Phytochemistry, 1975, Vol. 14, pp. 619–622. Pergamon Press. Printed in England.

EFFECTS OF GROWTH REGULATORS ON FATTY ACIDS OF SOYBEAN SUSPENSION CULTURES

EUGENE M. STEARNS, JR. and WILLIAM T. MORTON

The Hormel Institute, University of Minnesota, Austin, Minnesota, 55912, USA

(*Revised Received* 16 *October* 1974)

Key Word Index—*Glycine max*; Leguminosae; soybean suspension cultures; fatty acids; linoleic acid; linolenic acid; plant growth regulators; indole-3-butyric acid; kinetin; gibberellic acid.

Abstract—Soybean suspension cultures were grown for 24 weeks in the absence of plant growth regulators and in the presence of 1 ppm levels each of an auxin (indole-3-butyric acid), a cytokinin (kinetin) and a gibberellin (gibberellic acid), individually and in all possible combinations. Cells grown in the presence of the auxin with and without gibberellin contained relatively greater amounts of palmitic and smaller amounts of polyunsaturated acids than did cells grown under other regimens. The combination of cytokinin and gibberellin caused a higher proportion of linoleic and a lower proportion of linolenic acids than in cells of the other groups. Neither of these regulators by itself produced the effect, and addition of auxin to the other two diminished the effect.

INTRODUCTION

Seeds of common varieties of soybean, *Glycine max*, produce oil containing two major polyunsaturated fatty acids, linoleic (43–56%) and linolenic (5–11%) acids [1], although trace quantities of several other acids have been identified in developing beans [2]. The value of soybean oil for food uses is good, due to the high content of nutritionally essential linoleic acid; however, the value could be further enhanced by diminishing the amount of linolenic acid, which contributes to oxidative instability [3]. Attempts to lower the linolenic acid content of soybeans have been disappointing, primarily due to the lack of a strain truly low in linolenic acid [4].

In addition to genetic capability, environmental and nutritional factors are known to affect the degree of unsaturation of plant oils. Deficiencies in iron, manganese and N_2 are reported to depress the synthesis of polyunsaturated fatty acids by plants, while increased light intensity, increased oxygen tension or decreased ambient temperature produce the opposite effect [5]. Although environmental alterations of normal lipid patterns may result from direct effects on enzymes catalyzing desaturation, it has also been suggested that factors such as light and temperature may influence growth by controlling the types and amounts of plant growth regulators present [6].

To determine whether or not plant growth regulators exert effects on the unsaturation of lipids in plants apart from environmental effects, soybean callus cells maintained in suspension cultures were considered a useful model. Such cells can be maintained under identical conditions of environment and nutrition and the amounts of growth regulators can be closely controlled. The lipid and fatty acid composition of soybean callus is quite different from that of the seeds [7]. We have observed, however, that the pattern of incorporation of labeled acetate into the fatty acids of callus tissue is almost identical to that in developing cotyledons during experiments of up to 86 hr.

In the present study suspension cultures of undifferentiated soybean cells were maintained in the presence of growth regulators individually and in combination, and the fatty acids of lipids were examined.

Table 1. Characteristics of soybean suspension cultures in B5 medium containing growth regulators

Treatment*	Callus wet† wt (g)	Per cent lipid† (wet wt basis)	Morphological observations‡ Color	Aggregation
A	16·1 ± 0·4	0·06 ± 0·007	Y–B	Aggregates
C	15·8 ± 1·1	0·06 ± 0·010	Y	Small aggregates
G	14·7 ± 1·2	0·11 ± 0·016	Y–B	Finely divided
A + C	20·7 ± 2·4	0·06 ± 0·010	W–Y	Small aggregates
A + G	10·1 ± 0·9	0·07 ± 0·007	Y–B	Finely divided
C + G	8·7 ± 1·2	0·07 ± 0·014	W	Finely divided
A + C + G	13·9 ± 2·7	0·09 ± 0·006	W	Finely divided
None	11·6 ± 0·9	0·12 ± 0·003	Y–B	Aggregates

* Transferred at 4-week intervals for total of 24 weeks at 21–22° and 9 hr of incandescent light per day. A—Auxin (indole-3-butyric acid), C—cytokinin (kinetin), G—gibberellin (gibberellic acid), each at 1 ppm.

