

**DEVELOPMENT OF HYPERSENSITIVE RESPONSE BY
XANTHOMONAS CAMPESTRIS PV. *SESAMI* ON *LYCOPERSICON*
ESCULENTUM L., AND *SOLANUM TUBEROSUM* L., LEAVES**

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Abstract

Xanthomonas campestris pv. *sesami* (*Xcs*), causes bacterial blight disease which can significantly reduce the production of sesame. Previously collected isolate of *Xcs* differed in their ability to induce the hypersensitive response (HR) on tomato and potato. Tomato was most sensitive to HR induction by *Xcs*. Isolate *Xcs* caused an HR on tested plants. The minimal concentration of *Xcs* needed to induce HR was approximately 1×10^9 CFU (Colony Forming Unit) mL⁻¹. It was found that different temperatures and light treatments did not affect HR development, except that the HR did not develop in plants maintained continuously at 0°C. When plants were placed under natural light the tissues showed strong chlorosis within 2 days after infiltration. High relative humidity affected the development of HR, in the absence or relative low humidity HR did not develop.

Introduction

Bacterial blight, caused by *Xanthomonas campestris* pv *sesami* (*Xcs*), is one of the major bacterial disease of Sesame (*Sesamum indicum* L.) in Barani areas of Pakistan. The disease caused by *Xcs* was recorded from Pakistan by Mirza & Akhtar (1987). They identified the symptoms of bacterial blight as brown to black spots on the leaves, stem and pods of sesame. Severity of bacterial blight is related to soil moisture (30-40%) and relative humidity (75-85%) (Habish & Hammad, 1970). The highest production reported in Pakistan was during 1979-1980 as 19,300 tons since then its production has declined (Anon., 1995).

In addition to causing disease on host plants, several plant pathogenic bacteria, including *Xcs*, induce a hypersensitive response (HR) when inoculated to incompatible host or non-host plants (Goodman & Novacky, 1994). Plants are resistant to infection by many pathogens. Plants expressing a resistance (*R*) gene rapidly initiate defense responses following contact with a pathogen that expresses a corresponding avirulence (*avr*) gene (Flor, 1971). Isogenic plants lacking the *R* gene fall prey to the pathogen. The hypersensitive response (HR), a rapid cell collapse at the site of infection, is often dramatic evidence of resistance, particularly in bacteria-induced HR (Goodman & Novacky, 1994; Heath, 2000).

Many defense responses associated with bacteria-induced HR have been described since Klement *et al.*, (1964) who first demonstrated the total collapse of nonhost plant leaves following infiltration of pathogenic bacteria. They include ion fluxes, increased salicylic acid (SA) production, membrane depolarization, and pathogenesis-related (PR) gene induction (Goodman & Novacky, 1994; Dangl *et al.*, 1996; Hammond-Kosack & Jones, 1996; Dangl & Jones, 2001). Yet, the mechanism of HR induction is not well understood.

The HR results in a rapid, localized containment of the bacterium and death of plant cells at the site of infection and cells become necrotic 12 to 24 h after inoculation. The HR is thought to represent programmed cell death in plants and this is a form of plant defence against disease. Therefore, an understanding of the bacterium-plant interaction that results in the HR, and subsequent disease defence mechanisms in plants, may lead to the discovery of effective methods for disease control (Dangl *et al.*, 1996).

In this study the *Xcs* isolate that caused bacterial blight disease on sesame were evaluated for their ability to cause an HR on tomato and potato. The HR caused on tomato by *Xcs* isolate was characterized with regard to the minimum cell concentration necessary to cause the HR, and the effects of environmental conditions on development of the response.

Materials and Methods

Isolates and sources: The *Xcs* isolates used in this study were collected from NARC, Islamabad that caused disease on sesame. The bacteria were grown on nutrient glucose agar (NGA) at 28°C. The optical densities of bacterial suspensions were determined with a spectronic 20 spectrophotometer (spectronic Instruments Inc.) and the populations of cells at the various optical densities were determined by dilution plating.

HR development: The isolates were tested for their ability to induce an HR on tomato (*Lycopersicon esculentum* L.) and potato (*Solanum tuberosum* L.). Bacterial suspensions at $OD_{600} = 1$, which corresponds to a cell density of 1×10^9 CFU mL⁻¹, were infiltrated into the leaf mesophyll using a 1mL hypodermic syringe without a needle (Wei *et al.*, 1992). Infiltrated zones were observed for development of tissue collapse and necrosis for 48 h post-infiltration. To analyze bacterial growth in plant, the above mentioned suspension were infiltrated into leaves of tomato. Leaf discs 5mm in diameter were cut from the center of infiltrated zone at 0, 1, 2, 3, 5 and 7 days after inoculation (three discs for each day). Samples were placed individually in 1mL Sterilized Distilled Water (SDW), triturated and dilutions were placed on NGA medium. The experiments were repeated twice.

Effect of bacterial concentrations: The minimum cell concentration of *Xcs* necessary to cause the HR was also determined on tomato, as this value often differs between bacterial species. The minimum cell concentration required to cause the response was determined by infiltrating leaves with the bacterium cultured on NGA and suspended in SDW to concentrations ranging from $OD_{600} = 0.5-1$. Infiltrated areas were monitored for development of tissue collapse and necrosis for 48h post inoculation. For all experiments, at least three leaf panels per three plants were infiltrated, and experiment was repeated thrice.

Effect of temperature and light: Potato was grown in the growth chamber. The effect of temperature on HR was determined on potato leaves following infiltration and incubation at 0, 15, 30 and 35°C for 48 h post-infiltration. The effect of light was determined by constant light for 48h: constant dark for 48h: and light for 24h, dark for 24h. Infiltration was done as described above, and controls consisted of filtration with SDW.

