Growth Changes in Pea Plants Induced by Gibberellic Acid and their Effects on the Concentrations of Tissue Lipids

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Striking and pronounced growth responses to the gibberellins as plant regulators and hormones are well known and readily produced in suitable plants. It was our purpose to study the effects of such growth responses on the concentrations of plant lipids and on the concentrations of antioxidants that are synthesized in plants.

Growth changes were induced in pea plants by the application of gibberellic acid. Changes in the concentrations of lipids occurred in both treated shoots and treated roots, and these changes do not appear to be due to redistribution between shoots and roots. The concentrations of antioxidants, e.g. tocopherols, were not significantly changed by the alteration in growth. Fast-reacting sterols were present in the plants and were restricted to the esterified sterol fraction of the shoots. The protein concentration was decreased in treated plants and concentrations of carbohydrate fractions were increased reciprocally.

EXPERIMENTAL

Botanical. Three varieties of peas were used: Freezonia, American Wonder and Early Alaska. American Wonder is a true dwarf variety, the other two being low-height varieties. Seeds were first treated with Arosan (a fungicide), then planted (five seeds per pot, 30 pots per variety) in 4 in. glazed soil jars. The jars were plugged with glass wool and filled with quartz sand. Pots were watered with a complete nutrient solution (Hoagland & Arnon, 1938) containing iron as an EDTA complex. The pH of the solution was adjusted to 6.0–6.5 with NaOH or HCl.

The plants were grown in a greenhouse at the University of Oklahoma. No artificial light was used. Seeds were planted on 3 February. Pots were covered with paper sacks until emergence of the plants. Emergence was on 7 February except for American Wonder which emerged on 8 February. On 18 February plants were thinned to two plants per pot, and 15 pots of each variety were sprayed with a solution containing 100 µg. of gibberellic acid (Eli Lilly Co.)/ml. and 5 drops of aerosol/l. The spray did not adhere well to the plants and it is likely that some gibberellic

acid was absorbed through the roots. Initial height measurements were made on 18 February and plants were harvested on 7 March, 33 days after the planting (leap year).

At the time of harvest very little was left of the cotyledons and they were discarded. The shoots were separated from the roots by cutting at the cotyledonary node. Shoot height was measured in centimetres from above the first true leaf to the tip of the youngest leaf. The number of plants which bore visible flower buds were counted for each group and the individual shoots were weighed separately. The roots were washed free of sand with water and blotted dry, and the pooled roots from all plants of a single experimental group were weighed as one sample.

Chemical. Four pooled samples were obtained from 30 plants of each variety: control shoots, treated shoots, control roots and treated roots. These pooled samples were weighed to obtain the fresh sample weight. The samples were put separately in 1 quart fruit jars with about 1 pint of propan-2-ol at near-boiling point. The jars were then autoclaved at 82° for 35 min. with the solvent simmering. On removal from the autoclave, additional solvent was added to the jars to cover the plants completely. The jars were then tightly capped and refrigerated at 4°.

The lipids were extracted from the plant samples by transferring the jar contents to a Waring Blender and extracting with hot propan-2-ol and chloroform as described by Kates & Eberhardt (1957) except that three grindings in the blender were employed instead of two.

The extracted non-fat residues were dried overnight in an oven at 85°, cooled in a desiccator and weighed. These residues were then ground in a Wiley mill with a no. 20 screen to prepare the samples for subsequent analysis. There appeared to be very little sand in the root specimens.

The non-lipid residue was analysed for nitrogen (Ma & Zuazaga, 1942) and for two carbohydrate fractions. The samples were first extracted with water to remove soluble sugars and filtered through sintered glass, and then the residues were refluxed with 1.0N-HCl for 4 hr. and again filtered through sintered glass. The filtrates were analysed for carbohydrate by using the anthrone reaction. This procedure will include readily hydrolysable pentosans as well as starch, but will not dissolve cellulose, lignin and most structural carbohydrates.

