Industrial Crops & Products 122 (2018) 358–365

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Role of benzoic and salicylic acids in the immunization of oil palm seedlings-challenged by *Ganoderma boninense*

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ARTICLE INFO

Keywords:
Ganoderma boninense
Benzoic acid
Salicylic acid
Defence enzyme
Polyphenol oxidase
Lignin

ABSTRACT

Basal stem rot (BSR) infection of oil palm, caused by *Ganoderma boninense*, is one of the key constraining components in palm oil production causing considerable economic losses around the world. Although it was reported a century before, till date no effective controller has been identified, the alteration of lignin content in oil palm can control the BSR is one of the hypothesis. Since the degradation of lignin is the rate limiting step in the infection process of BSR disease. Two naturally occurring phenolic compounds such as benzoic acid and salicylic acid are known to play a key role in the plant defence as well as in the lignin synthesis. The goal of this research is to evaluate the effect of these two naturally occurring phenolic compounds against *G. boninense*. In this study, oil palm seedlings were immunized with various concentrations (1–15 mM) of benzoic acid and salicylic acid, prior to *G. boninense* inoculation. After challenging the oil palm seedlings with *G. boninense*, BSR progression along with the changes in defence enzymes (Phenylalanine ammonia lyase, Peroxidase, and Polyphenol oxidase), and the total lignin contents were examined and evaluated. The exogenous application of the phenolic compounds have not only suppressed the BSR infection but also promoted the plant growth significantly ($P \leq 0.01$). The disease suppression was due to the increased production of defence enzymes particularly polyphenol oxidase which had increased lignin content in the seedlings. An absolute disease reduction was registered in seedlings treated 10 and 15 mM benzoic acid with a significant ($P \leq 0.01$) increment in the production of lignin along with the defence enzymes. Whereas the salicylic acid exhibited an ambivalent behavior, at 1 mM salicylic acid induced the disease by 60% however as the concentration had increased to 5 mM suppression in the BSR was observed. The efficiency of phenolic compounds as an agent of disease reduction is found to be concentration dependent. The outcome of this study has found that benzoic acid at 15 mM concentration is a superior controller for the BSR disease suppression in oil palm. This study would pave the way towards a new management strategy to replace the chemical controls by naturally occurring phenolic compounds to control BSR effectively in oil palm ranches.

1. Introduction

Oil palm, an important cash crop in the tropics with high oil content, has an average yield of about 4 tons per hectare annually (Singh et al., 1991; Sumathi et al., 2008). The oil palm is susceptible to various diseases, so far sixty disease and disorders have been documented (Turner, 1981). Amongst all, Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* is a serious threat to oil palm plantations. Particularly, the Southeast Asian countries suffer severe losses due to BSR disease compared to the other countries such as Africa, Papua New Guinea and Thailand (Idris et al., 2004).

Chemical control is considered as an effective method against any kind of plant diseases. Although the chemical control in plantation crops particularly in tree crops is found to be cost forbidding with minimal effect. In oil palms, reports on the pressure injection of hexaconazole to mitigate BSR disease, suggest that it is not practically feasible because it requires cost-intensive equipment along with the skilled labour (Lam and Chiu, 1993). The cost of the pressure injection process is as expensive as 4.30 USD per tree (Tey and Mohd ahdly, 2007). Cost constrains apart, there are reports that hexaconazole's control over BSR in oil palm is very negligible or nil (Fee, 2011). The best practical approach to control the disease is to identify a potential

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https://doi.org/10.1016/j.indcrop.2018.06.005
Received 23 March 2018; Received in revised form 23 May 2018; Accepted 2 June 2018

compound that can induce resistance in the oil palm. Phenolic compounds are gaining grounds of acknowledgment as a legitimate compound to induce resistance in tree plants. The benzoic acid and their derivatives (salicylic acid) are involved in the signal transduction pathway in host-pathogen interaction and are known to possess antimicrobial activity against various pathogenic fungi (White, 1979). Hence, the exogenous application of phenolic compounds may induce disease resistance in oil-palm plants is the hypothesis.

The host-pathogen interaction relies on the chemical compounds such as lignin, subrin and phenolic compounds to protect the host cell wall (Miedes et al., 2015). Lignin is a naturally occurring aromatic heteropolymer synthesised via phenylpropanoid pathway which is formed with the deamination of L-phenylalanine into cinnamic acid by phenylammonialyase (PAL) enzyme and it is followed by the production of hydrocinnamylalchols. Subsequently, the hydroxycinnamylalcohols is polymerised by peroxidase (POD) and polyphenol oxidase (PPO) to yield a three dimensional, complex lignin structure (Barber and Mitchell, 1997; Higuchi, 1985). In the course of the pathogen attack these compounds are found in the elevated levels in order to eliminate the pathogen. This process is called as the systemic acquired resistance (SAR) (Kessmann et al., 1994). This suggests the importance of PAL, POD and PPO enzymes as effective defence agents in defence system of oil palm through lignification process.

The study is a part of an on-going project to develop an effective stratagem to control BSR disease in the oil palm plantation by identifying a biological control. Ten phenolic compounds were examined for their inhibitory potential towards *G. boninense*, of the ten phenolic compounds benzoic and salicylic acids proved effective (Surendran et al., 2017a, 2017b). In this study, the oil palm seedlings were immunized using different concentrations of benzoic and salicylic acids and challenged with *G. boninense*. The disease suppression ability of these two phenolic compounds was evaluated along with the changes in the pattern of key lignin biosynthesis enzymes such as PAL, POD and PPO and lignin content. Findings from this study will aid in developing an integrated biological control, which can be a potential replacement for the current chemical control methods.