For all experiments at least three leaf panels per three plants were infiltrated and experiment repeated twice. The development of HR was scored 48 h after infiltration.



Fig. 1a and b. Induction of hypersensitive response by *Xcs* on tomato and potato.



Fig. 2. Induction of hypersensitive response by *Xcs* on potato.

Results and Discussion

The *Xcs* showed HR on the leaves of Tomato and Potato. The HR was observed as a rapid localized death of plant cells with no apparent spread of the bacterium from the infiltrated zone. In case of tomato the infiltrated zone showed the first sign of tissue collapse after 36 h of infiltration (Fig. 1a), and within 3-4 days the zone became necrotic surrounded by strong chlorotic halos (Fig. 1b). In case of potato the first sign of tissue collapse was shown after 48 h and infiltrated zones became dry after 4-5 days (Fig. 2). Following infiltration, the *Xcs* cell count was 1×10^9 CFU mL⁻¹ in the mesophyll tissue of leaves. Population of *Xcs* in the infiltrated zone declined rapidly to about 5.6×10^4 CFU mL⁻¹ after 3 days, decreased to about 5.4×10^2 after 4 days and viable *Xcs* cells could not be detected after 7 days post-infiltration.

The hypersensitive response (HR) is a complex, early defense response that causes necrosis and cell death to restrict the growth of a pathogen. The HR occurs in an incompatible host-pathogen combination, whereas disease occurs in compatible interaction. Previous research on the HR elicited by *X. campestris* pv. *glycines* now *X. axonopodis* pv. *glycine* (*Xag*) showed that the bacterium does not induce the HR on tobacco, but efficiently causes the HR response in pepper, and is particularly inductive on tomato (Hwang *et al.*, 1992; Park & Hwang, 1999). Similarly, *Xag* 8ra and its mutants are unable to induce the HR on tobacco, but do on pepper and tomato (Oh *et al.*, 1999).

The minimum concentration of *Xcs* that consistently caused complete collapse of infiltrated tissue after 72 h was 1×10^9 CFU mL⁻¹. A cell concentration of 1.0×10^7 CFU mL⁻¹ resulted in small, collapse spots within the infiltrated zone and did not further increase in size with time. The HR did not develop when a cell concentration of $< 10^7$

CFU m L⁻¹ was infiltrated into the potato plants, but a *Xcs* concentration of 1.0×10^7 caused tissue collapse after 5 days post infiltration into tomato leaves. It was also found that development of HR is not light dependant. When Potato plants were infiltrated with *Xcs*, and placed in growth chamber for different duration of light and dark, it was found that typical HR developed within 72h post infiltration, however, during the experiment it was found that when plants inoculated with *Xcs* were placed under natural sunlight, leaves become chlorotic sharply within 36h. It showed that natural light affect HR development. Temperature 15, 30, 35°C also did not affect development of HR, except that the HR did not develop in plants maintained at 0°C. However, when plants were taken from 0°C and moved to 15 or 20°C in growth chamber collapse of the infected tissue was observed within 24h.

Two types of physiological evidence support the concept that the HR is an active plant metabolic process. First, HR development is profoundly influenced by environmental factors that affect plant metabolic process (Sequeira, 1979). Second, the HR is always associated with active plant resistance against pathogens on non-host plants. Resistance (R) genes detect the pathogen and change the membrane potential and ion permeability of the plasma membrane. In phase one of the response, the R genes trigger an increase in extra cellular pH and K⁺ (Orlandi *et al.*, 1992), while eliciting an influx of calcium and hydrogen ions into the cell. The outward K⁺ and inward Ca⁺² and H⁺ ion flux are dependent and trigger the HR, resulting in cell death and formation of local lesions, which contain antimicrobial compounds. In phase two cells undergoing the HR produce reactive oxygen species (ROS; oxidative burst), including super oxide anions, hydrogen peroxide, and hydroxyl radicals (Baker *et al.*, 1993). Lipid peroxidation and lipid damage may be partially responsible for some of these cell changes and probably affect membrane function. Phenolics and phytoalexins, such as glyceollin (in soybean), and other compounds are synthesized in cells surrounding the lesion. Callose, lignin, are deposited and pathogen related (PR) proteins are induced and include 1,3-glucanase and chitinase.

The HR precedes the secondary resistance response, the systemic acquired response (SAR) and is characterized by necrotic lesions around the infection site; biochemical changes include generation of active oxygen species (oxidative burst), cell death, overproduction of lignin-related materials, and the induction of certain pathogen related (PR) proteins. Atkinson *et al.*, (1993) showed that the HR elicited by *Pseudomonas syringae* pv. *syringae* was inhibited by Ca⁺²-channel blockers. He *et al.*, (1994), using the purified harpin from *Erwinia amylovora*, provided direct evidence that the HR is an active plant process. Meadows & Stall (1981) determined the minimum inhibitory concentrations (MIC) of 12 antimicrobial agents to *X. campestris* pv. *vesicatoria* for growth in nutrient broth, and for the induction of the HR in pepper leaves. The MICs for growth and induction of the HR were similar for chloramphenicol, rifampicin and tetracycline, but were much lower for growth than for the induction of HR for the other antimicrobial agents.

Here it was determined that minimum concentration that completely collapse the tissues was 1×10^9 CFU mL⁻¹, a cell concentration of $<10^7$ CFU mL⁻¹ did not induce HR except in case of tomato that showed collapse of tissues after 5 days post infiltration. These results also showed that tomato is most sensitive than potato for the development of HR. In the absence of relative humidity hypersensitive response did not developed on all plant species.

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