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Enzyme activity changes in *Brassica juncea* (L.) Czern.& Coss. in response to *Albugo candida* Kuntz.(Pers.)

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ABSTRACT

The quantitative analysis of Peroxidase, α -amylase, Invertase, IAA-oxidase, Ascorbic acid oxidase activity were observed in healthy and diseased Brassica juncea leaves and inflorescence. Maximum Peroxidase activity observed in infected leaves and disease inflorescence as compared to healthy leaves and infected inflorescence. α -amylase was maximum in healthy leaves and minimum in infected inflorescence. Maximum invertase activity was found in infected leaves of Brassica juncea and minimum in healthy leaves of Brassica juncea. IAA-oxidase activity was higher in infected leaves and inflorescence as compared to healthy leaves and inflorescence. The activity of Ascorbic acid oxidase decrease with the increase in infection in plants.

Key Words: *Brassica juncea*, *Albugo candida*, Peroxidase, α -amylase, Ascorbic acid oxidase.

INTRODUCTION

Brassica juncea (L.) Czern. & Coss. is an important oil seed belonging to family cruciferae. *Brassica juncea* is commonly known as 'Indian Mustard' and is one of the most important oilseed crops of northern India. Its cultivation is mostly confined to Uttar Pradesh, Rajasthan, Punjab, Haryana and some parts of Madhya Pradesh, West Bengal and Assam. In Rajasthan, cultivated area of mustard is 2.6 million hectares, producing about 2.2 million tones of grains (Directorate of Agriculture, Rajasthan, Jaipur). Oilseed brassicas are widely cultivated through the world as condiment and spices for improved flavour of human diet and as fodder for live stock feeding. However, the largest cultivation of this crop is for edible vegetable oil production.

White rust caused by the fungus *Albugo candida* is an important disease of brassicaceae family. White pustules (sori) of variable size and shapes are formed on leaves, stems and inflorescence. Sori are initially discrete but later coalesce to cover the whole plant organs. Distortion and hypertrophy of affected host tissue is frequent. The leaves become thick, fleshy and enrolled

when infection is severe, the size of the leaves may be reduced. The floral parts persist and show hypertrophy or hyperperplasia. Structures and development of galls induced by *Albugo* in the inflorescence axis of *Brassica juncea* [1].

Mustard is a rabi crop, which requires relatively cool temperature, a fair supply of soil moisture during the growing season and a dry harvest period. The plant is a sparsely branched annual herb. It is often cultivated mixed with wheat or barley during the winter season. Loam soil is best suited for its growth. The plant bears beautiful flower in a terminal compound raceme. The fruits are long siliqua. The seed are small, rounded and brown, black coloured. The seed contain 30 to 45% oil.

Analysis of enzymes is an essential features of the biochemical organization of living things. A number of major biological problems such as evolution of population, transformation, regulation in differential tissues are understood in the of the enzymes. In the present study existence of variability of enzymes in resistance and susceptible *Brassica juncea* against white rust caused by *Albugo candida* is assessed the result are reported here.

EXPERIMENTAL SECTION

On the basis of susceptibility and resistance to *Albugo candida*, white rust disease. For the quantitative estimation of enzymes different protocol were used. Healthy leaves and inflorescence and infected leaves and inflorescence were collected from Agriculture research station Durgapura, Jaipur.

Enzyme extraction:-

Fresh collected (1g) plant material were ground in 3ml chilled phosphet buffer (0.02M, pH 6.0) in chilled pestle and mortar. The extract was centrifuged at 10,000 rpm for 15 minutes in cooling centrifuge at 4°C. The supernatant was mixed with the double amount of chilled acetone and incubated at 5°C for half an hour for precipitation of the soluble proteins. It was recentrifuged at 3600 rpm for 10 minutes at 4°C. The supernatant was discarded, while residue was resuspended in 10ml of phosphate buffer (0.02M, pH 6.4) and enzyme source. Enzyme extracted was thus prepared was assayed for Peroxidase, Alpha amylase, Invertase IAA-oxidase and Ascorbic acid oxidase.

