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Induction of Carbohydrate Metabolism in Relation to Leaf Blight in Barley (*Hordeum vulgare*)

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Abstract: Induction of carbohydrate metabolism in relation to leaf blight (*Bipolaris sorokiniana*) was studied in healthy and infected leaves of barley (*Hordeum vulgare*). Two genotypes with varying sensitivity to leaf blight viz. PL 426 (susceptible) and BL 4 (resistant) in the absence of or following infection with leaf blight at different stages (020 days after infection) were used. Both acid and neutral invertase activities increased in response to leaf blight and might be linked with defense mechanism against fungal infection. Low activities of sucrose synthase and sucrose phosphate synthase in infected leaves as compared to non infected leaves may be involved in retention of more sugars for utilization by the fungus for its own metabolic processes. The levels of total sugar, nonreducing sugar and starch were less in infected leaves as compared to non infected leaves. Irrespective of cultivar and stage of infection, activity of total amylase marginally increased with concomitant decline in starch content. Leaf blight leads to an alteration in the fluxes of carbon and represents a cost effective stress to the plant in terms of carbon production and utilization.

Key words: Barley · *Bipolaris sorokiniana* · Carbohydrate metabolism · Leaf blight

INTRODUCTION

Barley, the world's fourth most important cereal after wheat, rice and maize suffers huge yield losses due to disease leaf blight that severely affects aerial parts of the plant. Losses in severely affected plants of barley can be as high as 16% in India, 20% in Nepal and 23% in Bangladesh [1, 2]. As a consequence, much effort has been devoted to select genotypes resistant to leaf blight. Leaf blight is caused by the fungus *Bipolaris sorokiniana* which becomes more prevalent in warmer growing areas especially under high relative humidity [3]. Considerable yield losses also occur due to premature drying of foliage and storage of infected seeds that act as primary inoculums for the next seasoned crop. Soil borne inoculums also spread this disease and the visible appearance of this pathogen is in the form of leaf spots. Infected plant develops dark brown lesions on crowns and lower parts of leaf sheaths. Round to oblong dark brown spots with definite margins appear on lower leaves following warm, moist weather that favours the development of this disease.

Owing to the increase global demand for cereal-based products, cereal cultivation, which was earlier confined to rain-fed situation, has extended to irrigated systems [4]. Breeding efforts and irrigation have increased cropping intensity along with increase in the spread of pathogen. Although significant progress has been made to obtain genotypes resistant to leaf rust still literature is scanty on leaf blight which otherwise has become a major constraint for production in South Asia's intensive cropping system [5, 6]

Carbohydrates have been shown to play an important role during plant-pathogen interactions [7]. The greatest part of the carbohydrates generated during photosynthesis is stored in a suitable form in order to be available for dissemination in an energy-liberating reaction when the plant needs it. On the other hand, carbohydrates are the basic building blocks for the synthesis of various defense chemicals such as phenolics, phytoalexins and lignin. Hence the quality and quantity of sugars play an important role in disease resistance [8]. Specific carbohydrates like sucrose, glucose and galactose are correlated with disease

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resistance in some plant-pathogen interactions. Altering the sugar content of leaves has been shown to be a possible way to control diseases [9], indicating that interfering with the physiology of the host could potentially offer an exciting opportunity to control diseases. Since sucrose is the major translocatory form of carbon in plants, its metabolism gets seriously affected during stress conditions. Different kinds of sugars also act as a signalling molecule during plant pathogen interactions. It has been reported that total sugars significantly increased during downy mildew infection in sunflower [10]. However, there are no reports in the literature on how carbohydrate metabolism gets affected during leaf blight infection. For comparison purpose, two varieties of barley susceptible (PL 426) and resistant (BL 4) to leaf blight were taken in this study.

MATERIALS AND METHODS

Plant Material: Two barley genotypes, susceptible (PL 426) and resistant (BL 4) to leaf blight were planted in the fields of Punjab Agricultural University, Ludhiana under recommended agronomic practices. Leaves infected with blight were harvested at intervals of 0, 5, 10, 15 and 20 days after infection (DAI) for studying carbohydrate metabolizing enzymes (invertase, sucrose synthase, sucrose phosphate synthase and total amylase) in relation to free sugars and starch accumulation.

