

Propagation Methods for *Berberis* spp. Habituated in Nilgiri Hills

P NALLATHAMBI^{1*}, C UMA MAHESWARI¹, JAGDISH KUMAR²,
N PREETHI¹ AND RAJESH KUMAR MEENA¹

¹ICAR-Indian Agricultural Research Institute, Regional Station, Wellington,
The Nilgiris, Tamil Nadu - 643231, India

²ICAR-National Institute for Biotic Stress Management, Raipur, Chhattisgarh - 493225, India
*pthambhi@iari.res.in

(Received: October 2018; Revised: November 2018; Accepted: December 2018)

ABSTRACT: *Berberis* species are important concern in agriculture and especially on perpetuation and dissemination of *Puccinia* species. Pathogenesis studies involve uniform population of host as well as pathogen under favorable environmental conditions. Since large population of identical clones or species are essential required for our cross inoculations and pathogenesis studies between main (wheat) and probable alternate hosts (Barberry) systematic experiments were designed on propagation methods. Cumulative analysis of data revealed that the naked seeds expressed better germination (97.5%) seed germination than air layered plants. Seedlings uniformity and establishment were consistent in plants established from seeds compared to air layered plantlets. Seed germination percentage was ranging from 79.5 to 97.5 irrespective of the samples and locations in Nilgiri hills. However, we could select uniform seedlings at very young stage from seed germination experiments than air layered plantlets, wherein uniformity of flowering and seed maturity was not maintained. Present investigations conclude that pathogenesis of *Puccinia* species can be carried out in seedlings plants and rapid production of barberry fruits could be possible in air layered plants than seedling plants.

Keywords: Propagation, *Berberis* species, Seeds, Pathogenesis, Nilgiris

The Western Ghats of India enrich with diversified nature of flora and fauna. Southern hills cover with many peaks above 2000 meters. It is one of the eight hottest host spots having larger biodiversity [1]. Traditionally, forest species are conserved for maintaining ecosystem and to meet our domestic needs like fodder, fruits and timbers etc., Out of plant biodiversity, the species of barberry are perennial shrub and long-lived woody plants habituated in Nilgiri and Kodaikanal hills of Tamil Nadu. There are 55 species of *Berberis* reported in India and out of these, 21 species are found in Himachal Pradesh [2, 3]. Pertaining to relevance of *Berberis* species in agriculture and especially on perpetuation and dissemination of deadly cereal pathogens like *Puccinia* species, they play imperious role, which is under critical investigation at our station. Few species like *B. vulgaris* is well known alternate hosts for *Puccinia* species (*Puccinia graminis* f.sp. *tritici*, *Puccinia triticina* and *Puccinia striiformis*) to complete their life cycle while causing rusts of wheat. Since, barberry fruits are rich in vitamins, minerals and antioxidants, few incompatible species with these rust pathogens are grown for fruits. The local people in Himachal Pradesh, India use roots

and stem bark from different species of *Berberis* to prepare *Rasant* (the village folks prepare the traditional crude drug used to cure several ailments). The roots are also used in several *Ayurvedic* drugs [4]. According to distribution, they are known by different names according to area and habituates of local tribal communities. The fruits, locally known as Oosikala (Tamil) are consumed by tribes in southern hills. Sporadic distribution of *Berberis* species is common in Nilgiri and Kodaikanal hills of Tamil Nadu state. Therefore, we made recurrent surveys and surveillance to understand their role in perpetuation and completion of macro-cyclic stages rust pathogens [5-7]. Since large population of identical clones or species are essential for repeated experiments in cross inoculations and investigation on pathogenesis between main (wheat) and probable alternate hosts (Barberry) under controlled conditions and owing to lack of desired protocols for mass multiplication of this woody species, experiments were designed on propagation methods. Comprehensive results from glass house and field experiments are discussed for rapid multiplication of clonal population of Barberry.

MATERIALS AND METHODS

Collection and Propagation of *Berberis* species

Generally, seeds and other vegetative parts of plants are used for propagation of woody shrubs. Since there are no experimental evidences to choose appropriate growth stage and parts of barberry plants, we attempted to standardize propagation methods. Barberry groves which are habituated in Nilgiris and Kodaikanal hills were spotted and marked for samples collection. Selective locations were identified in Doddabetta, Arakadu, Mynala, Kenthorai, Thummanahatty and Hubathali area in Nilgiris hills and Mannavanur and Boomparai area in Kodaikanal hills and Matured fruits were harvested from June to August months by following random sampling methods in. Ripened fruits were sorted out and cleaned for direct sowing to evaluate influence of natural sowing conditions like in forests. In same season, seed and vegetative propagation methods were evaluated in a set of experiments laid out in completely randomized block design (CRD) under glass house conditions and randomized block design in field for three consecutive years 2014 to 2016. In first set, whole fruits were used for sowing in small pots with natural acidic soil of hilly area amended with decomposed farmyard manure (2:1). Soil mixture was fumigated (imbibition of formalin vapour by applying 1ml/kg v/w) and filled in paper cups (100 ml capacity). In second set; seeds were collected from ripened fruits. For this fruit were crushed, washed in tap water and seeds without pulp were extracted manually and again repeatedly washed in tap water. Since seeds were embedded with fruit pulp, approximately 100 ml per gram of pulp and washed repeatedly. Finally, seeds were shade dried before sowing. In each set of samples, as per norms, 400 seeds were used for germination and seedling establishment [8]. In third experiment, the air layering method was followed, which is commonly under practice for fruit crops. In this method, tenders branches of *Berberis* species were chosen and layered under natural forests conditions. In each layering, 10-15g of dried Sphag num moss was over layered. Branches were chosen for air layering according to availability in particular shrub. Germination percentage of seeds and initiation of rooting and survival of layered branches were monitored regularly throughout experiments.

