International Journal of Innovative Horticulture. 7(2):139-142, 2018 **Original Articles**

In vitro assay for screening of *Areca* spp. for *Phytophthora* resistance

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ABSTRACT

Fruit and crown rot, diseases caused by *Phytophthora meadii* in arecanut (*Areca catechu* L.), leads to huge yield losses to the growers and remains one of the greatest challenges to arecanut productivity. Developing an arecanut variety with *Phytophthora* resistance, therefore, is of prime significance. *Areca triandra* and *A. concinna,* which are close relatives of arecanut, are possible candidates to develop disease resistant inter-specific hybrids. It is thus imperative to have an effective screening assay for disease resistance. A detached leaf assay is often used to characterize disease susceptibility and screening for resistance to a particular pathogen. Influence of external biotic and abiotic factors in these assays pose potential challenge apart from the biosecurity risks which it carries. In the present study, two approaches were followed to screen for phytophthoral resistance in *A. triandra* and *A. concinna*. In the first approach, challenge inoculation was carried out in embryo cultured plantlets, whereas in latter, it was done on leaf segments of adult field grown plants cultured in Eeuwens Y3 media. Results indicate that *in vitro* assay, involving challenge inoculation on either entire plantlet or leaf segment, is an easy and rapid for disease screening.

Keywords: *Areca catechu*, *A. concinna, A. triandra,* challenge inoculation, disease resistance, embryo culture, *Phytophthora meadii*

INTRODUCTION

The damage caused by *Phytophthora meadii* on *Areca catechu*, the betel nut which is grown extensively in Southern and Eastern parts of India, remains an important concern. The pathogen causes crown rot and fruit rot (Mahali) in arecanut and resulting in significant yield loss (ICAR-CPCRI, 2018). Farmers take up prophylactic measures to control the disease caused by *Phytophthora meadii* through spraying Bordeaux mixture. However the disease could become severe during South West monsoon and result in heavy yield losses if proper prophylactic measures were not taken up. *Areca concinna* and *A. triandra* are close relatives of *Areca catechu*, the cultivated species. Even though these are grown rarely by the farmers, they find use as masticatory and also possess ornamental value (Murthy and Pillai, 1982).

Host defense responses are mediated through different mechanisms, and the timing and degree to which these are activated could determine the outcome of the interaction between a plant and pathogen (Tao *et al*., 2003). A factor driving compatible interactions has been revealed by several studies to involve a mass down-regulation of defense genes (Schlink *et al*., 2010). Pathways associated with these genes include the defense hormone salicylic acid (SA) which is associated with biotrophic defense responses, jasmonic acid (JA), and ethylene (ET) which are associated with necrotrophic defense responses, and abscisic acid (ABA) which is associated with abiotic stress as well as pathogen defense (Bari and Jones, 2009). Multiple pathogenesis related (*PR*) gene classes are differentially regulated against *Phytophthora* and are thought to be important for successful defense (Moy *et al*., 2004; Schlink, 2009; Attard *et al*., 2010). However to delineate this molecular basis of resistance to *Phytophthora meadii,* one needs to have a proper and rapid screening technique.

Challenge inoculation on harvested immature nuts of *A. concinna* and *A. triandra* showed their resistance to *Phytophthora meadii* (Prathibha *et al*., 2015). Developing an arecanut variety possessing resistance to *Phytophthora meadii* would be of great significance to arecanut growers because if proper prophylactic spraying is not undertaken at an appropriate time, it may result in huge losses to the growers. In the present study, two approaches were

followed to screen the resistance in *A. triandra* and *A. concinna,* to *P. meadii*. In the first approach, challenge inoculation was carried out in *in vitro* embryo cultured plantlets whereas in latter it was done on tender leaf segments of adult field grown plants cultured *in vitro*.

MATERIALS AND METHODS

Plant material

In the first approach, entire *in vitro* plantlets were utilized. For this, plantlets were raised from zygotic embryos obtained from green nuts of *A. catechu*, *A. triandra* and *A. concinna* following the standardized protocol (Muralikrishna *et al*., 2017). In the second approach, tender leaves from mature bearing palms of these three species were collected, surface sterilized by swabbing with 70% ethanol followed by washing in sterile distilled water. Leaves were cut into pieces (40 mm) and surface sterilized using 0.01% mercuric chloride (three minutes) followed by washing thrice with sterile distilled water in a laminar air flow chamber. The surface sterilized tender leaf segments were then inoculated on to hormone free Y3 media (Eeuwens, 1976).

