Journal of Plant Biology and Agriculture Science

Abstract
Vol. 3, Iss. 4
2022

Bio-chemical analysis for resistance to Alternaria alternata early blight disease in potato Solanum tuberosum

Neda Peymani[1] , Mehdi Nasr Esfahan[*2], Ahmad Reza Golparvar[1] , Esmaeil Mahmmodi[1] , Majid Shams[1] 1Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran; 2Plant Protection Research Department, Isfahan Agriculture and Natural Resource Research and Education Centre (AREEO), Isfahan, Iran

Abstract

Early blight disease of potato, caused by the potato path type Alternaria alternata (Fr.) Keissler (AA), is one of the most serious fungal diseases to affect potatoes globally. To develop an understanding of how potatoes respond to AA potato path type infection, we examined the host transcript accumulation over the period of a week post AA inoculation on three resistant and three susceptible potato genotypes, using marker genes, PR-2, ChtA, PR-5, PR1-b, PIN2, ERF3, PAL and LOX and enzymes activity, catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), polyphenol oxidase (PPOs) and phenylalanine ammonialyase (PAL) analysis. The results indicated expression of PR-2, ChtA, PR-5, PR1-b and PAL genes by qPCR was significantly increased up to 8.61 fold in inoculated resistant genotypes to susceptible and controls, not inoculated potato genotypes. Transcription levels of PIN2, ERF3 and LOX genes were significantly decreased in resistant inoculated potato plants. Activities of POX, SOD and PPOs enzymes were also significantly increased up to 7.40 fold in inoculated resistant potato genotypes, 10/33/R1, 3/33/R2 and 21/33/R2 compared to susceptible and controls. CAT enzyme in 21/33/R2 genotype and PAL enzyme activity in resistant 21/33/ R2 and 10/33/R1genotypes, showed a significant increase by 3.3 fold in susceptible and control plants. Biomass growth factors (BGPs) showed a decreasing trend in inoculated samples compared to control genotypes. The knowledge obtained from changes in gene expression levels and enzyme production in defines processes in infected potato plants can inform future studies to identify the defines mechanism and generate resistant potato cultivars.

Keywords: Alternaria alternata, Antioxidant enzymes, biomass, defines mechanism, qPCR, Solanum tuberosum

Introduction

Early blight, caused by the necrotrophic ascomycete's fungus Alternaria solani (Ellis & G. Martin) Sorauer, is one of the most common diseases of potatoes, especially where potatoes are grown under irrigation. The disease causes yield losses through defoliation of the plants and severe epidemics of early blight may reduce potato yields by as much as 20 to 30% (7.32). Early blight can also impact the quality of the harvested tubers. Fungicides used to control the disease are expensive and frequently inefficient. The development of potato varieties resistant to early blight remains one of the best strategies for long-term control of this disease. To date, no monogenic traits conferring resistance to A. solani have been found in potato. Resistance is thought to be correlated with plant age since susceptibility increases as plants grow older

