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Research article

Peach leaf curl disease shifts sugar metabolism in severely infected leaves from source to sink

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ABSTRACT

Peach leaf curl is a disease that affects the leaves of peach trees, and in severe cases all of the leaf can be similarly affected. This study investigated some effects of this disease on the metabolism of peach leaves in which all parts of the leaf were infected. These diseased leaves contained very little chlorophyll and performed little or no photosynthesis. Compared to uninfected leaves, diseased leaves possessed higher contents of fructose and especially glucose, but lowered contents of sucrose, sorbitol and especially starch. The activities of soluble acid invertase, neutral invertase, sorbitol dehydrogenase and sucrose synthase were all higher in diseased leaves, whereas, those of aldose-6-phosphate reductase and sucrose phosphate synthase were lower. The activities of hexokinase and fructokinase were little changed. In addition, immunblots showed that the contents of Rubisco and ADP-glucose phosphorylase were reduced in diseased leaves, whereas, the content of phosphoenolpyruvate carboxylase was increased. The results show that certain aspects of the metabolism of diseased leaves are similar to immature sink leaves. That is photosynthetic function is reduced, the leaf imports rather than exports sugars, and the contents of non-structural carbohydrates and enzymes involved in their metabolism are similar to sink leaves. Further, the effects of peach leaf curl on the metabolism of peach leaves are comparable to the effects of some other diseases on the metabolism of photosynthetic organs of other plant species.

1. Introduction

Peach leaf curl (PLC), caused by the ascomycetous fungus *Taphrina deformans* (Berk.) Tul., is a disease of world-wide commercial importance that mainly affects leaves of peaches, nectarines, and sometimes other stone fruits, such as almonds and apricots (Frisullo et al., 2000; Cissé et al., 2013). Peach leaves suffering from PLC become curled and either pale green or reddish in colour, however, these changes are often restricted to only parts of an individual leaf (Caporali, 1964). These changes reflect alterations in the structure of the leaf and the palisade layer becomes indistinguishable from the spongy mesophyll (Matuyama and Misawa, 1961; Marte and Gargiulo, 1972). Modifications to the structure of the cells in infected parts of leaves occur and these can include: a thinning of the plant cell wall at its interface with the fungus, invaginations of both the plasmalemma and the tonoplast, an increase in the number of plasmodesmata and a reduction in the size of the chloroplasts which also possess fewer grana (Marte and Gargiulo, 1972; Bassi et al., 1984). Further, both the physiology and metabolism of infected leaves is altered. Thus transpiration is increased in infected leaves, whereas, photosynthesis appears to be almost absent (Raggi, 1987, 1995).

In plant tissues infected by many other pathogens, only parts of the tissue often show symptoms of the disease, and the metabolism of this part of the tissue is different from that in which the symptoms are not apparent (Técsi et al., 1994a,b; Chou et al., 2000; Berger et al., 2007). This means that localisation techniques (eg dissection, tissue printing, immunohistochemistry) are required to study the effect of the disease on the metabolism of the plant tissue (Técsi et al., 1994a,b). By contrast, peach leaves suffering from PLC

Abbreviations: AGP, ADP-glucose phosphorylase; A6PR, NADP-dependent aldose-6-phosphate reductase; PLC, peach leaf curl; PEPC, phosphoenolpyruvate carboxylase; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; SDH, sorbitol dehydrogenase; SPS, sucrose phosphate synthase; SUSY, sucrose synthase.

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are often quite homogenous with respect to infection: that is large parts of the leaf appear to be similarly affected (Matuyama and Misawa, 1961). Hence, in this respect peach leaves suffering from PLC are a useful tissue with which to study the effects of infection by a plant pathogen on metabolism. In common with a range of other plant diseases (Técsi et al., 1994a,b; Berger et al., 2007), marked changes in the metabolism of peach leaves occur during the development of PLC disease (Raggi, 1967). Therefore, in the present study we studied the stage of development of the disease in which the leaves had fully developed their curled shape and red colouration but no fungal reproductive structures were visible.

Previous studies of the effects of PLC on the metabolism of peach leaves have investigated the effects on photosynthesis, photorespiration, respiration, nitrogen metabolism (Raggi, 1967, 1994, 1995). However, little is known about how carbohydrate metabolism is affected. Therefore, in the present study this aspect was investigated. We showed that peach leaves suffering from PLC have many characteristics of sink leaves. Further, we showed that the effects of PLC disease on the metabolism of peach leaves has marked similarities to the effects of some other diseases on the metabolism of the photosynthetic organs of a number of other plant species.

2. Material and methods

2.1. Plant material

Mature fully expanded leaves (both healthy and diseased ones) were collected from 5 year old peach trees (cv Red Haven) growing in an experimental field of the Department of Agriculture, Food and Environmental Sciences of the University of Perugia, in Deruta (PG), central Italy. Leaf samples were collected at the end of May 2006. Those used for carbohydrate measurements were harvested either at 8.00 a.m. or 2.00 p.m., and those used for enzyme measurements just at 2.00 p.m. Leaf material was immediately frozen in liquid N_2 and stored at −80 °C until required.