Challenge inoculation

In vitro grown plantlets and leaf segments were used to screen their resistance to *Phytophthora meadii*. *P. meadii* was isolated from arecanut fruit rot sample collected from Kasaragod district of Kerala State and pure culture was maintained in carrot agar medium (Fig. 1). In the first approach, each of the leaf segments inoculated in Y3 medium were uniformly pricked (4 pricks) using sterile entomological pin and were inoculated with zoospore suspension. A sterile wet cotton swab was kept over the inoculation area and the culture plates were incubated with 12 hr light/dark period at 27°C and 95% humidity in plant growth chamber (Panasonic MLR-352H-PE). Treatments were compared after three and six days post inoculation (dpi). The membrane stability index served as a viability indicator in leaf segment assay which was determined six dpi according to the method of Sairam (1994). Similarly challenge inoculation method was followed for *in vitro* grown plantlets of above three species. Challenge inoculation was performed on youngest leaf portion of embryo cultured plantlets maintained in hormone free Y3 medium supplemented with sucrose (30 g/l), agar (6.5 g/l) and charcoal (0.5 g/l) with 12 hr light/dark period at 27°C and 95% humidity in plant growth chamber (Panasonic MLR-352H-PE). Observations were made after three and six dpi and images were captured in stereomicroscope (Leica, Germany) fitted with a camera and in turn connected to the computer and controlled by Leica Application Suite (LAS) software.

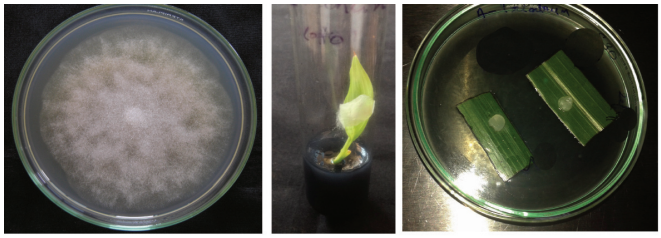

Fig. 1: Culture plate with profuse growth of *Phytophthora meadii* maintained in carrot agar medium and the plant material used in the present study

Statistical analysis

Data on MSI were compared among treatments and tested for their significance through DMRT.

RESULTS AND DISCUSSION

Challenge inoculation of *Phytophthora meadii* was carried out through physical injury on sterilized leaf segments obtained from mature field grown palm and also on *in vitro* grown plantlets of *A. concinna* and *A. triandra*. Observations during six days post inoculation (dpi) revealed the resistance of *A. concinna* and *A. triandra* to *P. meadii*, both in intact plantlet and detached leaf segments. The *P. meadii* infection was evident in *A. catechu* which showed the symptoms within 3 dpi. Infection in *A. catechu* was severe by six dpi both on embryo cultured plantlet as well as in detached leaf segments. In case of *A. concinna* and *A. triandra*, brown spots around the physical injury (prick) was visible within 3 dpi however further infestation was not observed 6 dpi (Fig. 2 & 3). Brown spot around the prick is suggestive of the hypersensitive response of *A. concinna* and *A. triandra* to

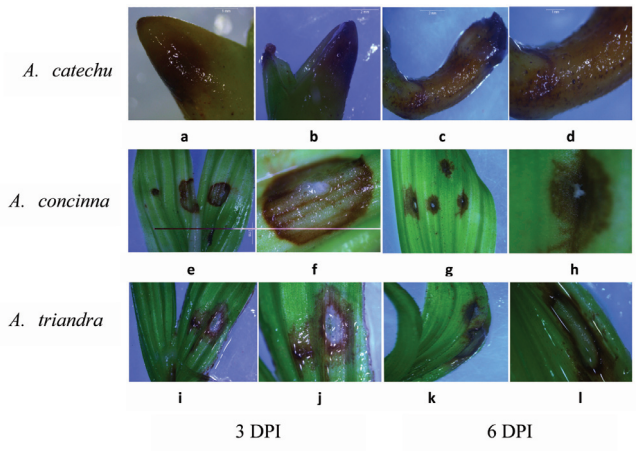

Fig. 2: Effect of challenge inoculation of *Phytophthora meadii*, on *in vitro* derived plantlets of *A. catechu* (a, b, c, d), *A. concinna* (e, f, g, h) and *A. triandra* (i, j, k, l). Plantlets in culture tubes were incubated at 27°C with 12 hr light/dark period and 95% humidity in a plant growth chamber. Treatments were compared three and six days post inoculation.

