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Vascular Wilt of Roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*) in the Humid Forest Region of South-western Nigeria

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Abstract: The etiology of vascular wilt of Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) was investigated at Ibadan, in the humid forest region of south-western Nigeria. Out of 250 Roselle plants examined, 26% exhibited symptoms of wilt. The vascular wilting of Roselle was associated with *Fusarium oxysporum* Schlecht. Emend. Snyd and Hans. Plant debris was found associated with the fungus.

Key words: *Hibiscus sabdariffa*, vascular wilt, Roselle, humid forest

INTRODUCTION

Roselle (*Hibiscus sabdariffa* var. *sabdariffa* L.) is a vegetable crop widely cultivated in tropical Asia, Australia, West and Central Africa[1]. Young shoots and leaves are consumed as a cooked vegetable and the swollen flower calyces, which have a tart flavor, are used to color and season other foods[2]. It serves as raw materials for preparation of beverages, jam and confectioneries in Malaysia[3]. The seeds contain 17% oil that is similar in properties to cottonseed oil[4]. When fermented, or sprouted, seed are used in sauces[5] and fibers from Roselle are used in making ropes[6].

Diseases have been reported as a limiting factor to the production of Roselle worldwide. These include leaf spot caused by *Cercospora hibisci* (Tracy and Earle) leaf blight caused by *Rhizoctonia solani* Kühn and fruit rot caused by *Phytophthora parasitica* Destor[1,7]. Ooi and Salleh[4] reported *Fusarium oxysporum* Schlecht. Emend. Snyd and Hans, as the causal agent of vascular wilt on Roselle in Malaysia. The causal agent of this disease was also isolated from plant debris and infected soil in Malaysia. Amusa *et al.*[8], however, associated stem blight of Roselle in southwestern Nigeria with *F. oxysporum.* Recent observations on Roselle plots at the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan and farmer's field located in Ibadan, Abeokuta, Akure, and Ile Ife, all within the humid forest agroecologies of western Nigeria, revealed wilting of some Roselle plants. A search into literature revealed that little or no information exists on the diseases of the crop in Nigeria. The aim of this study was to investigate the etiology and epidemiology of vascular wilting of Roselle, in the tropical rain forest of Southwest Nigeria.

MATERIALS AND METHODS

The Roselle plots used for this experiment were located at the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. Ibadan (7°20'N, 3°50'E: 200 mm above sea level) is in a transition zone between the humid forest and derived savanna agro-ecologies of Nigeria. It has a mean annual rainfall of 1300 mm and a mean daily temperature of 34°C (max) and 24°C (min). The Roselle plots planted in 2002 and 2003 were replicated 3 times. A total of 100 Roselle plants were planted in each plot at a spacing of 30x60 cm.

The percentage of wilted and non-wilted plants was recorded for each plot. The development of wilting was monitored regularly for four months. Twenty-wilted samples from the experimental fields were brought to the plant pathology laboratory of the IAR&T, Moor Plantation, Ibadan for pathogen isolation and identification. Soil samples (1-5 cm deep) of Alfisol Ibadan series were also collected randomly once a month from within the field at IAR&T between June and October using a hand trowel and kept in sterile sampling bags for transfer to the laboratory.

Isolation of pathogens: Affected parts of the infected plants were cut into 2 mm pieces, surface sterilized with 1% sodium hypochloride for 30 sec and rinsed in 4 successive changes of sterile distilled water. They were

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then plated on Acidified Potato Dextrose Agar (APDA) and incubated for 6 days at 26°C under a 12 h photoperiod. The fungus were identified using cultural, pathogenicity and morphological features and by comparison with standards[9].

Isolation of pathogen from soil: Soil samples (1 g) were placed in 9 mL of sterile distilled water in MacCartney bottles, shaken vigorously on a vortex mixer for 10 min and then serially diluted to $1x10^{-6}$ after which 1 mL of the suspensions from the $1x10^{-4}$ to $1x10^{-6}$ concentrations was plated on APDA. The plates were incubated for 6 days at 26°C and the fungus identified as described above.

