Arab Gulf J. scient. Res. **3** (2), pp. 461-469 (1985)

Comparison of Carbohydrate Content, Invertase and α-Amylase Activities in Leaves of *Medicago sativa* L. and *Tephrosia appolinea* (Del.) Link Infected by *Uromyces striatus*

M.O. Basalah, A.A.A. Suleiman and Sher Mohammad

Department of Botany, College of Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT. Healthy and diseased leaves of *Medicago sativa* L. contained less reducing sugars, sucrose, polysaccharides and total carbohydrates than those of *Tephrosia appolinea* (Del.) Link. After infection by *Uromyces striatus*, total carbohydrates increased in both *Medicago sativa* and *Tephrosia appolinea* leaves and reducing sugars were unchanged. Sucrose content decreased in *T. appolinea* and increased in *M. sativa*. The activity of invertase was found to be greater than that of α-amylase in both plant leaves. Invertase activity increased and α-amylase activity decreased after rust infection in leaves of both plants. Increased invertase activity and decreased sucrose content on infection suggest the occurrence of either phloem unloading or decreased export of photosynthetic products.

The influence of infection of green plants with obligate parasites on their carbohydrate content has been a matter of controversy. Some workers reported an increase of carbohydrate content with infection (Williams and Pound 1964, Holligan *et al.* 1973, Long and Cooke 1974, Long *et al.* 1975). Other workers reported a decrease in sugar content of infected leaves (Inman 1962, Daly 1967).

Studies on enzyme activities after infection showed that generally activites of enzymes were correlated with the concentration of their respective substrates (*cf.* hexokinase in leaves of wheat infected with rust; Lunderstad 1966). Long *et al.* (1975) reported an increase of reducing sugars accompanied by increase in invertase activity in rust infected *Seneico, Tussilago* and *Poa* species. Artificial wounding of plant tissues has also resulted in an increase in invertase activity of some plants like sweet potatoes (Matsushita and Uritani 1974).

In the present study, *M. sativa* L. which is cultivated and *T. appolinea* (Del.) Link which is wild-growing were chosen as experimental plants. Both belong to

the Leguminosae (Fabaceae) and *M. sativa* is of economic importance and used as animal fodder. Healthy and diseased leaves of both species infected by the rust fungus, *Uromyces striatus*, were analysed for levels of carbohydrates, invertase and α-amylase enzymes. The aim was to compare the two plants with regard to enzyme activities and carbohydrate content before and after rust infection.

Material and Methods

Plant material

The plants used for experimentation were cultivated plants of *M. sativa* L. and wild-growing plants of *T. appolinea* (Del.) Link (Leguminosae) collected from the Usfan Valley near Jeddah, Saudi Arabia in May, 1982. Cultivated and wild plants were at the same stage of growth when sampled. The plants were subject to attack by a rust fungus, identified, according to Laundon and Waterson (1965), as *Uromyces striatus*. The age of the plants was not known, but infected areas covered more than 80% of leaf area.

Sampling and extraction procedure for carbohydrates

Two gram fresh weight samples of green leaves from healthy and infected leaves of *M. sativa* and *T. appolinea* were used (3 replicates).

Extraction of soluble carbohydrates was carried out under reflux with three changes of hot 80% ethanol followed by evaporation of the alcohol and dissolution of the ethanol extract in hot water. The residue left was hydrolysed with 1.5 *N* H_2SO_4 for 6 hr under reflux, neutralised with 1.5 *N* NaOH, evaporated to dryness *in vacuo* and dissolved in hot water. Reducing sugars in the ethanol soluble fraction were estimated before and after hydrolysis with invertase by the method of Somogyi (1945), using a calibration curve of glucose. Sucrose content was calculated as the difference between the two estimations. Polysaccharides were estimated as reducing sugars measured by the method of Somogyi (1945) after acid hydrolysis.

Extraction and identification of enzymes

Invertase

The method employed was essentially that of Bacon *et al.* (1965). Leaf samples were crushed with ice-cold ethyl acetate to rupture cell membranes and then washed with ice-cold water. This removed most endogenous substrate and permitted exposure of exogenous substrate to the total cell invertase (Vieweg 1974). After blotting, each sample was transferred to a medium comprising 2 ml 0.5 *M* sucrose, 2 ml 0.1 *M* citrate buffer, pH 4.5, and 6 ml distilled water. Flasks were sealed with 'parafilm' and incubated in a shaking water bath at 35°C. Aliquots (1 ml) were removed after 90 min. Reducing sugars were assayed by the method of Somogyi

(1945) using a calibration curve of glucose. Invertase activity was expressed as mg glucose equivalents (gram fresh weight)$^{-1}$(hr)$^{-1}$.