2.2. Measurement of specific leaf dry weight

For both healthy and infected leaves the specific leaf dry weight (SLDW: i.e. dry weight per unit surface area of leaf) was determined as follows: 10 leaf discs (0.7 cm^2 each) were harvested (each disc was from a different leaf) with a cork borer, frozen in liquid N_2 and then dried to constant weight by freeze drying. Infected leaves varied in colour: from different shades of green, a mixture of green and red to totally red. To reduce the heterogeneity of the infected leaves, only those in which the whole lamina was red were used.

2.3. Measurement of photosynthesis and stomatal conductance

Measurements of net photosynthesis and stomatal conductance were carried out on leaves fully exposed to the sun in the field in the middle of the day (11:30–14:00). For both healthy and diseased leaves measurements were done on 5 leaves (and each of these leaves was present on a different shoot), by using a portable open system infrared gas analyser (LCA2, Analytical Development Company, UK).

2.4. Measurement of soluble sugars and starch

For measurements of carbohydrate contents, leaf discs (1.1 cm^2) were ground in glass-glass homogeniser containing 1.5 ml of ice-cold 80% ethanol/20% buffer solution (final concentration of buffer solution when diluted by 80% ethanol was: 20 mM Hepes-KOH [pH 7.1], 5 mM $MgCl_2$). The homgenates were incubated at 80 °C for 45 min with gentle shaking, cooled and then centrifuged (5 min at 14,000 *g*). Then supernatants were stored at −20 °C until required. The pellets (which contained the starch content of the samples) were washed by resuspending in 1 ml of 40 mM acetate buffer (pH 4.5), then recentrifuged 16,000 *g* for 5 min. This washing procedure was repeated 4-times. The final pellet was autoclaved for 45 min at 120 °C in 1 ml of 40 mM acetate buffer (pH 4.5) in order to solubilise the starch. After cooling 4 U of α-amylase and 40 U of amyloglucosidase were added, and the mixture was incubated for 1 h at 50 °C (and this procedure hydrolysed the starch). The samples were then centrifuged at 16,000 *g* for 5 min and supernatants were stored at −20 °C until required. Soluble sugar contents (including glucose originating from the hydrolysed starch) were measured using an enzyme-coupled method as described by Famiani et al. (2000). Sucrose was analysed after glucose and fructose, and following the addition of 100 units of invertase to the assay mixture. Sorbitol was measured in an enzyme-coupled reaction by following the reduction of NAD + by sorbitol dehydrogenase. The assay mixture was 100 mM Bicine pH 9.2, 5 mM $MgCl_2$, 0.01% (w/v) BSA, 1 mM NAD^+, 2U SDH. The measurements of all soluble sugars were done using an Anthos 2001 plate reader (Anthos Labtec Instruments, Salzburg, Austria) operating in dual-wavelength mode (340–405 nm). Mixing experiments, in which a known amount of metabolite was co-extracted with leaf material, showed that for all metabolites recovery was in excess of 90%. The contents of sucrose and starch are shown as glucose equivalents.

2.5. Extraction of enzymes

For all enzymes leaf discs (3 cm^2: equivalent to 100–200 mg of leaf material) were ground in a glass-glass homogeniser containing 1.5 ml of extraction buffer 50 mM Hepes (KOH) (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, 2% (w/v) PEG 20 and 5% (w/v) PVPP, centrifuged at 16 000 g for 5 min. The supernatants were then immediately desalted as described by Merlo and Passera (1991), but using the aforementioned extraction buffer, modified by the omission of both PEG and PVPP as desalting buffer. For the preparation of particulate invertase, the pellets were washed three times with the aforementioned desalting buffer. Then the final pellet was resuspended in the same buffer and enzyme activity in this was determined.

2.6. Sucrose synthase assay

The activity of SUSY in the direction of sucrose consumption was measured using a stopped assay (Dancer et al., 1990). Leaf extract (20 μl) was incubated for 20 min at 25 °C in 20 mM Hepes-KOH (pH 7.0), 100 mM sucrose, 4 mM UDP (100 μl final volume). The reaction was stopped by placing the assay tubes in boiling water for 3 min. Control assays were boiled immediately after the sample was added. The content of UDP-Glucose produced during the assay was measured using a spectrophotmeter by determining the amount of NAD^+ that was reduced by UDP-glucose dehydrogenase. The assay buffer used for this was 200 mM Glycine (pH 8.9), 5 mM $MgCl_2$, 2 mM NAD^+, and 0.02 U UDP-glucose dehydrogenase. Optimisation experiments showed that the assay was linear for at least 40 min, and with up to 60 μl of leaf extract. The amounts of both sucrose and UDP used in assays were saturating, and recovery of added UDP-glucose was close to 100%.

2.7. Invertase assay

The activities of both soluble and particulate acid invertases were measured in a stopped assay that was run for 40 min at 37 °C. The assay mixture contained 100 mM phosphate-citrate buffer (pH 4.8), 100 mM sucrose and 50 μl of leaf extract (final volume 200 μl).