Pathogenicity of the Isolate: The fungal isolate was grown on Potato Dextrose Agar medium in sterile petri dishes and incubated in a Gallenkemp incubator at a temperature of 27±2°C under alternating cycles of 12 h light and 12 h darkness for 8 days. Conidia of the fungus were dislodged with sterile distilled water and a stirring rod and the spores were counted with a heamocytometer and diluted to the desired concentration. The conidial suspension was then thoroughly mixed with oven-sterilised topsoil in plastic pots. Six-week-old seedlings were grown in oven-sterilised topsoil obtained from the IAR&T experimental field in 25 cm diameter plastic pots. The seedlings were transplanted into inoculated soils in plastic pots containing $2.5x10^{6}$ conidia of *F. oxysporum* g^{-1} of soil. Controls were oven-sterilized soil used without the pathogen. A total of 10 Roselle seedlings were inoculated with the isolates of the fungus

The inoculated and the control plants were incubated for 48 h in transparent polythene bags in a mist chamber at 80-85% relative humidity and at 23-26°C. The plants were then placed on benches in a greenhouse and observed for symptoms of the disease. The isolates from the infected plants were compared with the initial isolates using cultural and morphological features.

RESULTS

Symptoms of the disease in the field is the wilting of the whole shoot, while wilted plants have necrotic lesions on the stem, starting from the soil base extending upward affecting most of the branches (Fig. 1). The necrotic lesions (Fig. 2) were usually filled with pinkish colored conidia of the pathogen. The wilting affected young and mature plants and out of 300 plants examined in the field, over 26% were found wilted.

Stem tissues of the wilted plants were not discolored initially, but discoloration developed later, extending into the wood of the stem. Wilting of Roselle was associated

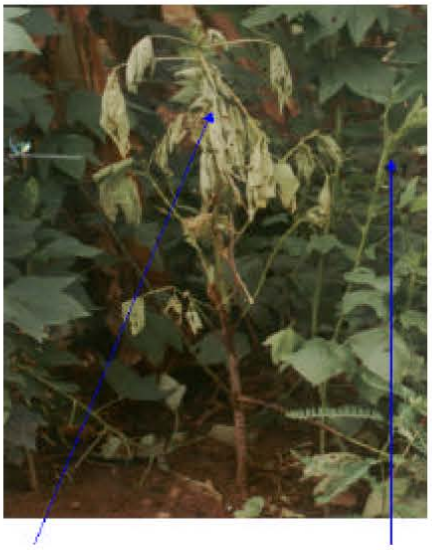

Fig. 1: Roselle plant showing complete shoots wilting. (A) Complete wilting and (B) Non wilted Roselle plant

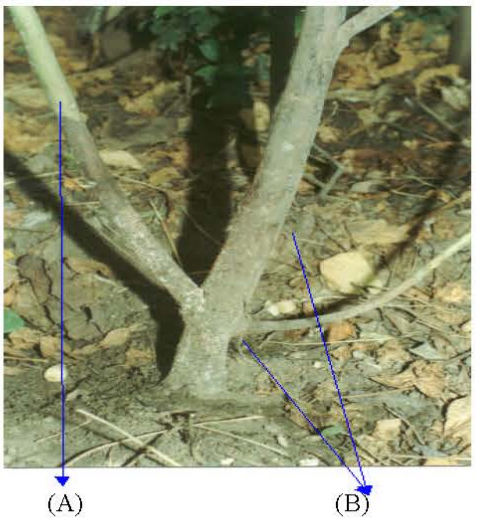

Fig. 2: Wilted Roselle plant showing stem blight disease symptoms (A) non Blighted stem and (B) Blighted stem portions

with *F. oxysporum*, which in culture produced whitish mycelium on PDA tinged with purple. The under-surface is dark purple and conidiophores are borne on branched and sometimes on unbranched short monophialides. Chlamydospores, which are generally abundant in cultures, are formed singly or in pairs. A few oval hyaline single micro-conidia were also produced in culture. Hyaline macro-conidia, with 3-5 septa are usually borne in sporodochia.