α-Amylase

The method employed was essentially that of Katsumi and Fukuhara (1969). Leaves were homogenised with 15 ml of $CaCl_2$ (0.03%) and centrifuged for ten min at 4000 rpm. The supernatant was collected and 6 ml of 1.5 *M* acetate buffer, pH 5.6, were added together with 15 ml of ethanol. The solution was left for half an hour in a refrigerator to precipitate. The precipitate was collected by centrifugation at 1000 rpm for 30 min at 10°C. The precipitate was homogenised and dissolved in 3 ml of acetate buffer and used as the enzyme solution. One ml was taken in a test tube, mixed with one ml of acetate buffer and shaken at 40°C. After ten minutes, one ml solution of substrate was added and shaken for 30 min at 35°C. To stop the reaction, 10 ml of 0.5 *N* acetic acid was added. Reducing sugars were estimated by the method of Somogyi (1945) and enzyme activity was expressed as mg reducing sugar (gram fresh weight)$^{-1}$(hr)$^{-1}$. The substrate was prepared by

Table 1. Carbohydrate content of healthy leaves of *Medicago sativa* L. and *Tephrosia appolinea* (Del.) Link and leaves infected with *Uromyces striatus*. Values are means of triplicates ± SD*.

Plant and condition of leaves	Carbohydrate content calculated as mg glucose (gram fresh weight of leaves)$^{-1}$				
	Reducing sugars (1)	Reducing sugars after invertase hydrolysis (2)	Sucrose content 2 − 1 = (3)	Hydrolysed polysaccharides (4)	Total carbohydrates 2 +4 = (5)
Healthy *M. sativa*	5.4 ± 1.7	8.9 ± 1.1	3.5 ± 1.0	35.3 ± 1.7	44.2
Diseased *M. sativa*	5.4 ± 1.0	9.4 ± 1.6	4.0 ± 1.1	38.1 ± 1.2	47.5
Healthy *T. appolinea*	8.7 ± 1.1	17.7 ± 2.1	9.0 ± 1.6	37.8 ± 1.6	55.5
Diseased *T. appolinea*	9.6 ± 1.2	17.9 ± 1.5	8.3 ± 1.6	39.1 ± 1.3	57.0

* Standard deviation from the means.

dissolving 200 mg amylose in 4 ml of 1 *N* NaOH and kept in a refrigerator overnight to dissolve completely. Then, the solution was diluted with 80 ml distilled water, adjusted to pH 7.5 with 1 *N* acetic acid and made to a final volume of 100 ml with water.

Results

Healthy and diseased leaves of *T. appolinea* contained more reducing sugars and more sucrose than did leaves of *M. sativa*. After infection, sucrose content decreased in *T. appolinea* and increased in *M. sativa* (Table 1). There was no significant difference in polysaccharide content of the two types of leaves. Leaves of *T. appolinea* had a higher content of total carbohydrates. There was a slightly higher polysaccharide content in diseased leaves of both species (Table 1).

Table 2 shows activities of invertase and α-amylase in healthy and infected leaves of *M. sativa* and *T. appolinea*. It can be seen that invertase activities were higher in both plants compared to α-amylase activities. With rust infection, invertase activity increased and α-amylase activity decreased in both plants.

Table 2. Invertase and α-amylase activities in healthy leaves of *Medicago sativa* L. and *Tephrosia appolinea* (Del.) Link and leaves infected with *Uromyces striatus*. Values are means of triplicates ± SD*.

Plant and condition of values	**Enzyme activity as mg reducing sugar (gram fresh weight)$^{-1}$ (hr)$^{-1}$**	
	Invertase	**α-amylase**
Healthy *M. sativa*	86.8 ± 1.2	18.3 ± 1.0
Diseased *M. sativa*	109 ± 1.5	13.4 ± 0.8
Healthy *T. appolinea*	171.9 ± 2.0	18.3 ± 1.0
Diseased *T. appolinea*	180.3 ± 1.7	11.1 ± 0.5

* Standard deviation from the means.

Discussion

Lewis (1976) suggested that pustules of rust fungi act as foci for the accumulation of many metabolites. Spore production results in rupture of host epidermis and increases water loss so that nutrients were made available by enhanced transpiration. Wolswinkel and Ammerlaan (1983) measured high values for sucrose hydrolysis in the upper part of *Vicia faba* stems, the release of sucrose and hexoses were strongly influenced by free space invertase activity.

