CHANGES IN CARBOHYDRATE CONTENT OF WHEAT PLANTS DURING THE PROCESS OF HARDENING FOR DROUGHT RESISTANCE[1]

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Introduction

The process of hardening for drought resistance results in many changes in the vegetative organs of plants. PRINGSHEIM (7) has noted that pumpkin seedlings, grown under conditions of insufficient moisture, had an increased osmotic pressure in the cells and a higher resistance to drought than check plants grown in a moist soil and humid atmosphere. Since then a number of investigations have been conducted dealing directly or indirectly with the physiology of hardening. FREY (1), for example, has established that under conditions of sufficient soil moisture, plants which previously had suffered from lack of water supply were transpiring more intensely than normal plants grown in a moist soil. Studies by TUMANOV (9) have shown that sunflower plants, which had been hardened by repeated wilting, transpired 73 per cent. more moisture than non-hardened plants; while buckwheat, which had suffered from periodic drought, had an increased transpiration of 35 per cent. The same investigator states that plants which had been exposed to recurrent wilting exhibited greater intensity of assimilation after their return to a normal state. For buckwheat this increase was 50 per cent. over normal plants.

TUMANOV observed also that hardened plants had smaller cells and consequently a larger number per unit leaf area and a denser network of veins. The reaction between various parts of the plant during development was likewise changed under the effect of hardening. The leaf blade was more developed than the stem, and the root system was larger than the aboveground portion of the plant. VASSILIEV (11) has noted that the stomata were fully open when wheat was grown with a continuous optimum soil moisture of 40 per cent. of the total moisture capacity of the soil. The same wheat grown in soil saturated to 70 per cent. of its capacity suffered from insufficient water supply and the stomata of the leaves were almost closed when the moisture content in the soil was decreased to 40 per cent.

These investigations present morphological evidence about the hardening process. But such gross studies of the nature of hardening, as for example the xerophytic structure, the increased capacity of gas exchange (through stomata), and a more intensive transpiration and assimilation,

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should be amplified by more detailed physiological studies of hardened plants.

The purpose of the present investigation was to determine the changes in carbohydrate content of plants during the periods of hardening and recovery. Through the aid of carbohydrates the osmotic pressure of the plant cell may be regulated markedly. Carbohydrates are important also as defense substances, protecting the protoplasm from coagulation when it is influenced by various harmful factors. Hence to characterize more definitely the hardening process for drought resistance, it seems desirable to consider first the carbohydrates.

This investigation represents at the same time a continuation of previously published studies (**11, 12**) on the relationship of carbohydrate metabolism in wheat to soil moisture and weather conditions.

Procedure

The experimental plants were various varieties of wheat differing in their resistance to drought. They were grown in pots containing 7 kilograms of soil, were fertilized (NPK) and watered daily to maintain approximately 70 per cent. of the moisture capacity of the soil. In each container were planted all the tested varieties. Reduction in soil moisture was obtained by discontinuing the water supply. The containers were weighed daily and the moisture content adjusted as desired. Samples were taken simultaneously from all the series under comparison. The plants were cut near the ground level and used promptly.

For the determination of moisture content the plants were weighed, dried at a temperature of 100° C., and reweighed. In the calculation of water content, the dry weight of 100 gm. was taken as the basis from which to compute departures. The material was prepared for analysis according to KISEL's (**3**) method, as follows. The cut plants were put in an autoclave and killed by exposing to steam for 15 minutes. They were then hung up in a greenhouse, air-dried, and ground to fine powder which would pass through a sieve of 0.25 mm. mesh. The powder samples were preserved in desiccators until analyzed.

The analyses were conducted according to the procedure described by VASSILIEV (**11**), with the following changes: After separation from the insoluble fraction the filtrate was cleared, while passing through the filter, with animal charcoal, as suggested by LEHMANN (**5**). In addition to sugars, there were also determined the hemicelluloses by the following method: After removal of sugars, the residue was extracted with 2 per cent. of HCl on a water bath for three hours. When cool, the solution was filtered, neutralized with NaOH, and then filtered with suction a second time through animal charcoal. Further procedure with this extract was as usual. In all cases the analyses were made on the total above-ground part of the plants. Samples up to June 1 were from plants in the stage of tillering. The carbohydrate data were calculated as milligrams per gram of dry tissue.