Extraction and Estimation of Sugars: Free sugars were extracted and estimated from the infected and non-infected leaves of barley and from the sugar free samples, starch was determined according to the earlier defined procedures [11, 12].

Enzyme Extraction: Enzymes were extracted from fresh tissue samples essentially by the earlier described method [13] by homogenizing the tissues in extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM sodium EDTA, 2.5 mM DTT, 0.5 mg ml^{-1} BSA and 0.5% (v/v) Triton X-100. Homogenates were centrifuged at 10,000 g for 15 min at 0-4°C. The pellet was resuspended in extraction buffer and centrifuged as before. The pellet was dissolved in minimum volume of extraction buffer and dialysed overnight against four times diluted extraction buffer without $MgCl_2$, EDTA, DTT and BSA.

Enzyme Assays: The activities of sucrose synthase (UDPglucose-fructose-2-α-D glucosyltransferase, E.C: 2.4.1.13) and sucrose phosphate synthase (UDPglucose: fructose-6-phosphate-2-α-D-glucosyltransferase, E.C: 2.4.1.14) were assayed by the erlier described method [14]. Activities of soluble acid invertase (pH 4.8), soluble neutral invertase (pH 7.5) (β-D-fructofuranoside fructohydrolase, E.C: 3.2.1.26/27) and total amylase (α-amylase/β-amylase: α-1,4-glucan 4-glucanohydrolase/ α-1,4-glucanmaltohydrolse, E.C: 3.2.1.1/2) were assayed essentially by the methods described previously by Singh and Asthir [11]. In all enzyme assays, the condition for linear rates with respect to substrate concentration, time, optimum temperature and pH were determined in preliminary assays.

Statistical Analysis: The recorded data were analysed in factorial CRD design by using CPCS 1 software programme. The correlation studies were performed by using MS Excel spreads sheet software for windows (version 2003).

RESULTS AND DISCUSSION

Invasion of pathogen leads to alterations in carbohydrate metabolism in leaves of susceptible (PL 426) and resistant (BL 4) genotypes of barley. Leaves infected with leaf blight showed significantly ($P<0.05$) higher activities of invertase as compared to non infected leaves (Fig. 1). Similar increase in invertase activities in response to fungal infection has earlier been reported in *Arabidopsis* and in wheat leaves by Fotopoulos *et al.* [15] and Sutton *et al*. [16]. It has been reported that both defense and invertase gene expressions were induced by pathogen attack in tomato [17]. The upregulation of invertase activity appears to be a common response to various biotic and abiotic stresses [18]. They further indicated that an increase in extracellular invertase activity leads to inverse regulation of photosynthesis and carbohydrates during pathogen infection. Our results are consistent with a role for invertase in the generation of hexoses, which may supply energy for defense reactions and/or may act as signals inducing defense gene expression. It has been found that powdery mildew fungi act as an additional sink, competing with host plant, resulting in considerable modification of photoassimilate production and utilization within the host tissue [15]. In general, at all stages of leaf infection, acid invertase activity predominated over neutral invertase activity.

The activity patterns of sucrose synthesizing enzymes viz. sucrose synthase (SS) and sucrose phosphate synthase (SPS) are given in Fig. 1 and 2. In comparison to SS, SPS activity was higher in accordance with the fact that major sucrose synthesizing enzyme was

