INTERACTION BETWEEN Botrytis cinerea and GRAPEVINE

IRDA SAFNI, SP, MCP.
Fakultas Pertanian
Jurusan Hama dan Penyakit Tumbuhan
Universitas Sumatera Utara

1. INTRODUCTION

The fungus Botrytis sp. causes a serious disease on many important crops, such as strawberry, grapevine, onion, apples, and many ornamental plants. On grapevines, the causal pathogen is the fungus Botrytis cinerea or Botryotinia fuckeliana, a saprophytic mold of the grouping Ascomycetes. This fungus infects the mature grapes (Agrios, 1997, Pearson and Goheen, 1988) and causes a grey mold or soft rot due to the extensive cell wall degradation (Reignault et al, 2000). The infection of the pathogen mostly occurs in the maturing stage of the grapevine.

In the process of infection of the host plant, B. cinerea secretes several chemical substances, such as enzymes (cellulose, polygalacturonase, pectin methylesterase) and polysaccharides to soften the cell wall, and therefore help the fungus to penetrate the plant tissue.

In response to the action of the pathogen infection, grapevine as the host plant defends itself either by forming specific physical and structural defenses or by means of chemical reactions. This process usually occurs after the infection of the pathogen or in the healthy tissue before the incidence of the disease. It can be seen with the structure of the epidermis and thickness and composition of the cuticle, and the presence of preformed antifungal compounds that inhibit the spread of the pathogen.

This essay will describe how B. cinerea attacks grapevine tissue and how grapevines defend themselves to limit the development of the infection, and how to manage and improve the disease resistance.

2. DISEASE DEVELOPMENT

The disease cycle of B. cinerea involves the change in grapevines and the plant symptoms, and it includes inoculation, penetration, invasion, growth and reproduction, dissemination, and the survival stage of the pathogen (Agrios, 1997).

2.1. Inoculation

The inoculation, which is helped by inoculum, is the process where the pathogen makes contact with the plant. B. cinerea utilizes three sources of primary inoculum that play a role in the epidemiology of the disease, namely mycelia,
conidia, and sclerotia (Nair and Hill, 1992). *B. cinerea* produces abundant gray mycelia which are important structures for the fungus to penetrate the plant tissue (Agrios, 1997). The prolific conidia, which are produced in humid weather after the germination of sclerotia, are utilized for dissemination (Agrios, 1997; Nair and Hill, 1992). In the survival stage, the fungus forms black, hard, flat, and irregular sclerotia, and is followed by apothecia formation after two to five months (Agrios, 1997; Nair and Hill, 1992).

2.2. Penetration

The infection of grapevines by *B. cinerea* occurs by direct penetration through lenticels in 20 °C as the optimum temperature (Dickinson and Lucas, 1977; Nair and Hill, 1992). The fungus penetrates the epidermis facilitated by pectinolytic enzymes. In the primary infection, mycelia penetrate the plant surface through natural openings in the cuticle. However, wounds caused by insects, powdery mildew, hail, or birds can stimulate the infection (Pearson and Goheen, 1988). The fungus could also infect flowers of grapevines at the primary infection following the period of latency, where the fungus is in a quiescent phase (Nair and Hill, 1992). It remains in the necrotic stigma and style tissue until the later season (McClellan and Hewitt, 1973). Soon after the fungus becomes active, it infects both the mature grapes and immature grapes in veraison (ripening) as a secondary infection (Nair and Hill, 1992), as a result; it causes bunch rot.

3. CHEMICAL SUBSTANCES ATTACKING PLANTS

Because the fungus is a tiny microorganism, it needs pressure to force an enter into the plant tissue. By secreting metabolites substances, such as enzymes, growth regulators, and polysaccharides, the fungus can weaken the cell walls that can cause maceration of the tissue (Agrios, 1997).

In the early process of infection, the fungus *B. cinerea* secretes several cell wall degradation enzymes, such as celluloses and pectinases, and polysaccharides. The enzymes involve in the activity of disintegrating the protoplast and the permeability of membrane cells whereas polysaccharides influence the translocation of water in the plants (Agrios, 1997).

3.1. Enzyme cellulose

Sasaki and Nagayama (1993) reported that cellulotyc enzymes, -Glucosidase, involved in the pathogenicity of *B. cinerea* on grapevines. The -Glucosidase showed a high activity intercellularly and extracellularly in infecting grapevines. Therefore, this enzyme might be important in the initial degrading the primary cell wall of grapevine tissue because -Glucosidase can hydrolyze glucosides with an aromatic compound.

3.2. Enzyme Pectinase

Two kinds of pectinolytic enzymes play important role in degradation the plant cell wall and providing nutrients for the pathogen, i.e. polygalacturonase (PG) and pectin methylesterase (PME). The effect of pectinolytic activities causes the
weakening of cell walls or tissue maceration and also provide nutrients to the pathogen (Agrios, 1997; Reignault et al., 2000).

