BIOLOGIA PLANTARUM 41 (1): 127-132, 1998

BRIEF COMMUNICATION

Changes in saccharide metabolism induced by infection of *Camellia sinensis* by *Exobasidium vexans*

P.K. PIUS*, K.V. KRISHNAMURTHY and R. NELSON

Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620024, India

Abstract

Changes in saccharide contents of tea leaves during infection with blister blight fungus *Exobasidium vexans* Masse was studied. Saccharose and glucose contents decreased in the blistered portions when compared to the normal regions until sporulation and remained constant during the entire period of sporulation. Fructose content increased abruptly during the initiation of sporulation and remained constant up to the end of sporulation in both blistered and non-blistered regions. Starch content continuously decreased in the blistered region. Peroxidase activity was highly enhanced during the final stages of leaf senescence. The activity of acid invertase was inversely proportional to the starch content and closely related to the changes in the saccharose and glucose contents. Protein and chlorophyll contents gradually decreased in the blistered regions.

Additional key words: blister blight, chlorophyll, fructose, glucose, host-parasite interaction, invertase, leaf senescence, peroxidase, saccharose, starch.

Starch, glucose, and saccharose accumulate around the site of infection until the end of the latent period (Owera *et al.* 1983, Kiessling and Hoffmann 1985). Direct assimilation of glucose was postulated by McKay and Maclean (1992). Grabo (1993) discussed the uptake of saccharose, either as a disaccharide or hydrolysed at the host-parasite interface. Saccharose and starch play an important role in satisfying the saccharide requirements of leaf and leaf-borne pathogens (Whipps and Lewis 1981, Manners 1989). As a result, the saccharose content decreases due to action of enzymes such as invertase (Mitchell *et al.* 1978, Greenland and Lewis 1983). Starch accumulation during sporulation has been demonstrated histochemically (Sziraki

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Received 15 August 1997, *accepted* 22 December 1997.
* Author for correspondence: e-mail: plant@info.bdu.ernet.in

et al. 1984). Starch is degraded in the vicinity of sporulating mycelium, while an accumulating ring surrounds the infected region (Inman 1962, Scholes and Farrer 1987).

Our experiments determine the changes in saccharide metabolism during different infection stages of tea plant by the fungal pathogen *Exobasidium vexans* Masse that causes blister blight disease (Agnihothrudu 1995). Studies on invertase activity, as well as on chlorophyll (Chl) and protein contents were carried out to assess the senescence of the leaves at different developmental stages of the fungus.

Camellia sinensis O. Kuntz clone UPASI-3 (B/5/63) of tea was grown for three years in a field at an altitude of 3000 m. The lower surface of the second leaf was inoculated with a suspension of basidiospores [approx. 5 mg in 10 cm^3 water with 0.01 % *Tween 80*, (v/v) to ensure uniform infection density]. Control plants were treated with a water/*Tween* solution. The plants were kept in pots with sand - soil mixture (3:1) in a well humidified chamber (air humidity 70 %, temperature 18 °C, irradiance 210 μmol $m^{-2}s^{-1}$, 16-h photoperiod) for further observations. The infected leaves were macro- and microscopically examined 1 h prior to collection for analysis. Ten 2-mm^2 leaf segments with one blister each were selected by punching out with a cork borer and sorted out by separate developmental stages. During the translucent spot stage (7 - 8 d after infection (d.p.i.) 2 samplings (F_1 and F_2) and at the outbreak of basidia (8 - 10 d.p.i.) the sampling B_1 were taken. B_2 characterised sporulating blister on both surfaces, whilst high sporulation took place at B_3 and B_4. Samples taken after the onset of basidiospore production (11 - 28 d.p.i.) were named N_1 to N_{10} according to the amount of basidiospores within the blister. The leaf discs were washed in distilled water and dried for determination of the dry mass.

For saccharide analysis, infected and healthy second leaves were detached 10 h after the beginning of the light period and immediately cooled to 4 °C. In infected leaves, segments without blister were cut out. The leaves were frozen to -30 °C, ground with pestle and mortar, and used for further analyses. All samplings ($n = 6$) were taken twice, each of 2 independent experiments.

Invertase activity was determined spectrophotometrically at 460 nm according to Bacon *et al.* (1965) and Long *et al.* (1975). For the extraction of ethanol-soluble and insoluble saccharides (mainly starch), a Soxhlet apparatus filled with 200 cm^3 ethanol (80 %) and 1 g (d.m.) leaf material was used. The ethanol fraction was retained for the enzymatic detection of glucose (Higgett and Nixon 1957, Bergmeyer 1988), fructose (Kakac and Vejdelek 1974), and saccharose (Van Handel 1968). Insoluble saccharides such as starch were hydrolysed in H_2SO_4 (75 %) and centrifuged. The resulting glucose in the supertanatant was estimated using the anthrone reagent as described by Herbet *et al.* (1971). Further on, plant material (250 mg) homogenised in a mortar and pestle with 5 cm^3 chilled phosphate buffer (0.1 M, pH 7.7) was centrifuged (10 min, 2000 *g*, 4 °C). The crude extract was used to measure the peroxidase activity (Braber 1980) and, following acetone precipitation, for the determination of proteins as described by Lowry *et al.* (1951). Chlorophyll (Chl) content was determined as described by Lichtenthaler (1987) using the above mentioned supernatant. For statistical evaluation (significance of differences between

comparable healthy and infected tissues), an *ANOVA* was performed using *Microsoft Excel*.

