Indian Journal of Weed Science **46**(2): 151–154, 2014

A new cost-effective method for quantification of seed bank of *Orobanche* in soil

Aditi Pathak[1] and C. Kannan*
Directorate of Weed Science Research, Jabalpur, Madhya Pradesh 482 004

Received: 16 October 2013; Revised: 27 March 2014

ABSTRACT

A simple technique to germinate *Orobanche* in the presence of live host seedlings under controlled conditions was developed. Surface sterilized seeds were preconditioned separately in Petriplates and were transferred on a bed made of sterilized sand, cotton and filter paper kept on a 1000 ml beaker. Host plant (mustard and tomato) seeds were germinated in Petriplates and transferred to the beaker containing the preconditioned *Orobanche* seeds. The beakers were then incubated at room temperature for 10 days and observed under stereo binocular microscope. This technique may be useful in many ways including quantification of the weed seed bank in the infested fields, screening of contaminated seed lots from infested areas for the purpose of quarantine and seed certification. Use of live host seedlings instead of synthetic stimulants, both reduced the cost and made it possible to use this technique.

Key words: Parasitic plant, *Orobanche*, Seed bank, Vegetable crops, Weed biology

Parasitism in higher plants is evolved as a result of competition for limited resources, primarily in the arid and nutrient-deficient habitats leading to the development of special adaptations like the production of haustoria, to derive nutrition from the neighboring plants (Atsatt 1973). *Orobanche* (broomrape) is an obligate root parasitic angiosperm, parasitizing several economically important plants world over. *Orobanche* spp. has a wide host range including agriculturally important crops such as tomato, tobacco, potato, brinjal, several legumes, sunflower and mustard (Parker and Riches 1993). The most common species in India are *O. cernua, O. crenata, O. ramosa* and *O. aegyptiaca,* causing a loss of about 20-80% in the tomato and tobacco and about 30-35% in brinjal and mustard (Ramachandraprasad *et al*. 2008). Life cycle of *Orobanche* has the following distinct stages, *viz.* seed germination, haustorial initiation, attachment, penetration and establishment in the host and emergence of the flowering stalk above soil (Graves 1995). Minute size of their seeds permits only few hours of life after germination and therefore must quickly attach to a host root before their resources are exhausted. However the seeds can remain dormant in the soil for about 20 years. The parasite attacks the host root and dev elops underground for about 25-40 days, after which depending upon conditions outside, produce the flowering stalks above ground. Thus by the time the parasite becomes visible above ground, sufficient damage had already been caused by development of the parasite at the cost of the host plant. Host crops infected with *Orobanche* are often contaminated with the minute seeds of *Orobanche,* which are difficult to clean and as such requires time consuming and laborious microscopic examination or costly germination tests to find the admixtures for the purpose of clean seed certification and quarantine. The management strategies would be more efficient if it is possible to determine the *Orobanche* seed bank in the soil with rapid and easy technique for germination method. This paper presents the findings of such work.

***Corresponding author:** agrikannan@gmail.com
[1]Department of Biosciences, Rani Durgavati University, Jabalpur, Madhya Pradesh 482 001

MATERIALS AND METHODS

Collection of seeds and viability test of *Orobanche*

Seeds were collected from fully matured flowers of *Orobanche* growing on tomato and mustard crops from the farmer's field in Gwalior district (Latitude: 26°17'N, Longitude: 78°13'E, Elevation: 617.0 ft) Madhya Pradesh, India, during March 2011. The seeds were sun dried and stored in plastic containers at room temperature (25±2^{0}C). Seed viability was assayed using 2, 3.5-triphenyl-tetrazolium chloride (TTC) staining (Granados and Torres 1999), where the seeds were placed in 1% solution of TTC, incubated for 72 h at 35 ^{0}C in the dark. The seeds were then observed microscopically and red or orange seeds were considered viable, while white seeds were considered dead.

Germination under controlled conditions

Surface sterilized seeds, about 100 in numbers were placed on autoclaved moist filter paper of size

Whatman No.1 in Petriplates of diameter 9 cm. The plates were covered with black polythene sheets and wrapped in aluminum foil to provide complete darkness and then incubated at 25±2 ^{0}C for 10 days for conditioning (Plakhine *et al*. 2009). The preconditioned seeds with filter paper were aseptically transferred on a bed made of sterilized sand, cotton and filter paper kept on a 1000 ml beaker. While sand was used for maintaining moisture for longer period, cotton and filter paper were used to keep the fine sand particles separate from the seeds.