Enzymes were determined following method described Alpha amylase was determined by the given in [2]. Invertase was determined using [3]. Peroxidase was determined using [4]. IAA-oxidase was determined using [5]. Activity of Ascorbic acid oxidase was determined using method of [6]. The experiments were replicated and repeated thrice.

RESULTS AND DISCUSSION

Enzyme activity changes of healthy and *Albugo candida* infected mustard plants revealed that in different parts as compared to healthy ones. α -amylase activity was higher in healthy *Brassica* leaves compared infected leaves and minimum activity found in healthy inflorescence. Higher invertase activity was found in infected leaves compared to its healthy leaves, infected inflorescence as compared to diseased inflorescence.



Fig:1 Healthy inflorescence of *Brassica juncea*.



Fig:2 Infected inflorescence of *Brassica juncea*



Fig:3 Healthy leaves of *Brassica juncea*



Fig:4 Infected leaves of *Brassica juncea*

Increased activity of the starch hydrolyzing enzyme (alpha amylase and Invertase) has been recorded in diseased tissues. The increased activity of these enzymes might be due to their enhanced synthesis by the host to meet the catabolic reactions in the enhanced state of host metabolism after infection [7].

Obviously high sugar levels were a result of starch hydrolysis by the activation of alpha amylase in the diseased tissue. Activation of Invertase is quite possible in diseased tissue.

The phenol oxidizing enzymes have been correlated with disease resistance in many crop plants. The production of lignin is catalysed by Peroxidase. Peroxidase activity was higher in diseased inflorescence and diseased leaves compared to healthy leaves and inflorescence. Maximum Peroxidase activity was found to be in infected leaves of *Brassica juncea*.

Presence of phenolics in high concentration in the plant cells gives them resistance to pathogens. After infection by a pathogen, plant cells synthesize phenol oxidizing enzymes that oxidize phenols to toxic quinones. Hence, the activity of these enzymes increases in infected cells. Higher activities of phenol oxidizing enzyme peroxidase in the rust and powdery mildew resistant lines of pea as compared to susceptible lines [8]. Increase in the activity of peroxidase after infection in different host-pathogen systems [9,10,11]. The results obtained in present studies are in agreement with those of this worker.

IAA-oxidase activity was maximum in diseased inflorescence compared to healthy inflorescence, healthy leaves and diseased leaves. Higher IAA-oxidase was found to be infected leaves compared to healthy leaves. During early stage of infection presence of less IAA oxidase activity was observed in tissues as compared to healthy tissue to maximum being in young staghead.

During late phase of systematic infection Auxin content decreases and records well below that the normal tissue level. This may be attributed to reduced concentration and conversion of Auxin precursors like tryptophan or increased synthesis of IAA oxidizing enzymes. Increased level of suggested that the enzymes oxidized IAA rapidly causing reduction in Auxin. This view is supported by hypertrophy produced by *Albugo candida* in *Brassica napus* [12].

Inhibition of IAA-oxidase due to accumulation scorpoletin was related with hyperauxiny in disease tissue recorded [13]. Similarly, chlorogenic and caffeic acid were considered responsible for IAA-oxidase inhibition [14]. Inhibition of IAA-oxidase by increased caffeic acid and m-coumaric acid in spiked sandal was recorded [15].

Infection due to *Albugo candida* in *Brassica juncea* var. varuna resulted in ascorbic acid metabolism. Ascorbic acid oxidase activity was highest in healthy inflorescence compared to diseased inflorescence. In leaves highest activity was found to be healthy leaves compared to infected leaves.

Increase in Ascorbic acid in diseased tissues was corresponding to the activity of Ascorbic acid oxidase [16,17].

