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Partitioning of Sugar between Growth and Nitrate Reduction in Cotton Roots

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JOHN W. RADIN, LINDA L. PARKER, AND CHARLES R. SELL[1]
Science and Education Administration, Western Cotton Research Laboratory, United States Department of Agriculture, Phoenix, Arizona 85040

ABSTRACT

The level of endogenous sugars was inversely related to nitrate availability in young cotton (*Gossypium hirsutum* L.) plants, with high nitrate causing a greater decline in sugar content of roots than of shoots. High nitrate (low sugar) plants also displayed relatively more shoot growth and less root growth than low nitrate (high sugar) plants. These data are consistent with the theory that roots are poor competitors for sugar, and that sugar supply is a major factor limiting root growth *in vivo*.

The effects of endogenous sugar level on root growth and on nitrate reductase activity in the root were different. When root sugar level was experimentally controlled by varying nitrate concentration in the nutrient solution, root growth was less sensitive than nitrate reductase activity to sugar deficiency. Also, in sterile root tips cultured on media containing a wide range of sucrose concentrations, growth rate was considerably less sensitive to endogenous sugar deficiency than was nitrate assimilation rate. Similarly, in plants which were detopped or girdled, nitrate reductase activity in the roots declined more rapidly than did root sugars, especially glucose and fructose. These results suggest that when sugar is deficient, cotton roots preferentially use it for growth at the expense of nitrate reduction.

Roots are considered poor competitors within a plant for photosynthetic assimilates (4, 20). When available carbohydrate decreases (*e.g.* with low irradiance or low atmospheric CO_2 levels) root growth is typically curtailed more than shoot growth. Conversely, when carbohydrate accumulates root growth increases more than shoot growth. It is evident that sugar supply is one of the most important factors limiting growth of the root system. The manner in which this sugar is partitioned among the various energy-requiring processes must therefore profoundly affect root functioning.

Assimilation of NO_3^- requires metabolic energy and C for amino acid skeletons, both of which are supplied by sugar in a heterotrophic organ such as a root. NO_3^- is an important competitor for sugar in roots (4, 20), and plants grown on high levels of NO_3^- display shoot to root ratios characteristic of low sugar plants (10, 15, 18, 19). The effect of NO_3^- on roots is particularly interesting and complex because it induces increased activity of NR[2] (the first enzyme of the NO_3^- assimilation pathway) at the same time that it depletes the roots' supply of sugar which supports NO_3^- assimilation. In this report we consider the question of whether sugar level affects growth and NO_3^- assimilation differently in roots of cotton plants.

MATERIALS AND METHODS

Plant Culture. Seeds of cotton (*Gossypium hirsutum* L. cv. Deltapine 16) were germinated in moist Vermiculite at 30 C and transferred after 3 days to buckets containing 16 liters of aerated nutrient solution. Each bucket contained six plants. The half-strength Hoagland solution was modified to contain as the sole N source either KNO_3 or urea at specified concentrations. The buckets containing the plants were placed in a growth chamber with a 14-hr daylength (240 μE m^{-2} sec^{-1} photosynthetically active quantum flux density) and maximum and minimum temperatures of 29 and 18 C, respectively. Plants were analyzed 2 weeks after transfer to the growth chamber. Nutrient solutions were not changed during the 2-week growth period. The solution pH was monitored but no adjustments were necessary.

Girdling and Excision. After 2 weeks, plants grown on 1 mM NO_3^- were selected for uniformity and divided into three groups. Plants in the first group were detopped just below the cotyledonary node, and the roots were left in the nutrient solution. Plants in the second group were girdled with a jet of steam below the cotyledonary node. The steam caused extensive necrosis within a 1-cm length of stem, but no wilting, and microscopic examination showed no damage to xylem vessels. Plants in the third group were maintained as controls. Plants were removed at intervals for 24 hr after treatment for assay and analysis.

Nitrate Reductase Assays. Procedures were similar to those described earlier (13). Whole root systems or shoot systems were weighed and incubated at 30 C in conical flasks containing 100 mM phosphate buffer (pH 7.5) with 30 mM KNO_3 and 0.13 M 1-propanol (1%, v/v). Before incubation the air was removed and replaced with N_2 in two cycles of evacuation. The reaction was stopped in an ice bath and aliquots were immediately removed for NO_2^- determination. Each value reported is the mean of three to six replicates.

