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Physiological behavior of cassava plants (*Manihot esculenta* Crantz) in response to infection by *Xanthomonas axonopodis* pv. manihotis under greenhouse conditions

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ABSTRACT

Cassava is one of the main sources of energy for human populations in tropical countries. Cassava production can be severely reduced by diseases, such as the vascular bacteriosis (bacterial blight) caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). In this study, the physiological behavior of plants from two cassava varieties, one resistant and one susceptible to infection by the *Xam* CIO151 strain was evaluated. The plants of the susceptible variety presented increased stomatal resistance, decreased leaf water potential and increased proline content compared with the resistant variety. On the other hand, the inoculated plants from the two varieties showed an increased accumulation of total sugars starting at 7 days post-inoculation compared with the non-inoculated plants.

1. Introduction

Cassava (*Manihot esculenta*) is one of the 100 species found in the *Manihot* genus, whose center of origin has been traced to the Brazilian Amazon [1]. Cassava is a perennial shrub with a height range of 1–5 m, is characterized as monoecious with sympodial branching and has a high tolerance to acid or degraded soils and droughts. The main harvested product is the tuberous roots, which have lots of starch reserves. Cassava is one of the more important crops in the world, contributing, along with corn, sugar cane and rice, as a principal source of energy for human populations in tropical countries [9]. Cassava is grown by poor farmers who depend on this crop as a source of subsistence [14].

The presence of diseases and the lack of technical crop management result in cassava production that does not meet its potential. One of the most destructive diseases is cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. manihotis (*Xam*), which can cause total loss in affected areas. This disease is distributed worldwide and, in particular, has been reported with high incidence in Latin America [36]. The causal agent produces characteristic symptoms in plants, such as angular and translucent spots on leaves that are initially small, but then combine and subsequently turn brown; afterwards, angular leaf spots are enlarged and often exudates a sticky, yellow gum observed also in young stems and petioles. Finally, defoliation, wilting of the shoot and dieback occurs [36]. *Xam* is a gram negative bacterium, so that it does not form spores or capsules, grows on media containing sucrose, producing non-pigmented colonies which differentiate it from other xanthomonads, are usually mobile with one polar flagellum; the physiological characteristics correspond to those found in the *Xanthomonas* group [36].

The epidemiological cycle of this disease starts during the rainy season with the establishment of the parasite on the foliage; this bacterium is carried by rainwater, insects or humans from infected plants or crop debris. The bacterium multiplies on the lower side of the leaves (abaxial), where it forms colonies that are protected by a mucous substance that promotes its subsequent epiphytic multiplication [35]. Afterwards, entry is gained through stomata and wounds, colonizing the intercellular spaces of the mesophyll in the

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leaves, multiplying and producing large amounts of exopolysaccharide matrix fibrils that, by increasing and in conjunction with the lysis of the middle lamella, favor the colonization of the leaf. This stage visually corresponds to the formation of angular and translucent spots [7]. As a result of the bacterium and its matrix, the gels produced by the plant or by specialized structures, such as tyloses, impede the flow of sap and produce wilting of the leaves and the shoot apex. The plant exudes a mixture of latex and bacteria on the surface of the aerial parts as a result of the lysis of adjacent tissues [35].

Activation of the immunity of plants depends on the recognition of the pathogen. This recognition occurs due to the presence of extracellular receptors known as PRRs (Pattern Recognition Receptors) or intracellular proteins called Resistance proteins (R) [12]. Once the pathogen is recognized, several immunity processes are activated, such as changes in ion flows, phosphorylation/dephosphorylation of proteins, hormone production (ethylene, salicylic acid and jasmonic acid), generation of reactive oxygen species, production of phytoalexins, synthesis of compounds that enhance cell walls, activation of transcription factors and expression of genes that encode pathogenesis-related (PR) proteins [6,11,16]. Activation of these mechanisms are associated with alterations in the metabolism, especially photosynthesis, that trigger disturbances in plant growth and development [22]. There are certain characteristics related to plant physiology that are regulated by the interaction with pathogens. It has been reported that the need to increase the production of assimilates occurs because the induction of defenses is highly demanding in terms of energy [29]. The pathogen can also modulate other processes, generating energy for its own growth. In this sense, there is a transition from source to sink in the plant, where the photoassimilates found in the infected cells provide nutrients to the pathogen [15]. It has also been demonstrated that foliar lesions caused by the pathogen lead to alteration of the foliar function and a decreased production of photosynthetic assimilates [6]. In some cases, it has been observed that infection generates a disturbance in the hormonal balance of the plants, which can accelerate or inhibit the senescence of tissues, affecting photosynthesis [5].