The lipid extracts were evaporated to small volume under reduced pressure in a warm-water bath. The lipids were taken up in chloroform-methanol (2:1, v/v), and these extracts purified by the procedure of Folch-Pi, Ascoli, Lees, Meath & LeBaron (1951). The purified lipid extracts were evaporated to small volume under reduced

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Growth measurements of pea plants											
Variety	Control				Treated				Difference in weight of roots (g.)		
	Shoots		Roots, pooled weight (g.)	Plants bearing flower buds (% of total)	Shoots		Roots, pooled weight (g.)	Plants bearing flower buds (% of total)			
	Height (cm.)	Weight (g.)			Height (cm.)	Weight (g.)					
Freezonia	47.5 ± 2.0 (6.3 ± 0.27)	3.8 ± 0.25	141.0	97	50.2 ± 1.8 (5.5 ± 0.27)	2.8 ± 0.2	103.9	20	1.02 (N.S.; <i>P</i> > 0.05)	3.30 (<i>P</i> < 0.01)	37.1
American Wonder	17.9 ± 1.0 (2.9 ± 0.14)	3.4 ± 0.3	110.9	64	46.2 ± 2.1 (2.9 ± 0.13)	3.2 ± 0.2	82.6	23	12.13 (<i>P</i> < 0.01)	0.79 (N.S.; <i>P</i> > 0.05)	28.3
Early Alaska	53.4 ± 1.6 (7.2 ± 0.23)	3.8 ± 0.2	118.6	100	63.5 ± 1.8 (7.3 ± 0.24)	3.3 ± 0.2	101.3	83	4.18 (<i>P</i> < 0.01)	2.11 (<i>P</i> < 0.05)	17.3

content for treated and control shoots. Treated roots had a slightly greater relative water content than did control roots. Total extractable material expressed as a percentage of dry weight was consistently lower in treated shoots than in control shoots, and higher in treated roots than in control roots. Most of the results indicate a small diminution, in both treated shoots and treated roots, of protein nitrogen content expressed as a percentage of the non-lipid residue. This decrease of protein nitrogen was associated with a reciprocal increase in concentrations of the carbohydrate fractions.

Values from chemical and chromatographic analysis of shoot lipids are given in Table 3. The major differences between control and treated groups were in the carotene, and phospholipid and pigment components. Since lipid phosphorus values were not changed by treatment, the diminutions in shoot lipids given in Table 2 are thus accounted for principally by diminutions of carotene and other pigments. Digitonin-precipitable Liebermann-Burchard-positive sterols as determined were substantially less in amount than chromatographically determined sterols. The toco-

Table 2. *Total extractable material, non-lipid residue, nitrogen and carbohydrate of pea-plant shoots and roots*

Experimental details are given in the text.

Variety	...	...	...	...	Freezonia		American Wonder		Early Alaska	
					Control	Treated	Control	Treated	Control	Treated
Shoots										
Total extractables (% of wet wt.)					1.079	0.998	0.980	0.934	0.999	1.011
Total extractables (% of dry wt.)					10.25	9.44	10.56	9.65	8.80	8.45
Extracted residue (dry wt. as					9.45	9.58	8.30	8.76	10.34	10.94
% of wet wt.)										
Total nitrogen (% of extracted					4.78	4.27	5.52	4.42	4.16	4.07
residue)										
Water-soluble carbohydrate (%					5.07	4.51	4.20	5.00	6.53	6.64
of extracted residue)										
Acid-hydrolysable carbohydrate					8.88	9.06	8.25	11.52	10.55	10.73
(% of extracted residue)										
Roots										
Total extractables (% of wet wt.)					0.188	0.218	0.211	0.208	0.207	0.219
Total extractables (% of dry wt.)					5.29	7.65	6.17	6.87	4.75	6.24
Extracted residue (dry wt. as %					3.38	2.65	3.25	2.82	4.14	3.29
of wet wt.)										
Total nitrogen (% of extracted					3.62	4.04	3.84	3.61	3.27	3.19
residue)										
Water-soluble carbohydrate (%					1.70	1.85	1.70	2.10	1.55	1.32
of extracted residue)										
Acid-hydrolysable carbohydrate					8.56	8.86	8.10	9.88	8.27	10.58
(% of extracted residue)										

