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ORIGINAL ARTICLE

RAPD markers for screening shoot gall maker (*Betousa stylophora* Swinhoe) tolerant genotypes of amla (*Phyllanthus emblica* L.)

Sethuraman Thilaga [a], **Raveendran Rahul Nair** [b], **Murugesan Rajesh Kannan** [a], **Doss Ganesh** [a,*]

[a] *Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Palkalai Nagar, Madurai 625 021, Tamil Nadu, India*

[b] *Aushmath Biosciences, No. 2/1. First Floor, Devaraj Corner, Vadavalli Post, Coimbatore 641 041, India*

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KEYWORDS

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Abstract *Phyllanthus emblica* Linn. is the most important medicinally useful tree crop in Asian Subcontinent and is severely infested by *Betousa stylophora* Swinhoe, known as shoot gall maker (SGM). This pest tunnels the shoots of seedlings and actively growing branches of trees and develops gall, leading to stunted growth, unusual branching and death of actively growing shoots. Our study revealed that trees possessing smooth bark were free from the attack of this pest than those with rough bark surface. Unfortunately, this character is not detectable either at seedling stage or during early growth of trees in the orchard. RAPD genetic fingerprinting of trees possessing smooth and rough bark revealed distinguishable and highly reproducible DNA banding pattern between the two genotypes. Of the 20 RAPD primers tested, five of them produced distinguishable RAPD bands between rough and smooth barked genotypes of *P. emblica*. Trees with smooth bark produced five unique RAPD bands with molecular weight ranging from 350 bp to 1500 bp and those with rough bark produced six RAPD bands (350 bp–650 bp) to utilize these DNA bands as potential DNA marker for screening tolerant genotypes of this crop against SGM. The utility of this finding in genetic improvement of this tree crop against SGM is discussed.

1. Introduction

Phyllanthus emblica Linn (Syn. *Emblica officinalis* Gaertn), commonly known as 'amla' and 'Indian Gooseberry' is one of the popular horticultural fruit crops in Asian Subcontinent for its rich source of vitamin C and medicinal values [1]. Establishment of orchards and sustainable yield is seriously hampered by shoot gall maker (SGM) as this pest attacks seedlings and trees of all the commercial varieties during June–December [2]. Severe infestation of *P. emblica* by SGM often leads to significant level of crop loss since this pest

* Corresponding author.
E-mail address: ganeshdsneha@yahoo.co.in (D. Ganesh).

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attacks young crop bearing branches [3]. Genetic improvement of Indian Gooseberry against SGM by conventional breeding methods is extremely difficult due to complex genetic nature of this tree in addition to very tiny flower [4–6]. Thus, search for natural selection is one of the approaches for developing new varieties with tolerance to SGM.

The types of bark texture among different genotypes of a species form well defined and differential microhabitats for pests to complete their life cycle. Smooth textured bark on tree was reported to be an important trait as an anatomical defense against epiphytic vegetation and insect pests [7]. Smooth bark makes it harder for epiphytes and insects to grip on the surface of the trunk, including the primary and actively growing branches [8]. The density and diversity of insects on the bark surface in trees species is primarily influenced by bark texture [9]. It was demonstrated that bark with rough surface has greater diversity of micro and macro-insects and contrastingly, smooth barked trees harbor either less or no insects [10]. It was reported that structure and color of the bark play a crucial role in tolerant mechanism of several tree species [11–13].

Thicker bark with crevices provides ambient microclimates for the pests to complete their life cycle than the thinner bark [14,15]. Trees with thinner bark receive strong solar radiation due to poor or lack of insulation, leading to unfavorable temperature for the survival of pest on the bark [16,17]. Natural forest comprises tree species with different bark types and they provide ambient microclimates for the survival of a variety of arthropod communities [8]. In our survey, population of *P. emblica* possessing smooth bark surface were generally free from the attack of SGM while trees with rough bark surface were severely infested. Unfortunately, this trait was not detectable either at seedling stage or at early establishment of plants in the field. However, visual differentiation of smooth and rough barked trees could be noticed only after 6–7 years of age after the field establishment. This study reports the influence of bark texture on the incidence of SGM. Trees possessing smooth and rough bark surface were evaluated for tree health and yield. RAPD markers were developed for discrimination between smooth and rough barked genotypes. Utilization of these DNA markers for management of SGM and genetic improvement of *P. emblica* is discussed.