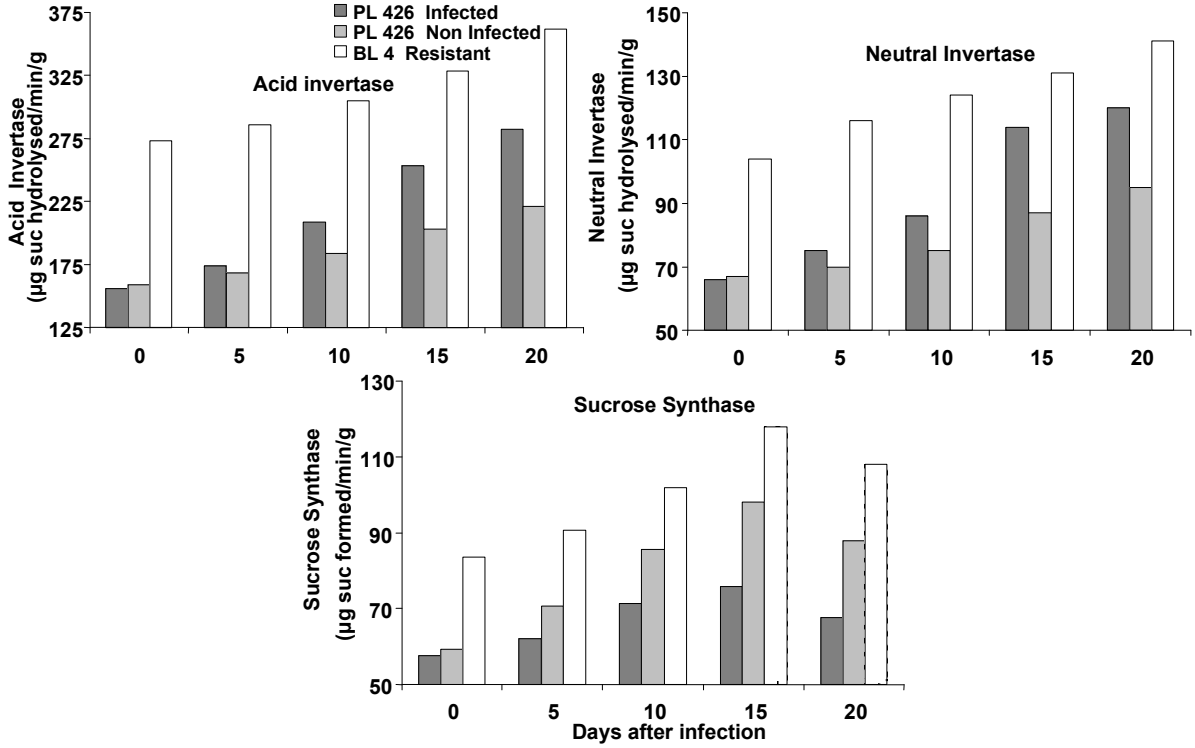

Fig. 1: Activities of acid invertase, neutral invertase and sucrose synthase at different days after infection in leaves of leaf blight susceptible (PL 426) and leaf blight resistant (BL 4) genotypes of barley raised in field. suc, sucrose