In a parallel setup, surface sterilized seeds of mustard and tomato were placed in separate Petriplate with moistened blotting papers for germination. After germination, about 10 seedlings were surface sterilized and transferred to the beaker containing the preconditioned *Orobanche* seeds. The beakers provided sufficient vertical space for the growth of host plant seedlings. A mild fungicide such as bavistin was sprayed to avoid fungal contamination. *Orobanche* seeds without host plant were maintained for the purpose of comparison. About 30 beakers with similar sets of *Orobanche* seeds and host plant seedlings were maintained. The beakers were then incubated at room temperature for 10 days after which, the filter paper with *Orobanche* seeds and host plant were removed gently for observation under stereo binocular microscope. The experimental set up was tested several times for confirmation of the credibility of the technique.

Screening of contaminated soils and quantification of seed bank

To quantify the seed load of *Orobanche* in the infested soil, about 1 g of infested soil was suspended in clean sterile distilled water, filtered to remove large soil particles and then transferred to the beaker with standard germination paper for conditioning of *Orobanche* seeds. The procedure described above was followed to estimate the number of seeds germinated. Analysis of soil samples was done collected from 10 farmer's fields in and around Gwalior district of Madhya Pradesh. Soils samples were collected on the basis of farmer's report of *Orobanche* infestation. Soil samples collected from the farmer's fields in Bahadurpur 1 and 2, Khokapura 1 and 2, Murena 1 and 2, Seetapur, Daulpur road, Billua 1 and 2, near Gwalior were used for quantification of weed seed bank and 5 replications were maintained for each location.

RESULTS AND DISCUSSION

Collection and identification of *Orobanche species*

Seeds were dark brown in colour, oblong to ovoid with surface reticulations. Size of stalk was 30-40 cm, inflorescence dense with pentamerous flowers, bluish violet colour at the top and white at base, corollas were strongly curved and lobed with bluish violet calyx 4 in number, 9- 12 mm in length, single pistil of length 16 mm, 5 corollas of 15-18 mm in length, the bract was dark in colour with hairy surface, stamens were 4 in number (8-12 mm length) and 10 mm style. Based on the above characters as also described by Foley (2004) and Plaza *et al.* (2004), the species under study was identified as *O. cernua*

Germination under controlled conditions

Seed viability was tested for the seeds collected from all different locations. Maximum viability of 67.8% was observed for the seeds collected from Murena 2 while a minimum of 44.4% from Seetapur location (Table 1). Periodical observation of the seeds of *Orobanche* indicated an overall germination percentage of about 70% on mustard and about 63% on tomato in a period of 20 days. Using the germination setup as described in methodology, we could germinate fresh seeds of *Orobanche* similar to the report of Abbes and Kharrat (2006), while according to Perez-de-Luque *et al.* (2004), under natural conditions preconditioning of seeds in soil is required for germination of *Orobanche*. Microscopic observation of the germinating seeds indicated the following important events in that sequence (i) seeds swell after absorbing moisture during the conditioning period (ii) color of the seed coat changes from light brown to dark brown on 2nd to 4th day upon exposure to the host seedlings. (iii) The proximal end of the imbibed seeds became more pointed and protruded from the base, (iv) the testa ruptured and (v) germ-tube elongates to reach the host root and attaches to the root surface by production of haustoria to enter the root tissues. After successful establishment of haustoria inside the host root, a globular tubercle is formed. The tubercle is densely surrounded by brown root meristem, growing into a flowering stalk of *Orobanche* which emerges above the ground to produce flowers.

Table 1. Viability of *Orobanche* seeds collected from the soils of different villages around Gwalior and tested by TTC staining method

Sample	Host crop	% Viability of *Orobanche* seeds
Bahadurpur 1	Mustard	65.8
Seetapur	Brinjal	44.4
Billua 1	Tomato	50.2
Khokapura 1	Mustard	49.2
Daulpur road	Brinjal	48.8
Billua 2	Tomato	55.6
Murena 1	Brinjal	44.8
Murena 2	Mustard	67.8
Bahadurpur 2	Mustard	56.6
Khokapura -2	Tomato	54.8

