Impact of Egyptian Broomrape (*Orobanche aegyptiaca* (Pers.) Parasitism on Amino Acid Composition of Carrot (*Daucus carota* L.)

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The relationship between the organic nitrogen status of Egyptian broomrape and one of its hosts, carrot, was studied by comparing amino acid profiles of leaf and root tissues of nonparasitized and broomrape-parasitized carrot plants and by analyzing amino acid profiles of broomrape at different growth stages. Total N concentrations, expressed as a percentage of the dry weight of the tissues, were similar in leaves of nonparasitized and parasitized carrot plants but were lower in parasitized roots than in nonparasitized roots. In both dry and germinated broomrape seeds, N concentrations were lower than or similar to those in broomrape tubercles, shoots, or callus. Individual amino acid concentrations in hydrolysates of leaves of parasitized carrot plants tended to be similar to or greater than those in hydrolysates of nonparasitized carrot plants. Roots of parasitized plants tended to have similar or lower amino acid concentrations than roots of nonparasitized plants. Dry and germinated broomrape seeds had similar amino acid profiles, but individual amino acid concentrations were lower than in the other broomrape tissues examined. The broomrape shoot tended to have lower amino acid concentrations than the tubercle and callus. Free amino acid profiles of leaves and roots of parasitized plants paralleled those of nonparasitized plants, respectively. Individual free amino acids tended to occur at similar or lower levels in dry and germinated broomrape seeds than in the tubercle, shoot, or callus. Free amino acid composition of the broomrape tubercle was similar to that of the parasitized root. Arginine and alanine concentrations in broomrape callus were dramatically higher than those of other amino acids in this or other tissues investigated. These results indicate that changes in the composition of both free and bound amino acids in carrot are associated with broomrape parasitism.

Keywords: *Egyptian broomrape; Orobanche aegyptiaca Pers.; carrot; Daucus carota L.; amino acid composition*

INTRODUCTION

Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is a phanerogamic holoparasite that attacks the roots of many dicotyledonous crops. It lacks chlorophyll (Baccarini and Melandri, 1967; Saghir et al., 1973) and obtains carbon, nutrients, and water through haustoria, which connect the parasite with the host's vascular system. Broomrape parasitism has a detrimental effect on the growth and yield of several economically important host crops, including carrot (*Daucus carota* L.), particularly in the Mediterranean region [reviewed by Foy et al. (1989), Sauerborn (1991), Parker and Riches (1993)].

Due to its close association with the host plant throughout its life cycle and its growth under the soil surface, many conventional weed control strategies targeted at broomrape have been futile. Anecdotal reports suggest that nitrogen, as either manure or inorganic fertilizer, has an inhibitory effect on broomrape parasitism. This observation is supported by findings from greenhouse and laboratory studies [reviewed by Sauerborn (1991) and Parker and Riches (1993)]. Effective and practical utilization of nitrogen as a control strategy depends on knowledge of the nitrogen status of broomrape and on understanding of the mechanism of nitrogen inhibition of broomrape growth.

Lee and Stewart (1978) were unable to detect nitrate reductase activity in broomrape, which indicates that the parasite is unable to utilize nitrate. Low activity of glutamine synthetase, the enzyme that catalyzes the incorporation of ammonium into organic compounds, was found in broomrape and another holoparasite, *Lathraea clandestina* L. (McNally et al., 1983a,b, 1984; McNally and Stewart, 1987). Additionally, nitrate reduction in *L. clandestina* has been shown to be incomplete or to proceed at very low rates (Thalouarn et al., 1987, 1988, 1990).

The absence of activity or low activity of nitrogen assimilating enzymes in broomrape may indicate that the parasite has access to organic nitrogen in the host plant. Translocation of amino acids and amides has been shown for broomrape (Aber et al., 1983) and other angiospermous parasites (Fer, 1979; Renaudin and Larher, 1981; McNally et al., 1983a; Thalouarn et al.,

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1986). Broomrape seeds were able to absorb and metabolize radioactive leucine (Leu) during both conditioning and germination, and a part of the Leu was metabolized (Bar Nun and Mayer, 1993).