Results

The experiments deal with three stages of the process of hardening of plants: (1) the end of the period of loss of water; (2) the succeeding day after water was applied; and (3) eight days after irrigation, when the plants should have recovered from drought.

I. Changes in carbohydrate content of wheat during period of water loss

Experiment A (May 24).—On May 6, four days after the appearance of the seedlings above-ground, irrigation of a selected number of cultures was discontinued, so that the soil moisture in these containers gradually decreased. As the number of plants per pot was not large and the weather was cool, the loss of water proceeded slowly. On May 23, at noon, the plants had lost their turgor for the first time. The soil moisture had now decreased from 70 to 36 per cent. of the total moisture capacity of the soil. On the following day, at 2:30 P.M., samples were cut from these plants and from check plants growing under conditions of sufficient water supply (watered daily to 70 per cent. of soil capacity). The results of the determination of moisture and carbohydrate content are presented in table I.

Table I shows that owing to insufficient soil moisture the water content of all plants decreased about 30–40 per cent. The total carbohydrates of the experimental plants appear to have increased in the majority of cases. Only in one instance, variety Kitchener, the least resistant type, was there a reverse condition. The total soluble carbohydrates (sugars) increased in all experimental plants and in some cases this increase was very marked, as for example in the variety Sarroubra. The two groups of sugars, monosaccharides and sucrose, which made up the total soluble carbohydrates, were with few exceptions higher in the experimental than in the check plants. The relative quantity of hemicelluloses, on the contrary, decreased in the experimental plants.

The general conclusions from this experiment are as follows: With the gradual drying up of the soil in which this wheat was raised the water content of the plants had decreased approximately 35 per cent. at the time of wilting. The total sugar content had increased in all cases, the hemicelluloses decreased, and monosaccharides, sucrose, and total carbohydrates increased in the greatest number of varieties (one exception).

TABLE I

CHANGES IN MOISTURE CONTENT AND CARBOHYDRATES OF WHEAT PLANTS DURING PERIOD OF DROUGHT, MAY 24 (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	mg.	mg.	mg.	mg.	mg.
Kitchener							
Check	667	100	118.8	29.9	78.9	9.9	20.0
Experimental	437	69	98.5	34.7	63.8	17.0	17.7
Gemtchoujina							
Check	675	100	97.8	22.6	75.3	12.8	9.8
Experimental	423	63	98.7	24.9	73.8	10.1	14.8
Caesium—0111							
Check	657	100	97.5	17.8	79.7	9.2	8.5
Experimental	418	62	108.0	29.0	79.0	12.5	16.5
Sarroubra							
Check	655	100	90.3	18.3	72.0	7.1	11.2
Experimental	437	65	107.9	51.4	56.5	15.3	36.1
Erithrospermum—341							
Check	669	100	85.1	14.8	70.3	7.1	7.7
Experimental	450	67	105.2	32.8	72.2	24.0	12.6

EXPERIMENT B (MAY 25).—This experiment was a continuation of the preceding one. The plants that began to wilt on May 23 were left without water for 24 hours. On May 25, at 2:30 P.M., or 24 hours later than the time of harvesting of the previous sample, another group of plants was cut for analysis. These specimens had a very wilted appearance and the tips of the leaves had begun to die. The results of analyses of this group are presented in table II.