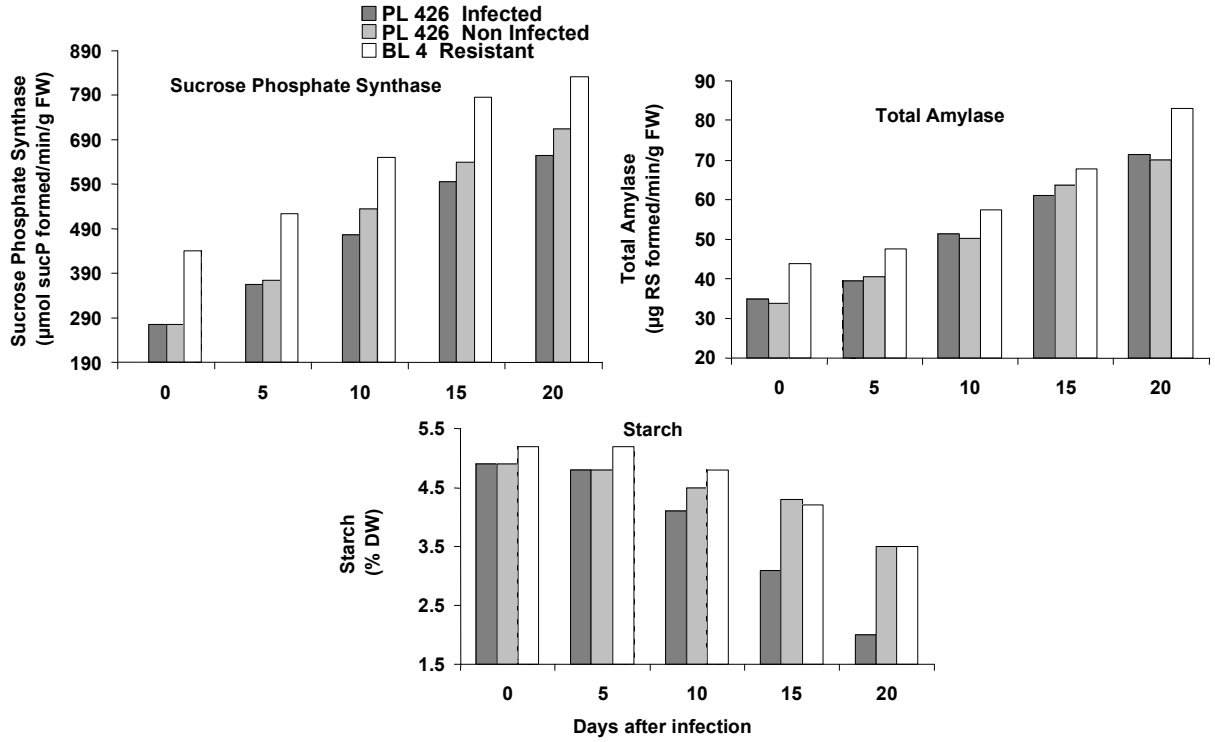

Fig. 2: Activities of sucrose phosphate synthase, total amylase and levels of starch at different days after infection in leaves of leaf blight susceptible (PL 426) and leaf blight resistant (BL 4) genotypes of barley raised in field. sucP, sucrose phosphate; RS, reducing sugars

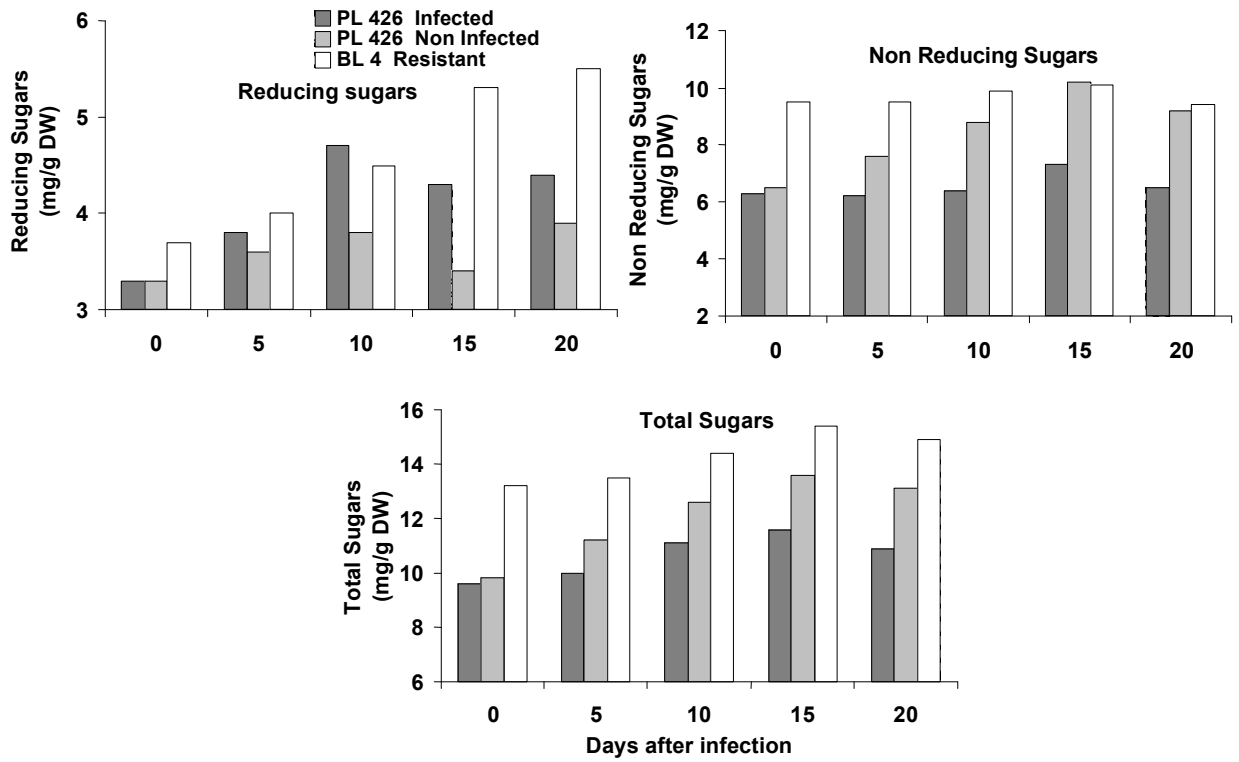

Fig. 3: Levels of reducing-, non reducing- and total sugars at different days after infection in leaves of leaf blight susceptible (PL 426) and leaf blight resistant (BL 4) genotypes of barley

