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Emerging Cassava Root and Stem Rot: A Challenge to Wetland Farmers in Kerala

Cassava (*Manihot esculenta* Crantz) native to Latin America, a drought tolerant, staple food crop is increasingly grown in tropical and subtropical areas, where it could be used as a valuable food source to overcome malnutrition (Bayata, 2019). Cassava is the main source of calories in the tropics and income for small-scale farmers. The mature storage roots can maintain their nutritional value even under poor soil moisture conditions for a long time. Thus, it represents the food security crop in some developing countries. In India, though the crop is practically cultivated in many states, the major cultivation is restricted to Tamil Nadu, Kerala and Andhra Pradesh. In India, cassava is being cultivated in 1.64 lakhs ha with the total production of 50.43 lakh tonnes (FAOSTAT, 2020). The contribution of Kerala in the area and production of cassava is 0.55 lakh ha and 17.26 lakh tonnes respectively (GOI, 2018). In spite of the relative progress in cassava annual yields around the world, the crop is plagued by pests and diseases. Cassava root rot has been reported from different cassava growing countries. The fungal root rot disease damaging storage root of cassava also infects stem, which can be found in humid or poorly drained soil. The disease was considered the second most common reason for reduced cassava yields in Cameroon, according to 36% of farmers (Messiga et al., 2004). Different diseases influence cassava production in Brazil, including cassava root rot disease (CRRD), a key constraint that accounts for up to 80% of yield losses. CRRD symptoms are classified as dry, soft or black rot depending on the causative agent (Bandyopadhyay et al., 2006). *Fusarium* spp. (*F. solani*, *F. oxysporum* and *F. verticillioides*), *Phytophthora* spp.(*P. nicotianae* and *P. drechsleri*), *Pythium scleroteichum*, which cause soft rot, and *Neoscytalidium hyalinum* and *Lasiodiplodia* spp., which cause black rot, are the most common pathogens in Brazil (Oliveira et al., 2013; Machado et al., 2014). In 2019, unlike tuber rot caused by *Phytophthora palmivora* reported previously in Tamil Nadu (Johnson and Palaniswami, 1999; Sankar et al., 2013), which lacks foliage symptoms, root rot causing wilting in cassava fields was noticed in wet lands of Kerala. Therefore, importance of the study was realized with the aim of characterizing the root rot disease and identifying the associated causal agents.

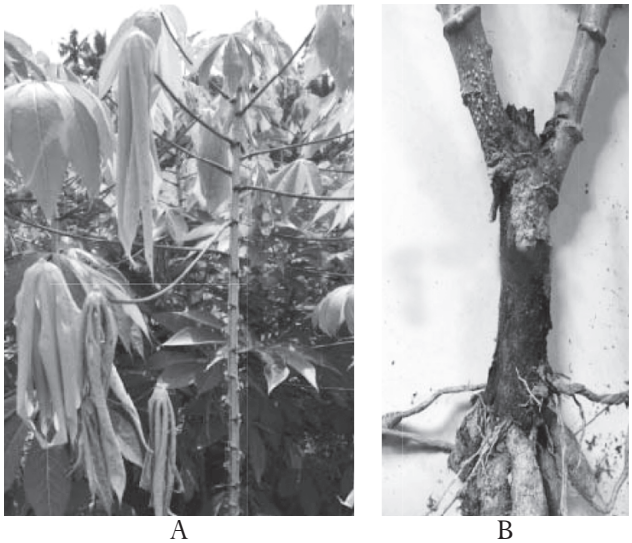
Survey was conducted in different cassava root rot infected areas during 2020 in the farmers' fields in wetland and the percent disease incidence was recorded. The symptoms expressed by infected plants were observed in various fields at different stages of the crop. Cassava stems, storage roots and roots of local varieties with rotting symptoms were sampled from each district viz., Kollam, Kottayam and Thiruvananthapuram from three fields and the associated pathogens were isolated using standard procedures in potato dextrose agar (PDA) medium and purified through single spore isolation method. The pathogen was grown in PDA for 7 to 9 days and the cultural and morphological characters were observed. Fungal isolation was performed on PDA supplemented with ampicillin (150 ppm) to arrest bacterial contamination. The infected roots and the collar region of the stem showing brown necrosis were washed thoroughly in tap water and around 3x 2 mm pieces were excised by covering necrotic as well as healthy portion and they were surface sterilized with 95% alcohol for one minute and sodium hypochlorite for 2-3 minutes. After that, the pieces were rinsed in sterile distilled water three times and dried on sterile blotting paper. The isolation procedure was carried out in a laminar flow chamber. These sterilized materials were incubated at 25°C in a BOD incubator on PDA ampicillin medium.

