THE NITROGEN CONTENT OF OAT CHLOROPLASTS

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I. *Background of the work.* Many techniques have been utilized to separate chloroplast material from the remainder of the plant cell. Chibnall (1) found that the protoplasmic suspension obtained by grinding spinach leaves in water could be fractionated by filtration through paper pulp. Nuclear and chloroplastic materials were retained on the filter, the cytoplasmic material passing through. Since nuclear material in leaves is very small in amount, the retained material was considered as essentially "chloroplastic material." Channon and Chibnall (2) ground cabbage leaves in water and warmed the suspension to flocculate the dispersed protoplasm. The ether extract of the precipitate was considered to be mainly of chloroplast origin, since cytoplasm contains little ether-soluble material. Menke (3) found that suspensions of spinach leaves ground in water would deposit chloroplastic material upon addition of ammonium sulfate.

All of these techniques may be criticized on the ground that they do not yield intact chloroplasts; hence some of the chloroplast material may remain unextracted and some cytoplasm may be carried along as an impurity in the extract. These objections are, to a large extent, removed by the technique of Granick (4). This experimenter ground turgid leaves of tobacco and tomato in 0.5 molar glucose and succeeded in obtaining intact chloroplasts in suspension, along with the remainder of the protoplasmic material. Short, slow centrifugation removed most of the non-green protoplasm, whereas longer, more rapid centrifugation deposited the chloroplasts. The plastids could then be washed by resuspending in water and flocculated by recentrifugation. Microscopic examination of the centrifugate revealed intact plastids almost entirely devoid of cytoplasmic granules. Furthermore, by colorimetric comparison of acetone extracts of leaf and chloroplasts, he was able to determine what per cent of total leaf chloroplasts he had in his suspension. This made possible the determination of total chloroplast nitrogen per leaf.

II. *Object of this work.* This work was undertaken: 1) To discover whether the technique of Granick is applicable to the leaves of cereal grasses, which contain much fibrous material. 2) To obtain information on the relative importance of chloroplasts and cytoplasm as synthesizers of protein in leaves of cereals. 3) To trace the course of protein synthesis in the chloroplasts and cytoplasm of a single leaf.

III. *The nitrogen content of green and chlorotic leaves and chloroplasts at various ages.* Kherson oats were sown in composted Flanagan silt loam in the greenhouse, and were allowed to grow until the fifth leaf was 1-3 cm. long. Leaves of the same size and physiological condition were then harvested in groups of 40. Ten of the leaves were dried for two hours in a forced-draught oven at 80° C., were then weighed to get the average dry weight per leaf, and finally were ground to a fine powder in a Wiley mill. This powder was analyzed to get the nitrogen content per leaf. The remaining thirty leaves were treated to extract the chloroplasts, according to the procedure of Granick (4). The extracted chloroplasts were analyzed for nitrogen simultaneously with the dried leaf material.

Nitrogen was determined by the microkjeldahl method of Pregl (6), all analyses, as well as blanks, being run in duplicate. The extracted chloroplasts, usually as a 2 cc. suspension, were pipetted into the digestion flasks. 20-30 mg. samples of the dried leaves were placed in other flasks. To the samples were added 2 cc. of $CuSO_4$-H_2SO_4 digestion mixture and a pinch of K_2SO_4. The mixture was then digested 15-30 minutes until clear. The digested mixture was then transferred into the still, made alkaline with excess NaOH, and steam-distilled into a flask containing 20 cc. of boric acid (1 lb. per 10 L.). The ammonia was titrated with .01 N HCl using

TABLE 1.—THE NITROGEN CONTENT OF OAT CHLOROPLASTS

Average length per leaf	Average dry wt. per leaf	Physiological Condition	Chloroplast N per leaf	Total N per leaf	N as % of dry wt. per leaf	% of total leaf N in the chloroplasts
7.2 cm	4.6 mg	Green	.084 mg	.285 mg	6.20%	30.1%
9.3	5.5	"	.119	.287	5.26	41.3
14.6	8.4	"	.137	.470	5.60	29.2
11.7	10.0	Chlorotic	.078	.261	2.61	30.0
17.6	17.7	"	.168	.495	2.79	34.2

bromcresol green as an indicator. Results were expressed as milligrams of nitrogen per leaf. The data are presented in Table I, all values being the means of two closely checking sets of results.

It can be seen that in both green and chlorotic leaves, at all stages of growth studied, the chloroplasts contain about 30-40% of the total leaf nitrogen. This checks well with the results of Granick (5), who considers further that about 80% of both leaf and chloroplast nitrogen is protein nitrogen. This would indicate that the chloroplasts synthesize large amounts of leaf protein.

IV. *The course of protein synthesis in a single leaf.* It was decided to use the first leaf of oat for this experiment, since this would avoid complications due to the translocation of soluble nitrogenous materials from other leaves. Oat plants grown in 2-gallon porcelain crocks were thinned until twelve uniform plants were left in each crock. The first leaf was harvested periodically at various stages of development, and analyzed for total and chloroplast nitrogen. The difference between these two values was called "cytoplasmic nitrogen." The last group of leaves was harvested when the ligule was visible and the leaves had apparently attained their maximum length. The results of the analyses are presented graphically in Figure 1. Again, it is found that the chloroplast nitrogen is about 30% of the total leaf nitrogen at all stages of development studied.

V. *Summary and conclusions.* **A.** The technique of Granick for extracting intact chloroplasts from leaves is applicable to oat leaves. **B.** The chloroplasts contain about 30-40% of the total leaf nitrogen in both green and chlorotic leaves at all ages studied. **C.** In any one leaf, chloroplasts produce about 30% of the total protein, the "cytoplasm" producing the remainder.

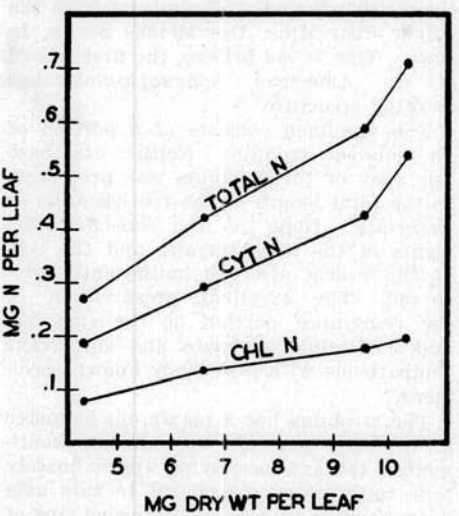

Fig. 1.—The nitrogen content of oat chloroplasts and entire leaf at various stages of development.

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