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THE RESTING METABOLISM OF DODDER SEEDS

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Key Word Index—*Cuscuta reflexa*; *Cuscuta campestris*; *Cuscuta indicora*; Cuscutaceae; Angiospermic parasites; seeds; dodder; enzymes of carbohydrate, amino acid and phosphate metabolism.

Abstract—Extracts of mature seeds of *Cuscuta reflexa* were examined for any deficiency in key enzymes. The activities of malate dehydrogenase, β-amylase and fructose 1,6-diphosphate aldolase exceeded 5.0 μmol substrate/min/g, while those of starch phosphorylase, α-amylase, acid phosphatase, phosphogluconate dehydrogenase (decarboxylating), aspartate aminotransferase, glucose 6-phosphate dehydrogenase, fructose 1,6-diphosphatase and alanine aminotransferase fell within the range 1 to 5 μmol/min/g and hexokinase, isocitrate dehydrogenase and alkaline phosphatase were below 1 μmol substrate/min/g seed powder. No activity of the following were found: acid invertase, alkaline invertase, phytase and glutamate dehydrogenase. Some of these observations were made also for seeds of *Cuscuta campestris* and *Cuscuta indicora*.

INTRODUCTION

Angiospermic parasitism is generally regarded as the result of a degenerative process by which plant species, which were once free-living and independent, lost their ability to carry out one or more of their physiological functions and have since been dependent for their existence on the host plant [1]. With a view to elucidating whether or not the seeds of the angiospermic parasites have any distinctive features, the authors undertook a study of some of the enzymes involved in the metabolism of starch, sugars, amino acids and phosphorylated compounds in mature *Cuscuta* seeds. In view of the known host preferences among *Cuscuta* species and the likelihood of species-dependent variation in enzyme activity, a comparative study was carried out, wherever possible, with the seeds of three different species of the dodder.

RESULTS AND DISCUSSION

The enzyme activities in the mature seeds of *Cuscuta* species are recorded in Table 1.

Amylolysis appeared to be the predominant route of starch degradation. The detailed characterization of the amylase activity was not attempted, but the distinctly wide pH optima (pH 5.0 and 7.2) suggested that both β- and α-amylase were active in the seeds of *Cuscuta*. The activity of hexokinase towards glucose was more pronounced showing the preferential use of this sugar in comparison with fructose. The value reported for glucose 6-phosphate dehydrogenase was likely to be the upper limit, since there might have been interference from phosphogluconate dehydrogenase (decarboxylating) in spite of the divergent peaks of activity.

Unlike the seeds of autotrophs, *Cuscuta* seed on germination is unable to abstract any nutrient from the soil and, as such, the seedling enjoys only a brief period of independent existence. The constitutive enzyme activity in the resting *Cuscuta* seed would be such as to rapidly mobilize, or initiate the mobilization of, the metabolites and the reserves and sustain the seedling with the aid of increased activities of the existent enzymes, or by the elaboration of additional enzymes. At the same time, the special need of the parasite seed for survival until appropriate hosts are encountered may require the suppression of some key enzymes in the resting state, with provision for the manifestation of their activities once the environmental conditions are favourable.

The absence of activity of invertase may not be surprising since mature seeds of many autotrophic plants do not have invertase activity, or have only negligible activity [2–5]. The broad pH optimum for aldolase and the absence of inhibition by EDTA and of stimulation by K^+ and NH_4^+ indicated that the enzyme belonged to Class I [6], resembling that of other higher plant aldolases [7]. The comparatively high activity of malate dehydrogenase in the two species of *Cuscuta* tested may be significant. Thorneberry and Smith [8] reported a correlation between malate dehydrogenase and seed viability in corn. Seeds of *Cuscuta* remain viable in the soil for years [9]; the high activity of malate dehydrogenase may be related to the viability of the dodder seeds. In spite of transamination being possible, the further utilization of α-keto glutarate stopped at the level of glutamate dehydrogenase because of the absence of this activity. Since *Cuscuta* seeds contain a fairly high concentration of inorganic phosphate [10], it was not clear whether phosphatase activity is an essential metabolic event in the resting metabolism of the seeds of *Cuscuta*.

