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# Phenols and peroxidase activity in *Pepper yellow leaf curl Thailand virus* (PepYLCThV) resistant and susceptible chili (*Capsicum annuum* L.) genotypes

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**Abstract** Chilli (*Capsicum annuum* L.) is most commonly cultivated species. *Pepper yellow leaf curl virus* (PepYLCV) causes serious leaf curl disease of chili. In order to understand plant defence mechanism to PepYLCV disease, the study reported a response of total phenols and peroxidase activity in two contrasting pepper genotypes (resistant and susceptible) against PepYLCThV. The total phenolic content of uninoculated plants in susceptible genotype (KKU-P31118) showed higher value than resistance genotype (9853-123) at both stages. However, the amount of total phenolic content in resistant genotypes was increased in juvenile and mature leaves in response to inoculation with PepYLCThV. In case of susceptible one, it was decreased as compared to uninoculated control. On the other hand, the peroxidase activity in chilli genotypes and at different stage of leaves was increased every week after inoculation and increased as a comparison to uninoculated plants. Hence, our finding suggested that the total phenols may act as defence mechanism in resistant genotype 9853-123.

**Keywords:** Disease response, PepYLCV, Peroxidase, Phenolic

## Introduction

Peppers (*Capsicum annuum* L.) are the most commonly grown worldwide as vegetable (seeet pepper) and spice (chili pepper) crops. Pods of chili pepeprs with various shapes, sizes and pungency (mild– hot) are used in traditional Thai food (Chiemsombat *et al*., 2018). Chili production is affected by abiotic and biotic stresses. A Begomoviruses *Pepper yellow leaf curl virus* (PepYLCV) is one of the most serious problems of the chili production in South and South

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East Asisan countries including in Thailand. The disease was first reported in Indonesia and spread thought in subtropic and tropics. In Thailand, PepYLCV has been observed in Kanchanaburi province, Thailand since 1995 (Hidayat *et al*., 1999; Chiemsombat *et al*., 2018). The use of resistant cultivar is a cost effective, environemental and health safer measure to manage losses caused by the disease. The understanding the plant defense mechanisms and plant response to the virus is crucial step to develop resistant cultivars.

Plant defense mechanisms to abiotic and abiotic stresseses were involved two main mechanisms such as pre-existing and infection induces defense mechanisms. The first defense mechanism is included cuticle, wax, stomata and plant hair, which is the structure of plant which produced before them exposted to the stress. However, periderm, reactive oxygen species, enzymes, lignin, phenolics, and peroxidase are related with biotic stress, especially for disease and insect resistances (Anand *et al*., 2009). In addition, the response of inducible reactions was involed with resistant gene action (Van Loon *et al*., 1998), which the phenolics, peroxidase, polyphenol oxidase and catalase were reported associated with resistance to anthracnose and PepLCV disease resistance in chili pepper (Anand *et al*., 2009 and Rai *et al*., 2010). Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants (Rai *et al.*, 2010). The term of phenolic can be precisely defined chemically as substance which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives. Phenol itself is a natural product but most phenolics have two or more hydroxyl groups (Lattanzio *et al*., 2006). Plant phenolics may be divided in two classes 1) preformed phenolics that are synthesized the normal development of plant tissues and 2) induced phenolics that are synthesized by plants in response to physical injury, infection or when stressed by suitable elicitors such as heavy metal-salts, UV-irradiation, temperature and phytoalexins. Induced phenolics may also be constitutively synthesized but, additionally, their synthesis is often enhanced under biotic or abiotic stress (Nicholson and Hammerschmidt, 1992). In addition, the peroxidase activity (POD) was associated with insect pests resistance (Privalle *et al.*, 1999) due to the biochemical has been shown in infected plant to biotic stress, which the degree of expression depends on plant genotype and age of plant leaves (Coley *et al*., 2006). In this precent study, we examined the response of resistant and susceptible chemicals to infection with PepYLCThV.

## Materials and methods

The experiment was designed by two factors factorial in randomized completely block design (RCBD) and three replications. Each replication

consisted of five plants. The first factor was two chili genotypes of *Capsicum annuum*, as resistant (9853-123) and susceptible (KKU-P31118) to PepYLCThV (9853-123). The second factor was different inoculation periods, consisted of 45 and 60 DAS. The experiment was conducted at Department of Plant Production of Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The chili germplasms were collected from two resources, one of the chili genotypes that colleted from The World Vegetable Center, Taiwan (Worldveg.) (9853-123) and another one was collected from Khon Kaen University (KKU-P31118).

