EFFECT OF IRON CHLOROSIS ON PROTEIN FRACTIONS OF CORN LEAF TISSUE[1, 2]

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Many concepts of the physiological changes in plants, as related to the organic and inorganic constituents, were determined by bulk analysis. Today a galaxy of new physical and chemical techniques is available to isolate and study individual components of cell tissue, thus permitting far more detailed work.

During the last few years the biochemistry, synthesis, and metabolism of plant proteins have been extensively investigated because of their association with enzymes and the fundamental role these molecules play in most biological phenomena (5, 9, 10, 11, 12, 14, 15, 16). The leaf is a seat of enzyme activity and leaf proteins are undoubtedly involved in a major way in photosynthetic reactions. The location of any protein relative to the architecture of the cell, and its separation in pure form from the heterogeneous substances making up the leaf, present extraction problems. Nevertheless, there is much interest in the physical, chemical, and enzymatic properties of cytoplasmic proteins as well as leaf proteins that are thought to be associated with enzymes in different particulate components inside the cell.

The present investigation considers the effect of iron chlorosis on the pattern of protein distribution in corn leaf tissue.

EXPERIMENTAL DETAILS & METHODS

After 7 days in a germinator, corn plants (*Zea mays* L.) were transplanted to Hoagland's No. 2 nutrient solution (6) and grown under controlled conditions of light intensity and temperature. The solutions were adjusted daily to pH 5.5, aerated continuously, and changed every 4 days. Seven days after transplanting, one treatment group received complete nutrients while the other received all nutrients except iron. Each treatment group consisted of five jars with two plants in each, all of which constituted one replication. A total of six replications was run. Leaves from ten plants (5 × 2) were sampled in each treatment per replication.

Three weeks after transplanting, or two weeks after treatment was begun, the plants were harvested. At this stage the main difference between the two groups of plants was the chlorosis in the minus iron treatment. For analysis the plants were taken to a cold room and the second, third, and fourth leaves from the top were harvested and pooled. Any surface dust was wiped off with a damp sponge. Along with the midrib, 4-inch portions from both the tip and the base of the leaves were discarded, and the remaining parts were torn into half inch segments. From this composited leaf material a 25 g sample was preserved in a deep freeze for fractionation analysis. Nitrogen and protein determinations were made on subsamples of the remaining fresh leaf tissue by the Kjeldahl (1) and folin phenol reagent (11) methods while phosphorus and iron were determined on a wet digestion of the dry plant tissue (8).

For ease of homogenizing, each sample was divided at the time of analysis by weighing into two equal portions. The tissue was allowed to thaw at room temperature for 15 minutes, taken to the cold room, and then transferred to a chilled stainless steel jar (200 ml) lined with teflon. The tissue was allowed to soak in 50 ml water for 5 minutes and then homogenized for 1½ minutes in an omnimixer (Servall model OM) operated at full speed. The jar was kept in a crushed ice bath to prevent a rise in temperature during homogenization. The resulting slurry was filtered through four folds of cheese cloth.

The homogenates from the two portions were mixed and subjected to fractionation by differential centrifugation in a cold room. Relative centrifugal forces up to 17,000 × g were obtained using nylon 50 ml tubes in a Servall type SS-34 super-speed centrifuge with remote speed control. For higher centrifugal forces, the refrigerated Spinco model L ultra centrifuge with rotor head No. 40 and 10 ml celluloid tubes were used. The scheme of fractionation procedure as well as the various particles identified under a phase microscope is summarized in figure 1.

After spinning the homogenate at the appropriate speed, the supernatant was transferred by means of a mechanical pipette to another tube for successive centrifugation. Each sediment was washed once with water and recentrifuged, and the wash then transferred to the succeeding fraction. The pellets obtained in different fractions were resuspended in water by homogenizing in a Ten Broeck homogenizer, and the final volume was made to 50 ml. The clear supernatant obtained in the last fraction was made to 150 ml.

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RESULTS & DISCUSSION

The total nitrogen and protein contents of the two sets of leaf samples are given in table I. There was no difference between the nitrogen contents of green and chlorotic leaves. The Kjeldahl method includes both protein and non-protein nitrogen. In contrast with nitrogen, the protein level for chlorotic leaves was 25 % below that of normal leaves.

Preparations from the various fractions were examined under a phase microscope using an oil-immersion lens. For this purpose the sediments in different fractions were suspended in water by gentle shaking. The particles in the prepared suspensions are described in figure 1.

The results for proteins in this experiment are much the same as for the corresponding fractions of McClendon's work (12). Although in the present investigation exactly the same centrifugal forces as those published by Gordon (5) were employed, results from the two studies do not agree except in the last fractions. This may be attributed to the difference in density of mediums used. Gordon (5) used 0.2 M phosphate buffer in 0.3 M sucrose solution.

No reference was available with which to compare the effect of iron chlorosis on the pattern of protein fractions in leaf tissue.

The cytoplasmic or soluble proteins did not change much with iron deficiency. It is suggested that further fractionation of this fraction by electrophoresis may show differences.