(9.28). In tomato, secondary plant metabolites correlated with early blight resistance include phenolic compounds (tannins, flavonols, and phenols) in leaves and stems. In addition, the fruit of resistant tomato varieties contain a higher amount of phenolic compounds than those from susceptible varieties. The constitutive expression of phenols, which are thought to function as preformed inhibitors, is associated with nonspecific basal resistance to multiple pathogens in all plant species. In addition to phenolic, the production of reactive oxygen species, such as hydrogen peroxide and superoxide, also has an integral role in pathogen defense. Peroxidase enzymes are important in the production of hydrogen peroxide and have been linked to increased disease resistance in plants. Peroxidase also shows affinity to substrates involved in cellular lignification and the products of its activity have direct antimicrobial activity in the presence of hydrogen peroxide. In Iran, early blight is a ubiquitous disease that can cause serious crop losses under optimum conditions including high temperature, humidity, and susceptibility and physiological ages of the cultivars. The primary objective of our study was to identify sources of potato early blight resistance and characterize biochemical responses to different isolates of A. solani. MATERIALS AND METHODS Cultural conditions. Potato seeds (Solanum tuberosum L.) were planted in plastic pots (30 cm diameter) containing soil and perlite under greenhouse conditions (18 to 25°C) at Tehran University. Tubers were treated with thiabendazole (tekto WP 60%, Golsam Gorgan Company, Tehran) before planting. A. solani isolation and spore production. Potato leaves infected with A. solani were collected in 2008 from different locations and potato cultivars from Arak Town (Markazi Province) in Iran. Five isolates of A. solani were recovered from sections of potato leaves with typical early blight lesions. The leaf sections were surface-sterilized in a 1% sodium hypochlorite solution for 1 min, rinsed with sterile distilled water, placed on potato dextrose agar (PDA), and incubated under continuous light at 20 ± 1°C. Hyphal tips of mycelium that grew from diseased tissue were transferred to petri dishes containing 15 ml PDA. Hyphal-tip sections were incubated for 7 days at 24 ± 1°C on PDA under 16 h/day near-ultraviolet light (310 to 400 nm). Single spores from hyphal-tipped fungal colonies were transferred to PDA dishes to obtain pure cultures of A. solani (N isolate, AM isolate, M isolate, an isolate, and H isolate). The isolates were maintained on PDA at 20 ± 1°C for 7 days. Plant inoculations and evaluations. Single spore isolates of A. solani were grown on PDA for 6 days at 24 ± 1°C under 16 h/day near-ultraviolet light (310 to 400 nm) in order to induce sporulation. Conidia from these in vitro cultures were collected by washing with sterile water and diluted to a concentration of 106 spore's ml–1 (3.25). Certified seed tubers of S. tuberosum 'Diamond', 'Granula', 'Agria', 'Milva', 'Lady Rozeta', 'Satina', 'Maradona', 'Sante', and 'Santana' potatoes were planted. Four plants (45 days old) of each cultivar were inoculated separately with each isolate of A. solani or a sterile water control. The plants were sprayed with the conidial suspension or sterile water and covered with clear plastic bags for 24 h to increase humidity and accelerate infection. The plants were grown under normal conditions in the greenhouse at 26°C (3.28). The intensity of infection was recorded using the scale described in Table 1 The two cultivars with the lowest and highest early blight infection scores and the two A. solani isolates with the least and greatest aggressiveness were selected for further evaluations. Phenol assay. Leaf tissue (0.5 g) was ground in liquid N2 with a mortar and pestle. It was extracted separately in 8 ml of 80%

methanol. The extracts were centrifuged at 4,000-× g for 5 min. To determine the amount of total phenols, 1 ml of leaf extract was placed in test tubes containing 5 ml of distilled water, 250 µl of Folin, and 1 ml of 20% sodium carbonate. The test tubes were stirred and readings were taken after 60 min using an absorbance spectrophotometer at 720 nm. Total phenol was expressed as the milligrams per gram of leaf fresh weight. Peroxidase assays. Leaf tissue was ground in liquid N2 with a mortar and pestle. Approximately 0.5 g of tissue was transferred to 1 ml of extraction buffer (0.1 M sodium phosphate, pH 6.0). Extracts were centrifuged at 14,000 × g for 20 min at 4°C and the supernatant (enzyme extract) was transferred to a new tube on ice until assayed. Protein concentration was determined according to Bradford with bovine serum albumin as a standard. Enzyme extracts (containing 30 µg of total protein) were added to 30 µl of 200 mM guaiacol and 25 mM citrate phosphate (pH 5.4). To each sample 30 µl of 30%, H2O2 was added and the absorbance was read at 470 nm in a UV-VIS spectrophotometer. The activity was calculated as the rate of increase of absorbance per minute, and expressed as unit per milligram of protein (14). Native PAGE. Isozymes of peroxidase were analysed by native polyacrylamide gel electrophoresis (PAGE) (31). Samples were prepared by homogenizing 0.5 g of leaflets from A. solani or water-inoculated plants. Alternaria-only samples were prepared from liquid-grown cultures and treated the same as plant tissue samples. Electrophoretic separation was performed under no denaturing conditions using a 6% stacking gel and 12% separation gel containing Tris-glycine buffer, pH 8.3. Gels were incubated in 25 mM citrate-phosphate buffer (pH 5.4) containing guaiacol for 30 min and enzyme bands were visualized with a 1% solution of H2O2. The gels were photographed and the Rf values of each band were calculated (distance run by isoenzyme/distance run by bromophenol blue). Analysis of results. All experiments were carried out using a factorial model of a completely randomized experimental design in four replicates per treatment. In greenhouse evaluations, the factorial model was 9 × 5, in total phenol determinations it was 2 × 3 × 4 (two potato cultivars × three treatments: two A. solani isolates and sterile water × 4 days), and in peroxidase assays it was 2 × 3 × 10 (two potato cultivars × three treatments × 10 days). Statistical analysis was performed using SAS version 9 (20). Analysis of variance was performed on all data sets. A Duncan test with a probability of 0.05 was used to show significant differences between treatments. All data are presented as means. Analyses of variance for greenhouse, peroxidase activity, and total phenol evaluations were significant at the 1% level ($P < 0.01$).