It is evident from the figures in table II that the water content of the experimental plants has decreased still further and is now about 50 per cent. of the normal amount. The total carbohydrates, on the contrary, have increased, except in the variety Kitchener. But the total soluble carbohydrates (sugars) show an increase only in three varieties, two of the less drought resistant ones (Kitchener and Gemtchoujina) indicating a decrease, which was due to a conspicuous reduction in their sucrose content. And since the increase in sugar of the variety Sarroubra is slight,

TABLE II

CHANGES IN MOISTURE CONTENT AND CARBOHYDRATES OF WHEAT PLANTS AFFECTED BY EXTREME DROUGHT, MAY 25 (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Kitchener							
Check	681	100	144.7	49.1	95.3	18.4	30.7
Experimental	365	52	139.8	38.8	101.1	20.7	18.1
Gemtchoujina							
Check	636	100	125.4	39.9	85.5	17.3	22.7
Experimental	338	53	131.8	34.4	97.4	19.6	14.8
Caesium—0111							
Check	642	100	133.3	42.9	90.4	15.2	27.7
Experimental	347	52	173.9	67.9	106.0	22.3	45.6
Sarroubra							
Check	637	100	126.1	46.9	79.2	17.1	29.8
Experimental	367	58	58.5	47.9	110.6	22.9	25.0
Erithrospermum—341							
Check	678	100	119.0	30.6	88.5	11.6	18.9
Experimental	387	57	138.3	43.7	94.6	20.2	23.5

only two varieties showed a greater amount of sucrose than the check plants. Monosaccharides and hemicelluloses were present in larger quantities in all the experimental plants. Whereas the hemicellulose content in the experimental plants of the previous group was lower, it is now higher in most varieties.

The principal conclusions are: An increased loss of soil moisture has caused further decrease in the water content of the plants. This has resulted in continuous decrease in sucrose, which was noted in the first experiment, and an increase in monosaccharides and hemicelluloses. But the total carbohydrate content has decreased in most instances.

II. CHANGES IN CARBOHYDRATE CONTENT RESULTING FROM IRRIGATION OF WHEAT PLANTS THAT HAD SUFFERED FROM DROUGHT

EXPERIMENT C (MAY 24).—A group of plants which had suffered from drought were irrigated on May 23 to the same extent that normal check plants were supplied with water (70 per cent. soil saturation). On May 24, at 2: 30 P.M., samples of these plants were gathered for analysis. Table III gives the comparative records on moisture and carbohydrate content of the irrigated and non-irrigated (drought affected) plants.

TABLE III

CHANGES IN MOISTURE AND CARBOHYDRATE CONTENT OF WHEAT PLANTS AFFECTED BY DROUGHT AND SUBSEQUENTLY IRRIGATED (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	mg.	mg.	mg.	mg.	mg.
Kitchener							
Wilting	437	100	98.5	34.7	63.8	17.0	7.7
Irrigated	584	133	126.4	38.3	88.1	7.0	31.2
Gemtchoujina							
Wilting	423	100	98.7	24.9	73.8	10.1	14.8
Irrigated	564	133	113.3	17.6	95.7	5.7	11.9
Caesium-0111							
Wilting	418	100	108.0	29.0	79.0	12.5	16.5
Irrigated	564	134	123.0	21.2	101.9	5.3	15.4
Sarroubra							
Wilting	437	100	107.9	51.4	56.5	15.3	36.1
Irrigated	560	128	....	15.0	....	5.2	12.8
Erithrospermum-341							
Wilting	450	100	105.2	32.8	72.2	24.0	12.6
Irrigated	581	129	146.8	23.6	105.4	5.2	9.8

The data of table III show that, as a result of irrigation, the moisture content, total carbohydrates, and hemicelluloses increased in all varieties while sucrose and total sugars decreased in practically every case and there was a decided reduction in the monosaccharides.

Comparison of the water and carbohydrate contents of plants which had received a deficient water supply, but had subsequently been irrigated, with that of normal (check) plants is given in table IV.

It is apparent from table IV that although the experimental plants had been irrigated on the previous day they were still deficient in moisture content, yet they indicate a remarkable augmentation in carbohydrates, all of them having increased in most groups excepting the monosaccharides.