Fungal colonies that developed from the infected material were subcultured separately on new PDA simple culture media after five to seven days of incubation. A total of 3 isolates were obtained. The pathogen was purified using a single spore isolation method using 2% plain agar. The pathogenecity of the fungus was tested *in vitro* in detached cassava storage roots and roots from six months old crop and also cassava plants in the net house. After culturing in PDA for nine days the morpho cultural characters were recorded. For identification through PCR, DNA was

isolated from the mycelium of three different isolates, from Pallichal (Thiruvananthapuram), Kottarakara (Kollam) and Thalavoor (Kollam). The fungal isolates were cultured on potato dextrose medium (Himedia) by incubating the plates at 28°C for 72h. The mycelia from pure fungal colonies were used for the DNA extraction by CTAB method. Fresh fungal mycelium (~80 mg) was ground using liquid N in a mortar and pestle. One ml of extraction buffer was added, homogenized well and transferred into a new 2ml micro-centrifuge tube. Four µl of Proteinase-K was added, mixed by inversion and incubated at 37°C for 15min. The samples were centrifuged at 12000 rpm for 15min and the supernatant was transferred to a new 2 ml micro-centrifuge tube and an equal amount of phenol:chloroform:isoamyl alcohol (25:24:1) was added and mixed well by inversion. The samples were centrifuged at 12000 rpm for 15min and the upper aqueous layer was carefully transferred into a new 1.5 ml micro-centrifuge tube and an equal volume of ice-cold isopropanol was added and mixed well by inversion, further incubated at -20°C for 1h. The tubes were then centrifuged at 12000 rpm for 15min and the supernatant was discarded and the pellet was washed with 70% ethanol, centrifuged at 10000 rpm for 5 min. The pellet was air dried at 37°C for 30min, suspended in 30 µl of TE buffer and stored in -20°C.

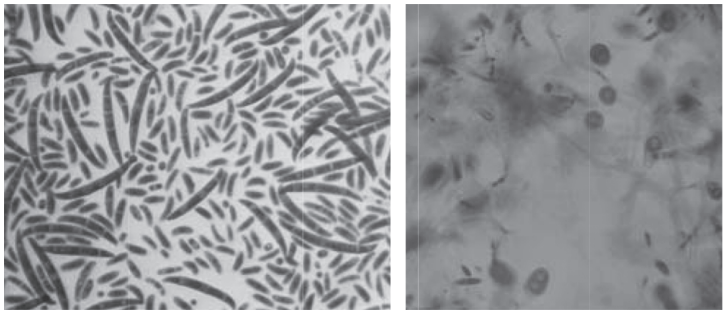
The quality and quantity of the isolated DNA was estimated using Nanodrop spectrophotometer and using agarose gel electrophoresis. Further, the DNA samples were amplified using the following Fusarium specific primers, FusaTef-F: ATGGGTAAGGAGGACAAGAC and FusaTef-R: GGAAGTACCAGTGATCATGTT available at the Molecular Plant Pathology laboratory, ICAR- CTCRI and PCR conditions (Initial denaturation at 95°C for 2 min; 30 cycles each of denaturation at 95°C for 30 sec; annealing at 53°C for 45 sec and extension at 72°C for 1min and a final extension at 72°C for 8 min. The purified PCR product was sequenced by Sanger's method using Applied Biosystems®, the sequence was identified by blasting the sequence in Fusarium ID database. Dual culture has been performed to select potential isolate of *Trichoderma* from ICAR-CTCRI microbial repository and a fungicide from selected fungicide through poisoned food technique against one virulent isolate (CTCRI-FT 1). Based on the result, a *Trichoderma* isolate and a fungicide were selected for future management studies.