† Mean ± s.e.m. for four cultures.

‡ W—white, Y—yellow, B—brown; Aggregates—0·5 to 2 cm dia; small aggregates—0·2 to 0·5 cm dia, finely divided—<0·2 cm dia.

RESULTS

Cells in all eight experimental groups, originating from equal aliquots of finely divided suspension, grew throughout the experimental period. Culture characteristics are shown in Table 1. Growth appeared best using a mixture of auxin and cytokinin, poorest using gibberellin combined with auxin or cytokinin or with no supplementation. Lipid yields, calculated as a percentage of wet weight, were similar throughout, but appeared slightly elevated in cultures containing gibberellin, all three growth regulators, or none. Soybean callus grown on B5 medium containing agar has been found to lose *ca* 96% of its weight on lyophilization. Cultures containing cytokinin alone or in combinations were lighter in color than other cultures and those grown in the presence of gibberellin by itself or in combinations remained finely divided. There was no apparent correlation between morphological observations and either the callus weight or lipid yield. In cultures exhibiting darkening or aggregation, development was progressive throughout. Cultures receiving no supplementation became somewhat green near the end of the experimental period.

Statistical data on the fatty acid compositions of cultures are presented in Table 2. Palmitic and palmitoleic acid values were combined because the latter appeared as a small and consistent shoulder on the trailing edge of the palmitic acid peak. Minor amounts (<1·0%) of myristic, pentadecanoic, pentadecenoic, heptadecanoic and eicosanoic acids were identified on chromatograms, but no correlation between these and experimental treatment was observed. A considerable overlapping of values for the major fatty acids shown on the table is noted when the standard error is added to or subtracted from mean values.

Palmitic acid was elevated in the presence of auxin alone or in combination with gibberellin;

Table 2. Fatty acid compositions of soybean suspension cultures in B5 medium containing growth regulators

Treatment*	Composition in relative percentage by GLC† 16:0 + 16:1	18:0	18:1	18:2	18:3
A	41·8 ± 0·7	9·3 ± 0·9	4·1 ± 0·5	3·8 ± 0·3	41·1 ± 1·9
C	31·2 ± 0·3	7·1 ± 0·2	6·9 ± 0·2	4·6 ± 0·1	50·2 ± 0·3
G	32·6 ± 1·5	6·5 ± 0·3	7·7 ± 0·5	4·0 ± 0·5	49·3 ± 1·3
A + C	34·8 ± 1·6	6·4 ± 0·2	5·2 ± 0·5	4·1 ± 0·2	49·4 ± 1·2
A + G	42·8 ± 0·2	7·2 ± 0·3	6·0 ± 0·3	5·2 ± 0·4	38·8 ± 2·8
C + G	34·4 ± 1·0	9·8 ± 0·5	6·8 ± 0·5	18·1 ± 0·5	31·0 ± 1·9
A + C + G	36·0 ± 0·6	6·9 ± 0·1	5·3 ± 0·6	8·3 ± 0·5	43·4 ± 0·7
None	36·9 ± 0·9	8·5 ± 0·6	6·6 ± 1·0	4·1 ± 0·4	43·8 ± 2·2

* Transferred at 4-week intervals for total of 24 weeks at 21–22° and 9 hr of light per day. A—Auxin (indole-3-butyric acid), C—cytokinin (kinetin), G—gibberellin (gibberellic acid), each at 1 ppm.

