

Defense reactions of bean genotypes to bacterial pathogens in controlled conditions

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Abstract. This study was focused on the role of antioxidant enzymes and total protein in imparting resistance against common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (Psp) in bean. Activities of Ascorbate peroxidase (APX), Catalase (CAT) and total protein were studied in resistant and susceptible bean genotypes. Five-day-old seedlings were inoculated with a bacterial suspension (10^8 CFU ml⁻¹) and harvested at different time intervals (0, 12, 24 and 36 up to 72 h) under controlled growing conditions and assayed for antioxidant enzymes and total protein. Temporal increase of CAT, APX enzymes activities showed maximum activity at 12 h after both pathogens inoculation (hpi) in resistant cultivar, whereas in susceptible it increased at 72 h after both pathogens inoculation for CAT and 12, 24 h for APX enzymes. Maximum total protein activities were observed at 12 h and 24 h respectively after Xap, Psp inoculation (hpi) in resistant and maximum activities were observed at 24 h and 72 h respectively after Xap, Psp inoculation (hpi) in susceptible. Increase of antioxidant enzyme and total protein activities might be an important component in the defense strategy of resistance and susceptible bean genotypes against the bacterial infection. These findings suggest that disease protection is proportional to the amount of enhanced CAT, APX enzyme and total protein activity.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a crop of major societal importance and is a major source of protein and essential nutrients. Worldwide, common bean is the most consumed legume, providing up to 15% of total daily calories and 36% of total daily protein in parts of Africa and the America. Turkey is an important bean producer. Bean production of 638.496 tons, is achieved by growing on 501.767 hectares of land in Turkey [1]. Beans may be affected by a number of distinct diseases, including bacterial diseases. Common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap), halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (Psp), are the most important bacterial diseases in bean. These bacterial diseases are observed in all the fields of bean production in Turkey. There is no specific pesticide to control bacterial spot disease of bean caused by *Xanthomonas axonopodis* pv. *phaseoli*. Therefore, genetic resistance is very important to control these bacterial diseases in bean plant. Plants have a variety of endogenous biochemical defense mechanisms that can be induced in response to attack by insects and pathogens [2]. Enzymes play an important role in plant defense mechanisms. For many years, the role of oxidative enzymes and of their metabolic products in the defense mechanisms of infected plants has been studied [3]. There is worldwide interest in



identifying safe and effective novel materials to induced resistance for the control of plant pathogenic bacteria [4]. The aim of this present study was to test activities of some plant defense related enzymes and proteins on susceptible and resistant bean genotypes.

2. Materials and methods

2.1 Planting material and bacterial inoculation preparation

Two different bean cultivars (resistance and susceptible) were obtained from Dept. of Field Crop, Ataturk University and Eastern Anatolia Agricultural Research Institute. All seed samples were surface sterilized with 3% (v/v) sodium hypochlorite solution for 4 min and were thoroughly washed with distilled water three times and surface dried. Plants were grown in 10 cm pots in a soil mix containing sand, perlite, and peat compost under 25°C with a 14 h/10 h light/dark conditions. *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* culture was provided by Dr. M. Figen Donmez, Dept. of Plant Protection, Iğdır University. Two days old cultures of Xap and Psp were harvested from NA plates, suspended in sterile deionized water, and adjusted to a concentration of 10^8 CFU mL⁻¹ as determined by a spectrophotometer at 660 nm.

2.2 Enzyme Assay

Sample collection: Resistance and susceptible bean plants were harvested 0, 12th, 24th, 48th, 72nd hours post inoculation (hpi) and stored at -80 °C for subsequent analysis [5].

2.3 Catalase assay

Bean seedlings (1 g) were homogenized in 5 ml of 50 mM potassium phosphate buffer pH 7.0 in a pre-chilled mortar and pestle on ice. The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C and the supernatant served as enzyme source. All experiments were carried out at 4 °C. Catalase was estimated by following the procedure of [6]. The 900 µL reaction mixture contained 50 mM potassium phosphate buffer and 30 mM H₂O₂. The reaction was initiated by adding 100 µL of enzyme extract and the decrease in A₂₄₀ nm was measured for 1 min. The enzyme activity was calculated by the absorbance values (mmole/g⁻¹FW/da⁻¹).