Bonner (3) has reported that chloroplastic proteins accounted for roughly 40 % of the total nitrogen in spinach leaves; our reduced protein levels in corn leaves are correlated with a reduction in chloroplasts. The iron that is active in chlorophyll formation is actually bound in organic combination in the chloroplasts and is essential for initiating synthesis of chloroplastic proteins in leaves (2, 9). Iron deficiency has therefore suppressed the protein synthesis in chlorotic leaves. However, nitrogen absorption continued in spite of low demand in the leaves for protein synthesis and resulted in the accumulation of non-protein nitrogen in chlorotic leaves, as indicated by a low protein to nitrogen ratio in these leaves when compared with the normal ones. Similar observations have been made by other investigators (2, 4, 7, 13). Bennett (2) reported that iron deficiency leads to an increase in amino acid nitrogen and a decrease in protein nitrogen. DeKock and Morrison (4) correlated free amino acids with phosphorus/iron (P/Fe) ratio in leaves; the acids are high when the ratio is high and decline as the ratio declines. This, they say, was true irrespective of the cause of chlorosis. In the present experiment also, the P/Fe ratio was higher in chlorotic than in green leaves (table I). This might have contributed to the higher concentration of non-protein nitrogen in chlorotic leaves.

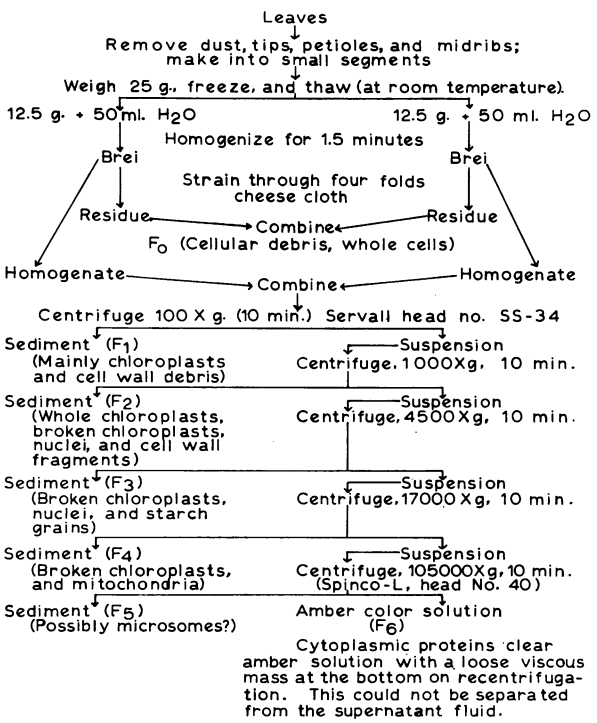

FIG. 1. Flow sheet for fractionation of corn leaf proteins.

TABLE I

NITROGEN, PROTEIN, PHOSPHORUS, & IRON IN NORMAL & CHLOROTIC CORN LEAVES

REP. NO.	PROTEIN	N	PROT./N	P	Fe	P/Fe
	% *on dry wt*			meq/100g *dry wt*		
			Green leaves			
1	29.5	4.3	6.9	4.9	0.23	21
2	34.2	4.5	7.6	4.5	0.22	20
3	29.0	4.4	6.6	4.2	0.20	21
4	32.4	4.2	7.7	5.2	0.25	21
5	32.4	4.3	7.5	4.3	0.23	19
Mean	31.5	4.3	7.3	4.6	0.23	20
			Chlorotic leaves			
1	21.1	3.9	5.4	3.4	0.02	170
2	22.8	4.1	5.6	3.4	0.02	170
3	25.2	4.2	6.0	3.3	0.02	165
4	23.7	4.1	5.8	2.6	0.02	130
5	25.1	4.1	6.1	3.6	0.02	180
Mean	23.6	4.1	5.8	3.3	0.02	165

Increases of free amino acids (7) and of aspartic and glutamic amino acids (13) in chlorotic leaves have been reported.

In both sets of leaf samples, the protein/N ratio is different from the conventional factor, 6.25. Incomplete recovery of total nitrogen in the Kjeldahl method may account for this, although salicylic acid and sodium thiosulphate were used for the reduction of nitrites and nitrates to ammonium salts. The reduction is incomplete, especially when the sample is wet

TABLE II

PROTEIN IN CORN LEAF FRACTIONS

REPLICATIONS	1	2	3	4	5	6	Avg.
Fractions			*% on dry wt*				
			Green leaves				
F_1	2.54	2.92	2.97	2.86	2.75	2.86	2.82
F_2	0.86	0.92	0.74	0.69	0.51	0.77	0.75
F_3	0.83	0.86	0.87	0.51	0.34	0.62	0.62
F_4	0.82	0.86	0.37	0.67	0.63	0.69	0.66
F_5	0.38	0.37	0.17	0.15	0.11	0.30	0.25
F_6	3.95	3.55	3.46	3.77	3.03	3.46	3.37
Total	9.38	9.48	8.38	7.74	7.37	8.70	8.47
			Chlorotic leaves				
F_1	0.57	0.54	0.49	0.37	0.49	0.54	0.50
F_2	0.63	0.74	0.54	0.48	0.57	0.54	0.56
F_3	0.51	0.51	0.57	0.66	0.54	0.49	0.55
F_4	0.57	0.54	0.31	0.37	0.43	0.48	0.45
F_5	0.09	0.23	0.20	0.23	0.23	0.23	0.20
F_6	3.40	3.43	2.95	3.11	3.20	3.29	3.23
Total	5.77	5.82	5.06	4.90	5.46	5.57	5 49

