Induced Biochemical Defence Mechanisms in Tea against Blister Blight

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Introduction

Blister blight is the major and most destructive leaf disease in tea that caused by *Exobasidium vexans*, fungi. It is a poly-cycle disease. The pathogen completes its life cycle in 11-28 days (Gadd *et al.*, 1948). There are no tea cultivars which have been found to acquire complete resistance to the disease. Tea cultivars in Sri Lanka have been categorized in to resistant and susceptible (Balasooriya *et al.*, 1996). Appropriate control measures are vital for the survival of the plant. Chemical fungicides are sprayed at recommended intervals throughout the disease season and an average of 24-28 rounds of fungicides spray is required to keep the disease under control (Ajay *et al.*, 2009). The large scale applications of fungicides pollute the environment and their residues can cause various health hazards to the human beings. An alternative strategy is to understand the tea plants induced biochemical defence mechanisms exploiting natural defenses against blister blight and using them in disease management. This approach would be an environment friendly, sustainable approach.

Methodology

Total proteins were extracted in phosphate buffer (20 mM, pH 7) from healthy leaves. Blister blight symptoms were collected from clone TRI 2025 and leaves with hypersensitive reactions (HR) and healthy leaves were collected from TRI 2043. Following assays were done to extract protein. Chitinase assay was done using gel diffusion assay (Zou *et al.*, 2002). Bradford assay was done for total protein (Bradford, 1976). β-1,3-glucanase and peroxidase were assayed according to the methods described by Dann and Deverall, (2006). Healthy, Translucent Spot and Blister Blight infected leaves were used for detection of O_2 and H_2O_2 according to the method described by Christensen *et al.* (1997). Artificial inoculation of *E. vexans* to the upper leaf surface of TRI 2024 was done through preparation of blister blight spore suspension ($1x10^6$ spores/ml) according to the methods described by Twizeyimana *et al.* (2006) using healthy shoot method and detached leaf.

Results and discussion

Total protein content increased with the blister blight infection in both susceptible (TRI 2025) and resistant cultivars (TRI 2043) (Figure 1). Peroxidase activity decreased in susceptible cultivars and increase in resistant cultivars. Induced defense mechanisms mainly involve in the oxidative burst, localized cell death, synthesis of pathogenesis-related (PR) proteins *etc.* PR protein biosynthesis and accumulation is considered a major defense mechanism of plant against fungal pathogen by exhibiting antifungal activity. Protein compound that are toxic to pathogen are produced and accumulate in a resistant plant relatively susceptible variety at a faster rate than infection. The target structures of the antifungal proteins range from the outermost part of the fungal cell, the cell wall, to the plasma membrane and finally to several intracellular targets. Therefore, these proteins

exhibit a very wide diversity of action mechanisms, including, for example, inhibition of the synthesis of the fungal cell wall or disruption of its structure and function, membrane channel and pore formation, damage to cellular ribosomes, inhibition of DNA synthesis and inhibition of the cell cycle.

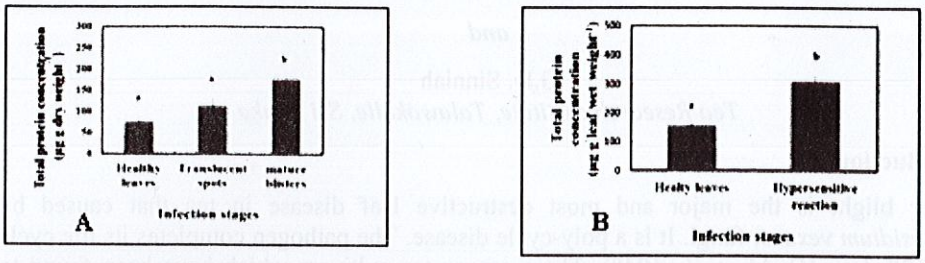

Figure 1: **A**: Changes in total protein content with blister blight infection in TRI 2025 **B**: Changes in total protein content with blister blight infection in TRI 2043