Source of *Pepper yellow leaf curl Thailand virus* (PepYLCThV) inoculums was collected from Plant Virology Laboratory of Chai Tai Seed Company. The inoculum was collected and mulitpied on susceptible chili genotype. The chili seeds were sown in polystyrene transplant flats filled peat moss (104 holds). The seedlings at 45 and 60 DAS were inoculated with PepYLCThV by grafting method. The disease score was evaluated at 7, 14, 21, 28 and 35 days after inoculation (DAI) which 6 disease score levels as follows: 0, 1, 2, 3, 4 and 5 (modified from Kumar *et al.*, 2006) where as 0 indicates no symptoms (symptomless, SL); (1) up to 5% curling and clearing ofupper leaves (highly resistant, HR); (2) 6–25% curling, clearing ofleaves and swelling of veins (resistant, R); (3) 26–50% curling, puckering and yellowing of leaves, and swelling of veins (moderatelysusceptible, MS); (4) 51–75% leaf curling, stunted plant growth andblistering of internodes (susceptible, S); (5) more than 75% curling and deformed small leaves, stunted plant growth with smallflowers and no or small fruit set (highly susceptible, HS). The disease index (DI %) was calculated using formula as below.

$$DI\ (\%) = [\Sigma\ (ni \times vi) / (N \times V) \times 100\%$$

With i: 0-5, ni = number of symptomatic plants to the value of a particular score, vi = value symptom score, N = the total number of plants were observed, and V= the highest score value.

The total phenolics content was determined from leave samples two stages at juvinile and muture stage of both resistant and suscepatble genotypes. The part of root stock of inoculated plants and uninoculated plants were compared. The total phenolics were analysed by Folin-Ciocalteu method as described by Cliffe *et al*. (1994). The leaf samples were harvested and extracted with adding 4.5 ml of distilled water, 0.5 ml of folin-ciocalteu stock reagent and 4 ml of $Na_2CO_3$ reagent (75 g/l). The reaction was incubated at room temperature for 1 h and the total phenolics contents were measured the absorbance by spectrophotomer at wavelength 765 nm.

The peroxidase activity (POD) was determined by Malik *et al*. (1980). A 0.2 g of leaf samples were collected from each treatment and homogenized in

chilled 1.5 ml 50 mM sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 15000 rpm for 20 min at 4 ˚C. The sample were mixed in a total volume of 3.0 ml contained 50 mM of sodium phosphate buffer (pH 7.0), 4 mM of $H_2O_2$, 20 mM of guaiacol and 200 µl of enzyme extract. The decomposition of $H_2O_2$ was at 436 nm by decreasing in absorbance per minute for calculation the POD activity.

## Results

The total phenolic content of uninoculated plants in susceptible genotype (KKU-P31118) showed higher value than resistance genotype (9853-123) at both stages i.e. at juvinile leaves (19.81 and 16.42 mg/100g) and mature leaves (19.65 and 17.68mg/100g), respectively. However, the amount of total phenolic content in resistant genotypes was increased in juvenile and mature leaves in response to inoculation with PepYLCThV. The increased in total phenolic content was observed in the following week after inoculation with the viral disease in resistant genotyes at both of plant age after inoculation (Figure 1A and 1B), In case of susceptible one, it was decreased as compared to uninoculated (Figure 1C and 1D). The relation of resistance to total phenolics response, the resistant genotype (9853-123) at 60 DAI (D2) of inoculated plant did not show any symptom of pepper yellow leaf curl disease (DI = 0%), while 45 DAI, it showed the disease incidence of 12.5%. The total phenolic content was increased in the infected plant at 45 DAI, but did not increase in the inoculated plant at 60 DAI in the $1^{st}$ to $4^{th}$ weeks after inoculation, but it was increased at the $5^{th}$ weeks after inoculation.