After infection, in *M. sativa* sucrose content slightly increased and decreased in *T. appolinea*. Reducing sugars were unchanged in *M. sativa* and slightly increased with infection in *I. appolinea*. Polysaccharides slightly increased in both types of leaves with infection. Starch synthesis in host cells is also thought to be enhanced by fungal sequestration of orthophosphate (McDonald and Strobel 1970, Bennett and Scott 1971). Low levels of orthophosphate in host cells would discourage phosphorolysis of starch (Chen-She *et al.* 1975). Gerwitz and Durbin (1960) found a slight increase in total carbohydrates with no change in reducing sugars at 20°C in stems of wheat after rust infection, but at 30°C total carbohydrates and reducing sugars increased markedly. Similarly, Holligan *et al.* (1973) noted an increase in the *Puccinia poarum* components, mannitol, arabitol and mannose containing polymers when infecting leaves of *Tussilago farfara* in which fructan had increased due to infection. Holligan *et al.* (1974) found that infection of leaves of *Tussilago farfara* by *Puccinia poarum* rust resulted in decreased export of photosynthetic products and, if infection was severe, in increased import.

In the present study, high sucrose content was accompanied by increased invertase activity in *M. sativa* while the opposite was found with *T. appolinea*. It is also noted that reducing sugars and polysaccharides slightly accumulated in *T. appolinea* while sucrose content decreased due to rust infection. Reducing sugars were unchanged, sucrose and polysaccharides content increased slightly. This may be due to decreased export of photosynthetic products, or to phloem unloading. Similarly, Long *et al.* (1975) reported that infection of *Senecio squalidus* by *Albugo tragapogonis* and *Tussilago farfara* and *Poa pratensis* by *Puccinia poarum* resulted in activity of an acid invertase at the sites of infection. They concluded that sucrose from the host is first hydrolysed and then absorbed by the fungus. They also suggested that invertase plays a key role in the provision of substrate for the typical accumulation of starch around pustules of biotrophic fungi in host species which store this polysaccharide.

Lunderstad (1966) reported increased invertase, glucohexokinase and fructohexokinase activities which were found to be correlated with their substrate levels in primary leaves of wheat infected by rust.

Hatch and Glasziou (1963) studied the role of invertase in the release of sucrose into sugar cane parenchyma cells which involved invertase activity. Basalah

(1984) postulated that invertase activity increased in leaves of *Solanum melongena* L. attacked by the fungus *Alternaria tenuissima*. This is supported by Long and Cooke (1974) and Whipps and Lewis (1981), who stated that the amount of sucrose varied in *Senecio squalidus* L. leaves attacked by *Albugo tragapogonis* (Pers.) S.F. Gray and other infected leaves were influenced by invertase activity.

Finally, the localization of invertase still needs some clarification. There is much evidence that the enzyme is present in cell walls of the fungus since the enzyme activity increased after rust infection in both *M. sativa* and *T. appolinea*.

References

Bacon, T.S.D., McDonald, I.R. and **Knight, A.H.** (1965) The development of invertase activity in slices of the root of *Beta vulgaris* L. washed under aseptic conditions, *Biochem. J.* **94**: 175-182.

Basalah, M.O. (1984) Effects of *Alternaria tenuissima* on the composition of carbohydrates in the leaves of *Solanum melongena* L., *Bot. Bull. Acad. sin.* **25**: 63-71.

Bennett, J. and **Scott, K.J.** (1971) Inorganic polyphosphate in the wheat stem rust fungus and in rust-infected wheat leaves, *Physiol. Pl. Path.* **1**: 185-198.

Chen-She, S.H., Lewis, D.H. and **Walker, D.A.** (1975) Stimulation of photosynthetic starch formation by sequestration of cytoplasmic orthophosphate, *New Phytol.* **74**: 383-392.

Daly, J.M. (1967) Some metabolic consequences of infection by obligate parasites. *In*: **Mirocha, C.J.** and **Uritani, I. (ed.)** *The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction*, Am. Phytophath. Soc., St. Paul, Minnesota: Bruce Publishing Co., p. 144.

Gerwitz, D.L. and **Durbin, R.D.** (1960) Some metabolic changes in wheat due to stem rust infection at different temperatures, *Phytopathology* **50**: 636 (ABs).

Hatch, M.D. and **Glasziou, K.T.** (1963) Sugar accumulation cycle in sugar cane. II.Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled environments. *Pl. Physiol., Lancaster* **38**: 344-348.

Holligan, P.M., Chen, C. and **Lewis, D.H.** (1973) Changes in carbohydrate composition of leaves of *Tussilago farfara* during infection by *Puccinia poarum, New Phytol.* **72**: 947-955.