That not only the suppressed activity of Ascorbic acid oxidase but also excessive synthesis through sugar precursors was responsible for accumulation of Ascorbic acid in diseased tissue catalyzation of Ascorbic acid synthesis by IAA has been reported [18]. *Albugo candida* pathogenesis may be attributed to the increased respiration rate, which may induce rapid oxidation of ascorbic acid. The decline may also be due to the production of the enzyme ascorbic acid oxidase, either by the pathogen itself or by the host-pathogen interactions[19]

Enzyme activity was found in the all plants. To function as a regulator of cell expansion IAA-oxidase affects cell elongation, probably, through its influence on cell wall extensibility. Since long, peroxidase are known to be involved in growth regulation and different biochemical pathways have been proposed to examine the mechanism of action [20]. Plant cell wall plays a critical role in water uptake. A wide range of growth regulating agents, including both hormones and environmental factors. These studies show that plant parts having enzymatic activity studies of some enzymes peroxidase, α -amylase, Invertase, IAA-oxidase, Ascorbic acid oxidase in *Brassica juncea*.

Table: Enzymatic activity from healthy and infected parts of *Brassica juncea*

S.No.	Plant Parts	Alfa-Amylase (µg/g)	Invertase (µg/g)	Peroxidase (µg/g)	IAA-Oxidase (µg/g)	Ascorbic Acid Oxidase (µg/g)
1.	Healthy Leaf	0.023	0.36	0.055	4.30	0.57
2.	Infected Leaf	0.033	0.13	0.076	2.75	0.32
3.	Healthy Inflorescence	0.031	0.52	0.36	2.55	0.67
4.	Infected Inflorescence	0.040	0.76	0.56	1.75	0.12

