Annals of Agricultural Science 62 (2017) 83–88

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Contents lists available at ScienceDirect

Annals of Agricultural Science

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

Distribution, pathological and biochemical characterization of *Ralstonia solanacearum* in Benin

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ARTICLE INFO

Article history:
Received 13 November 2016
Received in revised form 6 March 2017
Accepted 13 May 2017
Available online 2 June 2017

Keywords:
Agro-ecological zones
Bacterial wilt
Biovar
Solanum lycopersicon

ABSTRACT

In 2006 and 2007, 75 strains of *Ralstonia solanacearum* were collected from wilting tomato, pepper and eggplant in Benin. The distribution and the incidence of tomato bacterial wilt in the field were assessed by counting wilted tomato plants on 3 plots of 50 m^2 per field. The isolated bacterial strains, including the reference strain, were identified using ELISA, pathogenicity test and carbohydrate oxidation. Bacterial wilt is widely distributed in Benin and was found in five of the eight agro-ecological zones (AEZ) of Benin, which correspond to eight of the 12 districts of Benin. The disease was more severe in ferralitic soil (AEZ V), in valleys and lowlands (AEZ IV) and in highlands (AEZ I). The incidence of tomato bacterial wilt was up to 71%. No *R. solanacearum* strains were isolated from AEZ II, AEZ VII and AEZ VIII. Strains identified as *R. solanacearum* were more widely distributed in the south than the center and the north of Benin. Based on biochemical characteristics, Beninese *R. solanacearum* strains were grouped into biovar I/race1 and biovar III/race 1.

Introduction

Tomato (*Solanum lycopersicon*) is one of the most widely grown vegetable crops in the word (FAO, 1988). In Benin it is the most cultivated vegetable crop and important to the livelihoods of many people in *peri*-urban and rural areas (Agossou et al., 2001). It is used fresh and also processed as paste, juice, powder, or whole, providing a significant dietary source of vitamin A and C. Bacterial wilt, caused by the soil borne bacterium *Ralstonia solanacearum*, is regarded as a major limiting factor for tomato production in Benin, with yield losses of 100% in some area (Sikirou et al., 2001, 2009). Lebeau et al. (2011) reported that bacterial wilt inflicts severe economy losses in many crops worldwide.

Symptoms of *R. solanacearum* on tomato include wilting and necrosis as well as vascular browning (Swanson et al., 2005). Typically, stem and tuber cross-sections ooze whitish bacterial exudates (Genin and Boucher, 2002). The bacterium survives in infected plants, volunteer crops, susceptible weed hosts and infested soil (Hayward, 1994). It is disseminated mainly through use of infected plants, latently infected planting material, and contaminated irrigation water (Hayward, 1994).

The bacterium is widely distributed in tropical, subtropical and temperate regions worldwide on a range of host plants including food crops, cash crops, vegetables and fruits crops (Ji et al., 2005). Bacterial wilt of tomato has been reported in America (Denny and Hayward, 2001; Kim et al., 2003), Europe (Van Elsas et al., 2001), Asia (Fegan, 2005; Elphinstone, 2005) and Africa.

Strains of *R. solanacearum* are diverse in host range, pathogenicity, biochemical and physiological properties, geographical distribution, and epidemiological relationships (Poussier et al., 1999; Horita and Tsuchiya, 2001). The species was subdivided into six races according to host range (Buddenhagen and Kelman, 1964; Pegg and moffett, 1971) and into five biovars (Hayward, 1964; Hayward et al., 1990) based on carbon source utilization. There are numerous subtypes within the biovars that may be associated with particular geographical locations (Buddenhagen and Kelman, 1964). The classification of *R. solanacearum* into races and biovars is superficial and not give any phylogenetic information. Few years ago, based on sequence analysis of the internal transcribed spacer

Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.
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http://dx.doi.org/10.1016/j.aoas.2017.05.003
0570-1783/

region, Fegan and Prior (2005) proposed a new classification scheme. *R. solanacearum* was subdivided into four phylotypes.

Today, although bacterial wilt disease is known to be widespread in Africa, the distribution, the characterization, and the genetic diversity of *R. solanacearum* strains in the continent in general and in Benin in particular, is scarcely documented. Strains were reported from Ethiopia (Lemessa and Zeller, 2007), Nigeria (Osuinde and Ikediugwu, 2002), Uganda (Osiru et al., 2001), Kenya (Ateka et al., 2001), Cameroon (Mahbou Somo Toukam et al., 2009) and Benin (Sikirou et al., 2009). They were also reported from Burundi, Egypt, Libya, Rwanda, South Africa and Tanzania, (Elphinstone, 2005; Fortnum and Kluepfel, 2005). African strains from Reunion, Island, Madagascar, Zimbabwe, and Angola have been characterized (Poussier et al., 1999).