Holligan, P.M., Chen, C., McGee, E.E.M. and **Lewis, D.H.** (1974) Carbohydrate metabolism in healthy and rusted leaves of coltsfoot, *New Phytol.* **73**: 881-888.

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

Katsumi, M. and **Fukuhara, M.** (1969) The activity of α-amylase in the shoot and its relation to gibberellin-induced elongation, *Physiologia Pl.* **22**: 68-75.

Laundon, G.F. and **Waterson, J.M.** (1965) *C.M.I. Description of pathogenic Fungi and Bacteria: Uromyces striatus*, Commonwealth Mycological Institute. Kew, Surrey, England, Set No. 6, No. 59.

Lewis, D.H. (1976) Interchange of metabolites in biotrophic symbiosis between angiosperms and fungi, *In*: **Sunderland, N. (ed.)** *Perspective in Experimental Biology*, Vol. 2, Pergamon Press, pp. 207-219.

Long, D.E. and **Cooke, R.C.** (1974) Carbohydrate composition and metabolism of *Senecio squalidus* L. leaves infected with *Albugo tragapogonis* (Pers.) S.F. Gray, *New Phytol.* **73**: 889-899.

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

Lunderstad, J. (1966) Effect of rust infection on hexokinase activity and carbohydrate assimilation in primary leaves of wheat, *Can. J. Bot.* **44**: 1345-1364.

McDonald, P.W. and **Strobel, G.A.** (1970) Adenosine diphosphate glucose pyrophosphorylase control of starch accumulation in rust in infected wheat leaves, *Pl. Physiol.* **46**: 126-135.

Matsushita, K. and **Uritani, I.** (1974) Change in invertase activity of sweet potato in response to wounding and purification and properties of its invertases, *Pl. Physiol.* **54**: 60-66.

Somogyi, M. (1945) A new reagent for the determination of sugars, *J. Biol. Chem.* **60**: 61-68.

Vieweg, G.H. (1974) Enzyme des Saccharosestoffwechsels in Wurzeln, *Planta* **116**: 347-359.

Whipps, J.M. and **Lewis, D.H.** (1981) Patterns of translocation, storage and interconvertion of carbohydrates, *In*: **Ayers, P.G. (ed.)** *Effects of Disease on the Physiology of Growing Plant*, Cambridge University Press, Cambridge, pp. 47-83.

Williams, P.H. and **Pound, G.S.** (1964) Metabolic studies on the host-parasite complex of *Albugo candida* on radish, *Phytopathology* **54**: 446-451.

Wolswinkel, P. and **Ammerlaan, A.** (1983) Sucrose and hexose release from excised stem segments of *Viccia faba*: The sucrose-specific stimulating influence of *Cuscuta* on sugar release and the activity of acid invertase. *J. exp. Bot.* **34**: 1516-1527.

(Received 28/05/1984;
in revised form 17/02/1985)

مقارنة للمحتوى الكربوهيدراتى ونشاط انزيمى الانفرتيز وألفا أميلاز فى اوراق نباتى مدكاجو ستايفا (البرسيم) وتفروزيا أبولينا المصابة بالفطر يروماييسس استرايتاس

محمد عمر باصلاح - عبدالعزيز عبدالله سليمان وشير محمد صديق

قسم النبات - كلية العلوم - جامعة الملك سعود - الرياض - المملكة العربية السعودية

احتوت الأوراق الصحيحة والمصابة بالفطر فى البرسيم على كميات أقل من السكريات المختزلة والسكروز والمركبات الكربوهيدراتية عديدة السكريات والسكريات الكلية مقارنة بالأوراق الصحيحة فى تفروزيا أبولينا. بعد الإصابة بالفطر ازداد محتوى السكريات الكلية فى أوراق كلا النباتين ولم يتغير محتوى السكريات المختزلة بينما نقص محتوى السكروز فى تفروزيا أبولينا وازداد فى البرسيم. وجد أن نشاط انزيم الانفرتيز كان اكبر من نشاط ألفا اميلاز فى أوراق كلا النباتين. ولوحظ ازدياد نشاط انزيم الانفرتيز وقلة نشاط انزيم الفا اميلاز بعد الإصابة بفطر الصدأ فى اوراق كلا النباتين. ان زيادة نشاط انزيم الانفرتيز المصحوبة بقلة كمية سكر السكروز عند الإصابة بالفطر يمكن تفسيره إما بعدم النقل فى اللحاء أو قلة إبعاد نواتج عملية البناء الضوئى.