# **Research Article**



# BIOCHEMICAL CHANGES IN *BRASSICA JUNCEA* (L.) CZERN. & COSS. INFECTED WITH *ALBUGO CANDIDA* KUNTZ. (PERS.)

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### ABSTRACT

Carbohydrates, Proteins, amino acids, lipids, phenols and ascorbic acids have received considerable attention in relation to resistance in plants against disease. The biochemical estimation is carried out to study the host parasite relationship in *Brassica juncea* infected with *Albugo candida* respectively. Different parts of infected and healthy plants were taken in to consideration. Sugar showed higher amount in diseased and low in healthy parts while the starch content showed the reverse results as the amylase activity was directly proportional to disease percentage. Higher amount of proteins was in infected leaves and inflorescence as compared to healthy ones. High amounts of Amino acids were observed in healthy plant parts as compared to infected plant parts. Lipids and ascorbic acids were higher in normal counter parts as compared to infected leaves and inflorescences. Phenol activity was noted high in diseases leaves where as low in disease inflorescences.

Keywords: Brassica juncea, Albugo candida, Carbohydrate, Protein, Amino acid, Phenol, Lipid, Ascorbic acid.

#### INTRODUCTION

Indian mustard *Brassica juncea* (L.) Czern. & Coss. commonly known as "rai" is most important among oil yielding crucifer crop. It belongs to family cruciferae. The rapeseed and mustard group accounts for 24% of the total oil seed production of which Indian mustard alone covers about 70%. This oleiferous crucifer is grown extensively in the Indo-gangetic plains of India, particularly the state of Rajasthan, Maharastra, U.P., Gujarat and Punjab. However, it is cultivated in all districts of Rajasthan.

*Brassica juncea* is erecting much branched 3' to 6' high annual plant with slender and tapering root. The stem branches from the axil of the fourth or fifth leaf upward. lower leaves petioled, green, sometimes with a whitish bloom, ovate to obovate, variously lobed with toothed, scalloped or frilled edges, lyrate-pinnatisect, with 1-2 lobes or leaflets on each side and a larger sparsely sectose, terminal lobe; upper leaves subentire, short petioled, 30-60 mm long, 2-3.5 mm wide, the fruit is siliqua. The pods are bilocular with a false septum between two halve.

Unfortunately this crop of great economic importance sufferes from several fungal diseases every year. one of the most important disease is white rust of *Brassica juncea* caused by *Albugo candida* (pers.) Kuntz is an obligate parasite which belongs to family albuginaceae. It produced their zoosporangia in chains an unbranched zoosporangiophores of determinate growth. The fungus is known to produce abnormal growth on inflorescence in plant resulting in hypertrophy and hyperplasia and sterility of flowers. The stag head phase accounts for most the yield losses (up to 56%) in the individual plant. By controlling these diseases, substantial loss of revenue to the nation can be prevented. Primary metabolites are of prime importance and essentially required for growth of plants for example sugar, starch, protein, amino acid, lipid, phenol, ascorbic acid. Many primary metabolites lie in their impact precursors or pharmacologically active metabolites in of pharmaceutical compounds. The primary metabolites were studies in *Eclipta alba and Nerium indicum* earlier<sup>1,2</sup>. The present investigation was carried out for the study of primary metabolites in *Brassica juncea* infected with *Albugo candida* and was compared with healthy counterparts.

#### **MATERIALS AND METHODS**

For the quantitative estimation of primary metabolites different protocols were used. Healthy leaves and inflorescences and infected leaves and inflorescences (hypertrophied part) were collected, washed with distilled water shade dried and powdered. The powder was used for analysis of total sugar<sup>3</sup>, Starch<sup>4</sup>, protein<sup>5</sup>, lipid<sup>6</sup> phenol<sup>7</sup> amino acid<sup>8</sup> and ascorbic acid<sup>9</sup>.

### **RESULTS AND DISCUSSION**

The resistance of plants as a result of host pathogen interaction involves morphological and biochemical changes which depends upon the plant response to infection. The fungal infection induces the oxidative and hydrolytic reactions along with hormonal imbalance in the host tissue affecting the normal metabolism<sup>10</sup>.

Total carbohydrate estimation (table 1, fig 1-7) of healthy and *Albugo candida* infected mustard plants revealed that in different parts as compared to healthy ones. The total carbohydrate content decreased in infected plants as compared to healthy ones.



