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PHOSPHORUS, NITROGEN AND CARBOHYDRATE COMPOSITION OF THE SEEDS OF *CUSCUTA*

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Abstract—The seeds of *Cuscuta campestris* were analysed for phosphorus, nitrogen and carbohydrate components. The seeds apparently contained sufficient reserve nutrients to initiate germination under appropriate environmental conditions and to sustain the early phase of the non-parasitic existence of seedlings. There was no tendency to accumulate phosphorus and nitrogen, but starch and polyfructosan occurred in high amounts. Phytic acid and acid-insoluble polyphosphate constituted the reserve forms of phosphorus. A distinctive feature appeared to be a relatively high concentration of phospholipids. The occurrence of significant quantities of nucleotides and the abundance of hexoses and of the dicarboxylic amino acids in the parasite seed suggested a ready potential of metabolic activity.

INTRODUCTION

ANGIOSPERM parasites are dependent wholly or in part on their hosts for diffusible biochemical component(s). However, the exact nature of this dependence is not known. Some of the parasites have been grown in axenic cultures, but biochemical studies on such tissues have not been attempted. The vegetative parts of *Cuscuta reflexa* have been analysed for biochemical constituents,[1] and a seed analysis is another likely source of information on parasite metabolism. An essential component may be missing, or present in limiting amounts, shortening the independent existence of the germinated seed. In the present report the seeds of *Cuscuta* have been analysed for phosphorus, nitrogen and carbohydrate components.

RESULTS AND DISCUSSION

Total Phosphorus and Gross Phosphate Fractions

The results obtained are recorded in Table 1.

There was no evidence for a preferential accumulation of phosphorus in the seeds of the parasite; the total phosphorus content was comparable with that of the seeds of non-parasitic plants.[2,3] Acid-soluble phosphorus occurred in higher proportion than acid-insoluble phosphorus. Phospholipid was comparatively high and phosphoprotein low. The ratio RNA/DNA was about 5, which was lower than the ratio of 10 found for many non-parasitic seeds.[3] Insoluble polyphosphates occurred in higher concentration in the 'pH 9 fraction' than in the '2 N KOH fraction'. The polyphosphate fractions comprised about 15% orthophosphate phosphorus and about 16% not hydrolysable in 10 min with 1 N acid. There are few reports, in the literature on the occurrence of polyphosphates in seeds.

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TABLE 1. GROSS FRACTIONATION OF PHOSPHORUS IN SEEDS OF *Cuscuta*

	μg Phosphorus/g seed powder						
						Insoluble polyphosphates	
Total	Cold TCA-soluble	Phospholipid	RNA	DNA	Phospho-protein	pH 9·0	2 N KOH
3251	1781 (54·8)	236·8 (7·3) [18·8]	781·8 (24·0) [62·2]	153·4 (4 7) [12·2]	38 9 (1·2) [3 1]	29·6 (0·9) [2 4]	15·9 (0·5) [1·3]

The details of fractionation were as reported in text.
The values within parentheses are as % of total phosphorus in seed.
The values within square brackets are as % of TCA-insoluble phosphorus, the latter being calculated as the sum of the component fractions.

The values for RNA and DNA by different methods obtained for the solutions of nucleotides purified by adsorption on charcoal and elution are recorded in Table 2. A fresh sample of seed powder was used in this experiment. When standard RNA and DNA were subjected to the same treatment, there was 90% recovery for each by phosphorus determination and the results were corrected for this loss. The contents of RNA and DNA in the seed samples were calculated assuming that the nucleic acids in the seeds had the same composition as the standard nucleic acid samples in relation to total phosphorus, chromogenicity with orcinol and diphenylamine reagents, and UV absorption at 260 nm.

There was satisfactory agreement among the three methods used for the determination of nucleic acids. The values for phosphorus as determined in the crude fractions were about 6% higher for RNA and 13% higher for DNA in comparison with the corresponding values for the purified fractions.

Sub-fractions of Phosphorus in the TCA-Soluble Fraction

Data for P_0, P_{A10}, phytic acid and 'soluble polyphosphates' in the cold TCA extracts of the seeds are recorded in Table 3, calculated as μg/g seed and as % of the total phosphorus in seed and of total TCA-soluble phosphorus.

TABLE 2. ANALYSIS FOR RNA AND DNA IN THE PURIFIED FRACTION FROM THE SEEDS OF *Cuscuta*

	Nucleic acid content on the basis of		
	Phosphorus	Carbohydrate mg/g dry weight	Ultra-violet absorption
RNA	8·98	8 90	8·61
DNA	1·53	1·62	1·43

The analyses of nucleic acids were in the purified fractions isolated by adsorption on charcoal and elution.