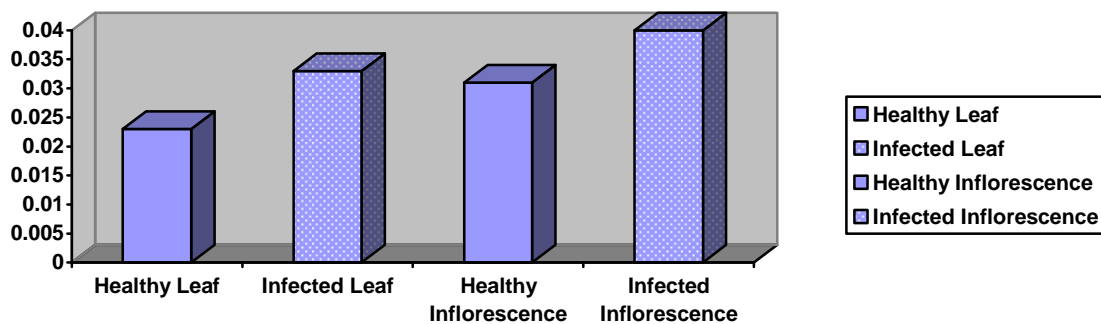


Fig. 1 Alfa-Amylase Activity (µg/g) of *Brassica juncea*

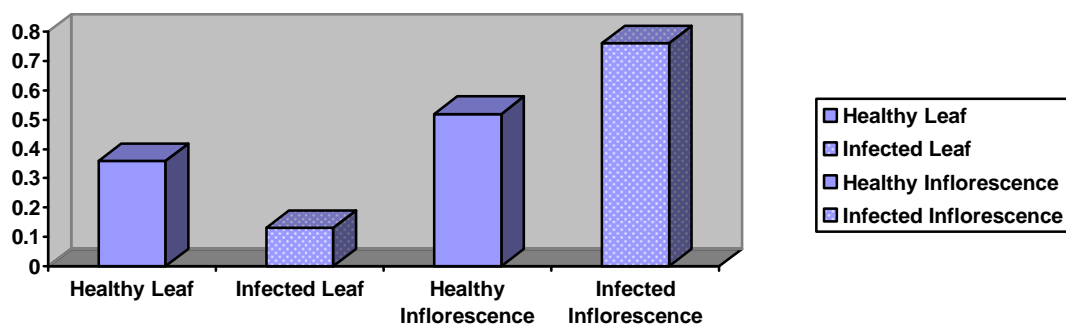


Fig.2 Invertase Activity (µg/g) of *Brassica juncea*

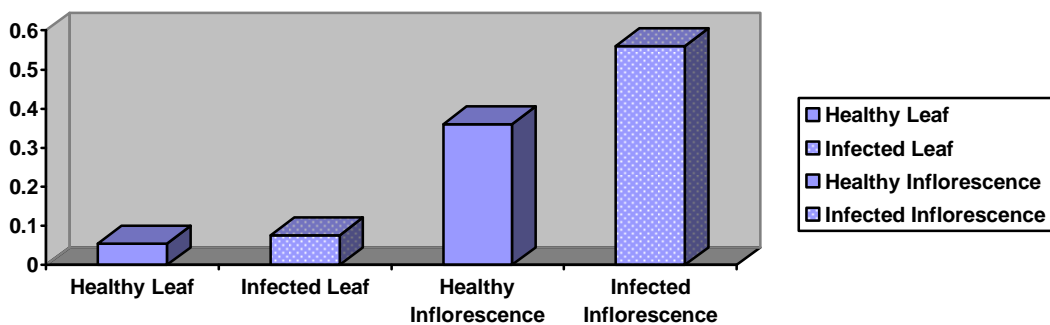


Fig.3 Peroxidase Activity (µg/g) of *Brassica juncea*

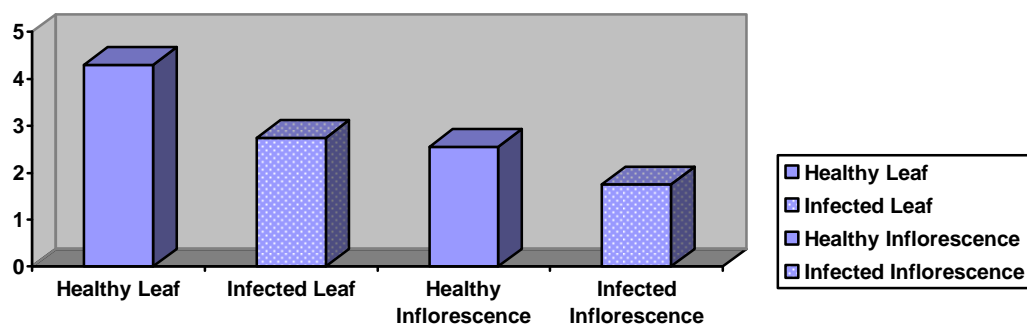


Fig. 4 IAA-Oxidase Activity (µg/g) of *Brassica juncea*

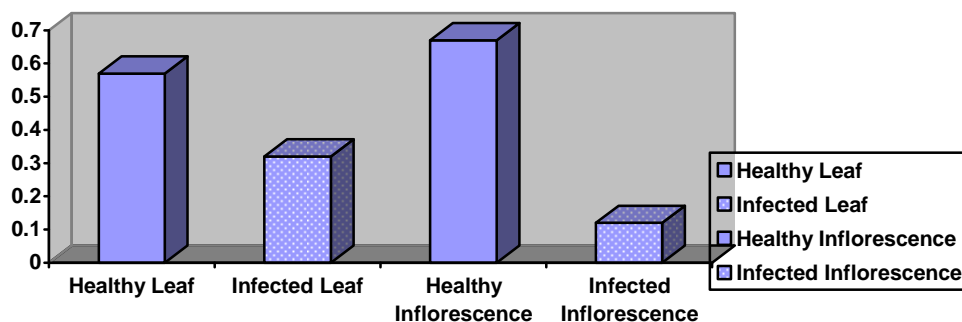


Fig. 5 Ascorbic Acid Oxidase Activity ($\mu\text{g/g}$) of *Brassica juncea*

CONCLUSION

Result obtained enzymatic activity was maximum in infected parts compared to healthy leaves and inflorescence. These results are suggestive of enzymes of commercial importance and may great interest in plant pharmaceuticals. Scientific information about the chemical composition of these plants and may be important both conceptually, to understand the physiological process of plant parts and technically by providing more information for agro industry.

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