The objective of this research was to determine the nitrogen status of broomrape in relation to a host, carrot, by investigating the effect of broomrape parasitism on the amino acid composition of carrot and by comparing amino acid profiles of broomrape at different growth stages.

MATERIALS AND METHODS

Plant Material. Carrot seeds of the variety Nantes Coreless (American Seed, South Easton, MA) were surface sterilized in a 1% solution of sodium hypochlorite (commercial bleach) and planted in vermiculite. After the appearance of the second true leaf, the seedlings were transplanted into polyethylene bags as described previously (Parker and Dixon, 1983; Goldwasser et al., 1997).

Broomrape seeds were surface sterilized, as described for carrot seeds, following a 20-s dip in 70% (by volume) aqueous ethanol. Seeds were conditioned and germinated according to procedures described by Mangnus et al. (1992). Seeds were considered to have germinated when the radicles had emerged. Embryos of both dry and germinated seeds were devitalized by soaking the seeds in a film of 100% ethanol for 8 h at 25 °C in 9-cm Petri dishes. Ethanol was allowed to evaporate overnight, and then seeds were harvested.

Two weeks after carrot seedlings had been transplanted, conditioned seeds of broomrape were spread on the roots with a spatula. Carrot plants were checked periodically under a microscope for broomrape germination and attachments on carrot roots. Initial attachments were found 7 days after conditioned seeds were spread on the roots. Tubercles were observed 4 weeks after formation of the initial attachments and were harvested 2 weeks after formation. Floral spikes of broomrape were collected 2 weeks after emergence from the tubercle. Leaves and roots from both parasitized and nonparasitized carrot plants were harvested 7 weeks after the seedlings were transplanted into the polyethylene bags.

Throughout their growth, plants were watered as required with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). The plants were placed in a growth room with light intensity of 300 μeinstein m^{-2} s^{-1} provided by fluorescent lights, and temperature was 24 °C during the 12-h light period and 20 °C during night.

Callus cultures of broomrape were developed and maintained according to the method of Ben-Hod et al. (1991). Calli were subcultured initially at 2-week intervals for 6 weeks and, thereafter, transferred to fresh medium every 4 weeks. Cultures were always placed in the dark. Friable calli were harvested every 4 weeks.

Immediately after harvest, all tissues were frozen in liquid nitrogen and lyophilized. Dried tissues, except dry and germinated seed, collected over several months and stored at −20 °C, were pooled, ground to pass a 1-mm screen using an Udy (Ft. Collins, CO) cyclone mill, and stored at −20 °C until analyzed. Subsamples were used for chemical analyses, and results are expressed on an "as is" dry weight (DW) basis.

Chemical Analyses. Total N, total C, and total S of duplicate subsamples of each tissue sample were determined simultaneously by combustion and gas chromatographic (GC) techniques using a Fison Instruments (Beverly, MA) C−H−N−S analyzer (Pella and Colombo, 1978). Amino acids were analyzed as phenylthiocarbamyl derivatives using a modification of the procedure of Bidlingmeyer et al. (1984). Protein amino acid concentrations were calculated as the differences between values obtained for whole-tissue hydrolysates and values obtained for free amino acid extracts.

Preparation of Samples for Amino Acid Analysis. For extraction of free amino acids, the procedure described by Foster (1990) was used. A 0.5-g subsample of each tissue sample was placed in a cellulose extraction thimble in a Soxhlet extractor, 250 μL of a 20 mM stock solution of norvaline (internal standard) was added to each thimble, and samples were extracted with 35% (by volume) aqueous ethanol. After passage of the extracts through activated C_{18} Sep-Pak cartridges (Waters, Milford, MA) and filtration through a 0.45-μm nylon syringe filter, extracts were ready for the derivatization procedure.