2.4 Ascorbate peroxidase assay

Bean seedling (1 g) was homogenized in 5 ml of 50 mM potassium phosphate buffer pH 7.0, containing a pre-chilled mortar and pestle on ice. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant served as enzyme source. All experiments were carried out 4°C. Ascorbate peroxidase activity was determined according to [7], by monitoring the decrease in the absorbance at 290 nm over 2 min. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7) with 0.1 mM EDTA, 0.5 mM ascorbic acid and 100 µL of enzyme extract. The enzyme activity was calculated by the absorbance values (mmole/g⁻¹FW/da⁻¹).

2.5 Total Protein Assay

One gram of leaf tissue was homogenized with 50 mM potassium phosphate buffer (pH 7.0), and centrifuged at 10, 000 rpm for 20 min at 4 °C and the supernatant was used as crude extract. Protein concentration was measured according to [8] serum albumin served as a standard.

3. Results and discussion

Seedlings of two different bean genotypes (resistant and susceptible) were analyzed for CAT and APX and Total Protein with or without pathogen inoculation. All the cultivars showed an increased level of enzyme activity after pathogen inoculation. Highest CAT and APX enzymes activities showed at 12 h after both pathogens inoculation (hpi) in resistant cultivar (Figure 1,2,5,6), whereas in susceptible it

increased at 72 h after both pathogens inoculation for CAT and 12, 24 h for APX enzymes (Figure 3,4,7,8).

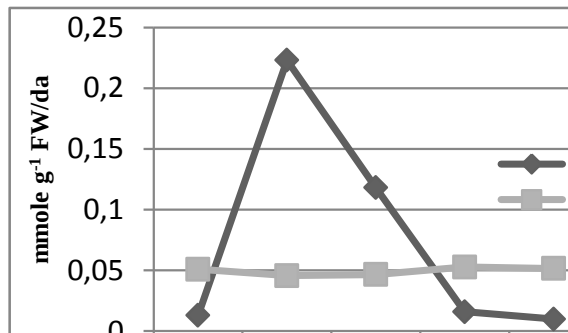


Figure 1. Effect of Xap treatments on CAT enzymes in resistance bean plants, inoculated with pathogen and control

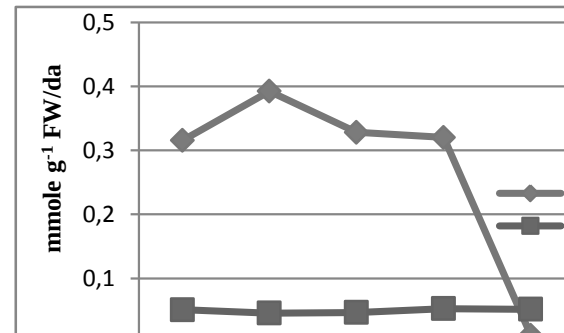


Figure 2. Effect of Psp treatments on CAT enzymes in resistance bean plants, inoculated with pathogen and control

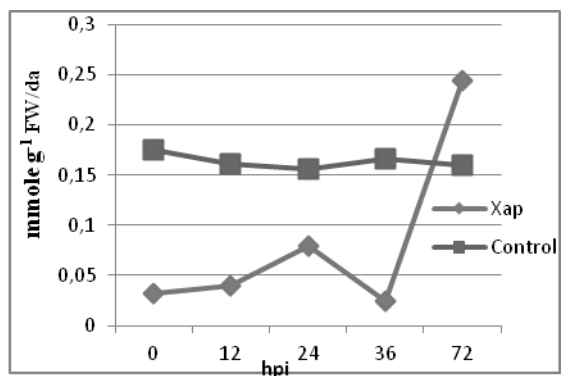


Figure 3. Effect of Xap treatments on CAT enzymes in susceptible bean plants, inoculated with pathogen and control