S. No	Plant Parts	Sugar	Starch	Protein	Lipid	Phenol	Amino acid	Ascorbic acid
		(mg/gdw)						
1.	Healthy Leaf	5.50	7.50	6.80	78	8.0	0.65	0.85
2.	Infected Leaf	9.45	5.52	13.80	200	10.20	0.49	0.95
3.	Healthy	5.70	6.45	6.60	100	8.80	1.10	1.25
4.	Inflorescence Infected Inflorescence	6.20	4.00	13.65	231	14.0	0.75	3.20

Table -1: Primary metabolites from healthy and infected leaf, inflorescence of Brassica juncea.





Figure 2: Starch Concentration mg/gdw of Brassica juncea





# Figure 3: Protein Concentration mg/gdw of Brassica juncea









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### Figure 5: Phenol Concentration mg/gdw of Brassica juncea





### Figure 7: Ascorbic Acid Concentration mg/gdw of Brassica juncea



Maximum amount of sugar was found in infected Brassica leaves (9.45mg/gdw) compared to be healthy leaves (5.50mg/gdw). The infected inflorescence show high sugar content (6.20mg/gdw) compared to be healthy inflorescence (5.70mg/gdw). Starch was maximum in healthy leaves (7.50mg/gdw) compared to infected leaves (5.50mg/gdw). It was also maximum in healthy inflorescence compared to diseased inflorescence. Increased activity of the starch hydrolyzing enzymes ( $\alpha$ amylase and Invertase) has been recorded in diseased tissues. Increased activity of these enzymes might be due to their enhanced state of host metabolism after infection<sup>11</sup> or by the pathogen itself<sup>12</sup>. The change of starch and sucrose by the enzymes in to easily assimilable substances would be favour the growth and proliferation of the pathogen in the diseased tissue<sup>13</sup>. Obviously higher sugar levels were result of starch hydrolysis by the activation of  $\alpha$ -amylase in the diseased tissue.

In the case of *Albugo candida* infected *Brassica juncea* plant tissues it appears high sugar levels were result of

starch hydrolysis by the activation of  $\alpha$ -amylase in the diseased tissue and explains the high levels of total soluble sugars and less starch content in diseased tissues.

The total protein amount was recorded high in infected leaves and inflorescence. The total protein content was recorded (6.80mg/gdw) in healthy leaves is compared to infected leaves (13.80mg/gdw). The healthy inflorescence shows high protein content (6.60mg/gdw) while infected with (13.80mg/gdw).

Total proteins were in higher amount in diseased tissues as compared to normal tissues. Total protein was found maximum in diseased tissue at the early stage of infection. However, it decreased to minimum level lower than normal inflorescence in mature staghead probably due to degradation of the tissues of the fungus at this stage.

Total protein content of the infected tissues increases initially. It is likely that due to less proteolytic enzyme activity, proteins were not broken down into amino acids



a completely resulting in decreased total amino acids in the diseased tissues at the early phase of infection as compared to healthy tissues<sup>14, 15</sup>.

Phenol content in infected leaves was higher as compared to healthy leaves. Total phenol content was 10.20mg/gdw in infected leaves as compared to 8.0mg/gdw in healthy ones. Maximum phenol present was healthy inflorescence compared to infected inflorescence. Total phenol content was 14.0mg/gdw in healthy inflorescence and 8.80mg/g. in infected inflorescence.

Presence of phenolics in high concentration in the plant cells gives them resistance to pathogens. After infection by pathogen plant cells synthesize phenol oxidizing enzymes that oxidize phenols to toxic quinines. Hence the activity of these enzymes increases in infected cell. Vir and Grewal (1974) reported increase in the phenolic content of gram cultivars after infection. Singh and Bedi (1976) reported increase in the amount of total phenols in both resistant and susceptible cultivars of gram infected with *Opercullela padwickii*. Various workers (Munshi et al., 1987; Sindhan et al., 1999 and Ramanathan et al., 2000) recorded increase in the amount of phenolic substances in different host pathogen systems after infection.

Amino acid content in healthy leaves and inflorescence was higher as compared to infected ones. Amino acid was (0.65mg/gdw) in healthy leaves as compared to (0.49 mg/gdw) in infected ones. Maximum amino acid was present in healthy inflorescence compared to infected inflorescence. Amino acid content was (1.10mg/gdw) in healthy inflorescence and (0.75mg/gdw) in infected inflorescence. Decreased amino acids during asexual phase of the disease might be due to utilization of these by the pathogen through haustoria. The fungus induces catabolic break down of amino acid and amides in to nitrogen<sup>16</sup>.

Ascorbic acid content is increase in infected leaves (0.95mg/gdw) compared to healthy leaves (0.85mg/gdw). Ascorbic acid content in healthy inflorescence shows (1.25mg/gdw) compared to be infected inflorescence (3.20mg/gdw). Infection due to *Albugo Candida* in *Brassica juncea* resulted in altering the ascorbic acid metabolism. Increased ascorbic acid contents were observed in diseased inflorescence stage over their normal counterparts. However old staghead showed maximum ascorbic acid contents. While in case of healthy and infected leaves, there was no appreciable change in the ascorbic acid contents<sup>16, 17</sup>.