For total amino acid determinations, triplicate subsamples of 0.02 g of each tissue were weighed into 5-mL glass ampules. After addition of 3 mL of 6 N HCl, ampules were immediately purged with grade 5 nitrogen and sealed; samples were hydrolyzed for 6 h in a Thermatic 60 (Castle Sybron, Rochester, NY) autoclave set at 132 °C. Hydrolysates were transferred to 5-mL volumetric flasks, 1 mL of internal standard solution (α-aminobutyric acid in 0.1 N HCl, 5 μmol/mL) was added, and the volume was brought to 5 mL with Milli-Q water (Millipore, Bedford, MA). Aliquots, filtered through a 0.45-μm Teflon syringe filter, were used for the amino acid derivatization sequence. Tryptophan and the S-containing amino acids, cysteine and methionine, which are partly or completely destroyed by acid hydrolysis, were not analyzed.

Table 1. Gradient Table for Analysis of Phenylthiocarbamyl Derivatives of Amino Acids Using a Perkin-Elmer Series 200 Quarternary Pump[a]

step	time	flow	% A[b]	% B[c]	curve
0	5.0	1.0	100	0	0
1	1.0	1.0	100	0	0
2	5.0	0.6	95	5	1
3	15.5	1.0	54	46	1
4	7.5	0.6	0	100	1
5	2.0	1.2	0	100	0
6	6.0	1.4	0	100	0
7	2.0	1.4	100	0	1
8	2.0	1.4	100	0	0
9	2.0	1.2	100	0	0

[a] Detector, Perkin-Elmer diode array model 235 C; column oven, Perkin-Elmer series 200; autosampler, Perkin-Elmer series 200 equipped with peltier accessory; pump, Perkin-Elmer series 200 quarternary gradient LC pump; data system, Perkin-Elmer Nelson Turbochrome 4. [b] Eluent A, 0.14 M sodium acetate, 0.05% (v/v) triethylamine, pH 6.40, containing 6% (v/v) acetonitrile. [c] Eluent B, 60% (v.v) acetonitrile in water.

Apparatus and Chemicals. High-performance liquid chromatographic (HPLC) analysis of phenylisothiocyanate (PITC)-derivatized samples was performed using a system consisting of a Perkin-Elmer (Norwalk, CT) series 200 quarternary gradient LC pump, a Perkin-Elmer diode array model 235 C detector (254 nm), a Perkin-Elmer series 200 autosampler equipped with a peltier accessory, and a Waters Pico-Tag free amino acid analysis column (3.9 mm × 30 cm). Column temperature was maintained at 36 °C with a Perkin-Elmer series 200 column oven. Eluents were laboratory-prepared and sparged with helium using a Perkin-Elmer solvent module. Separation of sample components was accomplished using the gradient listed in Table 1. Data acquisition and peak quantification were accomplished using a PE Nelson (Norwalk, CT) Turbochrome 4 chromatographic data system. Protein amino acid standard solution (standard H) and PITC were purchased from Pierce Chemical Co. (Rockford, IL). Homoserine and individual protein amino acids were obtained from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of the highest purity available.

Safety. The amino acid derivatizing reagent, PITC, reacts on contact with strong acids, emitting highly toxic cyanide fumes and/or oxides of sulfur.

RESULTS AND DISCUSSION

Elemental Analysis. Elemental analysis of carrot and broomrape tissues revealed differences in the C, N, and S composition of the tissues (Table 2). Total C concentrations, expressed as a percentage of the dry weight of the tissues, were similar in leaves of nonpara-

