Available online at www.sciencedirect.com
SCIENCE DIRECT
Scientia Horticulturae 109 (2006) 107–112
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

The effect of harpin protein on plant growth parameters, leaf chlorophyll, leaf colour and percentage rotten fruit of pepper plants inoculated with *Botrytis cinerea*

N. Akbudak [a,*], H. Tezcan [b], B. Akbudak [a], V. Seniz [a]

[a] *Uludag University, Faculty of Agriculture, Department of Horticulture, 16059 Bursa, Turkey*
[b] *Uludag University, Faculty of Agriculture, Department of Plant Protection, 16059 Bursa, Turkey*

Received 22 February 2005; received in revised form 13 March 2006; accepted 14 March 2006

Abstract

In this study, harpin protein was applied to the peppers (*Capsicum annuum* L. var. cvs. 'Demre', 'Yalova Charleston' and 'Sari Sivri') grown under natural conditions. These plants were subjected to artificial inoculation with *Botrytis cinerea*, which causes fruit spoilage in peppers. Changes in vegetative growth, total chlorophyll content in leaves, leaf colour and percentage of rotten fruits were determined after treatments. The number of leaves per plant value was quite low in all cultivars and the plant height value was low only in cv. 'Sari Sivri' treated with *B. cinerea*. Values obtained from vegetative growth parameters in the plants subjected to harpin protein + *B. cinerea* treatment were only higher than *B. cinerea* treatment. Leaf chlorophyll values exhibited significant decline in the plants subjected to *B. cinerea* treatment in all cultivars. However, the chlorophyll content in the plants subjected to harpin protein + *B. cinerea* treatment was low. The colour values obtained from leaves supported the chlorophyll findings. Fruit spoilage percentages were lower in the fruits picked from the plants of harpin protein + *B. cinerea* treatment compared with those picked from the plants only subjected to *B. cinerea* treatment.

Keywords: *Botrytis cinerea*; *Capsicum annuum*; Growth parameters; Leaf chlorophyll; Messenger; Percentage of rotten fruit

1. Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable crop. It is a vegetable with high consumption rate due to its importance in rich vitamin contents for human diet. Pepper is extensively grown both in the field and in the greenhouses. High rates of yield and quality losses occur in the plants because of a number of diseases. Gray mold caused by *Botrytis cinerea*, a ubiquitous fungus distributed worldwide and reported to be a pathogen of plants in more than 200 genera including *Capsicum*. The disease is common in pepper. It is generally more severe in plants grown in enclosures that maintain high relative humidity, such as plant beds or greenhouses, but it is also a threat to field-grown pepper crops. Furthermore, *B. cinerea* causes an important postharvest decay of pepper (Pernezny et al., 2003). It covers the decayed tissue with conidiophores, known as gray mold, causing blight or rot of leaves, flowers and fruits (Domsch et al., 1986). Young pepper seedling affected by gray mold may show damping-off with a soft tan to brown, water-soaked rot of the stem at or near the soil line or the cotyledons. Lesions on stems and leaves of older plants are covered with thick, matted masses of gray to brown conidiophores and conidia. But, the most common symptom is the sudden collapse of succulent tissue. Whitish-gray to tan powdery fungus spore masses frequently occur on the surfaces of dead plant tissues under cool and humid conditions. The control of gray mold in greenhouses starts with sanitation and first of all, greenhouses should be well ventilated, to keep the humidity low. In some cases, It may be necessary to use fungicides to manage gray mold. Several fungicides have been used effectively, but their use may also lead to residue-contaminated fruits when improperly applied, to appearance of resistant pathogens and to reduction of beneficial organism populations. Utilizing the harpin protein exhibits a high degree of environmental safety and it may also permit reducing fungicidal spraying frequency while at the same time increasing crop yields.

The use of plant activators has become common in managing plant diseases in recent years with the developments

* Corresponding author. Tel.: +90 224 442 89 70; fax: +90 224 442 90 98.
E-mail address: nakbudak@uludag.edu.tr (N. Akbudak).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2006.03.008

