

# Using biochemical markers to detect harmful bacterial wilt disease in potatoes and assess resistance in some cultivars

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## ABSTRACT

*Ralstonia solanacearum* causes bacterial wilt, which is one of the most serious potato diseases in the world's warm temperate zones, tropics, and subtropics. The study was carried out to isolate and identify bacterial infections, as well as to select the most resistant cultivars in order to avoid a drop in the overall value of Egyptian potato exports to the European Union (EU) owing to EU quarantine limitations on bacterial wilt-affected potato tubers. The five isolates were isolated and identified as *Ralstonia solanacearum* using standard identification methods such as morphological and serological tests. Furthermore, the results revealed that the RS5 isolate had the lowest percentage of disease incidence decrease on the three tested potato cultivars Bellini, Spunta, and Mondial, with 9.64 percent, 15.41 percent, and 34.12 percent, respectively, on the three cultivars. On all of the evaluated potato

cultivars, the RS8 isolate was the most successful in terms of disease control. In comparison to the healthy control therapy, this isolate lowered illness incidence by 60.60 percent, 63.21 percent, and 71.66 percent. The results of molecular identification show that the Taq-man (PCR) probe employed was of the type (B2), which could only identify biovar 2 of *R. solanacearum* bacterial wilt. Furthermore, the race 3 biovar 2 strain is detected using a particular primer and probe. With all five isolates, positive results were obtained in all tests employed, including IFAS, protein content, and SDS-PAGE. So the most virulent isolate was (RS5), followed by RS1, RS3, RS2, and RS8, indicating that the isolates examined were *R. solanacearum* race 3, biovar 2. The form of genetic distances and similarities based on pathogenic and plant growth characteristics were also studied. The closest genetic closeness (0.998) was observed between Bellini and Spunta cultivars, while the most distant genetic similarity (0.946) was found between Mondial and Bellini cultivars. These findings were based on the three potato cultivars' genetic distances and SDS-PAGE profiles.

**Key Words:** Biotechnology; Circular economy; Bio based economy

## INTRODUCTION

After wheat, maize, and rice, the potato (*Solanum tuberosum* L.) is generally ranked fourth among the world's most important staple food crops. In the underdeveloped globe, the potato is a consistent nutrition safety crop with enormous potential for difficulty reduction and malnutrition battle. The overall value of Egyptian potato exports is currently in a downward spiral due to the EU's quarantine restrictions on potato bacterial wilt. The bacterial wilt disease was initially identified in Egypt in potato tubers with rot signs. *Ralstonia solanacearum* Smith, which causes bacterial wilt of potato plants and brown rot on potato tubers, is one of the most dangerous diseases to potato production in Asia, Africa, Central and South America. Because these bacteria are soil-borne, they mainly attack plants via the roots and invade xylem vessels. As bacteria fill the vascular bundles of diseased plants, they obstruct the transport of water and nutrients, causing yellowing of the leaves, redness of the vascular bundles, necrosis, and eventually total wilt of the infected plants, which is followed by physiological changes in the diseased plants, such as increased respiratory rate and reduced transpiration and photosynthesis. The bacterium that causes wilt in potato plants is divided into five races based on host range and five biovars based on races' capacity to oxidize three hexose sugar alcohols and three disaccharides. As identified in the Andean area of South America, the race and biovar of *R. solanacearum* are responsible for attacking the potato crop.

R3bv2 was introduced to northern Europe in 1972, causing a surge in illness prevalence and severity, as well as severe harm. As a result, governments enacted quarantine legislation that mandated the destruction of the infected crops as well as the remainder of the impacted fields. Because of cross-reactions of polyclonal antibodies with different microorganisms, serological techniques might lack specificity and have specified susceptibility. Janse et al. employed immunofluorescent antibody staining and Tag-Man (PCR) (2004). To identify *R. solanacearum* strains, a fluorogenic (TaqMan) PCR technique was devised. In a multiplex response, two fluorogenic probes were used: one broad-range probe (RS) identified all *R. solanacearum* biovars, while a second more specialized probe (B2) was used to specifically identify

biovar 2A. Bacterial wilt affects around 200 plant species, representing more than fifty plant families. Because of its infestation, very wide host range, tenacity, and vast geographical dispersion, the bacterial wilt pathogen is the most damaging bacterial pathogen. The disease is abundant in wet equatorial areas, sub-equatorial areas, and certain temperate sections of the world, and it has been estimated to infect over 3.75 million acres in roughly 80 nations worldwide, with annual international damage estimates of over \$950 million. Bacterial wilt is the most serious bacterial illness that affects potatoes, and it was first reported in Egypt many years ago. Because of genetic changes across potato cultivars, disease incidence and severity varies.