Table 2. Elemental Composition of Tissues of Carrot and Broomrape[a]

tissue	total C[b], %	total N[b], %	total S[b], %
carrot			
unparasitized plant			
leaf	40.6 ± 0.2	4.3 ± 0.0	0.5 ± 0.0
root	33.4 ± 0.5	3.8 ± 0.1	0.4 ± 0.0
parasitized plant			
leaf	42.0 ± 0.1	4.2 ± 0.0	0.5 ± 0.0
root	39.4 ± 0.1	2.8 ± 0.0	0.3 ± 0.0
broomrape			
dry seed	52.1 ± 0.9	1.9 ± 0.1	0.2 ± 0.0
germinated seed	55.2 ± 0.2	1.7 ± 0.0	0.2 ± 0.0
tubercle	42.6 ± 0.2	2.8 ± 0.0	0.6 ± 0.0
shoot	43.2 ± 0.2	2.1 ± 0.0	0.2 ± 0.0
callus	45.3 ± 0.1	3.0 ± 0.0	0.5 ± 0.0

[a] Values are expressed on a dry weight basis. [b] Mean values and standard deviations are for pooled tissue samples; duplicate analyses were performed for each tissue.

sitized (40.6%) and parasitized (42.0%) carrot plants. Parasitized carrot roots had a C concentration of 39.4% compared to 33.4% in the nonparasitized roots. This difference may be due to the higher demand for organic compounds in the parasitized roots due to broomrape attachments. It also reflects the contribution of the broomrape attachments to the composition of the parasitized root samples. Total C concentrations in broomrape tubercles and shoots were 42.6 and 43.2%, respectively.

Seeds of broomrape tended to exhibit higher concentrations of C (52.1% in dry seeds and 55.1% in germinated seeds) than did other broomrape tissues (42.6−45.3%). Similar C concentrations in the two seed types suggest that the rate of metabolism may not be as high in germinating seeds as it is in the actively growing tubercle or shoot. The concentration of C in broomrape callus (45.3%) was comparable to concentrations in the tubercles and shoot, indicating that the parasite, in the form of callus, is able to sustain its carbon requirement in the absence of the host.

Total N and total S analyses gave similar results for leaves of nonparasitized (4.3% N and 0.5% S) and parasitized (4.2% N and 0.5% S) carrot plants. On a dry weight basis, both N and S concentrations tended to be lower in the parasitized roots (2.8% N and 0.3% S) than in the nonparasitized roots (3.8% N and 0.4% S). These values are consistent with a dilution effect associated with the presence of broomrape tubercles (2.8% N and 0.6% S) and shoots (2.1% N and 0.2% S) in the parasitized root sample, but may also reflect an adverse effect of the parasite on N and S incorporation by the host plants. Comparisons of S concentrations in tubercles with S concentrations in other tissues (carrot or broomrape) suggest that the tubercle is an actively growing stage of the parasite and has a high demand for N- and S-containing compounds. Similar N and S concentrations in the tubercle and the callus (3.0% N and 0.5% S) are consistent with an ability of broomrape to sustain its nitrogen requirement in the presence of an organic N source. Other researchers (Hall et al., 1987; Igbinossa and Thalouarn, 1996; Okonkwo, 1987) have reported that parasites grown on an artificial medium containing an organic N source performed better than those grown on an inorganic N source. Broomrape seems to lack, or to possess inefficient, nitrogen-assimilating machinery as observed earlier in other parasitic plants (Lee and Stewart, 1978; McNally et al., 1983a,b, 1984; McNally and Stewart, 1987; Thalouarn et al., 1987, 1988, 1990). Total N and S concentrations in dry (1.9% N and 0.2% S) and germinated (1.7% N and 0.2% S) broomrape seeds tended to be similar to or lower than those in all other tissues of both carrot and broomrape.

