Special Traits of Growth of the Agent of Powdery Mildew of Wheat Along and Across the Longitudinal Axis of a Leaf under the Action of Exogenous Zeatin

A. S. Ryabchenko, T. V. Avetisyan, and A. V. Babosha
Tsitsin Central Botanical Garden, Russian Academy of Sciences, Botanicheskaya ul. 4, Moscow, 127276 Russia
e-mail: phimmunitet@yandex.ru
Received October 21, 2008

Abstract—Scanning electronic microscopy was used to investigate the regularities of growth direction of infectious structures and colonies of the agent of powdery mildew of wheat Erysiphe graminis f. sp. tritici. The growth of appressoria with normal morphology in wheat leaves occurs predominantly along the long axis of the cell. Most anomalous appressoria grow perpendicularly. Treatment with zeatin changes the ratio of the directions of growth of normal appressoria and hyphae of the colonies. The dependence of these parameters and of the surficial density of colonies on the concentration of phytohormone is multiphasic. The hypothesis is suggested that the strategy of selection of the direction of growth of infectious structures on leaves with an anisotropic surface depends on the most probable position of the receptor cell and the action of cytokinins on their participation in redistribution of nutrients between the infected and noninfected cells of the host plant.

DOI: 10.1134/S1062359009050033

INTRODUCTION

The agent of powdery mildew Erysiphe graminis DC f. sp. tritici March belongs to obligate pathogens whose development takes place only upon interaction with living cells of the host plant. A special trait of powdery mildews is their attribution to epidermal tissue of the host plants and predominant development on its surface.

Infestation of the cells of the host plant is a complex process consisting of several stages. Under favorable conditions of temperature and humidity, the conidia of the agent of powdery mildew on the leaf surface germinate as the primary germ tube and then as an appressorium and get in contact with epidermal cells of the plant in at least two points. In the course of joint interaction, the appressorium produces an infectious outgrowth sent into the cell of the host plant. By the end of the first day, the haustorium is formed in the epidermal cell of the host plant, which is used for assimilation of nutrients and their transport to growing hyphae of the ectophytic mycelium (Serezhkina et al., 1996). The latter, in turn, form haustoria of the second order. The colony visible to the naked eye is usually formed on the fifth–sixth day.

As a rule, the quantity of normally formed infectious structures of the powdery mildew pathogen correlates with the surface density of colonies. By the latter it is possible to estimate the level of susceptibility of the host plant (Serezhkina et al., 1996, 1999). On leaves of resistant plants of barley and phlox, the part of infectious structures of powdery mildew fungus with deviations from normal development was noticeably higher in comparison with receptive plants (Mishina et al., 1988, 2002; Serezhkina et al., 1996). In this case, strongly elongated germ tubes were formed, their apices often separated from the substratum, lost orientation, and attached to the surface of hairs and to other uncommon surfaces (Serezhkina and Andreev, 1987; Serezhkina et al., 1990).

The development of the pathogen E. graminis tritici on leaves of plants differing in resistance also depends greatly on the level of its compatibility with the host plant (Ryabchenko et al., 2003). In the case of compatible partners, the colonies are formed by densely intertwined hyphae and the number of hyphal lobes is relatively low. At a low compatibility, the number of hyphal lobes increases. However, their adhesion to the leaf surface and ability to form efficient haustoria decrease. This provokes in the pathogen significant transverse growth of mycelium hyphae and occupation of a large area of the leaf surface in the search of other places of penetration to plant cells (Mishina et al., 2001). The colonies formed of sparsely situated mycelium hyphae and growing predominantly across the anticlinal walls of epidermal cells are characteristic of the growth of the powdery mildew pathogen on leaves of some forms of Aegilops speltoides possessing a certain level of resistance to this pathogen (Ryabchenko et al., 2003). On the other hand, the data of Serezhkina et al. (1999) indicate a possible correlation between the surface density of colonies and the size of colonies in the direction across anticlinal walls of the epidermal cells of the host plant.

Lobes of appressoria and hyphal lobes belong to mother cells of haustoria realizing alimentation of the
growing colony of the pathogen. A special trait of the agent of powdery mildew is that of two interaction points; the feeding haustorium develops only in the place of contact of the appressorium lobe. This raises the following question: what is the use for the pathogenesis on interaction with the plant of the primary germ tube producing no haustorium. Obviously, the number, position, and physiological state of the haustoria of the second order determine the size and form of pathogen colonies. In this case, the factors influencing the aforementioned parameters of development of haustoria to a considerable extent control the kind of pathogen development.