Sterile Root Culture. Cotton seeds were sterilized in 0.1% (w/v) $HgCl_2$, germinated, and the root tips transferred to liquid medium (13) under standard aseptic conditions. The KNO_3 concentration was either 0 or 3 mM, and the sucrose concentration was 0, 0.06, 0.6, 6, or 60 g/l. Each 50-ml flask contained 20 ml of medium and two roots, with 10 flasks/treatment. The roots were cultured on a rotary shaker for 5 days at room temperature before measurement and analysis.

Tissue Analyses. Plant tissues to be analyzed were dried at 70 C in a forced-draft oven and ground to pass a 40-mesh screen. Both amino acids and sugars were extracted with ethanol-water (70:30, v/v) containing 1% (v/v) concentrated HCl. The ninhydrin-reactive N (9) of the extracts was measured with a glycine standard curve, wth all values reported as glycine-equivalents. Total soluble sugars were determined colorimetrically with phenol-H_2SO_4 (5) and are reported as glucose-equivalents. In some experiments the sugars were extracted and individually determined by GLC (6). L-Arabinose was added to extracts as an

[1] Present address: USDA, SEA, Western Regional Research Center, 800 Buchanan Street, Berkeley, California 94710.

[2] Abbreviation: NR: nitrate reductase.

internal standard.

Samples of the sterile cultured roots were digested in concentrated H_2SO_4 and NH_3 in the digests was determined by a Conway microdiffusion procedure (17). The difference in reduced N content of the NO_3^--grown roots and the NO_3^--free controls was converted to a rate of assimilation (units: μmol hr^{-1} g fresh $weight^{-1}$) by the formula of Loneragan and Asher (8). Nitrate was extracted separately and determined (3).

RESULTS

When plants were grown on several concentrations of NO_3^- or urea as the sole N source, the shoot to root ratios were inversely related to soluble sugar concentrations in the roots (Fig. 1). For a given level of N in the nutrient solution, the NO_3^--grown plants contained less root sugar than the urea-grown plants, presumably because of the energy requirement for NO_3^- reduction. Even though high NO_3^- promoted relatively greater shoot growth than root growth, it also produced greater declines in sugar levels of roots than in shoots (Fig. 2). For this reason, the changes in sugar level could not have arisen simply from dilution by growth. These data are consistent with the classical theory that shoot to root ratios reflect sugar levels. However, urea-grown and No_3^--grown plants displayed rather different relationships between the shoot to root ratio and root sugar level, suggesting involvement of some other factor(s) also (Fig.1).

Nutrient NO_3^- level affected root and shoot NR activities differently (Table I). In the shoot, activity increased with increasing NO_3^- concentration, reflecting the greater availability of the inducer in the leaves. In the root, NR activity remained virtually constant. In an earlier study (15), plants grown on 5 mM NO_3^- had the same NR activity in the roots as plants grown on 1 mM NO_3^-, even though NO_3^- absorption, storage in the root, and transport from root to shoot were all much greater at 5 mM than at 1 mM. In contrast, in isolated root tips supplied with sugar, NR induction depended strongly upon external NO_3^- concentration up to 100 mM (12). There is little doubt that increasing nutrient NO_3^- leads to greater NO_3^- availability within the roots. The constancy of NR activities in roots of intact plants with respect to NO_3^- availability (Table I) probably resulted from the inverse relationship between NO_3^- and sugar (Fig. 2). At the same time, root growth was not constant with respect to NO_3^-, but rather increased at high NO_3^- (although to a lesser degree than shoot growth) (Table I).

The different responses to NO_3^- of root growth and root NR activity (Table I) suggested that under conditions of low sugar (high NO_3^-), growth was a better competitor for the available sugar supply than was NO_3^- assimilation. Interpretation of such data from roots of intact plants is difficult because NO_3^- caused changes in constituents other than sugar. Increased NO_3^- also caused large increases in extractable amino acids of both root and shoot (Table II). Because NR induction is apparently inhibited by amino acids in the root but not the shoot (13, 14), the different effects of NO_3^- on NR activities in shoot and root (Table I) could have been mediated by these changing amino acid levels. The following experiments were designed to test whether sugar level or amino acid level was the primary agent controlling NR activity in the roots.

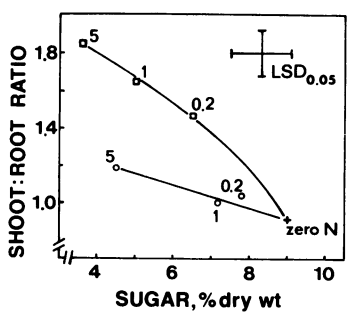

FIG. 1. Shoot to root ratio as a function of soluble sugar concentration in the roots. Plants were grown on either NO_3^- (□) or urea (○). Concentrations of actual N in nmol/l are listed next to each point.