† Fatty acids: 16:0—palmitic acid, 16:1—palmitoleic acid, 18:0—stearic acid, 18:1—oleic acid, 18:2—linoleic acid, 18:3—linolenic acid. Mean ± s.e.m. for four cultures and 3 GLC determinations.

however, the gibberellin by itself produced no effect. Other combinations of growth regulators had no effect on palmitic acid as compared with each other or with controls containing no supplementation. No effect on the content of stearic acid was discernible from the overlapping values of from 6·2 to 10·3%. Oleic acid was lowest in cultures containing auxin. However, all other values for oleic acid ranged from 4·7 to 8·2%. Linoleic acid was consistently low (3·5–5·6%) in all experimental groups but two. When all three growth regulators were present, each at 1 ppm, the content of linoleic acid was twice that in the other experimental groups and in the presence of cytokinin and gibberellin but no auxin, the elevation was four-fold. Neither cytokinin nor gibberellin by itself affected the content of linoleic acid. Linolenic acid was lowest in cells treated with the combination of cytokinin and gibberellin, the same combination which resulted in elevation of linoleic acid. The content of linolenic acid was also somewhat low in the cultures which contained elevated proportions of palmitic acid. While growth in the presence of auxin resulted in synthesis of a higher proportion of saturated acids, treatment with cytokinin or gibberellin elicited an opposite response and favored synthesis of unsaturated acids.

The ratio of linolenic to linoleic acid was 1·7:1 in the presence of cytokinin plus gibberellin and was increased in the presence of these plus auxin to 5·2:1. In other experimental groups the ratio ranged from 7·5:1 (A + G) to 12·3:1 (A + C). The elevation of linoleic acid in cells treated with cytokinin and gibberellin appeared to occur at the expense of linolenic acid.

The only difference noted between groups grown in the presence of all three growth regulators or with no supplementation at all was a higher relative proportion of linoleic acid in the former. Except for the effects of auxin already noted, only combinations of growth regulators appeared to affect fatty acid composition.

DISCUSSION

Since environmental and nutritional conditions were held constant and all suspensions originated from subcultures of a single callus culture, the differences in fatty acid patterns observed have been ascribed to the effects of administered growth regulators. Elevation of palmitic acid and depression of unsaturated acids in the presence of auxin suggests that the auxin either promotes the synthesis of the saturated acid from acetate or interferes with the synthesis of unsaturated acids. Similarly, the combination of cytokinin and gibberellin may inhibit desaturation of linoleic to linolenic acid, but to a lesser extent when auxin is also present. Some other effects of plant growth regulators on lipid metabolism have been reported. Application of a cytokinin has been shown to increase the incorporation of radioactivity of methionine-[methyl-^{14}C] into neutral and polar lipids [8, 9] and affect the synthesis of linolenic acid in leaves [10], while gibberellic acid is reported to increase the incorporation of labeled orthophosphate into phospholipids [11].

Several means of control exerted by plant growth regulators have been postulated. The substances may be responsible for gene repression or stimulation, may control the synthesis of *m*RNA or protein or may affect enzyme activity, either directly by an "allosteric" effect or indirectly by alteration of membrane permeability [12].

Callus cultures undoubtedly contain a mixture of cells with somewhat differing genetic characteristics [13, 14]. If the effects observed were due to stimulation or repression of genes controlling the synthesis of enzymes which catalyze specific steps in the biosynthetic pathway, such as the desaturation of linoleic acid, then perhaps chemical control of the lipid synthesized by existing plant varieties could be altered by application of mixtures of natural or synthetic growth regulating substances. If, on the other hand, the growth regulators favored growth of cells having genetic characteristics somewhat different from those of the original parent cells, then the altered fatty acid patterns reflect an increased population of cells with the altered characteristics. If the latter were true, then it might be profitable to clone cells from cytokinin and gibberellin-treated suspension with improved chances of obtaining a strain or strains with genetic characteristics favoring lowered proportions of linolenic acid in the oil.

EXPERIMENTAL

Cell growth. Gamborg's B5 medium for soybean root cells [15] without 2,4-dichlorophenoxyacetic acid, except where noted, was used throughout. Soybean callus derived from *Gly-*

