proof of function for both natural and induced variations.

Ways to alter chemical mechanisms in plants to confer resistance

It is well known fact that the chemical secreted by the plants induces the germination of the parasite. Lines with low stimulant can be used in the breeding process to overcome the problem. Wegmann [67] proposed the pursuit for inhibitors of the germ tube exoenzymes and germination inhibitors as resistance features. Polygalacturonase and pectin methylesterase are known enzymes secreted by the parasite to soften the host roots. The leucin-rich cell wall proteins are known to bind tightly to polygalacturonases and inhibit them [68]. The enzyme inhibiting the activity of these two exoenzmes paves a way to produce varieties against parasite attack. Once the germination is induced the next step can be prevention of the germtubes in entering the host plants. This could be achieved by thickening the cell walls of root through lignification by peroxidases, deposition of callose in the xylem wall by overexpression of callose synthase cDNA gene *HaGSLI* [69]. Phytoalexins are resistance molecules towards parasite first been identified by Wegmann [67] *et al.* for sunflower and chickpea. These phytoalexins are defence molecules produced only after the attack of the pathogen and therefore contribute to resistance against *Orobanche*. The sunflower phytoalexins scopoletin and ayapin showed germination inhibiting activity, when germination was induced by the synthetic stimulant GR24 [61].

Gene silencing

An important discovery in the last decade for suppressing gene expression is posttranscriptional gene silencing (PTGS) in plants using double-stranded RNA (dsRNA). A small double-stranded RNA molecule is the main element in the silencing process and that facilitate sequence-specific gene suppression by degradation of homologous mRNA sequences, thereby silencing the target gene. Gene silencing provides plants with defenses against various pathogens, such as nematodes and viruses, and is a tool of immense importance for research on plant development. Modern tool of silencing is virus-induced gene silencing (VIGS) which involves cloning a short sequence of targeted plant gene into a viral vector, which infects the plant tissue for degrading specific plant mRNA. These virus particles can be transferred into the parasite and silence the key gene involved in the parasite growth, as *Orobanche* is attached to the roots of the host plant and has xylem connection. The only important issue here is to see that the key gene in the parasite is different from that of the host gene. Here resistance to parasitic weeds requires either the identification of 1) genes whose products selectively inhibit parasite growth or 2) a target key-gene of the parasitic weed for silencing. This system also has disadvantages like (a) obtaining complete suppression of expression of a target gene through VIGS is difficult; and (b) virus can alter plant development when inoculated into the plant system. Studies have been conducted to silence Mannose 6-Phosphate Reductase (M6PR) gene which is essential for mannitol accumulation in the parasite grown on tomato plants [70]. *PaCCD7* and *PaCCD8* orthologous genes from *O. aegyptiaca* were silenced using a VIGS approach in *Nicotiana benthamiana*. The transient knock-down of PaCCD7 and PaCCD8 inhibited tubercle development and the infestation process in host plants [45]

Model plant approach

This approach is useful when there is no knowledge about the candidate genes in the host plant of target and the model plant is exhibiting resistance to the parasite. It begins with screening the germplasm for resistance genes in the modal plant such as *Arabidopsis thaliana*, *Medicago truncatula* and *Oryza sativa*. The next step will be functional analysis in through transcriptome/proteome/ metabolome and generating gene expression data. In *Medicago truncatula* upon *O. crenata* inoculation, alterations in the proteome corresponded to a general increase in the amounts of proteins belonging to the defense-related category, such as proteinase inhibitors, PRs, cell walls modifying, ROS detoxifying enzymes, and enzymes involved in the synthesis of secondary metabolites were categorised [66]. Candidate gene selection has to be made from the knowledge obtained the on the resistance mechanism of model plant in the target host namely tomato, tobacco, pea, faba bean, etc., followed by cultivar development through marker aided selection or transgenic approach.

Genetic engineering

Biotechnology provides a harmonising approach to traditional breeding for controlling parasitic weeds such as *Orobanche*. For example, genetic engineering enables the use of resistance mechanisms that are not available in the germplasm of a host. The progress toward development of a new *Orobanche* resistance strategy based on the inducible expression of an anti-microbial peptide, sarcotoxin IA, which derives from the flesh fly can pave the way to build resistance in the plants. In plants, bacterial peptides with two helices have not been reported [71] and the use of sarcotoxin under root-specific promoter (Tob promoter) in tobacco and tomato plants enhances host resistance to broomrape and did not have adverse effect on the growth of the host plant. In other case the genes present in related crops can also be transferred if there are no sexual barriers. For example, the genes conferring resistance to *Orobanche* in tobacco can be transferred to tomato provided that there are no crossability barriers operating between the genus.

Conclusion

Broomrape continues to be one of the most serious production constraints in many countries around the world for more than a century. Novel approaches to identify the resistance sources and to transfer it to the cultivars are needed. In spite of so many researches there is always a lag behind in tolerance breeding. The availability of resistance source in the wild relatives will be a boon to the breeding of tolerance crop. In the absence of resistance sources unlike sunflower, the candidate gene transfer across species or identification of key genes in host plant which could be silenced can pave way to control of these weeds. However, a deeper understanding and an integrated approach is always needed to address the issue of parasitic weed *Orobanche*.

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