TABLE IV

WATER AND CARBOHYDRATE CONTENTS OF WHEAT PLANTS GROWN WITH DEFICIENT (EXPERIMENTAL) AND AMPLE (CHECK) WATER SUPPLY

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	mg.	mg.	mg.	mg.	mg.
Kitchener							
Check	667	100	118.8	29.9	78.9	9.9	20.4
Experimental	584	88	126.4	38.3	88.1	7.0	31.2
Gemtchoujina							
Check	675	100	97.8	22.6	75.2	12.8	9.8
Experimental	564	84	113.3	17.6	95.7	5.7	11.0
Caesium-0111							
Check	657	100	97.5	17.8	79.7	9.2	8.6
Experimental	465	86	123.1	21.2	101.9	5.8	15.4
Sarroubra							
Check	655	100	90.3	18.3	72.0	7.1	11.2
Experimental	560	86	....	15.0	....	5.2	12.8
Erithrospermum-341							
Check	669	100	85.1	14.8	70.3	7.1	7.7
Experimental	581	87	146.8	23.6	105.4	5.2	9.8

TABLE V

WATER AND CARBOHYDRATE CONTENT OF WHEAT PLANTS APPARENTLY RECOVERED FROM DROUGHT (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	mg.	mg.	mg.	mg.	mg.
Kitchener							
Check	593	100	118.7	35.4	83.3	97.4	8.0
Experimental	545	92	135.8	38.9	96.9	22.7	16.2
Gemtchoujina							
Check	608	100	116.5	44.5	72.0	28.1	16.4
Experimental	537	88	164.6	61.1	103.0	20.6	40.5
Caesium-0111							
Check	591	100	117.8	47.6	70.4	28.4	19.2
Experimental	538	91	148.9	57.8	91.1	21.2	36.6
Sarroubra							
Check	593	100	176.2	52.2	124.0	26.0	26.2
Experimental	502	85	180.6	56.1	124.5	22.7	33.4

III. Water and carbohydrate content of wheat plants recovered from drought effects due to irrigation

Experiment D (June 1).—A group of plants which had been affected by drought were watered daily, beginning May 24, in the same way as the check cultures. On June 1, at 4:00 P.M., samples were gathered simultaneously from these and the check plants. The experimental plants were now of normal green color and were growing rapidly. The results of analyses are presented in table V.

These records (table V) permit the following conclusions: Plants which had been exposed to drought but later received an ample water supply, after eight days of irrigation were still deficient in moisture content. They were, however, much higher in most forms of carbohydrates, excepting the monosaccharides.

Discussion

The process of hardening plants for drought resistance may be divided into two phases: (1) the stage of water deficiency and (2) the stage of return to normal condition. During the period of water shortage, the plant is creating an internal state best suited for resistance to drought. The speed of this adjustment will depend on the rapidity of loss of water, but the trend is the same. The general direction of the changes in the vegetative organs is toward an increase of the suction tension of the cells and the water holding capacity of the tissues (Hauck **2**, and Lebedinzev **4**). This internal condition is maintained to a large extent even when the plants are restored to a normal supply of soil moisture. The results of the present studies will be considered on the basis of these statements.

The wheat plants exposed to drought in these experiments lost water slowly and began to wilt on the seventeenth day after irrigation was discontinued. On the following day the carbohydrate composition of these plants indicated an increased sugar (monosaccharide and sucrose) and a somewhat decreased hemicellulose content.

During the period of gradual loss of moisture the plants exhibited the following changes in their sugar content. First a decrease in both forms of sugars, which evidently was due to a decrease in photosynthesis. This change took place before the appearance of the first external signs of shortage of water, the wilting of the plants. The next step was the accumulation of sucrose and the consequent decrease in monosaccharides. The plants had now begun to wilt. This was followed by increase in monosaccharides and a simultaneous decrease in sucrose. Finally there was a gradual disappearance of the monosaccharides and the loss of sugar by the tissues. This stage began when the wheat suffered greatly from loss of water. It ended with the drying up and the death of certain parts of the plant. These results are in agreement with those previously reported (**11**).

The present investigation is of special interest in respect to the change in hemicellulose content. In our former work the hemicelluloses were not determined but a supposition was made that they were of the greatest importance in the carbohydrate balance of wheat plants. They are present in considerable quantity and seem to change in amount during the process of loss of water by the plant.