Total Amino Acid Composition. The amino acid composition of hydrolysates of carrot and broomrape tissues is presented in Table 3. Concentrations of individual amino acids in leaves of parasitized carrot plants tended to be similar to or greater than those in leaves of nonparasitized carrot plants, especially tyrosine (Tyr) (38 μmol g^{-1} in the parasitized leaves versus 25 μmol g^{-1} in the nonparasitized leaves). This, coupled with similar total N (expressed as percent, dry weight basis) in leaves of nonparasitized (4.3%) and parasitized (4.2%) carrot plants (Table 2), suggests that, due to greater demand for amino acids, more N has been incorporated into amino acids in parasitized carrot plants than in nonparasitized plants. Roots of parasitized plants tended to have amino acid concentrations similar to or lower than did roots of the nonparasitized plants, except serine (Ser) (52 μmol g^{-1} in parasitized versus 42 μmol g^{-1} in nonparasitized plants) and threonine (Thr) (45 μmol g^{-1} in parasitized versus 34 μmol g^{-1} in nonparasitized plants). Stewart (1987) has shown that activity of ribulose bisphosphate carboxylase and phosphoenolpyruvate carboxylase was reduced by 80 and 50%, respectively, in *Sorghum vulgare* L. parasitized by S*triga hermonthica* L., a hemiparasitic weed, compared to nonparasitized plants. Similarly, it is possible that broomrape parasitism has altered the activity of certain photosynthetic enzymes in carrot plants, which may have impaired the supply of carbon skeletons for the synthesis of amino acids needed for amino acid-synthesizing enzymes as well as enzymes of other metabolic pathways.

Concentrations of individual amino acids tended to be similar in dry and germinated broomrape seeds (Table 3) with the exception of Glu and Arg (70 and 36 μmol g^{-1} in dry seeds and 57 and 28 μmol g^{-1} in germinated seeds, respectively). Glu, which contributes carbon skeletons for the synthesis of several other amino acids, and Arg, a storage form of nitrogen, may have been metabolized during germination. It is noted that differences in levels of Glu between dry and germinated broomrape seeds could actually reflect a difference in glutamine (Gln) levels because the analytical procedures used here may have led to the formation of Glu from Gln.

The tubercle, shoot, and callus tended to have higher individual amino acid concentrations compared to the dry and germinated seeds. Lower levels of individual amino acids in the germinated seeds, at least in part, can be attributed to respiration. During germination, any plant seed actively metabolizes its carbohydrate reserves. Part of the carbon is lost by respiration. Bar Nun and Mayer (1993) demonstrated that broomrape seeds take up and metabolize radioactive Leu during germination. Concentrations of individual amino acids in both the tubercle and the callus tended to be higher than in the shoot. This reflects the difference in the biochemistry of two different growth phases of broomrape. Despite the absence of a host, broomrape callus had an amino acid composition that was generally similar to that of the tubercle, except Glu (114 versus 100 μmol g^{-1}), Arg (72 versus 51 μmol g^{-1}), Ala (91 versus 82 μmol g^{-1}), and Lys (60 versus 53 μmol g^{-1}).

Table 3. Total Amino Acid Composition of Tissues of Carrot and Broomrape[a]