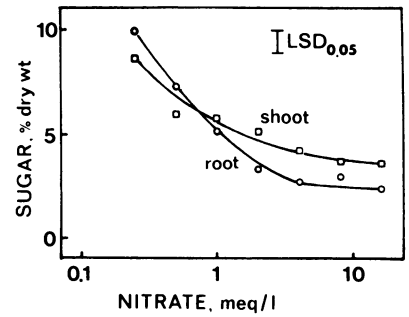

FIG. 2. Sugar concentrations in roots and shoots of plants grown at different nitrate concentrations.

Table I. Fresh weights and nitrate reductase activities of cotton plants grown on three NO_3^- levels. Plants were two weeks old at time of assay.

NO_3^- concentration	Fresh weight		Nitrate reductase activity	
	Shoot	Root	Shoot	Root
mM	g		μmoles hr^{-1} g fresh wt^{-1}	
0.2	1.08	0.79	2.95	0.83
1.0	1.53	0.99	3.71	0.83
5.0	2.00	1.17	4.80	0.84
$LSD_{0.05}$	0.44	0.25	0.77	NS

Table II. Amino acid concentrations of cotton plants grown on three NO_3^- levels. Values were determined as glycine-equivalents. Plants were two weeks old at time of harvest.

NO_3^- concentration	Amino acid concentration	
	Shoot	Root
mM	μmoles g dry wt^{-1}	
0.2	73	78
1.0	144	130
5.0	181	158
$LSD_{0.05}$	40	19

The NR activity of roots declines rapidly after excision of the shoot (Fig. 3). The rate of decline was considerably greater than that of sugars, especially glucose and fructose. In roots of steam-girdled plants, sugar level declined at the same rate as in excised roots, but NR activity dropped even faster than in excised roots (Table III). The greater loss of NR activity after girdling presumably resulted from the continuing rapid removal of NO_3^- via the xylem, a pathway largely unavailable to excised roots. Amino acid concentration declined slightly in girdled roots and increased slightly in excised roots, with neither change statistically significant (Table III). These changes in concentration bore no apparent relationship to NR activity in the roots. The relative changes in sugar levels and NR activities (Fig. 3, and Table III) again suggest that activity is quite sensitive to sugar availability.

In another approach, sterile root tips were cultured in media

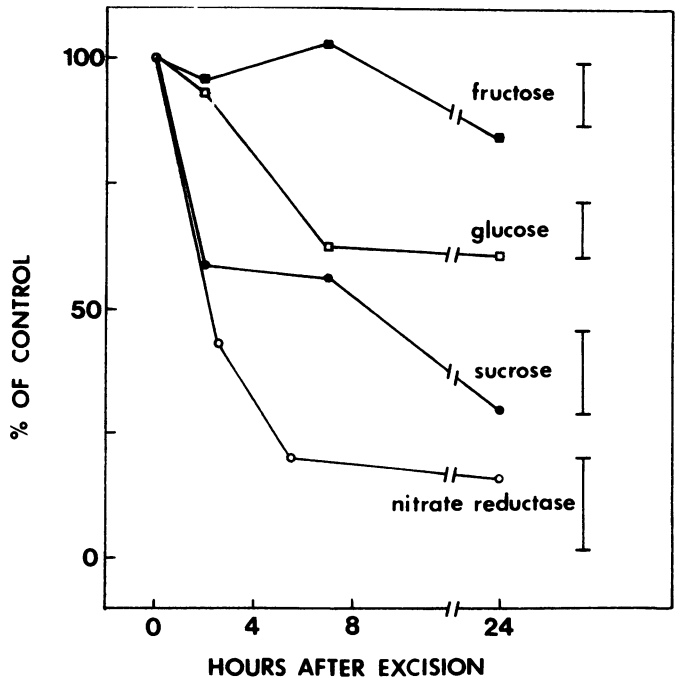

FIG. 3. Relative changes in sugar concentration and NR activity in roots after excision of shoot. Initial NR activity was 0.72 μmol hr^{-1} g^{-1} fresh weight. Initial sucrose, glucose, and fructose concentrations were 6.3, 0.6, and 0.5% of dry weight, respectively. Vertical bars represent $LSD_{0.05}$.

Table III. Nitrate reductase activities and amino acid and sugar concentrations of roots 4 hr after excision or girdling. Plants were grown on 1 mM nitrate. Amino acids and sugars were determined as glycine-equivalents and glucose-equivalents, respectively.