The POD activity in chilli genotypes and at different stage of leaves was increased every week after inoculation and increased as a comparison to uninoculated plants. The resistant genotype (9853-123) showed the increasing POD from 1.62 to 7.59 activity/minute/g in juvinile leaves and from 2.43 to 5.01 activity/minute/g in mature leaves. In case of KKU-P31118 (susceptible genotype), the activity of POD after inoculated for 5 weeks in chilli juvinile leaves and mature leaves that was increased by 2.09 and 1.79 activity/minute/g, respectively (Table 1.). The increased in POD activity of resistance genotype was observed in a week after inoculation with PepYLCThV (Figure 2A, 2B and Figure 3 and 4). It was increased by 41.71% in juvinile leaves and 56.02% in mature leaves as compared to uninoculated condition. While in susceptible genotype, the POD activity was increased lower percentage than resistant genotypes as 27.27% at young leaf and 28.49% at mature leaves stages.

**Table 1.** Total phenolic content and POD activity in Juvinile and mature leaves stages of two chili genotypes as compared to the inoculated and uninoculated plants after treated with Pepper yellow leaf curl Thailand virus

| Varietie | Total phenolic (mg per 100 g FW) | | Peroxidase activity (activity/min/g sample) | |
|---|---|---|---|---|
| | Juvinile leaves | Mature leaves | Juvinile leaves | Mature leaves |
| 9853-123 control | 16.42 | 17.68 | 1.9 | 1.24 |
| 9853-123 treated | 20.61 | 20.38 | 3.26 | 2.82 |
| **average** | 18.51 | 19.03 | 2.58 | 2.03 |
| **F-test** | ns | ns | ns | ns |
| **CV (%)** | 2.38 | 2.60 | 12.43 | 4.69 |
| KKU-P31118 control | 19.81 | 19.65 | 1.52 | 1.28 |
| KKU-P31118 treated | 18.07 | 17.7 | 2.09 | 1.79 |
| **average** | 18.94 | 18.67 | 1.81 | 1.54 |
| **F-test** | ns | ns | ns | ns |
| **CV (%)** | 1.61 | 6.32 | 26.34 | 2.61 |

ns = non – signification, means not followed by the same letter are significantly different at the 5% level of probability as determined by DMRT.

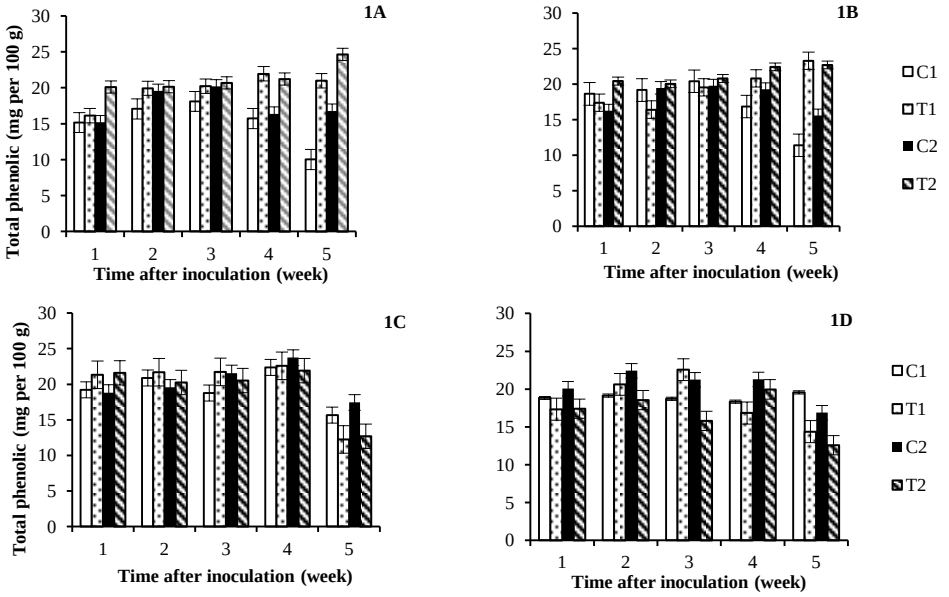


**Figure 1.** Changes in the total phenolic content in resistant and susceptible varieties inoculated for 5 weeks with PepYLCThV. A – juvinile leaves of resistant genotype (9853-123), B – 9853-123 (mature leaves), C – KKU-P31118 (juvinile leaves), D – KKU-P31118 (mature leaves); C1 – control of 45 days, T1 – treated for 45 days, C2 – control 60 days, T2 – treated for 60 days; Bars in each treatment is SD

