MECHANISMS MODULATING FUNGAL ATTACK TO AVOCADO FRUITS BY POSTHARVEST PATHOGENS AND NEW APPROACHES FOR THEIR CONTROL

D. Prusky and I. Kobiler

Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Bet Dagan, Israel dovprusk@agri.gov.il

Regarding pathogen, fruits and vegetables evolved an impressive array of defensive tools. At the same time, pathogens developed mechanisms to reduce fruit and vegetable resistance mechanisms in what must have been an evolutionary game. Observations indicate that postharvest host-pathogen interactions in avocado fruits are characterized by the following: i. multiple factors of fruit response affecting the resistance; ii. specific fungal factors modulating pathogenicity. Modulation of fungal pathogenicity can be obtained by activating the signal transduction mechanism, metabolizing inhibitory factors and changing the ambient pH where colonization takes place. Avocado fruit ambient pH and other nutritional factors are important since they determine the ability of the pathogen to successfully colonize and invade the targeted host, with the aid of secreted pathogenicity factors. Since pH is a critical consideration in the attack strategy of postharvest pathogens, they have developed environmental sensing mechanisms, enabling them to tailor in ambient conditions, by acidification and alkalinization, to best fit their offensive arsenal. What are the mechanisms used by the pathogen and how could them be modulated to affect fungal colonization? Recent achievements in the resistance of avocado fruits to fungal attack and in the mechanism of Colletotrichum pathogenicity will be summarized, and their use as new approach for postharvest disease control will be discussed.

Key Words: pathogenicity factors, susceptibility, decay.

MECANISMOS DEL AGUACATE CONTRA EL ATAQUE DE PATÓGENOS DE POSCOSECHA Y NUEVOS MÉTODOS PARA CONTROLARLOS

D.Prusky y I. Kobiler

Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Bet Dagan, Israel dovprusk@agri.gov.il

En relación a los patógenos, las frutas y verduras desarrollaron una impresionante serie de herramientas de defensa. A su vez, los patógenos generaron mecanismos para reducir los mecanismos de resistencia de frutas y verduras, lo que se ha convertido en un juego de evolución. Las observaciones señalan que las interacciones de poscosecha entre huésped y patógeno en aguacates se caracterizan por: i. factores múltiples que afectan la resistencia; ii. factores fúngicos específicos que moderan la patogenicidad. La regulación de la patogenicidad fúngica puede lograrse mediante la activación del mecanismo de transducción de señales, transformación por metabolismo de factores inhibidores y la alteración del pH ambiente donde ocurre la colonización. El pH ambiente y otros factores nutritivos del aguacate son importantes, ya que éstos determinan la habilidad del patógeno para colonizar e invadir con éxito el huésped objetivo, esto con la ayuda de factores de patogenicidad encubiertos. Ya que el pH es un aspecto decisivo en la estrategia de ataque de los patógenos de poscosecha, éstos han desarrollado mecanismos con sensores ambientales que les permiten adaptarse a las condiciones del ambiente, mediante acidificación y alcalinización para acomodar de mejor manera su arsenal ofensivo. ¿Cuales son los mecanismos que emplea el patógeno y como pueden ser regulados para afectar la colonización fúngica? Se resumirán los recientes avances en cuanto a la resistencia del aguacate a ataques de hongos y en relación al mecanismo de patogenicidad de Colletotrichum, y además se discutirá su uso como nueva estrategia para el control de enfermedades de poscosecha.

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Palabras clave: patogenicidad, susceptibilidad, enzymas pectolyticas

1. Introduction

Colletotrichum is one of the major postharvest pathogens in which quiescence has been studied. *Colletotrichum* spores adhere to and germinate on the plant surface, produce germ tubes, and the tip of the germ tube developing from the appressorium sends an infection peg through the cuticle. Following penetration, *Colletotrichum* initiates subcuticular intramural colonization (Perfect *et al.,* 1999) and spreads rapidly throughout the tissue with both inter- and intracellular hyphae that kill cells as they advance. After colonizing one or more host cells, the infecting hyphae, which can be described as biotrophic (Kramer-Haimovitch *et al.,* 2006), subsequently give rise to secondary necrotrophic hyphae (Bailey & Jeger, 1992; Coates *et al.,* 1993; Latunde-Dada *et al.,* 1996; Mendgen & Hahn, 2001; O'Connell *et al.,* 1985). *Botrytis* and *Monilinia* can penetrate through wounds and also breach the fruit cuticle by using an infection peg from an appressorium that then remains quiescent for long periods of time (Fourie & Holz, 1995; Pezet *et al.,* 2003). Depending on the physiological status of the organ, these hyphae may continue the infective process or remain quiescent.

