Pak. J. Phytopathol., Vol. 27 (01) 2015. 89-93

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

ASSESSMENT OF PHYSIOLOGICAL CHANGES IN ALTERNARIA DESTRUENS INFECTED CANOLA PLANTS

Aqsa Aftab, Amna Shoaib*, Naureen Akhtar, Nafisa Farooq
Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

A B S T R A C T

Canola (*Brassica napus* L.) is a major edible oil producing crop belongs to family Brassicaceae. Species of genus *Alternaria* have always been an increasing threat to Brassicaceae crops as pathogen of black spot diseases in plants. The present study was conducted to analyze the content of chlorophyll, protein and proline along with activities of peroxidase and catalase in canola plants infected with *Alternaria destruens*. Comparative analysis of physiology as well as biochemistry of diseased and healthy plants demonstrated that metabolism of disease plants was affected to a great extent. Content of chlorophyll, protein and proline was significantly declined by 60, 40 and 50%, respectively in diseased leaves as compared to healthy leaves. Activity of catalase was significantly increased and that of peroxidase was non-significantly different in diseased leaves in comparison to healthy ones. It was concluded that disturbed physiology and biochemistry of disease plants could lead to a disaster for the yield of this major oil producing crop.

Keywords: *Alternaria destruens,* canola, fungal spot disease, oil seed crop, physiology.

INTRODUCTION

Canola is an edible oil plant of rapeseed group belongs to the mustard family (Brasicaceae) along with 3,000 other species (Warwick, 2010). Seeds are harvested from the pods of canola plant and are crushed to create canola oil and meal. The high content of oil (44%) and protein (23%) make premium vegetable oil among the common edible oils (Aminpanah *et al*., 2013). Therefore, it is categorized at fifth position on production basis amongst the world's oilseed crops having global production of 57.5 million metric tons. Canola is a traditional crop in Pakistan, and is cultivated on 190.3 thousand hectares with net production of 162.2 thousand tons of both rapeseed and mustard. Province Punjab is the major canola producer contributing 96.3 thousand tons from 111.5 thousand hectares land (Anonymous, 2009). However, country edible oil consumption is much higher than production and it is expected that Pakistan can earn rupees 200 billion by canola cropping (Anonymous, 2012).

Alternaria blight is a serious disease of mustard family that has been reported from all the continents of the world causing significant average loss of 5-60% (Shrestha *et al*., 2005). *Alternaria* spp. usually cause black spots on leaves, pods and stems (Nowicki *et al*., 2012). On leaves, symptoms of the disease appear as brown to black circular spots that enlarge progressively under favorable conditions, become gray with concentric rings and purple or black border. Lesions on stems and pods are black or black with gray centers. During disease-favorable weather, severe defoliation can result in failure of pods development. Severely spotted pods are dried, shrunken and may split open prematurely and finally drop off (Schwartz and Gent, 2004). It has been further reported in oil seeds, infection caused by *Alternaria* spp. generally results premature seed ripening and siliquae dehiscence (Kumar *et al*., 2014). Besides, these pathogenic fungi are known to destroy chloroplast by their toxin metabolites that finally decrease the chlorophyll content (Lubaina and Murigan, 2013). However, interaction of pathogen with host plant generally induce signaling molecule in plants system. As a consequence plant antioxidant system activate through production of defense related enzymes like catalase and peroxidase (Parihar *et al*., 2012). The plants peroxidases

* Corresponding Author:
Email: aamnaa29@yahoo.com

induce defense action against pathogen through hypersensitive response, production of phenolics, glycoprotein and phytoalexin. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules thus avoiding cellular disintegration (Bolwell and Wojtaszek, 1997). Moreover, another important group of structural protein is plant proline that can help in mitigating stress of pathogen (Lubaina and Murigan, 2013). The present study was planned to investigate alteration in content of chlorophyll, protein and proline along change in activity of catalase and peroxidase of canola plants infected with *A. destruens* in comparison with healthy one.

MATERIALS AND METHODS

During survey in April, 2013, severs leaf spot disease was observed in canola field in a farm near Shiekupura, Pakistan. Diseased plants had symptoms comprised of elongated dark brown to black lesions of 0.1-0.5 cm in length with irregular margin on leaves, pods and stems. Healthy as well as diseased plant were brought into the laboratory. Leaves and stem were separately cut into small pieces, surface sterilize with sodium hypochlorite (0.1%) solution. The surface sterilized pieces were placed on glass Petri plates filled with two type of growth medium i.e. malt extract agar (MEA) and potato dextrose agar (PDA) and incubated at 25 ± 2 °C for 3-4 days. Isolated pathogen was identified as *A. destruens* on morphological basis (Shoaib *et al*., 2013).