The activities of the various enzymes in mature *Cuscuta* seeds were such as to sustain a low rate of respiration utilizing carbohydrates, with potential capacity for accelerating the metabolism on imbibition. The findings from the present study do not warrant a conclusion that the resting metabolism of the dodder *seeds* has features distinct from those of the seeds of autotrophs; the enzymes of the *seedling* may hold the clue to the need for parasitism by the plant.

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Table 1. Enzyme activities in the resting seeds of *Cuscuta* species

Enzyme	Activity (units/g air-dried seed) *C. reflexa*	*C. campestris*	*C. indicora*
Carbohydrate metabolism			
(a) *Glycolytic pathway and related amylases*			
β-	11.40	ND	ND
α-	4.48	ND	ND
α-glucan phosphorylase hexokinase:	4.50	1.84	2.12
(i) towards glucose	0.913	ND	ND
(ii) towards fructose	0.182	ND	ND
fructose-1,6-diphosphate aldolase	7.80	5.40	5.40
invertase:			
(i) soluble			
acid	NS	ND	ND
alkaline	NS	ND	ND
(ii) insoluble			
acid	NS	ND	ND
alkaline	NS	ND	ND
(b) *Oxidative pentose phosphate pathway*			
glucose-6-phosphate dehydrogenase	1.81	ND	ND
phosphogluconate dehydrogenase (decarboxylating)	3.62	ND	ND
(c) *Krebs cycle*			
malate dehydrogenase	173	290	ND
isocitrate dehydrogenase	0.724	ND	ND
Amino acid metabolism			
alanine aminotransferase	1.04	0.55	0.69
aspartate aminotransferase	2.64	2.10	1.47
glutamate dehydrogenase	NS	NS	ND
Phosphatases			
fructose 1,6-diphosphatase	1.21	ND	ND
phytase	NS	ND	ND
acid phosphatase	4.43	2.12	4.13
alkaline phosphatase	0.44	NS	NS

ND—not determined. NS—not significant.

EXPERIMENTAL

Seed collection and processing. Mature seeds of *Cuscuta reflexa* Roxb., were collected from parasite growing on *Lantana camara* Linn. (Verbenaceae), and those of *Cuscuta campestris* Yunck and *Cuscuta indicora* Choisy from parasites on *Medicago sativa* Linn. (Papilionaceae). Seeds were freed from the dried fruit wall and quickly washed 2× with tap H_2O, followed once with dist. H_2O. Moisture was removed by spreading on cloth and seeds were air-dried. Seeds of *C. reflexa* weighed on average 12 mg each; those of *C. campestris* and *C. indicora* 0.65 mg each. Seed powder, 60 mesh size, was prepared in a micro Wiley mill operated in a room maintained at 15°.

Preparation of dispersions, extracts and gel filtrates. A minimum of 5 min vigorous grinding with medium in a chilled mortar and pestle, with acid-washed sand, was necessary to effect proper dispersion of the seed powder (1–2.5%). Separate expts revealed that there was minimum interference by endogenous phenolics in the enzyme activity determinations in *Cuscuta* seeds and optimum enzyme activities were obtained when a reducing agent was present during extraction. Amylase activity was determined in the 15000 *g* supernatant of 1% dispersions prepared in 10 mM Pi buffer, pH 7, containing 10 mM 2-mercaptoethanol. Phosphorylase activity was determined in cloth-filtered seed powder dispersion, 2.5%, in 10 mM Tris–HCl buffer pH 7.2, supplemented with 10 mM cysteine. Hexokinase and the shunt dehydrogenases were determined in the 15000 *g* supernatant from 1% seed dispersions in 10 mM Pi buffer, pH 7, supplemented with 10 mM 2-mercaptoethanol and 0.10% Triton X-100. Acid- and alkaline-phosphatases were determined in 1% seed powder dispersions in 10 mM cysteine buffered with 10 mM Tris–HCl, pH 7.2. Fructose 1,6-diphosphatase and phytase were also determined in the seed powder dispersions (2% in 10 mM cysteine). Fructose 1,6-diphosphate aldolase activity was determined in 1% seed dispersions in 10 mM 2-mercaptoethanol. Invertase was tested in the gel filtrates from 15000 *g* supernatants of 1% dispersions of seed powder in 10 mM Pi buffer, pH 7, supplemented with 10 mM 2-mercaptoethanol and 0.10% Triton X-100; the enzyme activity was also tested in the 15000 *g* residue after suspending in a similar medium but which did not contain detergent. Malate dehydrogenase and glutamate dehydrogenase in *C. reflexa* seed were determined in the 800 *g* supernatants from 1% dispersions in 20 mM Pi buffer, pH 7.4, fortified with 10 mM 2-mercaptoethanol. Isocitrate dehydrogenase in *C. reflexa* and malate dehydrogenase in *C. campestris* were determined in the gel filtrate of 15000 *g* supernatants of 2% dispersions in 20 mM Pi buffer, pH 7, containing 20 mM 2-mercaptoethanol. Aminotransferase activity determinations were with 1% dispersions in 20 mM Pi buffer, pH 7.4, containing 10 mM 2-mercaptoethanol.