The present study is aimed at investigation of some regularities in the direction of growth of infectious structures at early stages of development of the powdery mildew agent. As phytohormones of the cytokinin type are an important factor influencing the pathogenesis, gradations in the zeatin concentration are used for measurement of the susceptibility of wheat plants.

MATERIAL AND METHODS

The objects of investigation were wheat Triticum aestivum L. of the susceptible variety Khakasskaya and disomal-supplemented wheat-aegilops line 56/99 from the collection “Arsenal” NIISKh TsRNZ) in some cases manifesting supersensitivity to the powdery mildew pathogen. The plants were grown at 20–22°C with 16 h photoperiod on Knop solution. In infestation, the population of the agent of powdery mildew used was supported on the susceptible wheat. Separated leaves of 10–12-day-old sprouts were inoculated with the pathogen and incubated on Petri dishes in the floating state, with the axial side upwards. Zeatin in concentrations of 0.25–4.5 µM was added to the nutrient solution directly after infestation.

The plant material for scanning electron microscopy was fixed in a 4% solution of glutaraldehyde and 2% solution of osmium tetroxide and then dehydrated in a series of solutions of ethyl alcohol of increasing concentration and in acetone, dried at the critical point, and sputtered with Au (Mishina et al., 2001). The samples were examined under a scanning electronic microscope LEO-1430 VP (Germany). The number of normal and abnormal (“grown out”) appressoria was counted 24, 48, and 68 h after inoculation and that of appressoria of young colonies after 48, 68, and 72 h growing along and across the long axis of epidermal cells. If the angle between the long axis of the epidermal cell (anticlinal cell wall) and the direction of appressorium growth was within 0°–30°, the appressorium was classified as growing along the long axis of epidermal cells. Accordingly, appressorium growing at an angle of 30°–90° were attributed to those growing across epidermal cells (Figs. 2, 3).

The form of colonies was estimated by superficialization of an oval stencil on photographs of colonies in the program Image J. The large and small diameter of the stencil were used for estimation of the size of a colony along (V) and across (P) the long axis of epidermal cells of the leaf, and determination was made in the same program (Fig. 1). The coefficient of the form of colonies was calculated by the formula V/P.

RESULTS

Certain tendencies in the direction and dynamics of growth of appressorial infectious structures are noted, both of conidia 24, 48, and 68 h after inoculation and of young colonies 48, 68, and 72 h after inoculation.

Six classes of appressoria were specified by directions and kind of growth:

1. normal appressoria growing along the long axis of the leaf (Fig. 2a).
2. normal appressoria growing across the long axis of the leaf (Fig. 2b).
3. anomalous appressoria growing along the long axis of the leaf (Fig. 2c).
4. anomalous appressoria growing across the long axis of the leaf (Fig. 2d).
5. appressoria of microcolonies directed along the long axis of the leaf (Fig. 3a).
6. appressoria of microcolonies directed across the long axis of the leaf (Fig. 3b).

Table 1 demonstrates that the characters of the kind of differentiation and direction of growth are interrelated. Growth of anomalous appressoria is directed predominantly across the long axis of the leaf and, accordingly, of the long axis of epidermal cells. The number of appressoria of this class on average exceeds by three–four times the number of anomalous appressoria growing along the long axis. Taking into consideration the applied method of determination of ratios of appressoria growing along or across the long axis of the leaf, under random selection of the direction of growth, the ratio of the former to the latter should be 1 : 2. Table 1 shows that the ratio observed in the experiment significantly differs from the aforementioned one. Twenty-four hours after infestation, the number of normal infectious structures directed along the axis in the control and in some variants treated with cytokinins exceeded by two times or more the number of appressoria growing across the axis. In the subsequent two days, the number of these classes essentially leveled though the tendency of the prevalence of infectious structures directed along the axis of the cell remained. It is possible to follow the direction of growth of the appressorium in most young colonies (48–72 h after infestation) (Table 2). In all cases, only the growth of colonies consisting of normal appressorial structures was observed, no colonies with anomalous appressoria were found. In all control variants, irrespective of the variety and time of observation, the appressoria directed along the long axis of epidermal cells prevailed in comparison with appressoria directed at angles over 30°. After treatment with cytokinins, depending on