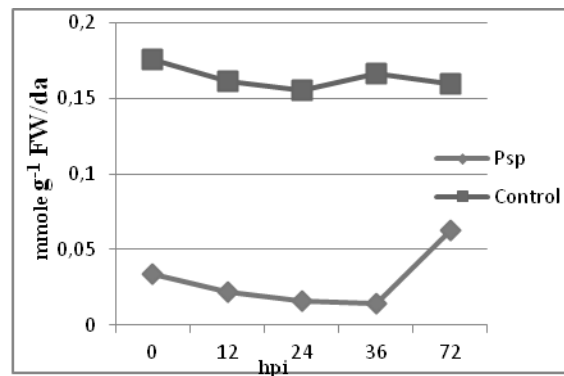


Figure 4. Effect of Psp treatments on CAT enzymes in susceptible bean plants, inoculated with pathogen and control

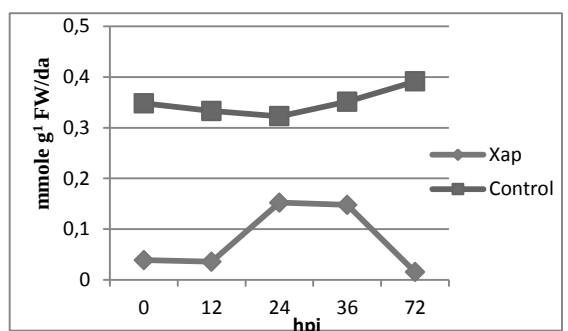


Figure 5. Effect of Xap treatments on APX enzymes in resistance bean plants, inoculated with pathogen and control

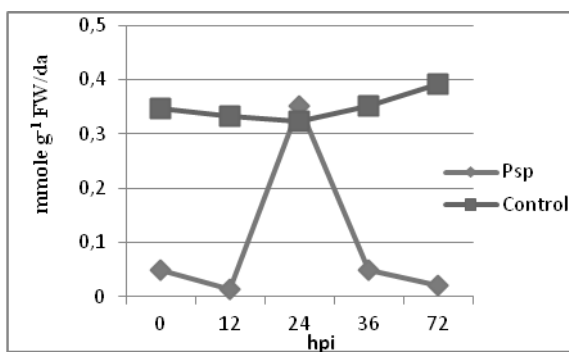


Figure 6. Effect of Xap treatments on APX enzymes in resistance bean plants, inoculated with pathogen and control

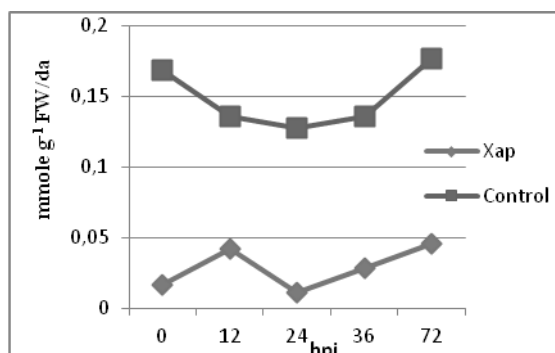


Figure 7. Effect of Xap treatments on APX enzymes in susceptible bean plants, inoculated with pathogen and control

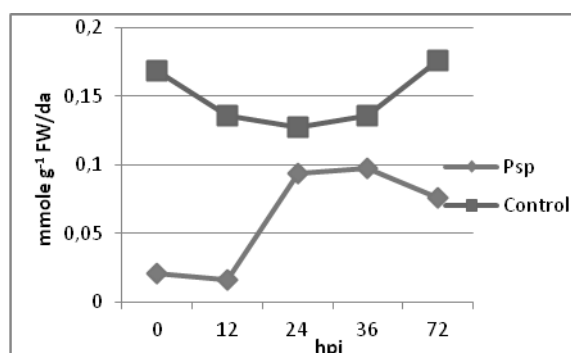


Figure 8. Effect of Psp treatments on APX enzymes in susceptible bean plants, inoculated with pathogen and control

The highest maximum total protein activities were observed at 12 h and 24 h respectively after Xap and Psp inoculation (hpi) in resistant (Figure 9,10) and also maximum activities were observed at 24 h and 72 h respectively after Xap, Psp inoculation (hpi) in susceptible (Figure 11,12).