RESULTS

Greenhouse evaluations. Greenhouse infection assays of multiple potato cultivars with different isolates of A. solani revealed significant differences between hosts. The intensity of the infection was determined by visual rating of inoculated plants using a scale from 0 to 100 based on the symptoms described in Table 1. Plants with a lower rating were considered more resistant than those with a high rating. S. tuberosum ‘Diamond’ exhibited the lowest disease severity with an average score (± standard error) of 16 (±2) among the five A. solani isolates (Fig. 1). Cultivar Granula showed the highest disease severity with an average score of 51 (±2). Analysis of variance indicated a significant difference between responses of cultivars to A.

solani infection. Other cultivars fell between ‘Diamond’ and ‘Granula’ with no clear separation between resistant and susceptible cultivars. Among A. solani isolates used in these assays, isolate A exhibited the highest aggressiveness with an average host disease severity score of 45 (±2). Isolate N was the least aggressive with an average host score of 24 (±2). The other isolates exhibited an intermediate level of aggressiveness (A > AM > M > H > N). No immunity to A. solani was observed in any of the cultivars used in this study. In addition, no significant interactions between a specific isolate and host were observed since the isolates exhibited consistent patterns of aggressiveness. However, since ‘Diamond’ exhibited significantly less disease severity than ‘Granula’, we used these two cultivars as well as A. solani isolates A (most aggressive) and N (least aggressive) to assay defense response differences. Total phenol. Total phenol content was significantly higher in pathogen-inoculated leaves when compared with healthy, water-inoculated controls (Fig. 2). Total phenol in ‘Diamond’ was higher than ‘Granula’ on all days tested. Significant differences in phenolic levels between the two cultivars were observed on all days of the assay (Table 2). In ‘Granula’, the level of phenolic compounds increased between the second and fourth day, and then decreased up to 8 days after inoculation. Total phenols decreased significantly in ‘Diamond’ between 2 and 8 days after inoculation. Differences in total phenol were also observed over time when using isolates of A. solani with different aggressiveness phenotypes (Fig. 2). The phenol levels in ‘Diamond’ and ‘Granula’ were averaged 2, 4, 6, and 8 days after inoculation with A. solani isolates A and N. There were significant differences between phenol levels elicited by isolate A, isolate N, and the water control on all days (Table 2). The less aggressive strain N elicited more total phenol than the more aggressive isolate A. Both A. solani isolates elicited similar patterns of total phenol accumulation compared with the water control with an increase between 2 and 4 days after inoculation followed by a decrease over the next 4 days. Peroxidase activity. Peroxidase activities were slightly higher in ‘Diamond’ when compared with ‘Granula’ and this difference was significant ($P = 0.0098$; Table 3). Peroxidase activity in plants inoculated with either A. solani isolate A or N was higher than in control plants sprayed with water (Fig. 3). When peroxidase activity was monitored over time after inoculation with A. TABLE 1. Scale for evaluation of the foliar damage in potato caused by Alternaria solani Rating Description of symptoms 10 Spots on lower leaves 20 Spots on most of the lower leaves 30 Spots on all lower and some of the middle leaves 40 Clearly developed blight lesions in lower leaves 50 Blight lesions in lower leaves spread to some middle leaves 60 Blight lesions developed in all inferior and most of the middle leaves 70 Blight lesions developed in all lower and middle leaves 80 Blight lesions developed in all lower and middle leaves and spread to upper leaves 100 Total blight (death of the plant).

DISCUSSION

We have shown that resistance to A. solani in the resistant potato cultivar Diamond is correlated with increased host defense responses including accumulation of total phenol and elevated peroxidase activity. Resistant and susceptible cultivars Diamond and Granula, respectively, could be significantly separated not only by disease severity scores but also by quantification of total phenol and peroxidase levels.