2. Experimental

2.1. Study area

The selected study area comprised of six different geographical locations which includes Sengottai, Tenkasi, Ambasamudram, Kalakkad, Mundanthurai and Alangulam in Tirunelveli District of Tamilnadu which form an extreme part of southern part of India (Fig. 1). These areas are situated close to the Southern Western Ghats lying in the Northern half of Tirunelveli District, Tamilnadu State, between latitudes 8°30′–8°59′N and longitudes 77°10–77°35′E. The annual rainfall and temperature recorded in these places were highly variable from 85 to 215 cm and 26 °C to 36 °C, respectively. However rainfall pattern in these areas was not evenly distributed throughout the year. These areas receive maximum rainfall during September and October–December during South–West Monsoon and North–East Monsoon, respectively. Extensive field observation was carried out in all the study areas during June–December, which records highest incidence of SGM.

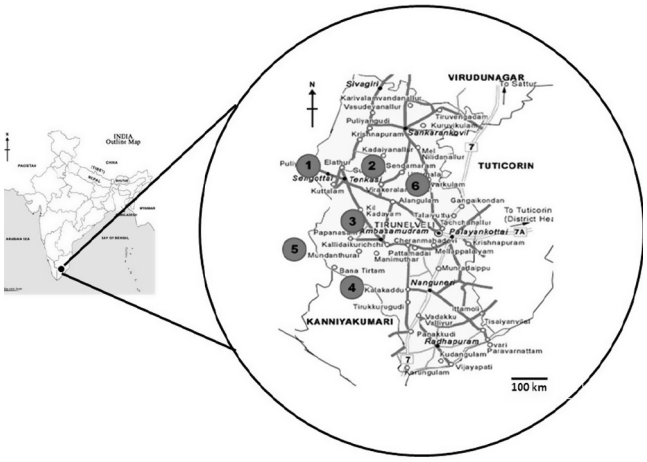

Figure 1 Study area: Map showing the location of sampling and study areas in Tirunelveli District, Tamilnadu in the Western Ghats region of Southern part of India (1. Sengottai, 2. Tenkasi, 3. Ambasamudram, 4. Kalakkad, 5. Mundanthurai, 6. Alangulam).

2.2. Field observations

The orchards subjected to field observations were randomly cultivated with *P. emblica* possessing rough and smooth bark surface (Fig. 2). Observation was carried out on the incidence of SGM in all the selected geographical locations, each comprising 400 well grown 15 years old trees. There were 17 parameters comprising vegetative and reproductive features were included in this study. Observation on tree shape, internodal length, leaf size, fruit size, fruit yield were recorded and analyzed in order to correlate these parameters with SGM incidence. In addition, percentage of SGM incidence, number of shoot galls per tree, defoliation, rejuvenation of young shoots after infestation, bark thickness with crevices and overall tree health was recorded. Data on the incidence of SGM were analyzed critically to associate with smooth and rough barked trees.

2.3. Isolation of genomic DNA

Genomic DNA was extracted from frozen leaf tissues of *P. emblica* by Cetyl Trimethyl Ammonium Bromide (CTAB) method with minor modification [18]. About 0.1 g of leaf tissue was ground into fine powder under liquid N_2 using sterile/chilled mortar and pestle. The powder samples were added into 1 ml of extraction buffer (100 mM Tris, pH 8.0, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% CTAB, 0.3% β - Mercaptoethanol and 1% PVP of Sigma-Aldrich, Mumbai, India) in a centrifuge tube and incubated at 60 °C for 60 min in heating block (Thermo Fisher Scientific, Mumbai, India). Samples were allowed to attain RT, and equal volumes of (24:1) chloroform and isoamyl alcohol (HiMedia Laboratories, Mumbai, India) were added and gently mixed to form an emulsion. Samples were spin at 12,000 rpm for 10 min in centrifuge (Kubota, Japan).