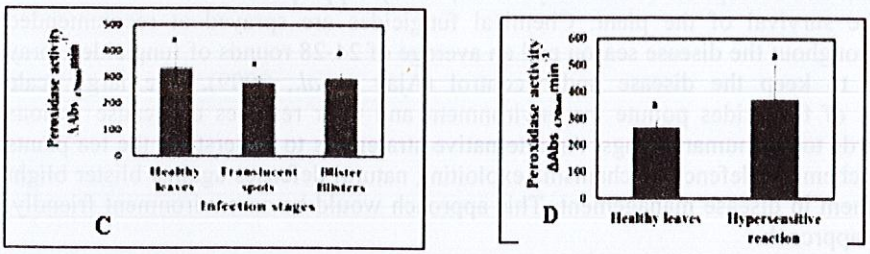

Figure 2: C-Changes in peroxidase activity with blister blight infection in TRI 2025, D-Changes in peroxidase activity with blister blight infection in TRI 2043.

The Peroxidase activity (Figure 2) initially decreased in the susceptible cultivar and increased in the resistant cultivar. Such decreases may arise from an inhibition of enzyme synthesis or activity or from relocalization to an environment such as the cell wall.

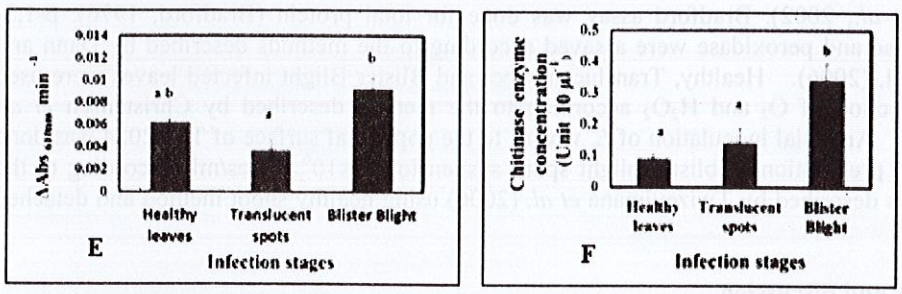

Figure 3: E Changes in β- 1, 3-glucanase with blister blight infection and F: Changes in chitinase concentration with blister blight infection in TRI 2025.

The Chitinase and β- 1, 3-glucanase activity (Figure 3) increased with the blister blight infection in the susceptible cultivar. According to the Yun (1997) a direct role for β-1,3-glucanases in defense against pathogens has been proposed because a substrate of these enzymes is a major component of the cell walls of many fungi. Chitinases show inhibitory activity to fungal spore germination and mycelial growth in disc plate diffusion. Combinations of chitinase and glucanase may be required for effective antifungal activity, presumably in order to mediate substantial enough fungal cell wall degradation to inhibit

the pathogen. Formation of O_2 and H_2O_2 occurred in both resistance and susceptible cultivars and formation of H_2O_2 delayed in susceptible cultivar than in the resistant cultivar. Superoxide and H_2O_2 play a central role in plant host as well as non-host resistance to fungal pathogens, including powdery mildew fungi. Among the functions of Reactive Oxygen Species (ROS) are induction cell deaths, membrane deterioration, protein cross linking, elicitor transduction and systematic acquired resistant. Highly localized O_2^- generation is associated directly with successful penetration and accumulates in living cells neighboring HR cells, H_2O_2 accumulation is linked to penetration resistance and the execution of HR cell death (Trujillo *et al.*, 2003).

In artificial inoculation of *E. vexans*, TS observed two week after inoculation. But inoculation to the healthy shoots and detached leaves were infected by other rotting fungi before blister blight infection and it should be further improved to extend shelf life of the detached shoot, eliminate other fungi, favor the germination and infection of blister blight.

Conclusion

Biochemical induced defence responses were increased with blister blight infection in both resistant and susceptible cultivar. Increasing amount was higher and prominent in resistant cultivar than that of susceptible cultivar.

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