Spunta and Bellini cultivars, unlike Mondial cultivars, are among the most vulnerable to the illness, according to studies. As a result of the tests, the closest genetic resemblance was revealed between Bellini and Spunta, while the most distant genetic similarity was observed between Mondial and Bellini. By assessing the protein contents, the genetic distance and similarity coefficient between the three potato cultivars were calculated. As a result, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is the most used method for separating proteins. This approach, which evolved from Laemmli's discontinuous SDS-PAGE technology, is most commonly employed to assess proteins in complicated extracts (1970). This approach uses two types of gels: a solvent gel (also known as running) in which the proteins are centered before entering the resolving gel and a stacking gel in which the proteins are centered before entering the resolving gel. Plants survive in nature because of genetic diversity, and higher yields are a result of it. It helps the breeder get knowledge on variety in plant genetic resources by examining the genetic variations across potato varieties, which raises the likelihood of improving and producing new varieties with desired qualities. As a result, the goal of the study was to identify the bacterial wilt pathogen and distinguish between three extensively grown potato types in Egypt in terms of sensitivity to infection and resistance to infection, so that breeding programs may profit from the findings.

The results show that the Taq-man probe was of the type (B2), which could only identify biovar 2 of *R. solanacearum* bacterial wilt. The most

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virulent strain (RS5) was followed by RS1, RS3, RS2, and RS8, indicating that the isolates tested were *R. solanacearum* race 3, biovar 2. Tag-Man is a molecular discovery technology that combines Polymerase Chain Reaction (PCR) with amplicon fluorescence detection. The RS primers and probe recognized all virulent *R. solanacearum* biovars and races, however the B2 primer and probe are specific for phylotype II sequevar I, which is comparable to Race 3 biovar 2 strain. The goal of this study was to determine the sensitivity of potato types to *R. solanacearum*. It used a range of Egyptian potato cultivars to screen for pathogen sensitivity as a foundation for recovering the administration of potato bacterial wilt. In the current study, no resistance to *R. solanacearum* was found in any of the three potato cultivars investigated, however sensitivity levels varied substantially among them. Because all known Egyptian potato cultivars are vulnerable, breeding efforts should look into resistance in wild solanum kinds that show tolerance or resistance to the disease in order to achieve favorable outcomes.

The study found that owing to the severity of the disease and the penetration of the bacterial infection into plant tissues, there are losses in potato yield in infected plants compared to healthy ones. *R. solanacearum* is a soil-dwelling bacterium that colonizes xylem channels and invades plants through roots. Potato production and yield losses of up to 100% owing to bacterial wilt have been documented in portions of tropical Africa, according to this study. The potato industry in Kenya is threatened by Bacterial Wilt (BW), which is caused by soils contaminated with the wilt-causing bacterium in most producing locations, resulting in yield losses of up to 50%.

Agricultural output losses owing to bacterial wilt ranged from 5% to 80%, according to the producers. According to recent investigations, the illness has been identified in all of Kenya's potato-growing areas, with 77 percent of the country's potato fields infected with tuber seeds imported from Europe. In Uganda, yield losses are projected to be over 30%, with more severe losses reaching 100%. Potatoes were one of Egypt's most exported crops. However, the total value of Egyptian potato exports fell from a high of US\$ 102.12 million in 1995 to \$US 7.7 million in 2000, owing primarily to the European Union's (EU) quarantine restrictions, which accounted for roughly 70–90 percent of Egyptian potato exports and resulted in a drop from 419,000 metric tons to 48,500 tons. The findings of the protein analysis (SDS-PAGE) of the three potato cultivars were used to create a phylogenetic tree based on the genetic distance of both the recorded data from the pathogen and plant growth factors, as well as the results of the protein analysis (SDS-PAGE). Clustering analysis revealed two groupings, dividing the examined items into two categories. The first group consisted of the Spunta and Bellini types, with Mondial forming an isolated group (Group II). As a result, their illness sensitivity was the closest while it was high, as evidenced by pathological tests.

Meanwhile, while planting potatoes in fields with a history of bacterial wilt, the potato types with the least sensitivity to the illness would be researched. As a result, we must develop efforts to find resistant and/or tolerant potato cultivars. SDS-PAGE is an electrophoresis technique for separating proteins by mass. This is a new approach of distinguishing various plants.