IECPS 2024
The 3rd International Electronic Conference on Plant Sciences
15–17 January 2024 | Online

Feasibility of household-scale dual culture bio-assay for in vitro screening of banana resistance against fusarium wilt

Listya Utami Karmawan*, Imelda Judith Kurniawan, and Graffy Intania
Atma Jaya Catholic University of Indonesia; listya.utami@atmajaya.ac.id

INTRODUCTION & AIM

Fusarium wilt disease is the most common type of disease found in the banana plantation, caused by soil born fungus, *Fusarium odoratissimum. F. odoratissimum* was already found in 17 countries, including several developing countries [1]. In Indonesia, *F. odoratissimum* has been found in 15 provinces from regions of Java, Kalimantan, Papua, Nusa Tenggara, Sumatera, and Sulawesi [2]. Unfortunately, some of these areas were in remote areas which has limited access to proper laboratory equipment. In this research, we propose to make an adaptation of dual culture bioassay method for banana plant tissue culture on household-scale to accelerate *Fusarium* wilt management research in remote areas, to improve further research either in remote areas of Indonesia or other countries.

METHOD

Cavendish banana *in vitro* plants were retrieved from local commercial laboratory. Explants from commercial laboratory were subcultured into new MS0 medium inside of test tube and subjected into dual culture bioassay [3]. Fungal (PDA medium) and plant culture media (MS0) was prepared using pressure cooker as household equipment (Fig. 1a). Aseptic techniques in transferring plants and *F. odoratissimum* were done inside glass aquarium/plastic container equipped with UV-C lamp (Fig 1c and d). Plants were cultured in room temperature under cool white tube lamps (Fig. 1b). Autoclave, laminar air flow, and culture room were used as control. Location A was using glass aquarium (Fig. 1c) whereas Location B was using plastic container (not shown).

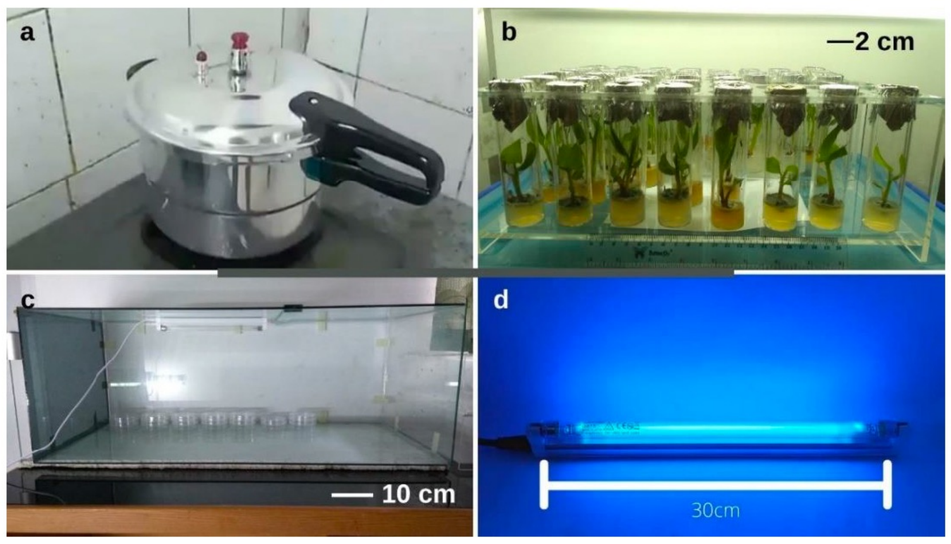

Figure 2. Adaptation of *in vitro* culture of banana plants in household scale. a. Pressure cooker for media sterilization; b. culture condition in room temperature; c. glass aquarium equipped with d. UV-C lamp as laminar for aseptic techniques as an alternative of laminar air flow.

RESULTS & DISCUSSION

The usage of a pressure cooker for MS0 preparation showed no significant difference compared to an autoclave (p-value: 0.287, Kruskal-Wallis test, Table 1), consistent with previous research [4]. Containers equipped with a UVC lamp increased the sterility of commercial PDA medium, the production of sterile non-commercial PDA medium, sterile *F. odoratissimum* subculture in commercial PDA, and sterile banana subculture up to 100%, 86.67%, 93.33%, and 64.29%, respectively. Overall, under a controlled environment, the dual-culture bioassay of banana plants at a household scale yielded similar results compared to a laboratory scale (Table 2, Fig. 2).

Table 1 Success rate in the preparation of planting medium and banana plant subculture

Sterilization Instrument	Treatment	Success Rate of Medium Preparation (%Sterile Medium) (Mean ± Std. Error)*	Success Rate of Banana Subculture (%Sterile Culture) (Mean ± Std. Error)*
Autoclave (laboratory scale)	Laminar Air Flow	100.00 ± 0.00^{a}	100.00 ± 0.00^{cd}
Pressure Cooker (household scale)	Glass Container +UV	100.00 ± 0.00^{a}	64.29 ± 7.15^{abc}
	Glass Container -UV	100.00 ± 0.00^{a}	23.81 ± 11.98^{ab}
	Plastic Container +UV	100.00 ± 0.00^{a}	64.29 ± 7.15^{bcd}
	Plastic Container -UV	97.78 ± 2.22^{a}	0.00 ± 0.00^{a}

*Different letters in the same column indicate a significant difference ($P < 0.05$).