Table 2. Comparison of some popular methods for weed seed bank estimation

Objective of the technique	Advantages	Disadvantages	Reference
In vitro germination of parasitic weed seeds	• Can provide valid information about the seed bank load of the parasitic weeds	• Root leachate collection and tissue culture involves costly facilities, expertise and time	Batchvarova *et al.* (1998)
Weed seed bank analysis	• Can provide weed seed density of non-parasitic seeds	• A general technique and not suitable for parasitic seeds	Espeland *et al.* (2010)
Sandwich model of *Orobanche* germination	• Can provide a general information about presence of *Orobanche* seeds	• Does not give complete information, time consuming and the glass-fiber may cause respiratory problems for the personnel involved	Losner-Goshen *et al.* (1998)
Petridish method using synthetic germination stimulants like GR24, Nijmegen-1 etc.,	• Widely used technique for the study of *Orobanche* seed germination • Gives better germination than any other technique under controlled conditions	• Germination stimulants are costly and not readily available in the market • No information about complete life cycle, only germ tube development can be observed but tubercle development and attachment cannot, because of absence of host root	Mangus *et al.* (1992)
1. Seed germination by using live host seedlings under laboratory conditions 2. Quantification of soil seed bank 3. Screening of crop seed samples from infested fields	• Germination of the parasitic weed seed, interaction between the host plant and weed and stages of development of the parasitic weeds can be studied in relatively less time • Uses locally available standard laboratory wares and thus very cheap and easy to setup when compared with other methods • Can be used to quantify the soil seed bank load of parasitic weed seeds which is not possible by other methods • Depending upon the sample size and representativeness, can give accurate prediction of the seed load in the soil • Screening of crop seed lots is less laborious and reliable than other methods	• Host plant may become week because of lack of food sources • Takes about 20-25 days including the preconditioning and germination period in the case of soil seed bank screening.	Our proposed technique

Batchvarova *et al.* (1998) demonstrated the *in vitro* germination of *Orobanche,* where the host root extract was used as stimulant for the development of the callus. This method requires the production of callus cultures which requires more technical facilities. Likewise in the "sandwich method" (Losner-Goshen *et al.* 1998), *Orobanche* seeds were placed between two layers of glass-fibre paper and the sandwich was buried in autoclaved sand in small pots as a modification of the method given Parker and Dixon (1983). This method uses glass-fibre which is not eco-friendly in their production and use. Similarly the use of Petri dish for the germination of *Orobanche* seeds was described by Sauerborn *et al.* (1987). However these methods were either costly or required some special skills or could not give an insight into the different stages of germination of the *Orobanche* seeds and for conducting the studies. In contrast (Table 2), the current method described in the study provides a very simple and effective technique to check the germination, quantify the viable seeds in a given lot and thereby estimate the weed seed bank load in the given quantity of infested soil and to study the stages in germination and development of *Orobanche* in the host. The germinated host plant seedling provides the essentially required host signals in a concentrated manner right near the pre-imbibed *Orobanche* seeds, thereby providing a perfect condition for the germination and growth of *Orobanche* seeds.

Quantification of soil seed bank

Results indicate that the quantity of viable *Orobanche* seeds varied from a maximum of 69.6 seeds per gram of soil in Bahadurpur region where mustard was host crop while minimum germination of about 6.8 seeds per gram of soil of *Orobanche* from the fields with brinjal in Seetapur regions. Bar chart (Fig. 1) showing the number of viable seeds of *Orobanche* per gram of soil indicates that host crop and the soil type influences the variations in the weed seed bank of the soil which may help to determine the severity of the infection in the succeeding cropping season and device management strategies accordingly. Quantification of weed seed bank is an important criteria in the management of weeds and it helps in predicting the weed dynamics (Allen and Nowak 2008) and intensity of crop weed competition for using appropriate management strategies (Creech *et al.* 2008). Understanding the factors behind germination of *Orobanche* and their actual seed load in the soil is crucial for the development of management strategies to deplete the soil seed bank of *Orobanche* (Joel 2000). Several tech-

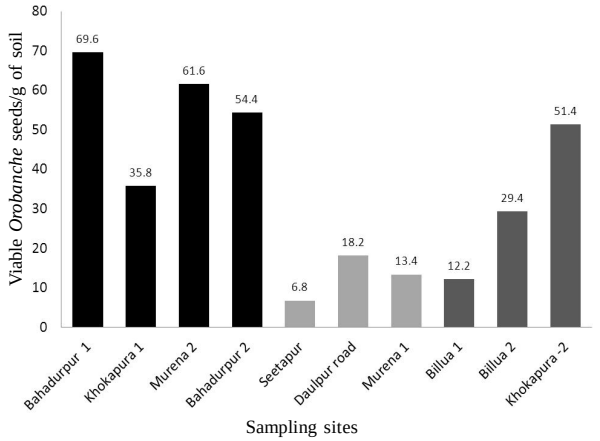

Fig. 1. *Orobanche* infestation in farmers fields in and around Gwalior district of Madhya Pradesh

niques have been proposed for estimation of non-parasitic weeds (Espeland *et al*. 2010). The general extraction and emergence method of germination used for non-parasitic weeds cannot be used for the parasitic weeds, especially *Orobanche*, because of their very minute size and obligate dependence on the host stimulants for germination. Further their similarity with other seeds of parasitic plants like *Striga* spp. and *Phelipanche* sp. poses difficulty in their identification. Germination of *Orobanche* is generally erratic (Batchvarova *et al*. 1998) and have the following stringent criteria *i.e.*, soil moisture and temperature during the preconditioning period, availability and reception of host signals as root exudates and finally the viability of seeds (Plakhine and Joel 2010). Thus the estimation of *Orobanche* has always been very tricky and not much work has been done on this earlier. The germination technique using live host plant seedlings described above has been demonstrated to fairly estimate the number of viable seeds present in a defined quantity of soil.