Saccharose (Fig. 1*A*) and glucose (Fig. 1*B*) contents differed between infected leaf segments and the control depending on the fungal development. Until the beginning of sporulation (B_1), the infected leaf segments had a higher content of the both saccharides than the control. As sporulation increased, the saccharose and glucose contents decreased in infected leaves to a level below that of the controls, and then remained nearly constant throughout the sporulating period. At the end of sampling period the content of both saccharides was nearly 50 % of that of the control. By contrast the fructose content (Fig. 1*C*) showed no statistically significant difference ($P \leq 0.05$) between infected and non-infected leaves. It increased abruptly in both infected and control leaves, concurrent with pronounced sporulation. This increased concentration remained nearly constant up to the end of sporulation (N_{10}) and until wilting of the non-infected plant material, respectively. At the end of the course of infection (N_7 to N_{10}), its level was slightly below that of the control.

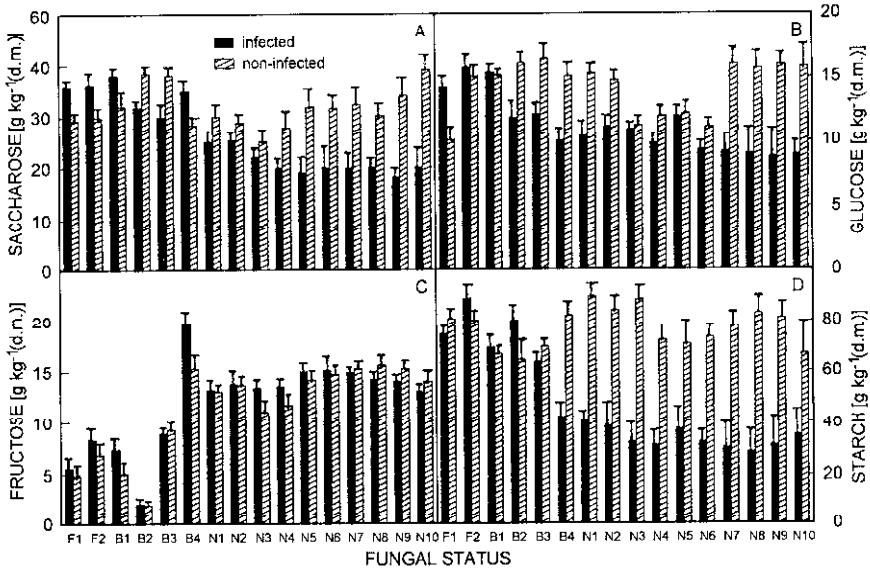

Fig. 1. Contents of saccharose (*A*), glucose (*B*), fructose (*C*), and starch (*D*) [g kg^{-1}(d.m.)] in infected and non-infected leaves at various development stages.

From the end of the incubation period until the onset of sporulation, infected leaves had a higher starch content than the control (Fig. 1*D*). During intensive basidiospore production (B_3), there was a sharp decrease in starch content in the infected leaf segments until the onset of necrosis. From N_1 onwards, the starch content was less than 50 % of that of the non-infected control.

The acid invertase activity was inversely proportional to the starch content and closely related to the changes in saccharose and glucose contents. The significantly

enhanced enzyme activity began with the onset of sporulation and reached a maximum at the stage of maximum basidiospore release, when it was nearly 10 times higher than in the control. At N_3, the senescence of the leaves commenced, as shown by the enhanced peroxidase activity. Invertase activity was much lower in non-infected leaves.

With the onset of necrosis (N_1), the Chl and protein contents decreased in blistered leaves as well as in the non-infected controls (Table 1). The protein content of the controls (Table 1) was, however, lower than that of the blistered segments. Leaf tissues between the blisters had the highest protein content at F_1. After high sporulation (B_4), it was reduced, and stagnated until the end. As the necrosis advanced (N_5), the infected leaf, as compared with the control, showed progressive senescence. This progress was, additionally, demonstrated by the decrease in Chl content (Table 1).

Table 1. Concentrations of chlorophyll (Chl) and soluble protein in blister-infected leaf segments between the blister without mycelium as compared to the non-infected control leaves (means of 5 replicates ± S.E.).