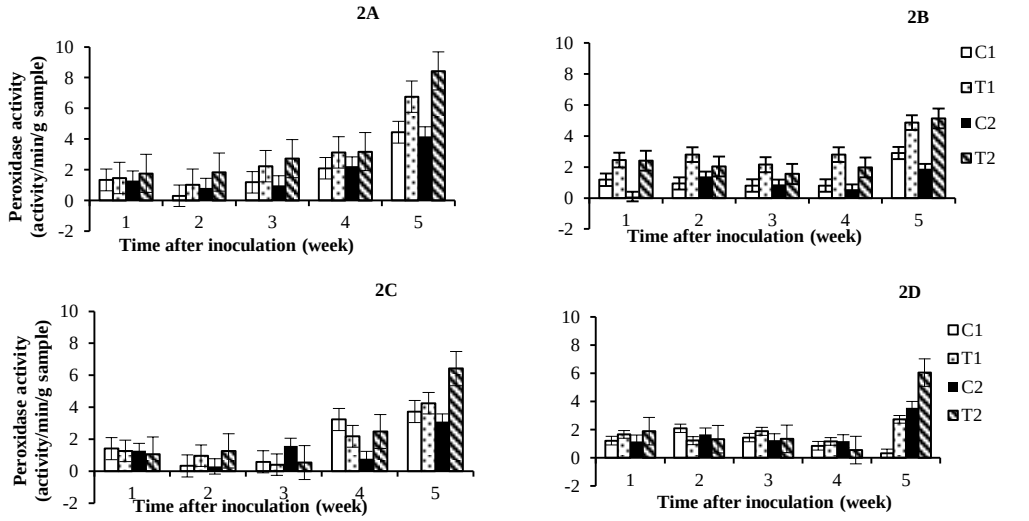


**Figure 2.** Changes in POD activity in resistant and susceptible varieties inoculated 5 weeks after inoculated with PepYLCThV (for detail see Figure 1.)

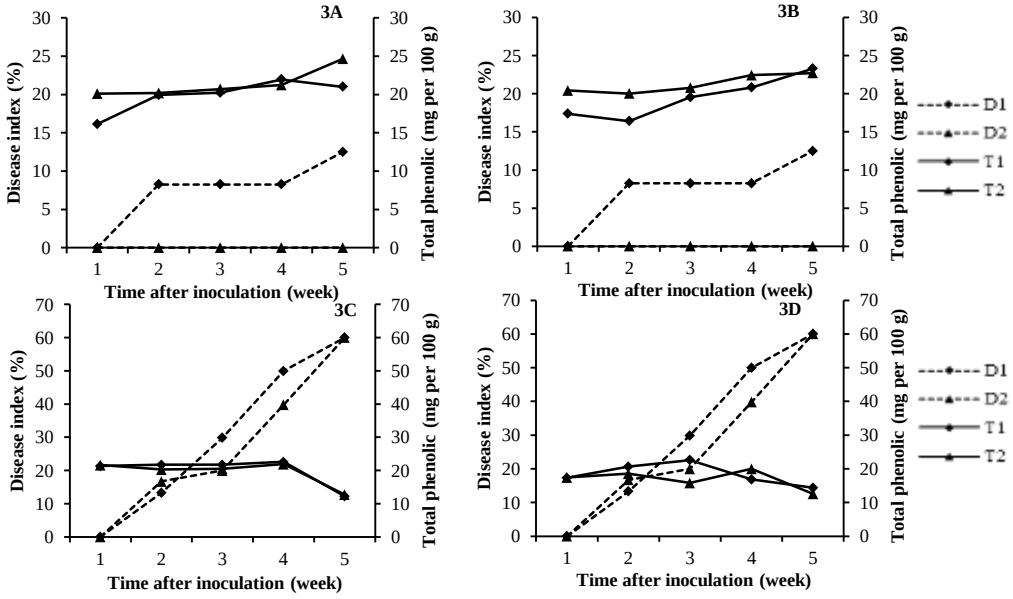


**Figure 3.** Changes in the total phenolic in resistant and susceptible varieties inoculated with PepYLCV. A – juvinile leaves of 9853-123, B – mature leaves of 9853-123, C – juvinile leaves of KKU-P31118, D – mature leaves of KKU-P31118; D1 – percentage of disease index of 45 days, T1 – total phenolic at 45 days, D2 – percentage of disease index of 60 days, T2 – total phenolic at 60 days