Table 3. *Amounts of lipid components in pea-plant shoots*

Experimental details are given in the text. Values are expressed as mg./g. dry wt. of extracted residue.

Variety	...	...	...	...	Freezonia		American Wonder		Early Alaska	
					Control	Treated	Control	Treated	Control	Treated
Lipid P					0.98	1.00	1.16	0.97	0.93	0.87
Esterified sterol*					0.97	1.28	1.20	0.90	1.09	0.90
Free sterol*					1.71	1.83	1.87	1.79	1.64	1.45
Tocopherol (Emmerie-Engel)					0.52	0.62	0.58	0.64	0.47	0.45
Tocopherol (phosphomolybdic acid)					0.54	0.60	0.58	0.60	0.47	0.45
Carotene					0.32	0.25	0.50	0.30	0.37	0.30
Silicic-acid-chromatography values (gravimetric)										
Triglycerides					5.91	6.17	2.57	5.15	5.32	4.47
Phospholipid and pigments					92.5	86.1	98.8	88.1	82.2	72.5
Esterified sterol†					2.90	3.25	2.74	4.00	2.54	2.48
Free sterol					4.64	4.06	2.70	3.86	2.92	2.33
Hydrocarbon					11.1	7.15	13.7	9.71	9.16	9.02

* Digitonin-precipitable Liebermann-Burchard-positive sterol.

† Values for esterified sterol were calculated from the gravimetrically determined sterol esters by multiplying by the factor 61.3%.

Table 4. *Amounts of lipid components in pea-plant roots*

Experimental details are given in the text. Values are expressed as mg./g. dry wt. of extracted residue.

Variety	Freezonia		American Wonder		Early Alaska	
	Control	Treated	Control	Treated	Control	Treated
Lipid P	0.91	1.11	1.29	1.37	0.64	1.04
Esterified sterol*	0.42	0.51	0.61	0.69	0.49	0.48
Free sterol*	2.46	3.67	2.53	2.83	2.15	2.58
Tocopherol (Emmerie-Engel)	0.16	0.17	0.33	0.43	0.12	0.29
Tocopherol (phosphomolybdic acid)	0.23	0.32	0.24	0.30	0.20	0.26
Silicic-acid-chromatography values (gravimetric)						
Triglycerides	2.65	5.94	2.16	2.94	1.98	2.59
Phospholipid and pigments	40.0	57.2	51.4	54.2	37.5	46.3
Esterified sterol†	0.68	2.09	0.83	1.40	0.92	1.67
Free sterol	4.65	6.23	7.41	6.18	4.08	6.37
Hydrocarbon	3.02	7.44	2.91	5.77	3.29	7.23

* Digitonin-precipitable Liebermann-Burchard-positive sterol.

† Values for esterified sterol were calculated from the gravimetrically determined sterol esters by multiplying by the factor 61.3%.

pherol content of the shoots was unchanged by the treatment.

Values from chemical and chromatographic analysis of root lipids are given in Table 4. Carotene was absent from root lipids. All of the lipid components measured were increased in treated groups on an absolute basis, namely mg./g. dry wt. of extracted residue.

DISCUSSION

The growth changes induced in the pea plants were accompanied by opposite changes in the lipid concentrations of shoots and roots. Since the concentrations of only carotene and other pigments appeared to be diminished in shoots, and the concentrations of all lipid components increased in roots, it is not likely that the changes in the concentrations of lipids in shoots and roots were due to redistribution of lipids as a result of the treatment with gibberellic acid. Differences in the overall composition of shoot lipids of control plants compared with those of treated plants could conceivably be due to different proportions of stem, leaf and flower-bud constituents in the samples of the two groups.