In Benin the distribution and the bacterial wilt are poorly documented and the *R. solanacearum* strains have not been characterized.

Understand local pathogen diversity is capital to succeed breeding and integrated pest management program (Sanchez Perez et al., 2008).

The objectives of the present work were to (i) assess the incidence of tomato bacterial wilt in the field, (ii) study the distribution of *R. solanacearum* in Benin, and (iii) determine the pathogenic and biochemical characteristics of strains of *R. solanacearum* collected from different locations in Benin.

Materials and methods

Survey and incidence of tomato bacterial wilt

A comprehensive survey of bacterial wilt incidence was conducted throughout the 12 districts of Benin grouped in 8 agro-ecological zones (Fig. 1). Three plots of 50 m^2 (5 m × 10 m) were delimited in each of the two fields within three villages in three townships per district; the number of plants per plot varied between 50 and 200 according to farmers' cropping systems. Bacterial wilt incidence was assessed as the number of wilted plants out of the total number of plants per plot.

Origin and collection of strains

In each of the 72 fields surveyed, five wilted tomato plants were uprooted at random for bacterial isolation. The collected diseased samples were treated each day as follow after the survey. The stems were cut at the collar and the leaves were removed. Each stem was surface sterilized with 70% ethanol and washed with sterile distilled water. Five centimeters of the stem were cut from the lowest part, transversely cut into two parts then vertically divided into two or four sections. The stem pieces were soaked in a capped bottle containing 5 ml sterilized distilled water for 30 min to allow bacteria to diffuse into the water (Wullings et al., 1998), then removed with sterilized forceps. Fourfold serial dilutions of each sample were made in sterile water and aliquots of 100 μl were plated on triphenyltetrazolium chloride (TTC) medium (Kelman, 1954). Plates were incubated for 48 h at 30 °C. Presumptive *R. solanacearum* colonies were purified by streaking on a new TTC medium and stored in sterile distilled water in 1.5 ml pipette tubes (Sarstedt D. 51588 Number Drecht) at room temperature [Wullings et al., 1998; Kelman and Person, 1961) and in 20% diluted glycerin at −86 °C.

Strain identification

Strains were initially selected based on similarity in appearance to *R. solanacearum* of known strains on TTC medium. Presumptive

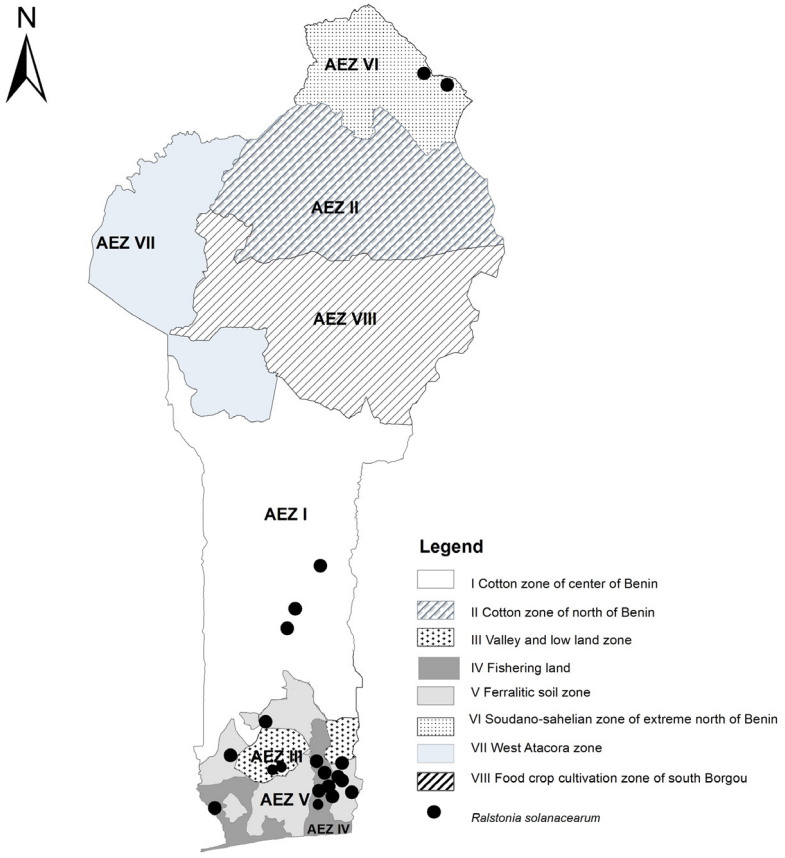

Fig. 1. Map of Benin showing the eight agro-ecological zones and the locations where *Ralstonia solanacearum* strains were collected.