Based on published reports, three major hypothetical modes for the activation of quiescent biotrophic pathogens have been suggested (Prusky, 1996): i) deficiency in the host nutritional resources required for pathogen development; ii) the presence of preformed or inducible fungistatic antifungal compounds in resistant unripe fruits, and iii) an unsuitable environment for the activation of fungal pathogenicity factors.
During fruit ripening and senescence, pH levels change as part of the ripening process: for instance, the pH of avocado fruit increases from 5.2 to 6.0 during ripening (Yakoby *et al.,* 2000). Pathogens can also alter the pH at the vicinity of the infection site and the change in pH can modulate the expression of pathogenicity factors (Denison, 2000; Yakoby *et al.,* 2000; Prusky *et al.,* 2001; Eshel *et al.,* 2002; Prusky & Yakoby, 2003). Expression of the endoglucanase

gene *AAK*1 by *Alternaria alternata* was found to be maximal at pH levels above 6.0, values that are characteristic of decayed tissue. *AAK*1 was not expressed at the lower pH values at which the pathogen was quiescent (Eshel *et al.,* 2002). In the pathogen *C. gloeosporioides*, the gene *pel*B was expressed when the pH was above 5.7, a value similar to that of decaying tissue (Yakoby *et al.,* 2000, 2001). The transcription factor *PACC,* which is involved in pH regulation, has a similar expression pattern to that of *PEL*B*,* suggesting that they are co-regulated or that *pac*1 regulates the expression of *PEL*B (Drori *et al.,* 2003). Disruption of *pac1*, the ortholog of *PACC*, of *C. gloeosporioides* resulted in loss-of-function mutants with severely attenuated virulence, suggesting that pH responsiveness is critical to the pathogenicity of this in this fungus. In another example expression of the *Fusarium oxysporum PG*1 and *PG*5 was enhanced, in acidic environment and a sequence that controls the positive activation of genes by pH, the PACC recognition site, was found in *PG*1 (Caracuel *et al.,* 2003). Fine-tuning of enzyme expression in response to the ambient pH in the host by the fungus further highlights the importance of the specific regulatory system that is activated under a changing environmental pH which can lead to the activation of quiescent infections (Prusky & Yakoby, 2003).

The ability to modify pH may be expressed as the alkalinization of the host at the infection point. Alkalinization of the infection site by fungi is achieved by active secretion of ammonia, which is largely the product of protease activity and deamination of amino acids. The pathogenicity of *C. gloeosporioides* and expression of the virulence factor PL-B both depend on raising the ambient pH.

2. New approaches for disease control of postharvest pathogens

Mango and persimmon are two important agricultural produce which require stringent postharvest handling to avoid disease and preserve the quality of the produce (Prusky et al., 2006). This can be achieved by integration of safer synthetic fungicides, biological antagonists and physical treatments. The studies that revealed host environment alkalinization by ammonia secretion during *A. alternata* and *C. gloeosporioides* colonization of fruits have opened a new approach to the modulation of disease development and control. For example, postharvest HCl treatment of mango fruits reduced the incidence of *Alternaria* rot after storage and shelf life (Prusky et al. 2006). These results suggest that acidic solutions may reduce the incidence of rots by modulation of the pH at the infection court. In vitro and in vivo studies also demonstrate that acidic solutions were also very efficient in inhibiting spore germination and germ-tube elongation. Prusky et al. (2006) suggested that acidification of the fruit surface provided an attractive approach for control of alkalizing postharvest pathogens, such as C. gloesporioides and *Alternaria alternat*a.

3. Summary

It has become clear in recent years that the activation of quiescent infections is not a simple process whereby a decline in host resistance results in the activation of fungal attack. Rather, activation of quiescent biotrophic infections seems to involve a coordinated series of events. One component in this complex process can be attributed to the physiological and biochemical changes that occur in the host during ripening and senescence and that lead to decreased host response and increased susceptibility. In parallel, activation of quiescent fungal infections consists of processes that compromise host defenses directly or indirectly by detoxification of antifungal agents. The physiological changes that accompany fruit ripening and host senescence, e.g. host pH, sugar content, cell-wall components and oxidation of wounded tissue, trigger responses by the infecting fungus. The alkalization by ammonia and the possible modulation of the host ROS response and fungal ROS production, may contribute to rapid necrotization of the tissue. Further amplification of the decay can result from activation of gene expression and release of cell-wall-degrading enzymes.

Despite intensive research, we still do not have sufficient knowledge of the spectrum of tools utilized by postharvest pathogens to invoke the transition from a quiescent biotrophic stage to an active necrotrophic one. In *C. gloeosporioides*, alkalization resulted in significant changes in the expression of transcription factors, sugar transporters and a wide range of primary metabolic genes (Miyara *et al.,* 2005). Further clarification of the role of putative signals (pH, nitrogen and sugar) in postharvest pathogenesis during fruit ripening is clearly needed. Nevertheless, the current state of knowledge of fungal modulation of host pH has already opened new avenues to the control of postharvest pathogens (Prusky *et al.,* 2006).

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