ACKNOWLEDGEMENT

The authors are thankful to Madhya Pradesh Biotechnology Council, Bhopal, M.P. India for financial assistance and Indian Council of Agricultural Research, New Delhi for providing necessary infrastructure facilities for the above study.

REFERENCE

Abbes Z and Kharrat M. 2006. Study of the interaction between *Orobanche foetida* and faba bean at root level. *Tunisian Journal of Plant Protection* **1**(1): 55-64.

Allen EA and Nowak RS. 2008. Effect of pinyon-juniper tree cover on the soil seed bank. *Rangeland Ecology & Management* **61**: 63-73.

Atsatt PR. 1973. Parasitic flowering plants: how did they evolve? *American Naturalist* **107:** 502-510.

Batchvarova RB, Slavov SB and Bossolova SN. 1998. *In vitro* culture of *Orobanche ramose*. *Weed Research* **39:** 191-197.

Creech JE, Westphal A, Ferris VR, Faghihi J, Vyn TJ, Santini JB and Johnson WG. 2008. Influence of winter annual weed management and croprotation on soybean cyst nematode (*Heterodera glycines*) and winter annual weeds. *Weed Science* **56**: 103-111.

Espeland EK, Perkins LB and Leger EA 2010. Comparison of seed bank estimation techniques using six weed species in two soil types. *Rangeland Ecological & Management* **63**: 243-247.

Foley MJY 2004. *Orobanchaceae* of the Arabian Peninsula. *Candollea* **59**: 231-254.

Graves JD. 1995. Host-plant responses to parasitism,pp. 206-225. In: *Parasitic Plants* (Eds. Press MC and Graves JD), Chapman & Hall, London,

Joel DM. 2000. The long-term approach to parasitic weeds control: manipulation of specific developmental mechanisms of the parasite. *Crop Protection* **19**: 753-758.

Granados LPF and Garcý´a Torres L. 1999. Longevity of crenata broomrape (*Orobanche crenata*) seed under soil and laboratory conditions. *Weed Science* **47**: 161-166.

Losner-Goshen D, Portnoy VH, Mayer AM and Joel DM. 1998. Pectolytic Activity by the Haustorium of the Parasitic Plant *Orobanche* L. (Orobanchaceae) in Host Roots. *Annals of Botany* **81**: 319-326.

Mangnus EM, Stommen PLA and Zwanenburg B. 1992. A Standardized Bioassay for Evaluation of Potential Germination Stimulants for Seeds of Parasitic Weeds. *Journal of Plant Growth Regulation* **11**: 91-98

Parker C and Dixon N. 1983. The use of polyethylene bags in the culture and study of Striga spp. and other organisms on crop roots. *Annals of Applied Biology* **103**: 485-488.

Parker C and Riches CR. 1993. *Parasitic Weeds of the World: Biology and Control. CABI*, 332 p

Perez-de-Luque A, Jorin J, Cubero JI and Rubiales D. 2004. *Orobanche crenata* resistance and avoidance in pea (*Pisum* spp.) operate at different developmental stages of the parasite. *Weed Research* **45**: 379-387.

Plakhine D and Joel DM. 2010. Ecophysiological consideration of *Orobanche cumana* germination. *Helia* **33**: 13-18.

Plakhine D, Ziadna H and Joel DM. 2009. Is Seed Conditioning Essential for *Orobanche* Germination? *Pest Management Science* **65**(5): 492-496.

Plaza L, Fernaandez I, Juan R, Pastor J and Pujadas A, 2004. Micromorphological Studies on Seeds of Orobanche species from the Iberian Peninsula and the Balearic Islands, and Their Systematic Significance. *Annals of Botany* **94**: 167-178.

Ramachandraprasad TV, Kiran Kumar VK, Denesh GR, Mishra JS and Prabakhar Setty TK. 2008. Current status of parasitic weeds and their management in India, pp. 49-56 In: *ISWS Biennial Conference on Weed Management in Modern Agriculture*: Emerging Challenges and Opportunities. Feb 27-28, Bihar Veterinary College, Patna.

Sauerborn J, Masri H, Saxena MC and Erskine WA. 1987. Rapid test to screen lentil under laboratory conditions for susceptibility to *Orobanche*. *Lens News* **14**: 15-16.