cine max L., strain PI-194-656, grown on B5 medium containing 0·1 ppm 2,4-D and 10 g/l agar, was used to prepare a suspension culture in the same medium but without agar. Experimental treatment commenced on transfer of equal aliquots of finely divided material to 50 ml portions of B5 medium containing growth regulators, each at 1 ppm, in 250 ml flasks. Four replicates of each experimental treatment were maintained on a reciprocal shaker (5 cm strokes, 66 cpm) at 21–22° with 9 hr/day of incandescent ceiling lighting at 300 lx. Five transfers to fresh medium at 4-week intervals were performed. The experimental period was 24 weeks. Cells were harvested by filtration on a suction funnel and wet wt determined for comparison of growth. Experimental treatments consisted of indole-3-butyric acid (A), kinetin (C), gibberellic acid (G), A + C, A + G, C + G, A + C + G and no growth regulator supplementation. Indole-3-butyric acid was selected as the auxin for experiments because it is less subject to biological oxidation than the natural hormone IAA [16] and possibly less likely to affect the metabolism and/or cytology than the commonly used synthetic auxin 2,4-D [13]. Levels greater than 1 ppm were avoided to decrease the possibility of differentiation occurring during the experimental period.

Lipid analysis. Lipids were extracted by homogenization × 3 in $CHCl_3$–MeOH (2:1). Filtrates were taken to dryness and made up to vol. in $CHCl_3$. Aliquots were used for gravimetric determination of lipid yields and for preparation of Me esters for GLC. Triplicate analyses of ester preparations were performed by GLC on a 0·32 × 245 cm column packed with 15% EGSS-X on Gas Chrom P at 170°. FID was used and peaks were integrated for quantitation. Identification of compounds was by comparison with reference standards.

Acknowledgements—The authors thank Dr. E. John Staba, Department of Pharmacognosy, University of Minnesota, for the gift of callus cells used in this study and Dr. A. C. Hildebrandt, Department of Plant Pathology, University of Wisconsin, for helpful suggestions. This investigation was supported by PHS Research Grant HL 08214 from the Program Project Branch, Extramural Programs, National Institutes of Health, and The Hormel Foundation.

REFERENCES

1. Swern, D. (1964) in *Bailey's Industrial Oil and Fat Products* (Swern, D., ed.), 3rd edn., p. 226. Wiley, New York.
2. Stearns, E. M. Jr. (1970) in *Progress in the Chemistry of Fats and Other Lipids* (Holman, R. T., ed.), Vol. 9, pp. 453–516. Pergamon Press, Oxford.
3. Smouse, T. H. and Chang, S. S. (1967) *J. Am. Oil Chem. Soc.* **44,** 509.
4. White, H. B. Jr., Quackenbush, F. W. and Probst, A. H. (1961) *J. Am. Oil Chem. Soc.* **38,** 113.
5. Hitchcock, C. and Nichols, B. W. (1971) *Plant Lipid Biochemistry*, pp. 152, 259. Academic Press, London.
6. Galston, A. W. and Davies, P. J. (1969) *Science* **163,** 1288.
7. Shin, B. S., Mangold, H. K. and Staba, E. J. (1971) in *Proc. 2nd Int. Conf. Plant Tissue Culture* (Hirth, L., ed.), p. 51. C.N.R.S., Paris.
8. Schaeffer, G. W. and Sharpe, F. T. Jr. (1971) *Physiol. Plant.* **25,** 456.
9. Schaeffer, G. W., St. John, J. B. and Sharpe, F. T. Jr. (1972) *Biochim. Biophys. Acta* **261,** 38.
10. Kull, U. and Büxenstein, R. (1974) *Phytochemistry* **13,** 39.
11. Koehler, D. E. and Varner, J. E. (1973) *Plant Physiol.* **52,** 208.
12. Wareing, P. F. and Phillips, I. D. J. (1970) *The Control of Growth and Differentiation in Plants*, p. 286. Pergamon Press, Oxford.
13. Staba, E. J. (1969) in *Recent Advances in Phytochemistry* (Seikel, M. K. and Runeckles, V. C., eds.), Vol. 2, p. 75. Appleton-Century-Crofts, New York.
14. Sievert, R. C. and Hildebrandt, A. C. (1965) *Am. J. Botany* **52,** 742.
15. Gamborg, O. L., Miller, R. A. and Ojima, K. (1968) *Exptl. Cell Res.* **50,** 151.
16. Van Overbeek, J. (1966) *Science* **152,** 721.