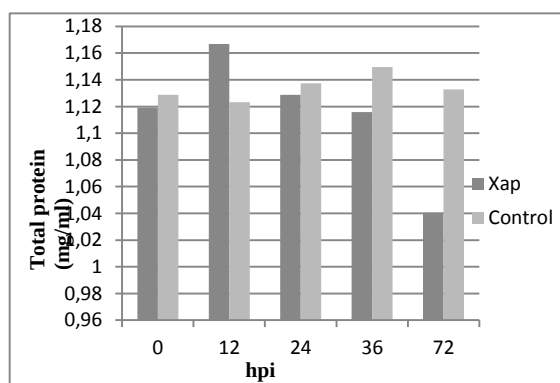


Figure 9. Effect of Xap treatments on Total Protein in resistance bean plants, inoculated with pathogen and control

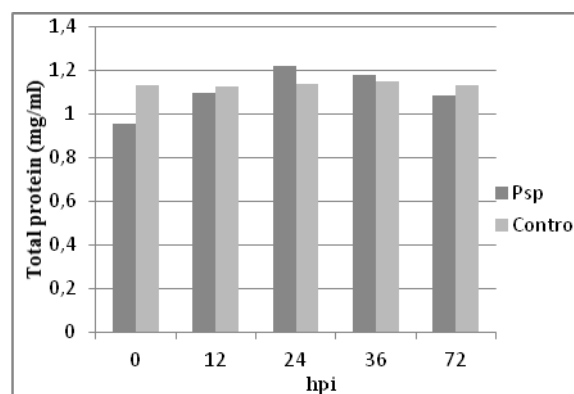


Figure 10. Effect of Psp treatments on Total Protein in resistance bean plants, inoculated with pathogen and control

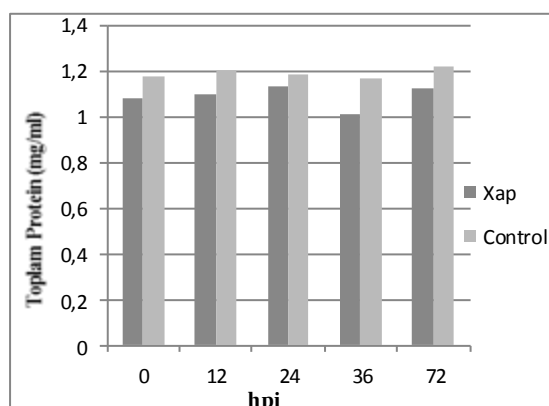


Figure 11. Effect of Xap treatments on Total Protein in susceptible bean plants, inoculated with pathogen and control

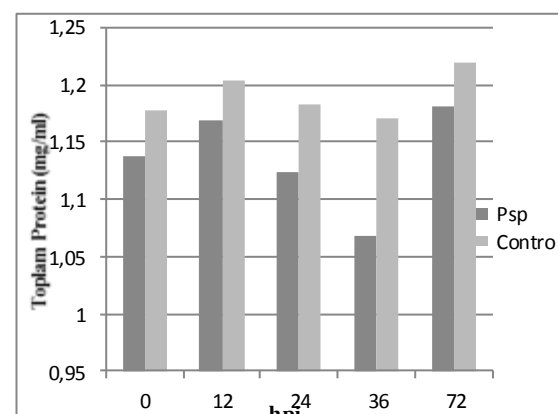


Figure 12. Effect of Psp treatments on Total Protein in susceptible bean plants, inoculated with pathogen and control

Our studies indicated that CAT and APX enzyme plays an important role in the defense system of the infected resistant bean plants pathogen inoculation compared to uninoculated control and also the susceptible under controlled growing conditions. Similar studies were observed increased APX and CAT enzymes [9].

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