Field survey showed that cassava root rot is a new emerging fungal disease in Kollam, Kottayam and Thiruvananthapuram districts of Kerala from 2019 onwards. The infection occurred throughout the crop period. It causes rotting of roots, stem, storage roots and finally wilting of the plants (Fig. 1). The disease incidence varied from 40 to 80 % in Kollam district and 10 to 100% in Thiruvananthpauram and 20 to 80% in Kottayam and the yield loss was upto 100%. All the cultures CTCRI-FPa 1 (Pallichal), CTCRI FKo 1 (Kottarakara) and CTCRI FT 1(Thalavoor) were observed in PDA plates. The cultures were fast-growing with floccose off white to pale violet aerial mycelium. Conidia are generated in sporodochia with fusiform macroconidia which were multi-celled, and have a foot-shaped basal cell with a pointed apical cell which was hyaline and microconidia were single-celled and hyaline. They also formed chlamydospores most commonly under suboptimal growth conditions, produced in pairs or individually (Fig. 2). They were abundant, have rough walls, and are brown and round. In pathogenicity test 100% of the detached roots and storage roots produced symptoms but only 10% of plants in net house produced wilting symptoms which need a detailed study. The TEF 1α gene amplification and sequencing (Applied Biosystems) (Unpublished) revealed that *Fusarium* sp. observed from the samples belong to FSSC (*Fusarium solani* species complex) clade 3, which has to be investigated further to identify the specific species associated with this disease. The interaction with the farmers and department personnel revealed that the



A B

Fig. 1. A) Wilting of cassava plants B) Cassava stem and root rot



A B

Fig. 2. A) Micro and macro conidia B) Chlamydospores of the pathogen, *Fusarium* sp.

disease first appeared in 2018 after heavy rainfall and deluge in Kerala and increased when followed by high temperature (30-34° C). *Fusarium solani* species complexes were generally reported to be the primary species resulting in root rot (Arias et al. 2013). *Fusarium* species have been characterized as geographically distributed soil fungi because the weather has a influential role in the abundance and activity of this species (Doohan et al., 2003).

Under *in vitro* studies *Trichoderma asperellum* (Tr 15-CTCRI) showed highest inhibition (85%). Carbendazim showed maximum inhibition among the fungicides even at 10 ppm concentration (100%). Since the disease is very severe in wetlands in traditional cassava cultivation, the farmers were dejected and for saving the crop urgently, an adhoc integrated strategy was recommended for managing the disease after discussion with the department staffs, farmers and literature on *Fusarium* management, viz., Strict sanitation of the field, removal and burning of highly infected plants, crop rotation with suitable crops for two years, avoiding water stagnation, ensuring good drainage in the plot, application of lime @ 150 to 250 g per plant at 10-15 days before planting with adequate soil moisture during application, when pH of the soil is 4-5, using only healthy setts and avoid setts from infected fields and application of neem cake @ 20 g per plant, application of *Trichoderma asperellum* enriched FYM @ one kg per plant (Prepared by mixing 2.5 kg *Trichoderma* with 100 kg of farmyard manure and then mixed with 12 tonnes of FYM for one hectare (or) 50 g of *Trichoderma* enriched manure (1kg *Trichoderma* mixed with 100 kg of FYM or vermicompost), sett treatment with Carbendazim (0.1 %) (or) combination fungicide contains Carbendazim and Mancozeb (0.2%) for 10 minutes, drenching with the same fungicides starting from planting three times at 15 days interval. The disease incidence was reduced in two farmers' fields in Thiruvananthapuram district (63% and 55% respectively), who followed the strategy as per the recommendation.

Cassava stem and root rot is emerging as serious threat to cassava cultivation in wetlands of Kerala, which may lead to a reduction in the area and production of cassava. The symptomatogy and the causal organism were studied in different fields of Kollam, Kottayam and Thiruvananthapuram districts of Kerala. Potential bio control agents and fungicides were selected for further application. The soil health and planting material need to be taken care of to avoid this problem. During conducive conditions of rainfall followed by high temperature, prophylactic treatment with fungicide needs to be pursued. Identifiying species of *Fusarium* causing the disease needs to be confirmed and a specific management strategy has to be developed.

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