RESULTS AND DISCUSSION

The role of alternate and collateral hosts is indispensable in perpetuation of *Puccinia* species in the absence of Gramineae hosts. *Berberis* species have been studied extensively in developed countries and they had Barberry eradication programmes, like USA to contain the proliferation of new pathotypes and races of stem rusts of wheat and allied crops. In India, although there is no systematic works on characterization of existing *Berberis* species. Barberries were recorded from Shimla hills first time [9]. Later on, few species like *Berberis vulgaris*, *B. aristata*, *B. lycium* and *B. umbellata* were documented from the Himalayas [10]. These species were investigated to relate them with rusts epidemiology. It is clear that there are no literature evidences on different species of *Berberis* species from southern hills with reference to Nilgiri and Kodaikanal hills of India. These are no reports on rapid multiplication of *Berberis* species. Therefore, efforts were made to collect, characterize and augment the same from southern hills. Systematic surveys were conducted in Southern hills particularly in Kodaikanal hills of Tamil Nadu during the month of November and January. Different species of Barberry bushes were identified in forests located in an elevation of 2421 m (AMSL). Appearance of aecial and pycnial (spermatogonial) stages of rust was recorded between the months of August to February every year.

Cumulative analysis of data revealed that the naked seeds expressed better germination. Seed germination percentage was 97.5 from Mynala area followed by 95% germination in seeds collected from Kenthorai area and 93% germination in seeds collected from Theetukal (dense forest) area of Nilgiris. Less germination percentage was recorded from seeds of Doddabetta area (Figure 1). This variation may be due to maturity

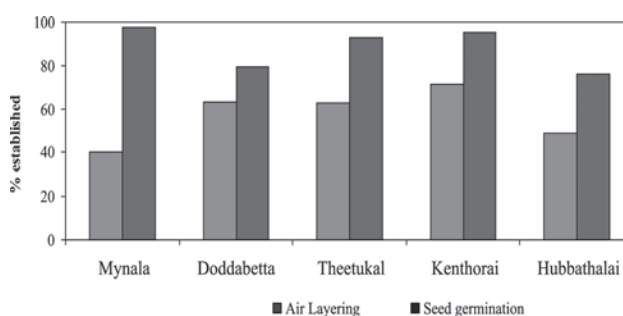


Figure 1. Percentage establishment of *Berberis* plantlets from seeds and air layering

stages in each location because of variation in altitudes and temperature of these locations. Weather conditions of these locations are variable very frequently and these factors may influence the seed maturation and viability of embryo. Frequent varying most of the time. Doddebetta area is always cloudy and cool as compared to Hubbathali area. Germination was poor in the seeds entrenched along with fruit pulp (whole fruits) and hence data was not considered. Rapid germination was noticed in bold seeds extracted from relatively larger fruits than small fruits. Seedling vigour and uniformity were positively correlated with the size of the fruits and seeds. Overall germination percentage was ranging from 79.5 to 97.5 irrespective of the samples and locations tested. Seedlings survival was cent percent without any mortality, perhaps common phenomenon in most of the *Berberis* species. Based on these results, seedlings were multiplied large scale and transplanted in field conditions (Plate 1a-c). Cent per cent success was recorded in transplanted plants than air layered plants. Air layered plants resulted 40 per cent survival, although, all were having profuse rooting after 45 days of layering under forest conditions. The temperature is a critical requirement and high fluctuations in hourly temperature may influence the seed setting and viability even after maturity. Earlier workers suggested that water stress and temperature can influence seed germination [11]. It was also concluded that high-temperature tolerance in seeds is a prerequisite for species to colonize bare grounds and abandoned areas [12]. However, in case of Barberry seeds, these two weather factors have to be investigated further because seeds collected from some of the locations expressed poor germination percentage.