Enzyme activity determinations

These were in the proportionality range with respect to the amount of enzyme and period of incubation and at the predetermined pH optima. Appropriate controls were included in every assay. Substrates and any supplements added to the assay were at near neutrality. When colorimetric comparisons were involved, the reaction was at 30° for 30 min. Incubations in spectrophotometer cuvettes were at room temp.; these reactions, except in the case of malate dehydrogenase, were for 5 min and $A_{340\ nm}$ read every min. When malate dehydrogenase was assayed, readings were taken every 15 sec for the first min. The values obtained were calculated to correspond to 30° by assuming a Q_{10} value of 2.

The *α-glucan phosphorylase* assay, in the direction of starch synthesis, was after Ref. [11] by estimating the Pi liber-

ated [12]. The *α-amylase and β-amylase* assay was after Ref. [13]. The final colors were matched against the color developed from maltose. The *hexokinase* assay was after Ref. [14] using coupling enzymes. The *fructose* 1,6-*diphosphate aldolase* assay was after Ref. [15] and color was developed according to Ref. [16]. The readings were converted to μmol of substrate transformed on the basis of predetermined relationship between the color intensity and the alkali labile phosphate formed in the reaction mixture. The *invertase* assay was after Ref. [17] and reducing sugar formed was determined according to Ref. [18] as modified Ref. [19]. The *glucose* 6-*phosphate dehydrogenase and phosphogluconate dehydrogenase* (*decarboxylating*) assays were after Ref. [20] with activity of the former measured at pH 7.5 and the latter at 9 pH. The *malate dehydrogenase* assay, in the direction of L-malate formation, was after Ref. [21]. The *isocitrate dehydrogenase* assay was after Ref. [22]. The *glutamate dehydrogenase* assay system, in the direction of L-glutamate formation, was similar to the one for malate dehydrogenase, with the difference that 2-ketoglutarate replaced oxaloacetate and NH_4Cl was added. The assay was carried out also in the direction of glutamate oxidation, linked to NAD/NADP. The *aspartate aminotransferase and alanine aminotransferase* assay was after Ref. [23] and the keto acid formed was estimated according to Ref. [24]. The *acid and alkaline phosphatases* assay was after Ref. [25]. β-Glycerophosphate was used as substrate and Pi liberated at pH 5 (acid phosphatase) and, in the presence of Mg^{2+}, at pH 8 (alkaline phosphatase) was determined. The *hexose diphosphatase* assay was similar to the above with the difference that fructose diphosphate replaced β-glycerophosphate and the pH was 6. The *phytase* (6-*phytase*) assay was after Ref. [26]. An extinction coefficient of 6.22×10^3 l./mol/cm for NADH/NADPH [27] was employed for calculations. One unit of enzyme activity caused the transformation of 1 μmol of substrate in 1 min at 30° and under the conditions of the assay. In the case of amylase, one unit corresponded to the liberation of the reducing equivalent of 1 μmol of maltose in 1 min.

Reporting of data. Analytical data are reported in units of activity per g seed powder. Each enzyme activity was determined at least twice with different batches of seed powder. Variation in activity never exceeded 10–15% from batch to batch; in some cases the deviation was of the order of 2–5%. The values recorded are those of a typical experiment.

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