TABLE 3. SUB-FRACTIONS IN COLD TCA EXTRACTS OF THE SEEDS OF *Cuscuta*

μg/g Seed powder							
Whole extract			Nucleotide fraction		Phytic acid	Soluble polyphosphates	
P_0	$P_{\Delta 10}$	P_{total}	$P_{\Delta 10}$	P_{total}		pH 2·5	pH 4·5
815	370	1781	131	193	365	526	271
(25·0)	(11·4)	(54·8)	(4·0)	(6·0)	(11·2)	(16·2)	(8·3)
[45·7]	[20·7]	[100]	[7·3]	[10·8]	[20·4]	[29·5]	[15·2]

The analysis of the TCA-soluble fraction was as reported in text. P_0 represents the orthophosphate phosphorus initially present in the sample, $P_{\Delta 10}$ the orthophosphate phosphorus formed on heating the sample for 10 min with 1 N sulfuric acid at 100°, and P_{total} the total phosphorus content of the sample as determined by wet digestion.

The values within parentheses are as % of total phosphorus in seed.

The values within square brackets are as % of the TCA-soluble phosphorus.

The content of the stable form of phosphorus (obtained as the difference between the total phosphorus and the orthophosphate phosphorus at the end of 10 min hydrolysis with 1 N acid) was about twice that of the labile form in the cold TCA extract. A tenth of the total phosphorus in the TCA-soluble fraction was present as nucleotides. The ratio acid-labile/stable phosphorus in the nucleotide fraction was nearly 2. A fifth of the phosphorus in the TCA extract was in the form of phytic acid.

The fractions precipitable as the barium salt at pH 2·5 and 4·5 together contained about 45% of the total phosphorus in the TCA extract. $P_{\Delta 10}$ estimation revealed that only 6% of the total phosphorus in the pH 2·5 fraction and 14% in the pH 4·5 fraction were acid-hydrolysable, suggesting heavy contamination of the fractions with non-polyphosphate material.

Total Nitrogen, Protein and Free Amino Acids

Total nitrogen was 18·5 mg/g dry wt. and total protein (N × 6·25) was 114·1 mg/g dry wt. Total soluble protein (20·0 mg/g dry wt.) was composed of globulins (14·8 mg/g dry wt.), and albumins (5·0 mg/g dry wt.). The predominant insoluble protein was probably a reserve material. The content of the free amino acids are shown in Table 4. The finding that the seeds did not contain any accumulation of the reserve amino acids—proline and arginine—was of particular interest, γ-aminobutyric acid and the amides were absent.

Carbohydrate Fractions

The total ethanol-soluble carbohydrates (as glucose equivalent) were 44 mg/g dry wt., the ethanol-insoluble polyfructosans (as fructose) were 7·5 mg/g dry wt. and starch was 26·8 mg/g dry wt. The results obtained on chromatography of the ethanol-soluble fraction are recorded in Table 5.

Pentose was absent. Glucose and fructose were present in equivalent amounts, suggesting an origin from sucrose. There was a high content of fructose-oligosaccharides, stachyose and raffinose.

TABLE 4. FREE AMINO ACIDS IN THE SEEDS OF *Cuscuta*

Amino acid	Content, as % of total amino acids recovered from chromatogram
Alanine	12·6 (0·64)
β-Alanine	—
γ-Aminobutyric acid	—
Arginine	+
Asparagine	—
Aspartic acid	5·1 (0·25)
Citrulline	3·0 (0·14)
Cysteic acid	—
Cysteine	6·0 (0·30)
Cystine	—
Glutamic acid	13·2 (0·66)
Glutamine	—
Glycine	8·3 (0·41)
Histidine	6·1 (0·30)
Hydroxyproline	+
Isoleucine	6·3 (0·31)
Leucine	4·1 (0·20)
Lysine	5·0 (0·25)
Methionine	3·6 (0·18)
Ornithine	—
Phenylalanine	—
Proline	+
Serine	6·1 (0·30)
Threonine	9·3 (0·46)
Tryptophan	—
Tyrosine	3·1 (0·15)
Valine	8·6 (0·46)

The separation of the amino acids and determination were as reported in text. Values in parentheses are the amount of the amino acids in mg/g dry seeds. The symbol + shows presence in traces and — absence in demonstrable amounts.

Other Analyses

Total lipids were 22·5 mg/g dry wt., total phenolics were 16·0 mg/g dry wt. and ash was 31·0 mg/g dry wt. The data obtained in the present investigation suggested that the parasite seeds belong to the class of seeds accumulating carbohydrates rather than protein or lipids.

TABLE 5. CARBOHYDRATE COMPONENTS IN THE ETHANOL-SOLUBLE FRACTION OF THE SEEDS OF *Cuscuta*

Sugar	Content, as % of total carbohydrate recovered from chromatogram
Xylose	—
Fructose	13·7 (5·93)
Glucose	13·9 (6·12)
Sucrose	17·1 (7·40)
Raffinose	39·7 (17·19)
Stachyose	15·6 (6·74)

Values within parentheses are in mg glucose-equivalent of component per g dry seed. The symbol — shows absence in demonstrable amounts.

EXPERIMENTAL

Seeds

The seeds of *Cuscuta campestris* Yunck growing on *Medicago sativa* L. were collected from the departmental garden. The seeds were washed with distilled water, surface dried with cloth and air dried under a fan. The seeds (50–100 g) were powdered in a Wiley mill and the powder dried in a desiccator.