Strong activity of polygalacturonase was found in the pathogenicity of grapevine leaves infected by *B. cinerea* (Reignault et al, 2000). They detected three acidic PG isozymes produced from the wild strains of grapevine, which able to colonize and induce rotting of wounded host tissue. Have et al. (1998) also found the presence of polygalacturonase and endopolygalacturonase in the infected area of grapevine due to infection of *B. cinerea*. The enzyme polygalacturonase was predominantly found in the outer zone of the infected area, whereas the enzyme endopolygalacturonase was in the central zone of the infected area.

Similarly, the enzyme PME plays an important role in the pathogenicity of *B. cinerea*. Reignault et al (2000) found a single strongly basic isozyme of PME activity in infected grapevine tissue of *B. cinerea*. Likewise, a strong band of PME in electrophoretic indicated that PME is an important protein of *B. cinerea* (Reignault et al, 1994). The presence of this enzyme in the plant cell wall involves in the cell division and pectin degradation during the early steps of plant invasion by the fungus.

### 3.3. Polysaccharides

*B. cinerea* also produces extracellular polysaccharides including glucanes and rhamno-galacto-mannans. From an experiment, Fanizza et al (1995) reported that the presence of polysaccharides caused a reduction of fresh and dry weight of grapevine shoot and showing a yellowish colour of grapevine leaves. The symptom of yellowing leaves is the evident for the activity of polysaccharides which mainly obstruct the translocation of water in the host plant.

### 4. HOST-PATHOGEN INTERACTION

#### 4.1. Susceptibility of Host Tissue

The susceptibility of the host tissue differs between the vegetative and generative organs. Mature leaves of grapevine tend to be more resistant than the young leaves that are known to be very susceptible (Nair and Hill, 1992). Infection of the leaves normally occurs during spring season where the duration of leaf wetness is long, and therefore facilitates the penetration of mycelium. Due to the lack of water on the leaf surface, the growth of necrotic of the pathogen stops. However, conidia are produced abundantly on the leaf-infection site during the wet season.

In contrast, in the generative stage, young and immature grapes are highly resistant to *B. cinerea* and will develop susceptibility as the plant develops (Hill et al., 1981). Grape are susceptible soon after the start of the ripening process when the contain of sugar concentration is 6-8% (Nair and Hill, 1992). In their experiment, Hill et al (1981) found that the resistance principle of matures grape is in the grape skin, but not in the fruit flesh. It occurs because of the presence of inhibitor substances in grape skins that restrain the activity of enzyme polygalacturonase of the fungus. Other factors such as a loose of grape architecture and cracks in the skin of mature grapes may contribute the susceptibility of grapes (Hill et al, 1981; Nair and Hill,
The cracks often occur due to the destruction of caterpillar and light brown apple-moth.

4.2. Defense Reactions of Host

Grapevine defends itself against fungus *B. cinerea* with by means of structural characteristics acts as physical barriers that inhibit the spreading of the pathogen infection and by biochemical process where the plant secretes toxic substances to the pathogen.

4.2.1. Structural defense

Grapes have been known to inhibit the infection of *B. cinerea* with three kinds of methods, i.e. secreting exudates from the surface, performing morphological barriers, and forming physical barriers. The increasing amount of sugar exudates from the grapes may contribute the resistance of the grape. The addition of sugar exudates as nutrients for the germination of the pathogen only enhances the infection slightly (Nair and Hill, 1992).

Another way rot grapes to protect themselves from the spreading pathogen is by the formation of morphological barriers. The structure and thickness of the cuticle and the epidermal layers largely contribute the resistance of grape against Botrytis infection. These morphological barriers are more likely to play an important role in the mature berries, when they are susceptible to *Botrytis* infection (Nair and Hill, 1992).

In addition, the grapevine can form physical barriers, i.e. suberazation of the cell wall. After the infection of the pathogen, the healthy cells surrounding the infected area begin the suberazation of the infection site. This process is usually followed by formation of a cork layer that blocks the spread of the infection and toxic substances from the pathogen, and also the flow of nutrients and water translocation from the healthy area to the infected area. Furthermore, this process also enhances the secretion of fungal metabolic compounds (Nair and Hill, 1992).

4.2.2. Biochemical defense

Biochemical defenses of grapevines against *Botrytis* infection include enzyme inhibitor substances, phytoalexin synthesis, and the accumulation of defense-related proteins such as pathogenesis-related proteins (PRPs).

The resistant berries, young and immature berries, usually do not secrete toxic substances against *Botrytis*, but only secrete inhibitors substances, such as proanthochyanidins and tannins that are present in the skins of berries (Hill *et al.*, 1981). These enzymes act as inhibitors against the enzyme secreted by the fungus, particularly polygalacturonase (PG) that contributes to the cell maceration. However, *Botrytis* can break down tannins, thus the role of inhibitors substances may contributing a minor factor to the resistance (Nair and Hill, 1992).