	concentration, μmol/g of DW								
	carrot								
amino acid	unparasitized		parasitized		broomrape				
	leaf [b]	root[b]	leaf [b]	root[b]	dry seed[b]	germinated seed[b]	tubercle[b]	shoot[b]	callus[b]
Asx	117 ± 16	69 ± 11	118 ± 9	73 ± 9	49 ± 4	46 ± 2	101 ± 6	83 ± 2	88 ± 1
Glx	116 ± 14	71 ± 7	121 ± 6	72 ± 6	70 ± 7	57 ± 3	100 ± 7	71 ± 2	114 ± 2
Ser	71 ± 9	42 ± 5	72 ± 3	52 ± 2	36 ± 3	35 ± 1	63 ± 3	44 ± 1	58 ± 1
Gly	124 ± 17	77 ± 4	133 ± 5	76 ± 4	57 ± 5	55 ± 3	91 ± 4	64 ± 1	79 ± 2
His	28 ± 4	17 ± 1	29 ± 2	15 ± 3	12 ± 1	9 ± 0	18 ± 1	13 ± 1	18 ± 1
Arg	64 ± 8	40 ± 3	66 ± 2	38 ± 2	36 ± 3	28 ± 2	51 ± 2	36 ± 0	72 ± 0
Thr	70 ± 11	34 ± 5	75 ± 4	45 ± 2	30 ± 2	31 ± 2	50 ± 2	35 ± 2	43 ± 1
Ala	114 ± 16	80 ± 2	120 ± 3	72 ± 4	43 ± 3	45 ± 2	82 ± 4	57 ± 1	91 ± 2
Pro	70 ± 10	48 ± 1	75 ± 2	41 ± 2	29 ± 2	30 ± 1	49 ± 3	37 ± 0	45 ± 1
Tyr	25 ± 4	13 ± 1	28 ± 1	12 ± 1	11 ± 1	8 ± 0	21 ± 2	16 ± 0	20 ± 0
Val	88 ± 13	65 ± 1	92 ± 2	56 ± 3	34 ± 3	36 ± 2	66 ± 2	46 ± 1	62 ± 1
Ile	65 ± 10	49 ± 1	67 ± 2	40 ± 2	25 ± 2	25 ± 2	48 ± 2	34 ± 1	44 ± 1
Leu	116 ± 17	75 ± 2	120 ± 3	63 ± 3	40 ± 3	41 ± 2	78 ± 2	54 ± 1	70 ± 1
Phe	61 ± 9	34 ± 1	62 ± 1	31 ± 2	20 ± 2	20 ± 1	35 ± 2	25 ± 0	31 ± 0
Lys	78 ± 13	51 ± 4	70 ± 0	41 ± 3	23 ± 2	20 ± 2	53 ± 3	41 ± 1	58 ± 1

[a] Amino acids in tissue hydrolysate are listed according to the elution sequence of phenylthiocarbamyl derivatives separated by HPLC on a Waters Pico-Tag free amino acid column (3.9 mm × 3.0 cm). DW, dry weight. [b] Mean values and standard deviations are for pooled tissue samples; triplicate analyses were performed for each tissue.

Table 4. Free Amino Acid Composition of Tissues of Carrot and Broomrape[a]

	concentration, μmol/g of DW								
	carrot								
amino acid	unparasitized		parasitized		broomrape				
	leaf [b]	root[b]	leaf [b]	root[b]	dry seed[b]	germinated seed[b]	tubercle[b]	shoot[b]	callus[b]
Asx	5.4	7.6	7.7	5.0	0.4	0.7	3.3	2.3	1.3
Glx	2.6	2.6	3.6	1.5	0.7	0.7	3.4	2.5	2.1
Ser	3.7	2.8	3.2	2.6	0.4	1.0	2.4	1.0	5.0
Gly	0.4	0.8	0.4	0.7	0.2	0.6	0.3	0.2	0.4
His	0.4	0.5	0.5	0.6	0.4	0.3	0.6	0.2	1.3
Arg	10.7	9.2	11.1	8.5	1.6	3.4	6.7	1.5	27.3
Thr	1.2	0.9	1.6	0.9	1.0	0.8	1.3	1.0	1.9
Ala	4.2	3.7	5.3	3.9	0.8	1.9	3.9	1.9	17.7
Pro	1.4	1.0	2.8	1.5	0.3	1.3	0.4	0.3	0.7
Tyr	0.3	0.2	0.5	0.0	0.5	0.4	0.4	1.1	0.5
Val	1.1	1.5	1.8	1.3	0.5	0.0	1.5	1.0	2.6
Ile	2.7	1.1	2.9	0.9	0.5	0.7	0.9	0.1	0.5
Leu	1.6	1.2	1.8	1.1	0.2	0.9	1.1	0.8	1.0
Phe	0.2	0.5	1.5	0.6	0.2	0.3	0.4	0.2	0.2
Lys	1.0	1.8	0.3	0.4	1.8	2.3	0.2	0.2	0.6

[a] Amino acids in tissue hydrolysate are listed according to the elution sequence of phenylthiocarbamyl derivatives separated by HPLC on a Waters Pico-Tag free amino acid column (3.9 mm × 3.0 cm). DW, dry weight. [b] Values are for pooled tissue samples; single analysis were performed for each tissue.