Developmental stage		Chl *a* [g kg^{-1}(dm)]	Chl *b* [g kg^{-1}(d.m.)]	Chl *a+b* [g kg^{-1}(d.m.)]	Protein [g kg^{-1}(d.m.)]
F_1	control	6.82 ± 0.03	2.25 ± 0.04	9.40 ± 0.02	164 ± 14
	infected leaf	7.04 ± 0.02	2.51 ± 0.01	9.49 ± 0.01	213 ± 19
B_4	control	6.40 ± 0.03	2.09 ± 0.02	8.45 ± 0.01	159 ± 21
	infected leaf	7.11 ± 0.01	2.31 ± 0.01	9.42 ± 0.01	132 ± 8
N_1	control	4.20 ± 0.03	2.70 ± 0.20	6.90 ± 0.18	109 ± 12
	infected leaf	3.88 ± 0.11	2.79 ± 0.04	6.47 ± 0.15	151 ± 6
N_7	control	3.48 ± 0.12	1.24 ± 0.08	4.98 ± 0.37	87 ± 3
	infected leaf	3.51 ± 0.27	1.26 ± 0.10	4.86 ± 0.45	71 ± 6

Consumption of saccharides during fungal sporulation is high and thus a deficit of saccharides in plant tissues is usually observed (Schipper and Mirocha 1969). Long *et al.* (1975) observed an increased saccharide content in infected areas as a result of changes in the starch content (Scholes and Farrar 1987 Wagner and Boyle 1995). This implies a strong relationship between the onset of fungal growth and sporulation and mobilisation of saccharides in host tissues. The parasite incorporates these saccharides in its growing mycelium. Additionally, photosynthetic activity may be reduced (Scholes *et al.* 1994).

These observations can be verified by determining saccharose and glucose contents, linked to acid-invertase activity (Fig. 1). According to Inman (1962) as well as to our results, initial infection results in an enhanced glucose concentration. However, the onset of basidiospore release causes a decline of glucose concentration below that of the control as a result of the fact that *ca.* 6.4×10^6 basidiospores can be produced per day (Huysmans 1952) which represent an enormous consumption of metabolites and energy.

McKay and Maclean (1992) have reported glucose as the main metabolite transported between host and pathogen. Reduction in the invertase activity of the host might result in a decrease in production of glucose, thus causing a reduction in the further metabolic activities of the fungal cells. Although the localisation of invertase is not yet clear (Storr and Hall 1992, Scholes *et al.* 1994), its increasing activity during sporulation may be due to a fungal enzyme, as Xiu *et al.* (1993) have proposed.

We think that the decrease in glucose is not a direct effect of the transfer and efflux of saccharides during sporulation and at the onset of necrosis (N_1), because no further sharp decrease in glucose concentration was detectable. Fructose accumulates, as it is not transported between host and fungus, while the content of substrate, *i.e.* saccharose, decreases.

As only 2 basidiospores are produced per basidium, the amount of spores delivered per blister is generally reduced. This permits the maintenance of reduced basidiospore production over a long period. Slow sporulation seems to prevent a premature exhaustion and necrosis of the infected leaf, including an early death of the fungus, which ensures the most effective utilisation of resources.

According to Miidla *et al.* (1987), the contents of Chl and proteins decreased in both control and infected leaves as the end of sporulation is in close connection with necrosis. The differences in saccharide metabolism as compared with the control and exhaustion of saccharides can be the primary indicator for the onset of necrosis.

References

Agnihothrudu, V.: Some more important fungal disease of tea. - Indian Phytopathol. **48**: 229-253, 1995.

Bacon, J., Macdonald, T.R., Knight, A.H.: The development of invertase activity of root of *Beta vulgaris* washed under aseptic conditions. - Biochem. J. **94**: 175-182, 1965.

Bergmeyer, H.U.: Methods of Enzymatic Analysis. - VCH, Weinheim 1988.

Braber, J.M.: Catalase and peroxidase in primary bean leaves during development and senescence. - Z. Pflanzenphysiol. **97**: 135-144, 1980.

Daly, J.M., Inman, R.E., Livne, A.: Carbohydrate metabolism in higher plant tissue infected with obligate parasites. - Plant Physiol. **37**: 531-538, 1962.

Friedrich, S., Boyle, C.: Wirkung unterschiedlicher Luftfeuchten auf die Produktion und Keimung der Konidien von *Erysiphe graminis* f. sp. *tritici in vitro*. - Z. Pflanzenkr. Pflanzensch. **100**: 180-188, 1993.

Grabo, P.: Physiologische Untersuchungen zum Verständnis der Wirt-Parasit-Beziehung des Pathosystems Erbse (*Pisum sativum*) Achter Mehltau (*Erysiphe pisi*). - Ph.D. Thesis. Universität Braunschweig, Braunschweig 1993.