SPS in leaves. Activities of both SS and SPS were increased in the non infected leaves during development while leaf blight led to reduction in these enzyme activities. It was observed that SPS activity in leaves of barley gradually declined during brown rust infection to 32% of uninfected controls. Reduction in sucrose synthesis which otherwise gets translocated helps in retention of more glucose and fructose in infected leaves for its onward utilization by the growing fungus [19]. A blockage of intercellular sugar transportation in response to pathogen infection has also been reported by Scharte *et al*. [20]. Thus fungus promotes its own growth via lowering the activity of both these sucrose synthesizing enzymes. High SS and SPS activities of resistant genotype suggest that more sucrose is synthesized for translocation as well as for providing substrate for invertase. Translocation and storage of sucrose (as in vacuole) will cause food scarcity to fungus while hexoses resulted from invertase action may induce defense reactions, thus imparting resistance to genotype. No clear cut differences were observed for the activity of total amylase in the infected and non infected leaves of leaf blight susceptible genotype (Fig. 2).

A gradual increase in the levels of total free sugars till 15 DAI was observed in both genotypes and thereafter sugar level declined which may be correlated to the utilization of sugars from leaves. Leaves infected with leaf blight showed lower contents of endogenous total- and non-reducing sugars but higher reducing sugars (consistent with decline of SPS and induction of invertase activities) as compared to healthy leaves (Fig. 3). The reduction level of soluble sugars in the leaves with infestation of *Bipolaris sorokiniana* would mean that fungus acts as an additional sink and utilized these carbohydrates for its own metabolic processes. Similarly, the reduction of sugar contents upon infection of tomato plants with *Botrytis cinerea* [21] and of *Helianthus annuus* plant with *Sclerotinia sclerotiorum* [17] has also been reported. Likewise, the lowest level of starch in infected leaves (Fig. 2) suggests that the starch was transitory reserve which probably gets hydrolysed into simple sugars to meet the energy requirements of fungus. Lower levels of total sugars, non-reducing sugars and starch from fungal infected leaves compared with those of healthy leaves have been reported in various other crop species [10, 22].

As leaf blight disease is elevated by high temperature, correlation studies were carried out between disease severity and air temperature in fields. These studies indicate significant correlation ($p<0.05$) between disease severity and temperature in case of acid invertase (r= 0.97086), neutral invertase (r=0.96651), SPS (r=0.99001), total amylase (r=0.97238) and total sugar (r=0.91423) contents, indicating that these parameters are influenced by both leaf blight and temperature. However no such correlation exist in SS, reducing sugars, non reducing sugars and starch contents, indicating thereby that these parameters are influenced only by leaf blight.

In conclusion, this study showed that fungal infections represent a cost to the plant in terms of carbon production and utilization. Lower yields of infected host plants may be due to reduced photosynthesis and alteration in the fluxes of carbon within the infected leaves. As invertase activities respond to leaf blight and might be involved in defense mechanism against fungal infection, modulation of these activities can confer resistance against leaf blight.