Some comparative studies have shown that there is no differential physiological response (inoculated vs. non-inoculated) in plants that are resistant to a pathogen (incompatible interaction), while susceptible plants infected with a virulent pathogen (compatible interaction) do present significant changes in various physiological parameters [2,24]. On the other hand, infected barley plants with either broad or specific resistance to *Blumeria graminis*, have presented decreases in values of physiological variables although with less intensity than those recorded in susceptible plants [33]. Statistically significant differences were observed in physiological and biochemical variables in an incompatible system with tobacco plants infected with *Phytophthora nicotianae* [28]. According to these results, the response at the physiological level in different plant-pathogen interactions is not univocal and is specific to particular pathosystems. In the specific case of a resistance interaction between cassava-*Xam*, through transcriptome analysis, a repression of genes related to photosynthesis was observed 24 h after inoculation (hpi), with a decrease in intensity at 7 and 15 days post-inoculation (dpi) [18]. These results suggest that some molecular components of photosynthesis are affected during *Xam* infection.

Although some cassava varieties have been reported to be resistant to some strains of *Xam*, many of these varieties do not have complete resistance and are not adapted to the different agro-ecological regions where cassava is grown. Additionally, in most cases, these materials do not possess acceptable culinary characteristics. In recent years, significant efforts have been made to elucidate the basis of molecular resistance in cassava to CBB in order to incorporate this knowledge in cassava breeding programs [19]. However, knowledge of the physiological responses of cassava to infection with *Xam* is scarce or nonexistent.

This study evaluated the physiological behavior of cassava plants, resistant and susceptible, infected by *Xam* under greenhouse conditions. This allowed establishing the effect of the bacterial infection in aspects that included the fluorescence of chlorophyll, the leaf water potential, the stomatal resistance, the accumulation of sugars and the proline content.

2. Materials and methods

2.1. Plant material

The stakes of the TMS30572 and CM6438-14 cassava varieties were obtained from the material grown on the Los Bugambiles Farm (La Vega, Cundinamarca, Colombia). The stakes were grown in plastic containers, 15 cm diameter and 20 cm length, and were maintained under greenhouse conditions at 28 °C, 65% relative humidity, photoperiod of 12 h of light and 12 h of darkness, and a photosynthetically active radiation (PAR) of 200 μmol of photons m^{-2} s^{-1}. The plants were periodically fertilized each month in accordance with the nutritional requirements of the cassava plants. The soil was maintained at field capacity condition. This trial was carried out twice under the greenhouse controlled conditions.

2.2. Inoculation and evaluation of the disease

Sixty-day-old plants of each variety were inoculated with the CIO 151 *Xam* strain. This bacterium was grown on a solid YPGA medium (Yeast 5 g/L, Peptone 5 g/L; Glucose 5 g/L, Agar 10 g/L) for 24 h at 28° C before inoculation. From this culture it was prepared an inoculum in YPG liquid medium, adjusting the optical density OD_{600nm} to 0.002 (corresponding to 1.43×10^6 CFU/mL). The inoculation was done by puncturing the second internode below the apical meristem, as previously described [34]. The disease was assessed at 7, 14, 21 and 30 days after inoculation, employing a well establish scale of symptoms from 0 to 5 [31], where 0 = no symptoms, 1 = necrosis at the inoculation point, 2 = stem exudates, 3 = one or two wilted leaves, 4 = more than three wilted leaves and 5 = plant death. Based on the notes of this scale, the disease progress curve was estimated for each replicate by calculating the area under disease progress curve (AUDPC), following the equation: $AUDPC = \Sigma ((Xi + X\,i + 1)/2)\ x\ ((T\,(i + 1) - Ti))$. Where: Ti = is the number of days from inoculation to the date of sampling, Xi = is the disease scale value based on the symptoms (1-5), (T (i + 1)-Ti) = time in days between two readings [30,34]. The experiment was established with a completely randomized design with four treatments: inoculated TMS30572, non-inoculated TMS30572; inoculated CM6438-14 and non-inoculated CM6438-14. Five biological replicates were used to evaluate the gas exchange, chlorophyll *a* fluorescence and AUDPC, and three replicates were used for the biochemical tests and measurement of the leaf water potential. The presence of the inoculum bacterium was confirmed in the plants (completely healthy plants) before the test, at the start of the test (immediately inoculated plants) and at the end of the test. For the latter, the bacterium was isolated and plated on YPGA plates.

2.3. Measurement of the gas exchange and chlorophyll fluorescence *a*

The PAR (Q; μmol s^{-1} m^{-2}), relative humidity, stomatal resistance (Rs; s cm^{-1}), transpiration rate (E; μg H_2O cm^{-2} s^{-1}) and leaf temperature (T, °C) were obtained with an LI-1600 steady-state