In addition, the infected grape can produce several fungitoxic compounds that are directly involved in the early stage of infection. These antifungal compounds are phytoalexins and other phenolic compounds, such as pterostilbene, glycocid acid, viniverins, and other hydroxystilbene derivatives. Phytoalexins are low weight molecular compounds that are synthesized by and accumulated in plants against pathogens (Nair and Hill, 1992). The presence of phytoalexin is initiated soon after
the recognition of the pathogen by the plant, which was proved by the presence of significant amounts of resveratrol, a major component of phytoalexin, before the infection occurs (Jeandet et al, 1995; Langcake and McCarthy, 1979). The presence of stilbene phytoalexin can be determined with emission of bright blue fluorescens around the lesions of green tissues (Nair and Hill, 1992). However, the fungitoxicity of resveratrol is quite low, which can be indicated by the less amount of resveratrol in the more susceptible lesions than in the less susceptible one (Langcake and McCarthy, 1979).

The high concentration of phytoalexin that is found in the grape skins of healthy plant before the ripening process determines the resistance rate of grapevine. Abiotic factor such as UV irradiation can enhance the concentration of phytoalexin (Jeandet, 1995). Systemic signals synthesized by the plant in response to the infection can also be a factor that influences the concentration of phytoalexin in the plant tissue.

However, the concentration of phytoalexin decreases from ripening to maturity. This may be due to a detoxification of phytoalexin and/or a laccase-like stilbene oxidase activity and a competition between chalcone synthase and stilbene synthase (Bavaresco et al, 1997).

In short, stilbene phytoalexin and their oxidation products play important roles in the defense system of the grapevine, by showing the antifungal activity and enhancing other phenolic compounds around the infected area.

Besides those compounds, pathogenesis-related proteins (PRPs) induced by salicylic acid and B. cinerea also contribute the resistance of grapevine. Some experiments reported the role of defense-related proteins, such as chitinase and 1,3-glucanase, solely or combination of both, can decrease the development of grey mould disease of grapevine (Derckel et al, 1998; Renault et al, 1996). Salicylic acid is a substance involved in many plants in regulating expression of their defense machinery, where in grapevine it controls the production of several chitinase isoforms in leaves and berries (Demsey and Klessig, 1994; Derckel et al., 1998a). Derckel et al. (1998b) showed that the accumulation of PRPs was found around localized of dead cells due to hypersensitive response. Many studies suggest that grapevine leaves undergo a hypersensitive response that is associated with localized cell death of the host plant to inhibit the growth of the pathogen (Agrios, 1997).

5. GENETICS OF RESISTANCE

The infection of B. cinerea is due to the interaction between the fungus and grapevine as the host plant, and their genetic material, the DNA, determine the properties of both of them (Agrios, 1997). Recently, the inheritance of host reaction and the inheritance of pathogen infection have been conducted to breed and to distribute varieties resistant to pathogen. The inheritance of host reaction includes the degree of resistance susceptibility to some pathogen carried by letter R and r respectively, and the inheritance of pathogen infection comprises the degree of Avirulence and virulence of the pathogen allele carried by A and a respectively.

This theory can be applied with the molecular model of gene-for-gene concept, where the specific Resistance gene in grapevine must interact with a
specific Avirulence gene in *B. cinerea* to give disease resistance or incompatible interaction (Agrios, 1997).

The susceptible cultivars of grapevine against *B. cinerea* differ based on the compactness of their clusters, the thickness and anatomy of the berry skins, and their chemical defense substances, such as phytoalexin and other phenolic compounds. To protect the susceptible cultivars, it is important to combine cultural practices and chemical control (Pearson and Goheen, 1988). Furthermore, there have been resistant grapevine cultivars introduced recently (Nair and Hill, 1992)

6. CONCLUSION

The interaction between grapevine and fungus *B. cinerea* cause grey mould or bunch rot disease is specific due to the response between the pathogen and the host. *B. cinerea* secretes several toxic enzymes, namely cellulose, pectinase including polygalacturonase and pectin methylesterase, and polysaccharide, which result in cell wall weakening and cell maceration.

Interestingly, grapevine also gives response due to their defense against the infection of pathogen. The defense mechanisms comprise structural defenses, which form morphological and physical barriers, and biochemical defenses including enzyme inhibitor substances, phytoalexin synthesis, and the accumulation of defense-related proteins such as pathogenesis-related proteins (PRPs).

To manage and improvement of disease resistance, molecular model of gene-for gene concepts have been introduced. It can improve the incompatible or resistant interaction between the fungus and grapevine.
REFERENCES