Table 2 Success rate in the dual culture bioassay of banana plants and *Foc* TR4

Location	Treatment	Necrosis area (cm^2) (Mean ± Std. Error)*
Laboratory	Control	6.18 ± 1.13^{a}
Laboratory	Bioassay	26.62 ± 2.51^{c}
Location A	Control	10.43 ± 1.12^{ab}
Location A	Bioassay	51.06 ± 8.15^{d}
Location B	Control	6.15 ± 0.78^{ab}
Location B	Bioassay	13.32 ± 1.63^{b}

*Different letters in the same column indicate a significant difference ($P < 0.05$).

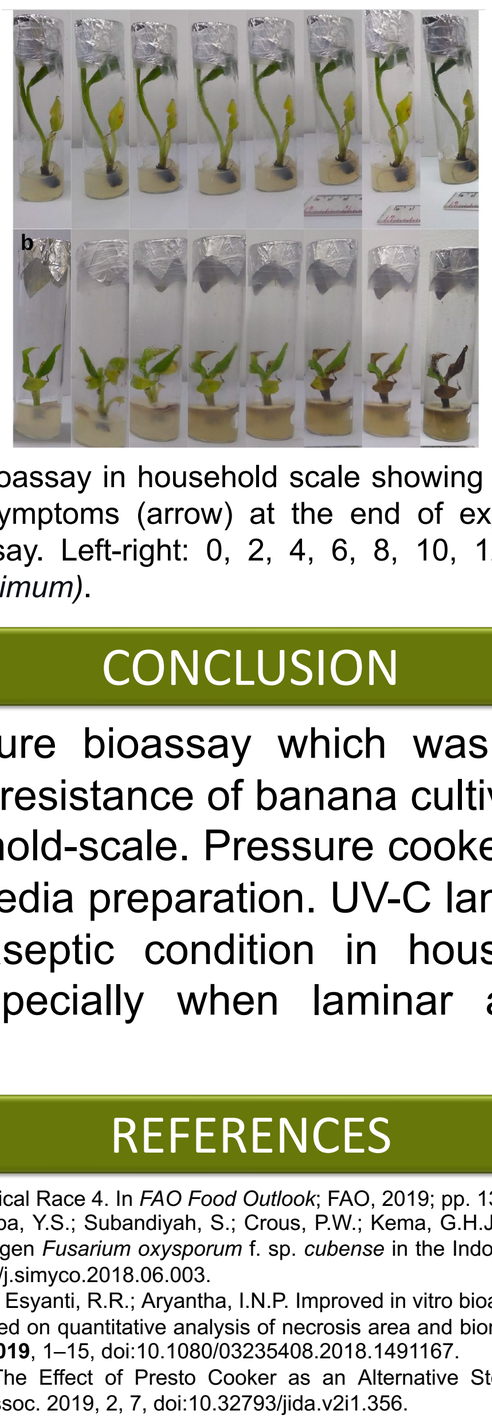

Figure 2. Dual culture bioassay in household scale showing leaf necrosis as one of Fusarium wilt disease symptoms (arrow) at the end of experiment. Top: control, Bottom: dual culture assay. Left-right: 0, 2, 4, 6, 8, 10, 12, 14 dpi (day(s) post inoculation of *F. odoratissimum)*.

CONCLUSION

In vitro dual culture bioassay which was used to analyze pathogenicity and resistance of banana cultivar was feasible to be done in household-scale. Pressure cooker was comparable to autoclave for media preparation. UV-C lamp was mandatory for maintaining aseptic condition in household-scale plant tissue culture especially when laminar air flow was not available.

REFERENCES

1. Fao Banana Fusarium Wilt Tropical Race 4. In *FAO Food Outlook*; FAO, 2019; pp. 13–20.
2. Maryani, N.; Lombard, L.; Poerba, Y.S.; Subandiyah, S.; Crous, P.W.; Kema, G.H.J. Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. *Stud. Mycol.* **2019**, *92*, 155–194, doi:10.1016/j.simyco.2018.06.003.
3. Karmawan, L.U.; Dwivany, F.M.; Esyanti, R.R.; Aryantha, I.N.P. Improved in vitro bioassay for *Musa acuminata* cv. Pisang ambon kuning (AAA group) based on quantitative analysis of necrosis area and biomass changes during Foc4 infection. *Arch. Phytopathol. Plant Prot.* **2019**, 1–15, doi:10.1080/03235408.2018.1491167.
4. Djais, A.A.; Theodorea, C.F. The Effect of Presto Cooker as an Alternative Sterilizer Device for Standard Dental Equipment. J. Indones. Dent. Assoc. 2019, 2, 7, doi:10.32793/jida.v2i1.356.