Seeds germination and successful establishments of seedlings are primary events [13]. Vigorous and uniform

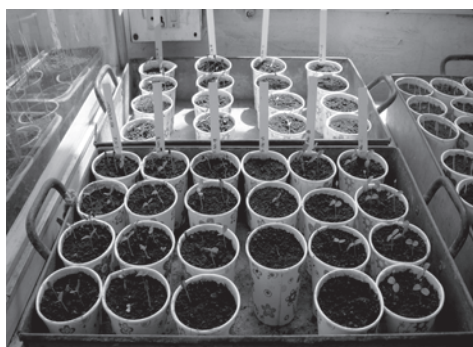
plants are essential for any systematic experiments. However, various environmental conditions encounter before or after sowing of seeds which, decrease or prevent seed germination rate. In present studies also, germination and establishment of seedlings were poor in direct sowing of dry fruits of barberry in field conditions. This was done to imitate the natural conditions in forests. Perhaps due to high soil moisture content under low atmospheric temperature under field conditions, there was poor germination and subsequent survival of seedlings. This agrees with view of earlier report [13] that low temperature coupled with high soil moisture may decrease germination and further establishments of seedlings in large seeds. We also noticed that few seeds were shriveled and seed coat was not even opened to emerge radicle. The physical characteristics and seed viability is mediated by water potential. Nonviable embryo of seed leaks more solutes than viable embryos and hence osmotically active molecules accumulate in the extra embryonic-fluids in non-viable seeds. This increased leakage creates water potential gradient between soak water and seed and enhance water uptake. The additional water uptake by nonviable seeds increases seed swelling and decrease in seed density than viable seeds [13].

ACKNOWLEDGEMENTS

The paper is an outcome of recurrent surveys in Nilgiri and Kodaikanal hills of Tamil Nadu with cooperation for local people and officials of different organizations. The officials of IARI Regional station and Pusa Campus New Delhi-110012 immensely supported during this investigation. All are gratefully acknowledged.



1a. Berry and seeds



1b. Germination



1c. Seedlings

Plate 1 (a-c). Seedlings established for pathogenesis studies on *Puccinia* spp.

REFERENCES

1. NORMAN MYERS, RUSSELL, AMITTERMEIER, G CHRISTINA, MITTERMEIER GUSTAVO, AB DA FONSECA AND JENNIER KENT (2000). Biodiversity hotspots for conservation properties, *Nature*, **403**: 853-858.
2. CHOWDHERY HJ AND BM AWDHWA (1984). Flora of Himachal Pradesh; Analysis, (Botanical Survey of India, Howrah). In Proceedings; *Symposium on "Emerging Issues in Plant Health Management"* organized by Indian Phytopathological Society held at Dr YS Parmar University of Horticulture & Forestry, Nauni, Solan (HP) from September 28-29, 2012.
3. RAO RR, T HUSAIN, B DUTT AND G ARTI (1998). Revision of the Family Berberidaceae of India-I. *Rheedea*, **8**(1): 1-66.
4. RAJASEKARAN AND NILAY KUMAR (2009). Rasont-A traditional crude drug prepared from *Berberis* sp and its uses. *Indian Journal of Traditional Knowledge*, **8**(4): 562-563.
5. NALLATHAMBI P, JAGDISH KUMAR C UMAMAHESWARI, SANDEEP KUMAR, JOHN PETER AND K JAYAKUMAR (2012a). Occurrence of wheat rusts on Barberry grove in Nilgiris Hills. *Nilgiri Wheat News*, **4**(2): 4-5.
6. NALLATHAMBI P, JAGDISH KUMAR, C UMAMAHESHWARI, M SIVASAMY, P JAYAPRAKASH, VK VIKAS AND K JAYAKUMAR (2012b). Standardization of seed germination and seedling establishment of *Berberis* spp. from Nilgiri Hills. *Nilgiri Wheat News*, **4**(2): 5.
7. NALLATHAMBI P, JAGDISH KUMAR, C UMAMAHESWARI, EP VENKATASALAM, A KUMAR, P JAYAPRAKASH, M SIVASAMY AND VK VIKAS (2013). Occurrence of Aecial stage of stem and leaf rusts on *Barberry* species in Nilgiris. *Nilgiri Wheat News*, **5**(1): 4.
8. ISTA (1999). International Rules for Seed Testing. *Seed Science and Technology*, **27** Supplement. pp: 1-333.
9. BARCLAY (1887). A descriptive list of Uredinales occurring in the neighborhood of Shimla (western Himalayas); *Journal of Asiatic Society of Bengal*, **56**(3): 350-375.
10. BULTER EJ AND GR BISBY (1931). *The Fungi of India*, pp:1-237.
11. WEN B, P XUE, ZHANG N, YAN Q, JI M (2015). Seed germination of the invasive species *Piper aduncum* as influenced by high temperature and water stress. *Weed Research*, **55**: 155-162.
12. YUAN X AND B WEN (2018). Seed germination response to high temperature and water stress in three invasive *Asteraceae* weeds from Xishuangbanna, SW China. *PLoS ONE* **13**(1):e0191710. <https://doi.org/10.1371/journal.pone.0191710>.
13. TAYLOR AG, J PRUSINSKI, HJ HILL, AND MD DICKSON (1992). Influence of seed hydration on seedling performance. *Horticulture Technology*, **2**(3): 336-344.