Phosphate Fractionation

This was based on the methods of Schmidt and Thannhauser[4] and Schneider,[5] as outlined by Volkin and Cohn,[6] with slight modifications. Determination of total phosphorus in the seeds or isolated fractions after wet digestion, or of orthophosphate phosphorus in a given fraction, was by the method of Fiske and Subbarow,[7] as modified by LePage,[8] unless otherwise specified. Aliquots of cold-TCA extract were employed for the determination of initial orthophosphate phosphorus (P_0), orthophosphate phosphorus at the end of 10 min hydrolysis with 1 N sulfuric acid (P_{10}) and total phosphorus (P_{total}) by wet digestion. Acid-labile phosphorus ($P_{\Delta 10}$) was calculated as the difference between P_{10} and P_0, and acid-stable phosphorus (P_{stable}) as the difference between P_{total} and P_{10}. Total acid-soluble nucleotides in the TCA extract were determined by adsorption on Norit A and elution according to Tsuboi and Price.[9] Other aliquots of the extract were used for phytic acid determination according to McCance and Widdowson,[10] as modified by Pons *et al.*[11] For acid-soluble polyphosphates, the procedure was according to Wiame,[12] the precipitation of the barium salts being conducted at pH 2·5 and 4·5.

The acid-insoluble, phospholipid-free residue was digested with alkali, acidified and centrifuged to recover the precipitated DNA. The P_0 content of the supernatant, as determined on aliquots by the method of Martin and Doty,[13] measured phosphoprotein and P_{stable} RNA. Independent estimates of RNA were made on the basis of the orcinol reaction[14] and UV absorption at 260 nm. To arrive at a more precise value for the nucleic acid, the ribonucleotides were isolated by adsorption on Norit and eluted with ethanolic ammonia prior to

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determination. The resulting solution was analysed for P_{total}, for pentose content by the orcinol reaction and for UV absorption at 260 nm. A sample of yeast RNA (Schwarz) was used as reference standard after alkaline degradation to the nucleotides and isolation of the nucleotides by adsorption on charcoal and elution. The DNA fraction was extracted with hot TCA and aliquots of the extracts were employed for determination of P_{total} according to Bartlett,[15] deoxyribose by the method of Burton[16] and UV absorption at 260 nm. For more precise evaluation of DNA content, the nucleotides were isolated by adsorption on Norit, eluted with ethanolic ammonia, and the estimations repeated. A sample of thymus DNA (B.D.H.) was put through the same operations and used as the reference standard.

The fractionation for insoluble polyphosphate was according to Miyachi,[17] by extracting the cold-TCA-insoluble, phospholipid-free seed residue at pH 9·0 followed by 2 N KOH. The precipitation with Ba^{2+} was carried out at pH 4·5, since no precipitate was formed at pH 2·5.

Nitrogen Fractions

Amino acids were extracted according to Kliewer[18] Samples were subjected to bidimensional chromatography according to Smith *et al.*[19] and unidimensional (ascending) chromatography according to Reland and Gross.[20] The colored spot of each amino acid was cut out and extracted at room temp. for 25 min with 7 ml 0·75% ethanol containing 5 mg % $CuSO_4 \cdot 5H_2O$, and the absorption read in a Klett–Summerson colorimeter using filter No. 42 for proline and hydroxyproline, and No. 54 for all the other amino acids, against similar spots from reference chromatogram[21] The recovery of the amino acids from the chromatogram ranged from 90 to 100% of the amount applied.

Total nitrogen was determined by the micro-Kjeldahl method and crude protein was calculated by applying the factor 6·25. The fractionation of seeds for soluble proteins and distribution into globulin and albumin was based on the method of Millikan and Mann.[22] Protein determination was by the colorimetric method of Lowry *et al.*[23]

Carbohydrate Fractions

Ethanol-soluble carbohydrates were extracted according to Bell,[24] the components separated by descending chromatography with *n*-butanol–acetic acid–water (4:1:5),[25] and the spots visualized with the diphenylamine reagent of Buchan and Savage.[26] The sugars were eluted from unsprayed chromatograms according to Bacon and Edelman[27] and estimated as total carbohydrate with the phenol–sulfuric acid reagent of Montgomery,[28] using glucose as standard. The recovery of carbohydrate from the chromatograms ranged from 85 to 105% of the amount applied. Polyfructosans were estimated in the ethanol-insoluble residue by extraction with hot water according to McRay and Slattery[29] and estimation as fructose with reagents of Roe and Papadopoulos.[30] Starch was separated from the original seed powder according to Pucher *et al.*[31] and estimated by the method of Montgomery.[28]

Other Estimations

Lipid was extracted with petroleum (b.p. 40–60°) and determined crude by drying aliquots of the extract. Phenolics were determined according to Folin and Denis,[32] as modified by Goldstein and Swain,[33] using tannic acid as standard. The ash of the seed was determined by incineration to a constant weight at 600°.

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Replication of Analyses

The validity of every analysis was verified by determination with duplicate samples of seed powder. In some estimations as many as 4 different samples were analysed.

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