During analysis of the plant lipids for sterols by the Sperry & Webb (1950) procedure, the total sterols of shoots but not of roots exhibited fast-reacting characteristics (Moore & Baumann, 1952; Idler & Baumann, 1953) to the Liebermann-Burchard reagent. Free sterols of neither shoots nor roots reacted in this manner. The fast-reacting sterols obviously were not produced as artifacts during the saponification step of the total sterols since the reaction was not given by total sterols from roots. The fast-reacting sterols were thus shown to be localized in the sterol esters of the shoots. Frantz, Dulit & Davidson (1957) reported

that fast-reacting sterols in rat-skin lipids were distributed in very much the greater proportion as esters. From these similar independent observations in both plant and animal tissues it is concluded that occurrence as esters is a general phenomenon for fast-reacting sterols and that esterification must be intimately related to their metabolism.

Our findings in treated plants of decreased root weight and reciprocal changes of nitrogen and carbohydrates are in accordance with findings reported by Brian, Elson, Hemming & Radley (1954). These workers also reported chlorosis as an effect of the treatment, and this is borne out by diminutions of carotene and other pigments in the study reported above. Dahlstrom & Sfat (1961) have listed responses of plants to gibberellic acid as: alteration of carbohydrate constituents, decrease of chlorophyll content, dislocation of nitrogen metabolism, promotion of respiration in seeds, variation in the activity of certain enzymes and reversal of inhibition by maleic hydrazide. Stowe (1961) reported efforts to study pea lipids *in situ*, in the hope of determining whether the activation of hormones by lipid substances is a natural method of growth regulation.

SUMMARY

1. In studies with three varieties of pea plants, treatment with gibberellic acid resulted in several growth changes. Height increases in shoots were accompanied by weight decreases. Root weights were markedly decreased. The flowering of plants was inhibited.

2. Total extractable material was decreased in concentration in treated shoots and increased in treated roots. In shoots decreases were accounted for by decreases of carotene and other pigments.

In roots all lipids were increased. A number of lipid substances in the total extractable materials were measured.

3. Protein concentration was decreased in treated plants and carbohydrate fraction concentrations were increased reciprocally.

4. The esterified sterols of the shoots exhibited fast-reacting characteristics to the Liebermann-Burchard reagent.

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Ascorbic Acid and Aging in the Rat

UPTAKE OF ASCORBIC ACID BY SKIN AND BONE MARROW, AND ITS CONCENTRATION IN VARIOUS ORGANS

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Ascorbic acid participates in several metabolic reactions, and has been considered to be of particular significance in the aging process. Even though it is not an essential dietary requirement for mammals other than primates and guinea pigs, its metabolic functions are the same in all. In man its concentration in the blood cells declines with age in the adult (Kirk & Chieffi, 1953). The hepatic ascorbic acid concentration in rat increases rapidly during the first 3 weeks of extra-uterine life, declines sharply during the next 5 weeks and then remains relatively constant (Mourhouse & Guerrant, 1952). Several workers (Piez & Likins, 1957; Sinex, Van Slyke & Christman, 1959; Stetten,

1949; Stone & Meister, 1962) have shown that it is involved in the mechanism of hydroxylation of collagen. The administration of ascorbic acid to deficient guinea pigs enhances collagen synthesis (Gould, 1960; Robertson, Hiwett & Herman, 1959), but there is no evidence that it is required for the synthesis of other proteins. It has been suggested by Gross (1959) that ascorbic acid may be required for the maintenance of collagen. In view of the above observations, the uptake of ascorbic acid in the skin and bone marrow and its concentration in different organs of rats of various age groups have been studied to elucidate its role in the aging process.