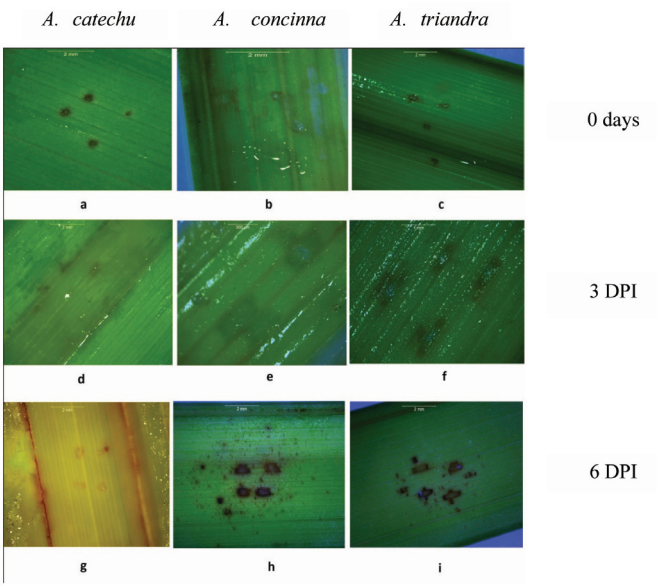

Fig. 3: Effect of challenge inoculation of *Phytophthora meadii* on detached tender leaflets of *A. catechu* (a, d, g), *A. concinna* (b, e, h) and *A. triandra* (e, f, i).

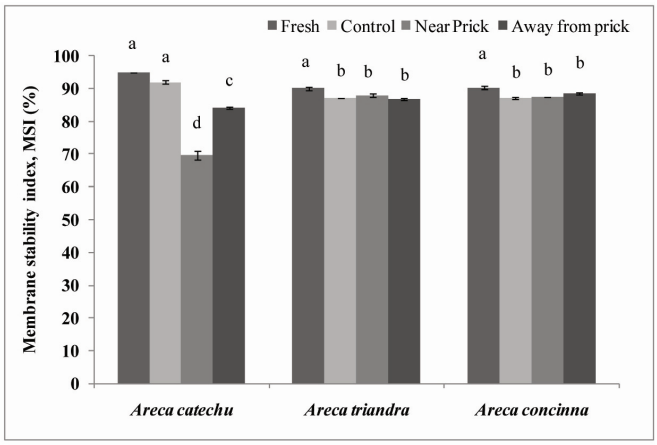

Fig. 4: Effect of challenge inoculation of *Phytophthora meadii*, on to leaf segments cultured *in vitro* in Y3 basal medium, on membrane stability index, MSI. Different alphabets in individual plant species indicate significant difference according to DMRT.

P. meadii; consequently the growth of the mycelium would have arrested in the leaf tissue. The hypersensitive response, involving induction of cell death at the site of pathogen attack in plants, is considered as a typical feature of disease resistance in plants (Pontier *et al*., 1998). Genetic interactions controlling the disease resistance in plants was reported by Flor (1955). The hypersensitive response has been reported to be felicitated by the increased production of reactive oxygen species (Naton *et al*., 1996; May *et al*., 1996; Rao *et al*., 1997).

Results revealed that membrane stability index (MSI) remained constant in the leaf segments during the experimental period indicating the viability of the explants. However, with the spread of infection in *A. catechu*, MSI declined significantly (Fig. 4). Intact plants grown in artificial nutrient medium inoculated with fungus have been effectively used for screening and selection (Lebeda and Buczkowski, 1986; Lebeda and Švábová, 1997; Luhová *et al*., 2002). Similarly excised plant organs such as leaves, stems, shoots, fruits and roots have frequently been used for resistance screening in crop-pathogen interactions (Barlass *et al*., 1986; Lebeda, 1986; Saindrenan *et al*., 1990; Remotti and Löffler, 1996).

Our study highlighted the resistance in wild relatives of arecanut *viz A. concinna* and *A. triandra* to *P. meadii* which is in agreement with previous report where inoculation was done on harvested nuts (Prathibha *et al*., 2015). Through *in vitro* method of screening, one could study the plant pathogen interaction over a period of time without cross contaminations or biosecurity risks, which could occur if the screening is carried out in open fields.

CONCLUSIONS

The present study confirms the existence of resistance in *A. concinna* and *A. triandra* to *Phytophthora meadii*. Challenge inoculation of *in vitro* grown plantlet would provide a window to study real time molecular basis of resistance to *P. meadii*.

ACKNOWLEDGMENT

The authors are grateful to Indian Council of Agricultural Research, New Delhi for the financial assistance.

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