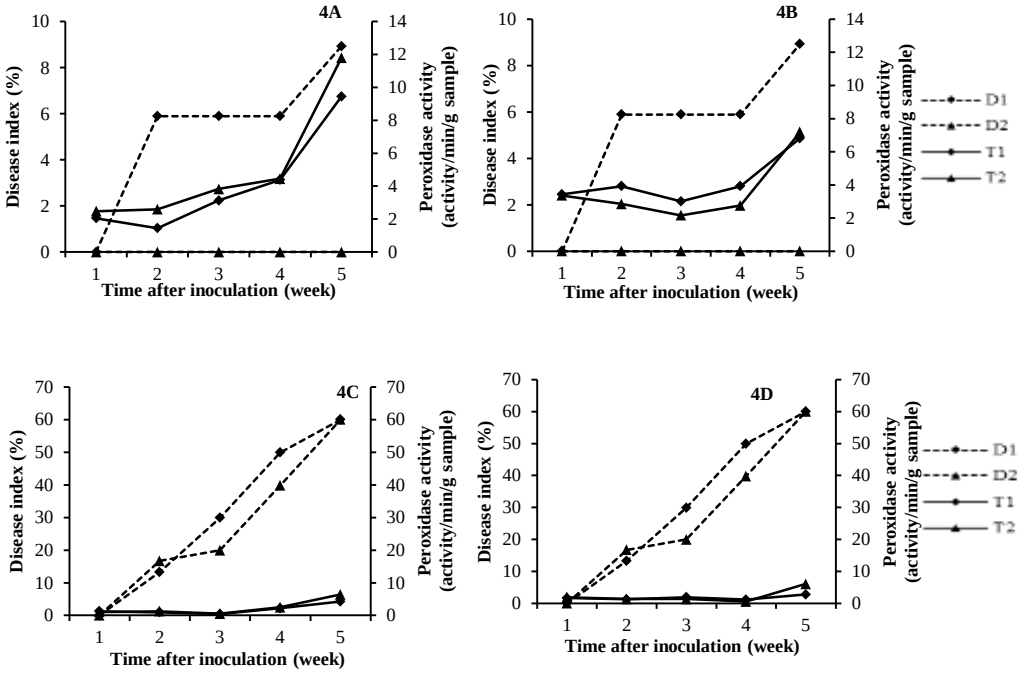


**Figure 4.** Changes in peroxidase activity in resistant and susceptible varieties inoculated with PepYLCThV (for detail see Figure 3)

## Discussion

The total phenolic contents play an important role for defense mechanism of resistance to plant disease, this study the resistant genotype (9853-123) showed resistance to PepYLCThV. The total phenolic contents were increased after infected plant of resistance genotype (9853-123) compared to uninoculated plant, but was decreased in susceptible genotype (KKU-P31118) in response to inoculation with PepYLCThV. The changes in the enzyme activities indicated that total phenolic contens was strongly correlated with resistance to PepYLCThV disease in *Capsicum annuum* (9853-123). The increasing of total phenolic content also found responses to other diseases like anthracnose disease (Anand *et al*., 2009), Peper leaf curl virus disease (Rai *et al*., 2010), Fusarium wilt (Jabeen *et al*., 2009) and Pepper yellow mosaic virus disease (Goncalves *et al*., 2013). The total phenolic contents in plant are used as substrated to produce lignin in cell wall, thereafter the plant cells against diseases (Nicholson and Hammerschmidt, 1992). In addition, the POD activity in infected plants produced higher content than uninoculated ones in both chili genotypes due to usual defense mechanism. However, in resistant genoypes are

rapidly increased in POD activity more than susceptible genotype thereafter the resistant plant could overcome disease infection. In addition, the present study also revealed that young leaves had higher content of POD than the mature leaves due to stressed that mature leaves may be contained the substances that decreased in digestibility, whereas young leaves were low in defensive chemicals (Coley *et al*., 1980). Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989), which it is reported to direct associated with resistance to disease and involved in various reactions such as polysaccharide bond, wound healing, phenol oxidation, defense against pathogens and regulation of cell elongation (Passardi *et al*., 2004, Bhavani *et al*., 2011). Addition, peroxidase activity was reported that assocaited with resistanct to anthracnose (Bharathi *et al*., 2004). In conclusion, this study found that the increased of total phenols and POD was considered association with resistant mechanism to PepYLCThV disease in *Capsicum annuum* (9853-123).

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