Table 1: Mean±SD of inoculum density of *F. oxysporum* found in soil in which Roselle was present at IAR&T Ibadan in 2002 and 2003 planting season

	Soil	
	2002	2003
Months	Mean±SD inoculum load$x10^6$	
May	3.5±0.21	3.6±0.41
June	3.8±0.45	3.9±0.20
July	4.1±0.43	4.2±0.35
August	4.2±0.62	4.3±0.31
September	4.3±0.26	4.5±0.42
October	4.5±0.38	4.7±0.41
Mean	4.1±0.39	4.2±0.35

The soil assay revealed an inoculum density of *F. oxysporum* ranging from $3.5\pm0.31x10^6$ and $3.6\pm0.31x10^6$ colony forming units cfu g^{-1} of soil to $5.1\pm0.31x10^6$ cfu g^{-1} of soil and $5.3\pm0.42x10^6$ cfu g^{-1} of soil in which Roselle was planted, with the mean inoculum load of $5.62\pm0.40x10^6$ cfu g^{-1} of soil, respectively, between May and October 2002 and 2003 (Table 1). The plant debris also harbored the fungal isolates.

Results revealed that *F. oxysporum* isolated from the wilted Roselle plants induced vascular wilt. When re-isolated the fungus was identical to the initial isolate.

DISCUSSION

The fungal pathogen *F. oxysporum* is an ubiquitous pathogen infecting several vegetable crops causing several disease conditions[3,10]. Ooi and Salleh[4] had earlier reported *F. oxysporum* as the causal agent of vascular wilt of Roselle in Malaysia, while Amusa *et al.*[8] reported the pathogen as responsible for the stem blight of Roselle in Nigeria.

The prevalence and the rapid spread of these diseases during the peak of the rainy season could be due to the humid condition prevailing at that time of the year, which supports the rapid production of conidia. Reasons for the above observation might be related to the fact that rainfall or rain-splash probably played an important role in the dispersal of the pathogen's propagules in the field. Egan *et al.*[11] reported the dispersal of the conidia of *F. oxysporum* by water, hurricane wind or excessive rainfall.

The presence of the pathogen in the infected stems, along with high levels of inoculum of the pathogen in the soil, indicates that soil and debris are probably the potential sources of the primary inoculum. *F. oxysporum* was also isolated from the Roselle roots, rotten stems and seeds in Malaysia[4]. While Amusa *et al.*[8] reportedly isolated *F. oxysporum* inducing stem blight of Roselle from soil and plant debris. This is in agreement with the soilborne nature of the pathogen[12]. The Roselle-cultivated soil was also found to harbor the pathogen with mean population of $4.4\pm0.38x10^6$ and $4.7\pm0.35x10^6$ cfu g^{-1} of soil in 2002 and 2003, respectively. It has been reported that *F. oxysporum* persists in soil as dormant chlamydospores that are capable of initiating fresh infection on susceptible host[13]. Ooi and Salleh[4] reported *F. oxysporum* as a soil borne fungus found in agricultural soils throughout the world.

The pathogenic ability of *F. oxysporum* in inducing stem blight, wilting and total death of the whole shoot might be directly related to its ability to produce mycotoxins. *F. vertcillioides* (Sacc.) Nirenberg has been reported to produce Fusarin C and Fumonisin B_1, which probably play major roles in its pathogenicity[14]. The sudden death syndrome of soybean caused by *F. solani* has been attributed to a phytotoxic polypeptide produced by the pathogen[15].

From the foregoing, the severity of vascular wilt of Roselle can be reduced if the site on which the crop is to grow is tested for the presence of pathogenic *F. oxysporum* before planting. General sanitation and proper plot management, which includes elimination and burning of infected plant debris is also advocated to prevent further spread of the disease. Breeding for resistance to this disease is advocated, so that the potential of Roselle in the area can be fully exploited.

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