Treatment	Nitrate reductase activity	Amino acid concentration	Sugar concentration
	μmoles hr^{-1} g fresh wt^{-1}	μmoles g dry wt^{-1}	% dry wt
Control	0.77	107	4.8
Excised	0.27	113	2.4
Girdled	0.15	95	2.4
$LSD_{0.05}$	0.08	NS	1.0

containing a wide range of sucrose concentrations and 0 or 3 mM KNO_3. As expected, the reduced N content of NO_3^--free roots was constant with respect to both sugar level and age (data not shown). The reduced N content of NO_3^--grown roots was strongly affected by sugar level (Fig. 4). Assimilation of NO_3^- in these roots was clearly more sensitive to sugar deficiency than was growth. At low sugar levels the production of reduced N was essentially zero even though sugar supply was enough to support a growth rate of up to 0.3 mm/hr (Fig. 4). Tissue NO_3^--N concentrations ranged from 2.9 to 1.2% of dry weight at the lowest and highest sugar levels, respectively, indicating that NO_3^- absorption was not limiting assimilation.

DISCUSSION

Two separate observations—changes in NR activity of roots after blockage of the phloem or removal of the shoot (Fig. 3 and Table III), and the effects of sugar supply on growth and NO_3^- assimilation in sterile cultured roots (Fig. 4)—support the initial suggestion that NO_3^- reduction in cotton roots is extremely sensitive to sugar supply. Of the root sugars, glucose and fructose showed moderate or small changes after excision of the shoot, and

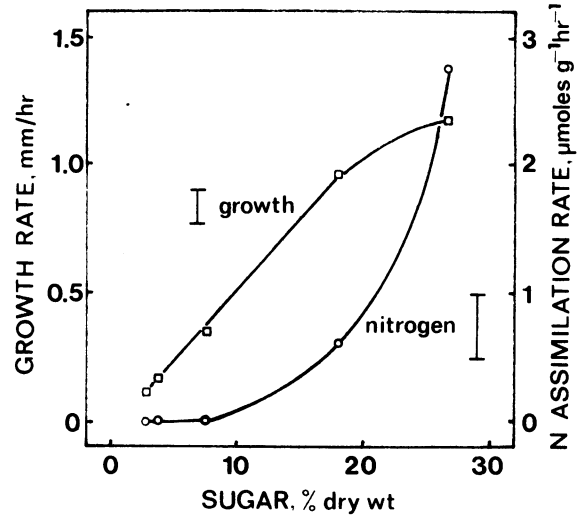

FIG. 4. Growth and nitrate assimilation rates of sterile root tips as a function of their endogenous sugar concentration. Roots were cultured in media containing sucrose concentrations of 0, 0.06, 0.6, 6, or 60 g/l. Vertical bars represent $LSD_{0.05}$.

only sucrose declined substantially (Fig. 3). Because the first two sugars are essentially intermediates between sucrose and the production of energy by respiration, it is unlikely that the sugar sensitivity of root NR activity results simply from a general deficiency of substrate for respiration. Rather, the diversion of root sugar into the process of NO_3^- reduction is probably regulated in some fashion. The lesser sensitivity of root growth to sugar deficiency implies that it maintains priority over NO_3^- reduction for available sugar.

NR induction in cotton roots is inhibited by some amino acids, notably glutamine and asparagine (13, 14). As the NO_3^- supply is increased, cotton roots become less dependent upon N assimilated in the root itself and more dependent upon amino acids imported from the shoot (15). Do the amino acids imported at high NO_3^- levels limit NO_3^- reduction in the root, and thereby direct sugar toward other processes? Although this attractive mechanism may operate in intact cotton plants, the regulated partitioning of sugar persisted even after isolation of the root system (Figs. 3 and 4, and Table III). At least one additional mechanism must therefore be suppressing NO_3^- reduction in roots at low sugar levels. This additional mechanism makes the possible involvement of feedback inhibition of NR in the partitioning phenomenon still a moot question.

It has long been theorized that sugar supply can control ion uptake (11). Two observations discount the possibility that such an effect on nitrate uptake could account for the present results. First, sterile root tips cultured with low sugar contained ample NO_3^- for assimilation. Second, in earlier work (15) the ratio of NO_3^- to reduced N in bleeding sap of detopped roots increased with time after detopping, implying that NO_3^- assimilation declined faster than NO_3^- uptake and transport into the xylem. This idence does not preclude the suggestion of Aslam and coworkers (1, 2) that glucose deficiency can divert incoming NO_3^- into the storage pool, thereby rendering it unavailable as either inducer or substrate of NR.