Ascorbic acid contents increased during pathogenesis whereas enzyme activity decreased. It is therefore, that decrease was due to destruction of ascorbic acid by the enzyme whereas in diseased leaves, there was no appreciable change in the ascorbic acid content.

Lipid content in infected leaves and inflorescence was higher as compared to healthy leaves and healthy inflorescence. Lipid content in healthy leaves was (78mg/gdw) compared to infected leaves (200mg/gdw). Lipid present in infected inflorescence (231mg/gdw) compared to be healthy inflorescence. Increased lipid content was observed in diseased inflorescence and leaves stage over their normal counter parts. Lipid content increased during pathogenesis from asexual phase of disease to sexual phase of disease.

### CONCLUSION

Higher amount of sugar, protein and amino acid was found in healthy parts compared to infected ones while highest starch, phenol and lipid present in infected parts compared to healthy ones. These study show that plants parts having rich primary metabolites could be used industrially as raw materials. Therefore, economic use depends partly on the quantitative and qualitative aspects of their organic reserves, especially carbohydrates, protein, phenol, amino acids, ascorbic acids and lipids.

# REFERENCES

- 1. Dhingra M 1988. Studies on growth and differentiation in tissue cultures of some economically important plants. Ph.D thesis. University of Rajasthan, Jaipur, India
- 2. Audichya M 1999. Regeneration studies on oil containing seeds through tissue culture. Ph.D thesis University of Rajasthan, Jaipur,
- 3. Loomis W.E. and Shull C .A. 1973 Methods in Plants Physiology, New York: Mc: Graw Hill Book co.
- 4. Lowery OH, Rosebrough NJ, Farr AL & Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 265-275.
- 5. Jayaraman J 1981 Laboratory Manual in Biochemistry. New Delhi: Wiley Estern Limited, New Delhi.
- 6. DuboisM,Gills K, HamiltonJK,Rebers PA&Smith F 1951 A colorimetric method for the determination of sugar. Nature.167-168.
- 6. Bray HG&Thorpe WV 1954 Analysis of phenolic compounds of interest in metabolism.Meth *Biochem. Anal.* 1 27-52.
- 7. Lee, Y.P.&Takahashi, J. 1966 An improved colorimetric determination Amino acids with the use of ninhydrin .Annal. Biotech. 34: 72-77.
- 8. Roe R.A. and Kuethe C. A. 1943:The determination of ascorbic acid in hole blood and urine through 2,4 –dinitrophenyl hydrazine derivative of dehydro ascorbic acid.J. Biol. Chem.147:399
- 9. Sakhare, R.K. and A.N. Thite, 1986. Difference in levels of hydrocy peroxidases, IAA-Oxidase and acid phosphatese in healthy and white sust infected leaves of *Amaranthus olium var oleracea*. *Ind. J. Mycol. Pl. Path.*, 16(2):176-177.



- 10. Nonaka, F. 1959. On the relation between the stem rot woused by leptosphaeria salvini) and the rice nematode disease, white tip, and on the changes of the respiration rate and activities of respiratiory enzymes of the cohite tep plants. *Sci. Bull. Fac. Agric. Kikyshu Uni*.17. 1-8.
- 11. Kosuge. T. and D.G.Gilkrist, 1979 Metabolic regulation in host parasite interaction. Pp 679-702.
- 12. In R. Heitefuss and P.H. Williams (eds.) Encyclopedia of plant physiology IV. 'Physiological plant pathology' springer – verlag, Berlin.
- 13. Dropkin, V.H. 1972. Pathology of meloidogyne, galling, giant cell formation, effects on host physiology, *Eur. Pl.Bull.* 6 : 23.

- 14. Howell, R.K. and L.R. Krushberg, 1966. Changtes in concentration of nitrogen and free and bound amino acids in alphalpha and pea infected by *ditylenchus dipsaci. Phytopath.*, 56 : 1170
- 15. Uritani I. 1971. protein changes in diseased plants. Ann Rev. phytopath. 9 : 211 – 234
- 16. Goyal BK 1989. Studies on fungal abnormalities of *Brassica juncea in vivo and in vitro*. Ph.D thesis. University of Rajasthan, Jaipur, India.
- 17. Rao DV 1989. In vivo and In vitro studies of green ear disease of *Pearl millet* (Bajara) caused by *Sclerospora graminacola* (Sacc.) Schrot with special reference to host parasite interaction, biochemistry and control. Ph.D thesis. University of Rajasthan, Jaipur, India.

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