(1). In the present experiment green samples containing more than 80 % moisture were used directly for nitrogen estimation. Published values for the nitrogen contents of purified proteins range from 14 to 19 %, and use of the traditional factor, 6.25, depends upon the assumption that all proteins contain 16 % nitrogen. In chlorotic leaves the low protein/N ratio is due to the presence of non-protein nitrogen, as already explained above.

Protein analysis of various fractions (table II) revealed F_6 contained the greatest amount, with a gradual decrease in protein from F_1 to F_5. The pattern of leaf protein distribution was not the same in plants from both treatments. It is interesting that while there is an appreciable difference in protein contents of the corresponding fractions of the two kinds of leaves from F_1 to F_5, very little difference is noticed in the soluble fraction, i.e. in F_6. While the fractions from F_1 to F_5 were sediments of solid particles, the F_6 fraction represented the soluble proteins of the leaf. These are considered as cytoplasmic proteins. The lower protein/N ratio in chlorotic leaves was attributed to the proportionately large amounts of soluble nitrogenous compounds not utilized in the synthesis of conjugated proteins.

Gradual decrease in protein content in the fractions of green leaves is mainly due to the decrease in the bulk of the sediment obtained at different centrifugal forces. From this data it is not possible to infer the actual protein content of particles representing these fractions. The data in table II simply show relative distribution of these fractions in the whole leaf.

SUMMARY

The effect of iron chlorosis on the pattern of protein distribution in corn leaf tissue was studied. Corn plants were raised under controlled conditions in solution culture, and leaf samples were collected from green and chlorotic plants. Leaf homogenates prepared from the samples were subjected to fractionation by centrifugation at varying speeds and the fractions were separated and analyzed for their protein and nitrogen contents.

There was no appreciable difference in total nitrogen from green and chlorotic leaves. A 25 % reduction was found in the protein content of chlorotic leaves, which gave a reduction in the protein to nitrogen ratio.

The fractions were examined under a phase microscope with chloroplasts found mainly in the first fraction, nuclei and starch grains in the second and third fraction, mitochondria in the fourth fraction, and possibly microsomes in the last fraction sedimented.

Iron chlorosis caused an 82 % reduction in the protein content of the chloroplastic fraction of the leaf. The quantity of proteins of the supernatant from the last fraction sedimented (cytoplasmic proteins) was apparently not changed by chlorosis.

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GERMINATION INHIBITORS RELATED TO DORMANCY IN WHEAT SEEDS [1, 2]

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Dormancy of seeds at maturity and for an extended period of time following harvest is of great importance to the life cycle of plants. Of the many investigations of this subject, those few which pertain to wheat indicate that varieties with red kernels are more resistant to sprouting than those with white kernels (1, 5, 6, 7, 10, 12). All wheat varieties with white kernel color readily sprout at maturity (3); no exception has been found to date. In the varieties with red kernel color, varying degrees of dormancy ranging from strong to no dormancy at maturity and in the post harvest period have been observed. We initiated this study to determine the nature of dormancy in wheat and the possibility of breeding white wheat varieties with some of the dormancy characteristics of the red wheats.

Many investigators have suggested numerous causes for dormancy in wheat. We have reinvestigated the following main hypotheses: A: impermeability of the seed coat to oxygen, B: impermeability of the seed coat to water, C: immature or dormant embryo, D: mechanically tough seed coat, and E: the presence of germination inhibitors in the seed coat. Some of these hypotheses arise from observations of morphological differences between kernels of wheat with red and those with white seed coats (2, 6). The seed coat of the red wheats tightly covers the embryo whereas the seed coat of the white wheats is often separated from the embryo. This difference suggests that water might enter the embryo of the white wheat more easily than that of the red wheat varieties. Wellington (12) reported that the behavior of the covering or bran layers during germination indicated a difference in the mechanical properties of these layers in red and in white wheat kernels.

After presenting data to disprove the first four hypotheses for wheat dormancy, we report data on the presence and partial purification of some of the germination inhibitors in the seed coat of wheat. Mosheov (9) first demonstrated the presence of germination inhibitors in wheat kernels. Miyamoto and Everson (8) investigating dormancy in wheat found a parallelism between the degree of pigmentation and the amount of catechin and catechin-tannins in the seed coat during the late dough stage of maturity. They suggest that the precursors of the seed coat pigments inhibited the germination of the embryo.

MATERIALS & METHODS

Names, seed coat color and dormancy at maturity of the varieties of *Triticum vulgare L.* used in this study, are included in table I. The newly harvested red-seeded varieties which did not germinate on moistened filter paper after at least 8 days were classed as

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