2. Materials and methods

2.1. Preparation of inoculum

Rubber wood blocks (6 × 6 X 6 cm) were obtained from Klang Kayu Gethah Wah Heng Sdn. Bhd, Semenyih, Selangor. The rubber wood blocks were air-dried in an oven for about 48 h, till it reached a constant weight. The dried rubber wood blocks were sterilized at 121 ºC and at 103.4 kPa for 15 min. The sterilization procedure was repeated twice. Each block was placed in a separate heat resistance polypropylene bags before sterilization. 50 ml of potato dextrose agar (PDA) is added to each bag, with even dispersal on the surface of wood blocks. The wood blocks were autoclaved again at 121°C and at 103.4 kPa for 15 min and allowed to cool overnight at room temperature. The 7 days old *G. boninense* strain PER 71 obtained from MPOB was used as an inoculum. Each rubber wood block was inoculated on all the sides of wood blocks using six mycelia plugs (3 mm). The inoculated blocks were then stored in a dark chamber for 4 weeks at room temperature (28 ± 2 °C) for the complete growth of mycelia. Only the blocks completely colonised by *G. bonineses* were used in the disease trial.

2.2. Immunization of oil palm seedlings

Three-months-old oil palm seedlings of commercial variety Dura X Pisifera (DXP) were obtained from Sime Darby Plantation, Selangor, Malaysia. The seedlings were maintained in the greenhouse in polybags (20 X 20 cm) containing sterilized soil mixture (3:2:1 v/v/v topsoil: peat: sand). This setup was maintained in the shade inside the net house for a month for seedling acclimatization. The healthy seedlings with at least three leaves were selected for the study. The oil palm seedlings were pre-treated (soil drenching) weekly for a month with different doses of treatments (250 ml/plant) as listed in Table 1. After pre-treatment, the seedlings were carefully uprooted and inoculated with *G. boninense* colonised rubber wood blocks according to the sitting technique (Idris 2004). The fully colonised rubber wood blocks were placed in contact with the roots of oil palm and then filled with the soil mixture. In case of the negative control the rubber wood blocks without *G. boninense* were placed and treated with distilled water. The oil palm seedlings were treated with respective treatments monthly till the termination of the trial (8 months after inoculation). The experiment was designed in a randomised complete block design (RCBD), two blocks with each treatment consists of 12 replicates and the experiment was conducted thrice. The plants were watered daily. NPK fertilizer (10 g/bag) was applied to all the plants at a monthly interval.

Table 1
List of treatments used to treat oil palm seedlings in this study.

Treatment	Phenolic compounds
T1	Negative control (Distilled water)
T2	Positive control (*G. boninense)*
T3	Benzoic acid (1 mM) + *G. boninense*
T4	Benzoic acid (5 mM) + *G. boninense*
T5	Benzoic acid (10 mM) + *G. boninense*
T6	Benzoic acid (15 mM) + *G. boninense*
T7	Salicylic acid (1 mM) + *G. boninense*
T8	Salicylic acid (5 mM) + *G. boninense*
T9	Salicylic acid (10 mM) + *G. boninense*
T10	Salicylic acid (15 mM) + *G.boninense*

2.3. Plant growth and disease assessment

The plant growth parameters and disease assessment were measured monthly for eight months. The growth parameters such as height of the plant was recorded using measuring tape, SPAD chlorophyll content (Chlorophyll Meter -SPAD-502,Minolta Co. Ltd., Japan), stem (bole) diameter (Digimaticvernier calliper, Mitutoyo, Japan), and the root and shoot weight using digital weighing scale (Mettler Toledo ML-214).

The internal and external disease symptoms were recorded based on the disease rating scale and disease severity (DS) calculated according to MohdZainudin and Abdullah 2008.

$$\text{DS}\ (\%) = \frac{\sum \text{Number of seedlings in the rating X rating number}}{\text{Total number of seedlin assessed X highest rating}} \times 100$$

The data was further used to calculate the area under the disease progression (AUDPC) and percentage of disease reduction (DR).

2.4. Determination of enzymes activities

The second leaf from the top of the seedling was excised and stored in icebox before transferring to the lab. The enzyme activity was assessed, for phenylalanine ammonia lyase (PAL) activity. One gram of leaf was homogenised at 4 °C in 25 mM Tris-HCl buffer (pH8.8) at 8000 RPM (Eppendorf 58,110 R) for 30 min. The reaction mixture contains 1 ml of enzyme extract, 0.5 ml of L-phenylalanine, 0.4 ml of 25 mM Tris-HCl buffer (pH 8.8) incubated for 2 h at 40ºC. The reaction was stopped by the addition of 0.06 ml of 5 N HCL. The observation was read at 290 nm using a spectrophotometer (Parihar et al., 2012).

Total enzyme activity (U/g of fresh tissue) = [(Optical density X dilution factor)/ g of tissue assayed] *100 (3)

To get the crude extract of the enzyme one gram of tissue was homogenised in 5 ml of 50 mM sodium phosphate buffer (pH 6.8) and centrifuged for 1000 RPM at 4°C. For polyphenol oxidase (PPO) activity, to 100 µl of crude enzyme, 50 µl of 60 mM catechol and 2 ml of