This warrants further study on the biochemical and physiological processes occurring in broomrape callus.

Free Amino Acid and Protein Amino Acid Composition. Differences in free amino acid concentrations in tissues of carrot and broomrape were notable, whereas the protein amino acid composition (data not shown) of tissues of both carrot and broomrape reflected the respective total amino acid concentrations. Asx and Glx represent concentrations of Asn + Asp and Gln + Glu, respectively (Table 4). The amides Asn and Gln tend to convert to Asp and Glu, respectively, during sample analysis. In carrot tissues, the concentrations of individual amino acids in leaves of parasitized plants tended to be similar to those in leaves of nonparasitized plants, except Asx (7.7 versus 5.4 μmol g^{-1}), Glx (3.6 versus 2.6 μmol g^{-1}), Ala (5.2 versus 4.2 μmol g^{-1}), Pro (2.8 versus 1.4 μmol g^{-1}), Val (1.8 versus 1.1 μmol g^{-1}), Phe (1.5 versus 0.2 μmol g^{-1}), and Lys (0.3 versus 1.0 μmol g^{-1}) (Table 4). This indicates that broomrape parasitization caused a shift in the levels of mostly transport forms of organic N such as Asp, Asn, Glu, Gln, and Ala in carrot leaves. Concentrations of individual amino acids in parasitized roots tended to be similar to those in roots of nonparasitized plants, except Asx (5 versus 7.6 μmol g^{-1}), Glx (1.5 versus 2.6 μmol g^{-1}), Pro (1.5 versus 1.0 μmol g^{-1}), and Lys (0.4 versus 1.8 μmol g^{-1}) (Table 4). Differences in Asx and Glx concentrations may actually reflect differences in Asn and/or Asp and Gln and/or Glu, respectively.

In tissues of broomrape, certain trends were apparent. Concentrations of individual amino acids tended to be similar or lower in the dry and germinated broomrape seeds compared to those of the tubercle, shoot, and callus except Lys (1.5 μmol g^{-1} in dry and 1.0 μmol g^{-1} in germinated seeds versus 0.2−0.6 μmol g^{-1} in the other broomrape tissues). Lower amounts of free pools of amino acids in the germinated seeds indicate a rapid incorporation of amino acids into other secondary metabolic compounds. Free amino acid concentrations of the tubercle tended to be similar to those of the roots of parasitized carrot plants, with the exception of Asx (3.3 versus 5.0 μmol g^{-1}), Glx (3.4 versus 1.5 μmol g^{-1}), Thr (1.3 versus 0.9 μmol g^{-1}), and Pro (0.4 versus 1.5 μmol g^{-1}) (Table 4). This is evidence of the close

association between the root and broomrape tubercle. The tubercle could be selectively accumulating Asx and Pro. Also, the host may be sythesizing Glx and Thr in excess to replenish their free pools. The concentrations of Asx and Glx of broomrape tissues (tubercle and shoot) (Table 4) were generally higher than those of the other amino acids except Ser in callus (5 μmol g^{-1}), Arg and Ala in the tubercle (6.7 and 3.9 μmol g^{-1}, respectively), and callus (27.3 and 17.7 μmol g^{-1}, respectively). Analysis of xylem sap of *Striga hermonthica* parasitizing *S. vulgare* indicated that the main nitrogen components were Asp, Asn, and Glu (Stewart 1987). The concentrations of Arg and Ala in broomrape callus were higher than those of other amino acids in any tissue.

In conclusion, broomrape caused certain changes both in protein and in free amino acid pools of the host, carrot. Future studies should focus on assaying the activity of selected amino acid synthesizing enzymes in parasitized and nonparasitized host plants. Following the fate of ^{14}C-labeled or ^{15}N-labeled organic N substrates, Ala, for example, in host plants parasitized by broomrape should give some interesting insights into nitrogen metabolism by broomrape. Much is left to be learned about the biochemistry and physiology of broomrape, which may be a primary reason for lack of convenient methods to manage this destructive parasitic weed.

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