REFERENCES

1. Dubin, H.J. and V. Ginkel, 1991. The Status of Wheat Diseases in Warm Areas of South Asia. In: Wheat in Heat Stressed Environments: Irrigated Dry Areas and Rice-Wheat Farming Systems Saunder, D.A. and G.P. Hettel (Eds), Maxico DF, Maxico: CIMMYT., pp: 353-359.
2. Saari, E.E., 1998. Leaf blight disease and associated soil borne fungal pathogens of wheat in South and Southeast Asia. In: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Duveiller, E., H.J. Dubin, J. Reeves and A. McNab (Eds), Maxico D F, Maxico: CIMMYT., pp: 37-51.
3. Kumar, J., P. Schaffer, R. Huckelhoven, G. Langen, H. Baltruschat, E. Stein, S. Nagarajan and K.H. Kogel, 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: Cytological and molecular approaches towards better control. Mol. Plant Path., 3: 185-195.
4. Dubin, H.J. and S. Rajaram, 1996. Breeding disease-resistant wheats for tropical highlands and lowlands. Annu. Rev. Phyto. Path., 34: 503-526.
5. Nagarajan, S. and J. Kumar, 1998. Foliar blights of wheat in India: germplasm improvement and future challenges for sustainable, high yielding wheat production. In: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Duveiller, E., H. J. Dubin, J. Reeves and A. McNab (Eds.), Maxico D F, Maxico: CIMMYT. pp: 52-58.
6. Singh, R.V., A.K. Singh and S.P. Singh, 1998. Distribution of Pathogens Causing Foliar Blights of Wheat in India and Neighboring Countries. In: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Duveiller, E., H. J. Dubin, J. Reeves and A. McNab (Eds.), Maxico DF, Maxico: CIMMYT. pp: 59-62.
7. Vidhyasekaran, P., 1974. Finger millet helminthosporiose, a low sugar disease. Zeit.f.Pflanzenkrankheiten u. Pflanzenschutz 81: 28.
8. Vidhyasekaran, P. and D. Kandasamy, 1972. Carbohydrate metabolism of phaseolus aureus infected with obligate and facultative parasites. Indian Phytopathology, 25: 48.
9. Lukens, R.J., 1970. Melting out of Kentucky bluegrass, a low sugar disease. Phytopathology, 60: 1276-1280.
10. Kumar, B.R.P., M.J. Kulkarni, B.N.V. Rao, K. Chandrika, V.R.B. Gowda and T. Prasad, 2000. Biochemical and histochemical changes associated with Downy Mildew (*Plasmopara halstedii* [Farl] Berl and de Toni) infection in sunflower (*Helianthus annuus* L) Helia 23: Nr 33 pp: 1-18.
11. Singh, R. and B. Asthir, 1988. Import of sucrose and its transformation to starch in the developing sorghum caryopsis. Physiol. Plant, 74: 58-65.
12. Asthir, B. and R. Singh, 1995. Flouride-induced changes in the activities of sucrose metabolizing enzymes in relation to starch accumulation in sorghum caryopsis, raised through liquid culture. Plant Physiol. Biochem., 33: 219-223.
13. Stommel, J.R., 1992. Enzymic components of sucrose accumulation in the wild tomato species *Lycopersicon peruvianum*. Plant Physiol., 99: 324-328.
14. Morell, M. and L. Copeland, 1985. Sucrose synthase of soybean nodules. Plant Physiol., 78: 149-154.
15. Fotopoulos, V., M.J. Gilbert, J.K. Pittman, A.C. Marvier, A.J. Buchanan, N. Sauer, J.L. Hall and L.E. Williams, 2003. The monosaccharide transporter gene, AtSTP4 and the cell-wall invertase, At beta fruct1, are induced in Arabidopsis during infection with the fungal biotroph *Erysiphe cichoracearum*. Plant Physiol., 132: 821-829.
16. Sutton, P.N., M.J. Gilbert, M.J. Williams and J.L. Hall, 2007. Powdery mildew infection of wheat leaves changes host solute transport and invertase activity. Physiol. Plant, 129: 787-795.

17. Berger, S., M. Papadopoulos, U. Schreiber, W. Kaiser and T. Roitsch, 2004. Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiol. Plant, 122: 419-428.
18. Roitsch, T., M.E. Balibrea, M. Hofmann, R. Proels and A.K. Sinha, 2003. Extracellular invertase: Key metabolic enzyme and PR protein. J. Exp. Bot., 54: 513-524.
19. Tetlow, I.J. and J.F. Farrar, 1992. Sucrose-metabolizing enzymes from leaves of barley infected with brown rust (*Puccinia hordei Otth*.) New Phytologist, 120: 475-480.
20. Scharte, J., H. Schon and E. Weis, 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. Plant, Cell & Environ., 28: 1421-1435.
21. Jobic, C., A. Boisson, E. Gout, C. Rascle, M. Fevre, P. Cotton and R. Bligny, 2007. Metabolic processes and carbon nutrient exchanges between host and pathogen sustain the disease development during sunflower infection by *Sclerotinia sclerotiorum*. Planta, 226: 251-265.
22. Ghosh, L., M.S. Alam, M.R. Ali, A.M. Shohael, F. Alam and R. Islam, 2003. Changes in some biochemical parameters of Mulberry (Morus sp.) leaves after infected with leaf spot disease. J. Biol. Sci., 5: 508-514.