in biological agriculture practices. Use of plant activators to reduce plant diseases is relatively new and very few activators are commercially available. One of these bioactivators is the messenger consisting of harpin protein. Harpin protein, marketed by the Eden Bioscience Corporation, is biotechnologically developed bio-activator that eliminates undesirable effects of pesticides and may be used as alternative to control insects and fungi as well as and to increase yield and quality (Mayer et al., 2001). It is isolated from *Erwinia amylovora*, the bacterial pathogen that causes disease fire blight and its ability to activate these growth and defense systems provides an alternative for Integrated Pest Management Programs (Wei et al., 1992; Anon., 2000). When harpin protein is applied to a plant, it binds to plant receptors. This binding process induces a cascade of responses that activate the expression of hundreds of genes, stimulating several of many genes that stimulate distinct biochemical pathways responsible for the enhancement of growth and pest resistance within the plant. Harpin protein activates natural plant pathways that control certain growth mechanisms, including the salicylic acid dependent pathways and the jasmonic acid induced pathway (for plant defense) (Hunt and Ryals, 1996; Ryals et al., 1996). Harpin protein may also aid in the suppression of insects and diseases and improve plant vigor, growth, increase plant height, biomass, fruit size, fruit quality, overall yield and stress tolerance (Copping and Menn, 2000; Anon., 2004).

This study aimed at determining the effects of treatments of harpin protein. With this purpose, plants of three pepper cultivars in active growing period were treated with harpin protein and inoculated with *B. cinerea*; treated and non-treated plants were examined and compared for growth parameters, percentage of rotten fruit, leaf chlorophyll content and colour.

2. Materials and methods

2.1. Plant materials, growth medium and growth conditions

The study was conducted during the 2003–2004 growing season at the Uludag University, Faculty of Agriculture, Department of Horticulture, Research and Training Greenhouse (glasshouses with automatically temperature and humidity control). Seedlings produced from surface disinfected seeds (with a 60% solution of commercial bleach for 10 min and rinsed four times with distilled water) were grown from 5 January to 15 May in 1.5 l capacity pots (one seedling per pot) containing soil and sand mixture (soil:sand, 75:25, v/v). Growing conditions consisted of a day/night temperature regime of $22 \pm 2/18 \pm 2$ °C and 16 h photoperiod. Overall 180 plants were used (15 plants for each experiment). Plants were watered as needed and fertilized weekly with N–P–K formulations were 15–15–15.

The pepper cultivars 'Demre' (dark green colour, thick fruit wall, sweet, long type, pepper), 'Yalova Charleston' (yellow-green colour, thick fruit wall, sweet, charleston type pepper) and 'Sari Sivri' (yellow-green colour, sweet, long type pepper) which are intensively grown under protected cultivation in Turkey were used in the trial. Cv. Demre is a cultivar which bloom later and develop more slowly compared with the other two cultivars (Anon., 2003).

Plants used in the study were divided into four groups within each cultivar:

(1) *Harpin protein*: The plants treated only with harpin protein.
(2) *B. cinerea*: The plants inoculated with only artificial pathogen.
(3) *Harpin protein + B. cinerea*: The plants treated with both harpin protein and artificial the pathogen inoculum.
(4) *Control*: The plants treated only with water.

2.2. Harpin protein treatments

The harpin protein was applied as foliar sprays, three treatments at 50 g 100 l^{-1} (3% a.i.) water while plants were actively growing in the greenhouse. The first treatment started when the plants were 15 days old (seedling period), and the other treatments were applied at 14-day-intervals (135 mg a.i. harpin protein was applied to each cultivar) (second treatment—seedling period; last treatment—first flowering period) (Gang and Liu, 1999; Akbudak et al., in press).

2.3. Artificial inoculation of the plants with B. cinerea

One pathogenic isolate of *B. cinerea* obtained from pepper was cultured in petri plates containing PDA medium at 25 °C for 10 days. After spraying, the plant leaves were inoculated with a standardized volume of 10 ml conidisil suspension (10^6 conidia ml^{-1}) of the pathogen per plant using a small-calibrated hand sprayer (1.5 l capacity). When the plants were 2 months old, plants (harpin protein-treated, non-treated) were inoculated with *B. cinerea*. After inoculation, the plants were held in closed polyethylene bags for 1 day and the disease caused by the pathogen progressed over the 2 months period. After all of them, the disease severity and quality parameters of the plants were scared during the growing period.

The growth parameters, total chlorophyll content in leaves, chlorophyll *a/b* ratio and L, a, b leaf colour values were determined 2 months after the fungal inoculation.

2.4. Plant growth parameters

Plant height (*cm*): In all treatments, shoot lengths of the plants forming each replicate were measured and means were taken. The distance from the first true leaf pair to the shoot tip was taken as basis in the measurements.

Stem diameter (*cm*): Stem diameters of the plants forming each replicate were measured and averaged in all treatments. Measurements were done just above the first true leaf pair.

Number of leaves per plant: This parameter was determined by counting the leaves in the plants forming each replicate, and the average values were obtained.

Plant height, stem diameter and leaf number values were obtained by taking the means of measurements made in all the plants forming the replicate.