Results of the first experiment (experiment A) show a decreased quantity of hemicelluloses and an increased sugar content in most of the experimental plants as compared with the check plants. It is very probable that a correlation exists between the decrease in hemicelluloses and the increase in sugars in these plants, and that the hemicelluloses were at least one of the sources for the formation of sugars. Several investigators have shown that starch is present in the vegetative organs of wheat in but very small amounts and that it has no importance as a carbohydrate storage product. One may presume, therefore, that in wheat the hemicelluloses take the place of starch.

Results of experiment B present evidence of the conversion of sugars—the disappearance of sucrose. The hemicellulose content has also altered. But instead of a decrease, there is an increase in its quantity. This interesting fact may be interpreted in the following way: It is well known that hemicelluloses belong to a group of carbohydrate compounds the characteristic of which is a marked capacity to swell (the pentosans). Rosa (**8**) has assigned to them a decisive rôle in the increase in frost resistance of hardened plants. In a recent investigation by Wood (**13**) on the carbohydrate balance of Australian succulent xerophytes, an unusually large accumulation of pentosans was noted in these plants when the aridity of the environment increased. The significance of the hydrophyllous colloids in the drought resistance of plants, especially wheat, is emphasized by Newton and Martin (**6**). From this and other studies we may conclude that the accumulation of hemicelluloses during the stage of water loss represents a means of resistance and a natural reaction of wheat plants to drought.

Let us now consider the carbohydrate balance of our plants during the period of recovery from drought. As soon as the plants were irrigated fundamental changes in the water and carbohydrate balance were brought about. The water content increased but the sugars, especially monosaccharides, decreased, while the hemicelluloses increased very markedly, thus producing an increase in total carbohydrates. These changes are not analogous to those existing in normal check plants. Compared with the latter, the experimental (irrigated) plants had a lower water and monosaccharides content but they were high in hemicelluloses, sucrose, and total carbohydrates. The same differences were still obtaining during the eighth day of recovery. Apparently the condition had become fixed in these plants.

The present data are inadequate to explain why the plants which had been exposed to drought did not return after irrigation to the state of the check plants in their chemical composition. One may only suggest that the condition established during the drought period had become more or less irreversible.

In general, the most conspicuous feature by which a wheat plant that has been hardened to resist drought differs from a normal one is the greater accumulation of hemicelluloses and sugars, chiefly sucrose.

Summary

1. The object of this investigation was the determination of changes in carbohydrates of five varieties of wheat during the process of hardening for drought resistance.

2. Hardening was induced by growing the plants with insufficient water supply in suitable containers until permanent wilting had set in and the tips of the leaves had begun to die. Recovery was brought about by irrigation from this point on.

3. Representative numbers of plants were cut and analyzed for moisture content and carbohydrates at four periods: (1) the time of beginning of wilting, (2) the stage of permanent wilting, (3) twenty-four hours after resumption of irrigation, and (4) eight days after recovery. The carbohydrate analyses consisted of quantitative determination of monosaccharides, sucrose, and hemicelluloses, the most important constituents of the wheat plant.

4. On the day following the beginning of wilting the carbohydrate content of the plants of most varieties studied was as follows: An increased concentration of both forms of sugar (monosaccharides and sucrose) and a somewhat decreased concentration of hemicelluloses. With further loss of water, when the points of the leaves commenced to yellow, there were the following changes: Decrease in sucrose, increase in monosaccharides, and a decided increase in hemicelluloses; the contents of the latter were now greater than in the check plants. On the day following the first irrigation, the water content in the tissues had increased but not to the amount present in the check plants. The monosaccharides had decreased and sucrose was also at lower concentration, and in most cases less than in the control plants. After recovery (eight days of irrigation following maximal drought effects), the experimental plants, when compared with normal plants, still had a decreased water and monosaccharide content and an increased concentration of sucrose and hemicelluloses. This condition had become more or less "fixed."

5. The significance of the hemicelluloses in drought resistance of wheat is discussed.

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