Cotton is a species in which most NO_3^- reduction occurs in the shoot (15). In those plants (*e.g.* pea) in which the shoot depends upon the root for most of its N, partitioning of root sugar between growth and NO_3^- assimilation might be expected to differ from that in cotton, as discrimination against NO_3^- metabolism would affect the plant's major source of reduced N. Species appear to respond to NO_3^- in either of two ways: root growth increased with increasing NO_3^- in plants which reduce most NO_3^- in the leaves

(*Xanthium* [19], cucumber [10], cotton), whereas root growth decreased with increasing NO_3^- in plants which reduce most NO_3^- in the roots (field pea [19], garden pea [10]). Thus, there is some indirect evidence against regulated partitioning of root sugar in the latter group.

The effects of sugar supply on NO_3^- reduction in roots need to be viewed in the context of the entire network of NO_3^--processing steps, *i.e.* absorption, efflux, movement between pools, transport into the xylem, etc. Considerable evidence suggests that the various steps respond differently to substances imported via the phloem (7). In the present experiments, NO_3^- absorption was probably not sugar-limited to the same extent as root NO_3^- reduction (see earlier discussion). There seems to be little basis for the *a priori* assumption of equal priorities for root growth and NO_3^- absorption (*e.g.* 16) or for the assumption of any particular priorities for any sugar-dependent root function. For the most part, an experimental basis for such assignments has yet to be developed.

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LITERATURE CITED

1. ASLAM M, A OAKS 1975 Effect of glucose in the induction of nitrate reductase in corn roots. Plant Physiol 56: 634–639
2. ASLAM M, A OAKS, RC HUFFAKER 1976 Effect of light and glucose on the induction of nitrate reductase and on the distribution of nitrate in etiolated barley leaves. Plant Physiol 58: 588–591
3. CATALDO DA, M HAROON, LE SCHRADER, VL YOUNGS 1975 Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 6: 71–80
4. CURTIS OF, DG CLARK 1950 Introduction to Plant Physiology. McGraw-Hill, New York
5. DUBOIS M, KA GILLES, JK HAMILTON, PA REBERS, F SMITH 1956 Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350–356
6. GUINN G 1974 Abscission of cotton floral buds and bolls as influenced by factors affecting photosynthesis and respiration. Crop Sci 14: 291–293
7. JACKSON WA 1978 Nitrate acquisition and assimilation by higher plants: processes in the root system. *In* DR Nielsen and JG MacDonald, eds, Nitrogen in the Environment, Vol 2. Academic Press, New York, pp 45–88
8. LONERAGAN JF, CJ ASHER 1967 Response of plants to phosphate concentration in solution culture. II. Rate of phosphate absorption and its relation to growth. Soil Sci 103: 311–318
9. MOORE S, WH STEIN 1948 Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol Chem 176: 367–388
10. OLDAY FS, AV BARKER, DN MAYNARD 1976 A physiological basis for different patterns of nitrate accumulation in cucumber and pea. J Am Soc Hort Sci 101: 219–221
11. PITMAN MG, WJ CRAM 1977 Regulation of ion content in whole plants. *In* DH Jennings, ed, Integration of Activity in the Higher Plant (Symposium XXXI of the Society for Experimental Biology). Cambridge University Press, Cambridge, pp 391–424
12. RADIN JW 1974 Distribution and development of nitrate reductase activity in germinating cotton seedlings. Plant Physiol 53: 458–463
13. RADIN JW 1975 Differential regulation of nitrate reductase induction in roots and shoots of cotton plants. Plant Physiol 55: 178–182
14. RADIN JW 1977 Amino acid interactions in the regulation of nitrate reductase induction of cotton root tips. Plant Physiol 60: 467–469
15. RADIN JW 1977 Contribution of the root system to nitrate assimilation in whole cotton plants. Aust J Plant Physiol 4: 811–819
16. RAPER CD, LR PARSONS, DT PATTERSON, PJ KRAMER 1977 Relationship between growth and nitrogen accumulation for vegetative cotton and soybean plants. Bot Gaz 138: 129–137
17. SANFORD G, JN CARTER, EC SIMPSON JR, DE SCHWANINGER 1973 Nitrate determination by a modified Conway microdiffusion method. J Assoc Off Agric Chem 56: 1365–1368
18. TURNER TW 1922 Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. Am J Bot 9: 415–445
19. WALLACE W, JS PATE 1967 Nitrate assimilation in higher plants with special reference to the cocklebur (*Xanthium pennsylvanicum* Wallr.). Ann Bot 31: 213–228
20. WAREING PF, J PATRICK 1975 Source-sink relations and the partition of assimilates in the plant. *In* JP Cooper, ed, Photosynthesis and Productivity in Different Environments. Cambridge University Press, Cambridge, pp 481–499