From the survey points, samples of each healthy and diseased plant were collected and kept in liquid nitrogen, brought into laboratory for conducting physiological assays.

Chlorophyll content was determined by homogenizing fresh leaf material with 10 mL of chilled 80% acetone. Resultant homogenate was centrifuged at 800 rpm for 15 minutes and supernatant was analyzed on UV-spectrophotometer for total chlorophyll content (Baskaran *et al*., 2009).

Leaves tissues were homogenized with 1 mL phosphate buffer under ice-cold conditions, and the resulting homogenates were centrifuged at 0 ºC at 3,000 rpm for 10 min. Then 0.1 mL extract was added with 0.9 mL distilled water followed by addition of reagent C and D. The absorbance of sample was recorded at 650 nm and final concentration was calculated using Bovine Serum albumin as standard (Lowry *et al*., 1951).

For the estimation of proline content, leaf tissue was homogenized with 3% sulfosalicylic acid (w/v). The homogenate was filtered through Whatman's No. 1 and filtrate (1 mL) was mixed with glacial acetic acid and ninhiydrin reagent (1 mL each). The test tubes with the reaction mixture were kept in a boiling water bath (100 ºC) for 1 hour. The absorbance of fraction was determined at 546 nm. Standard curve was used to determine proline concentration and calculation were done on fresh weight basis (U mol proline g^{-1} FW) (Claussen, 2005).

For extracting antioxidant enzymes, leaves were ground with 50 mM phosphate buffer succeeded by centrifugation at 13000 rpm for 20 min at 4 ºC and supernatant was used for catalase and peroxidase activities according to method of Zhang *et al*. (2007). Data was analyzed through t-test using computer software Statistics 8.1.

RESULTS AND DISCUSSION

Chlorophyll content: Total chlorophyll content (0.66 mg g^{-1}) was significantly ($P \leq 0.01$) reduced by 60% in diseased plant in comparison to healthy plant (Figure 1 A). Alteration in chlorophyll contents in diseased plant could probably be correlated with stomata closure, reduction in CO_2 assimilation and transpiration. Thus, substantial enhancement in intercellular CO_2 concentration may lead to drop in Photosystem II quantum yields and total chlorophyll content in diseased plant (Petit *et al.,* 2006). Reduction in chlorophyll in the presence of pathogen indicate high level of lipid peroxidation mediating cell damage in canola tissues (El-Khallal, 2007).

Total soluble protein content: Protein content of diseased plant was found to reduce significantly ($P \leq 0.05$) up to 40% as compared to healthy one (1.44 mg g^{-1}) (Figure 1 B). Protein contents in organism are taken as an important indicator of reversible and irreversible changes in metabolism (Singh and Tewari, 2003). Low protein content could be due to proteolysis or denaturation of protein (Lubaina and Murigan, 2013). Moreover, reduction in the protein contents could also occur owing to the production of alternariol and tenuazonic acid by *Alternaria* in the infected tissues. Alternariol-induced cytotoxicity is mediated by activation of the mitochondrial pathway of apoptosis. Whereas, high concentrations of tenuazonic acid may inhibit protein synthesis thus negatively affect plant growth (Nowicki *et al*., 2012).