Greenland, A.J., Lewis, D.H.: Changes in the activity and predominant molecular forms of acid invertase in oat leaves infected by crow rust. - Physiol. Plant Pathol. **22**: 293-312, 1983.

Herbet, D., Phipps, P.J., Strange, R.E.: Chemical analysis of microbial cells. - In: Norris, J.R., Ribbons, D.W. (ed.): Methods in Microbiology. Pp. 209-272. Academic Press, London 1971.

Higgett, A.G., Nixon, D.A.: Enzymatic determination of blood glucose. - Biochem. J. **66**: 120, 1957.

Huysmans, C.P.: [Studies on the blister blight (*Exobasidium vexans*) in tea of Sumatra.] - Bergculture **21**: 419-464, 1952. [In Dutch.]

Inman, R.E.: Disease development, disease intensity and carbohydrate levels in rusted bean plants.- Phytopathology **52**: 1207-1211, 1962.

Kakac, B., Vejdelek, Z.: Handbuch der photometrischen Analyse organischer Verbindungen. Band 2. - VCH, Weinheim 1974.

Kiessling, U., Hoffmann, G.M.: Physiologische Effekte bei Interaktionen zwischen Echtem Mehltau (*Erysiphe graminis* f. sp. *hordei*) und Zwergrost (*Puccinia hordei*) an Gerste. - Z. Pflanzenkr. Pflanzensch. **92**: 407 - 416, 1985.

Lichtenthaler, H.K.: Chlorophylls and carotenoids pigments of photosynthetic biomembranes. - Methods Enzymol. **148**: 350-400, 1987.

Long, D.E., Fung, A.K., McGee, E., Cook, R.C., Lewis, D.H.: The activity of invertase and its revelance to the accumulation of storage polysaccharides in leaves by biotrophic fungi. - New Phytol. **74**: 173-182, 1975.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Manners, J.M.: The host-haustorium interface in powdery mildews. - Aust. J. Plant Physiol. **16**: 45-52, 1989.

McKay, D.B., Maclean, D.J.: Effect of glucose and glucitol analogues on respiration by mycelia of *Puccinia graminis*. - Exp. Mycol. **16**: 110-118, 1992.

Miidla, H., Padu, E., Kolok, U., Soosaar, A.: Biochemical changes in primary wheat leaves during growth and senescence. - Biol. Plant. **29**: 445-452, 1987.

Mitchell, D.T., Fung, A.K., Lewis, D.H.: Changes in the ethanol-soluble carbohydrate composition and acid invertase activity in infected first leaf tissues of susceptable crow rust of oat and wheat stem rust. - New Phytol. **80**: 381-392, 1978.

Owera, S.A.P.P., Farrer, J.F., Whitebread, R.: Translocation from leaves infected with brown rust. - New Phytol. **94**: 111-123, 1983.

Schipper, A.L., Mirocha, C.J.: The histochemistry of starch depletion and accumulation in bean leaves at rust infection sites. - Phytopathology **59**: 1416-1422, 1969.

Scholes, J.D., Farrar, D.H.: Development of symptoms of brown rust of barely in relation to the distribution of fungal mycelium, starch accumulation and localized changes in the concentration of chlorophyll. - New Phytol. **107**: 103-117, 1987.

Scholes, J.D., Lee, P.J., Horton, P., Lewis, D.H.: Invertase: understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildews. - New Phytol. **126**: 213-222, 1994.

Storr, T., Hall, J.L.: The effect of infection by *Erysiphe pisi* DC on acid and alkali invertase activities and aspects of starch biochemistry in leaves of *Pisum sativum* L. - New Phytol. **121**: 535-543, 1992.

Sziraki, I., Mustary, L.A., Faludi-Daniel, A., Kiraly, Z.: Alternatives in chloroplast ultrastructure and chlorophyll content in rust infected Pinto beans at different stages of disease development.- Phytopathology **74**: 77 - 84, 1984.

Van Handel, E.: Direct microdetermination of saccharose. - Anal. Biochem. **22**: 280-283, 1968.

Wagner, S., Boyle, C.: A method for simultaneous vital staining in obligate biotrophic interactions. - Z. Pflanzenkrankh. Pflanzensch. **102**: 184-190, 1995.

Whipps, J. M., Lewis, D.H.: Patterns of translocation, storage and interconversion of carbohydrates. - In: Ayres, P.G. (ed.): Effects of Disease on the Physiology of the Growing Plant. Pp. 47-84. Cambridge University Press, Cambridge 1981.

Xiu, W.T., Rolfe, S.A., Scholes, J.D.: Changes in invertase activity and isoform pattern in leaves of *Arabidopsis thaliana* infected with *Albugo candida*. - Abstracts. 6th International Congress of Plant Pathology. P. 242. Montreal 1993.