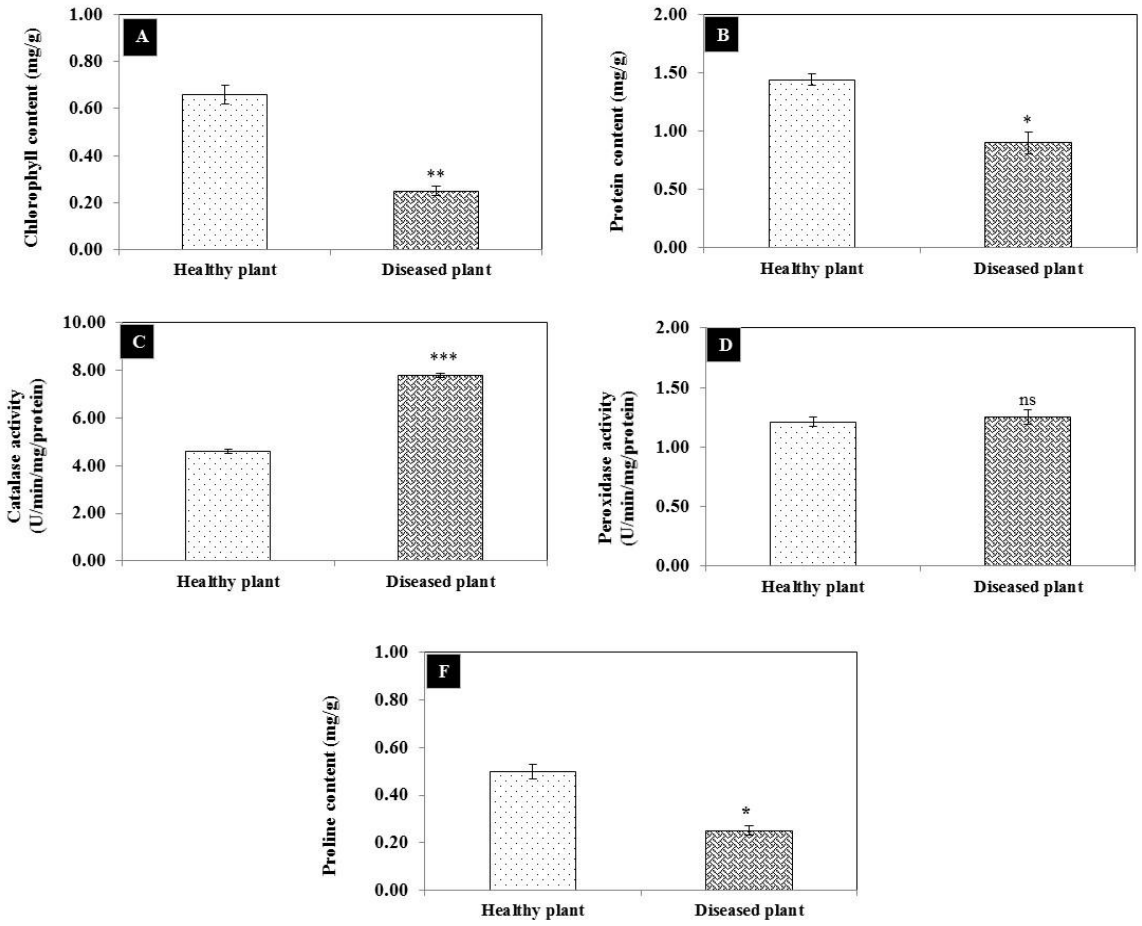

Figure 1 A-E. Effect of *Alternaria destruens* on physiology and biochemistry of canola leaves.
*, **, *** show significant difference between two corresponding treatments of healthy and diseased canola at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, level of significance, respectively as determined by t-test.

Antioxidant enzymes activity assays: The catalase activity of the diseased plant (4.7 U min^{-1} mg^{-1} protein) was significantly (P ≤ 0.001) increased over healthy one (7.8 U min^{-1} mg^{-1} protein).

However, the difference between peroxidase activity of diseased and healthy canola leaves was statistically non-significant (Figure 1 C & D). Antioxidant enzymes are very good biochemical markers of stress. Earlier work suggested that rise in antioxidant enzymes activity helps the plants to maintain their growth under stress conditions and may be regarded as an indicator of disease tolerance (Candan and Tarhan, 2003; Zembala e*t al*., 2010). It can be concluded that fungus enhanced oxidative stress in canola, and enable it to combat the disease stress via production of scavenging systems (Lubaina and Murigan, 2013). However, this resistance by plant does not meet with desired level resulting in loss in plant growth and health.

Proline content: The proline contents of diseased canola were drastically (P ≤ 0.05) reduced up to 50% in comparison to healthy one (0.5 mg g^{-1}) (Figure 1 E). Proline content of leaves is regarded as valuable mean to evaluate plant health status during growth (Grote *et al*., 2006). Low proline content of diseased plant might be correlated with reduction in free amino acid concentration in vacuoles that could result in destabilization of membranes and subcellular components, including the mitochondrial electron transport complex II and intracellular osmotic pressure (Bandehagh *et al*., 2008). It could be assumed that a large number of necrotic lesions on canola leaves possibly not have desired content of proline to prevent necrosis in response to attack of virulent.

CONCLUSIONS

Alternaria black spot disease adversely influenced cholorophyll, total soluble protein, proline and

antioxidant enzymes synthesis pathways in canola plant. Therefore, it is imperative to control disease by some suitable method to avoid loss in future. Paddock isolated from last years canola stubble could be selected as *Alternaria* spores are easily transported by wind and can spread into areas that have not had canola for several years.

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