Chemistry of Natural Compounds, Vol. 31, No. 4, 1995

COMPOSITION AND GROWTH-STIMULATING ACTION OF THE TOTAL SECONDARY METABOLITES OF COTTON LEAVES

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UDC 578.084+581.192+543.51

The composition of the total secondary metabolites (SMs) of the extractive substances isolated from the leaves of cotton plants of the lines L-249, L-15, and L-598, and the variety Tashkent-1 (T-1) has been studied. Quantitative mass spectrometric analysis has shown that a larger amount of important groups of SMs is synthesized in the selection line L-249 than in its parental forms and the control T-1.

For the development of the theory and practice of the use of plant growth regulators it is necessary to find new effective natural ecologically harmless compounds.

It is known that these substances are elaborated in every plant. The complex interaction of phytoregulators with different properties creates a hormonal balance specific for each plant species. The growth and development of each organism depend on this balance, which is genetically fixed. The isolation of secondary metabolites, associated in the plant with a definite gene responsible for growth and other physiological–biochemical processes and the manifestation of their roles is therefore of definite interest.

Investigations of the presence of SMs are being carried out with the aim of creating a bank of correlations between genetic characteristics and the level of SMs [2].

Plant material was obtained from the lower stages of plants of the control variety T-1 and from the selection line L-249 and its parental forms L-15 and L-598 [1] in the ripening phase. In the investigations we made use of the fact that the biosynthesis of SMs is controlled by genes [2].

We studied the unseparated sum of the extractive substances of the above-mentioned plants by the mass-spectrometric method of multicomponent monitoring (MMM) [3].

The absolute SM content (in the presence of standards of the groups of compounds we investigated) was determined in the total extractive substances of the leaves of a L-249 cotton plant in parallel with the totals obtained analogously from the leaves of their two parental forms and the control T-1.

The absence of model specimens of esters of sterols and β-amyrin with fatty acids permitted us to estimate their amounts by a relative method using a MMM comparison of the total heights of characteristic peaks of the self-pinching-out line and the parental forms with the same magnitudes for the control, T-1, taken as unity.

The deficiencies of this procedure are partially compensated by the fact that calculations of the absolute and relative amounts of the substances being analyzed are obtained from a single experiment for each line and variety.

The results, which are given in Table 1, showed that the levels of free sterols and β-amyrin in lines L-15 and L-598 were approximately the same; in L-15 the amount of α-tocopherol had increased, and in both lines the level of polyprenols was 1.5-2 times higher than in the control, T-1.

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*Deceased.

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0009-3130/95/3104-0514$12.50

TABLE 1. Amounts of SMs in the Leaves of Cotton Plants During Their Ripening, mg/g of the Sum of the Extractive Substances

SM	T-1	L-15	L-598	L-249
β-Sitosterol	0.25	0.29	0.32	0.90
Stigmasterol	0.15	0.14	0.19	0.75
Campesterol	0.05	0.04	0.08	0.39
24-Ethylidene-cholesterol	0.14	0.20	0.17	0.40
β-Amyrin	0.13	0.12	0.12	0.40
α-Tocopherol	1.37	2.48	1.40	3.88
β(γ)-Tocopherol	0.09	0.31	0.12	0.61
Polyprenols	22.0	43.0	34.0	14.4
Relative amounts of esters				
Sitosterol esters	1.00	0.94	0.18	1.85
Amyrin esters	1.00	0.83	0.23	1.99

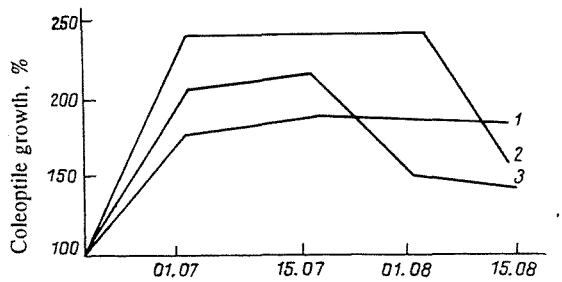

Fig. 1. Influence of endogenous IAA on the growth of wheat coleoptiles: *1*) 2% solution of sucrose; *2*) ΣSMs from L-249; *3*) untreated plants.

In the leaves of a cotton plant of the self-pinching-out form L-249 there was a fairly sharp increase (3- to 8-fold) in all the SMs, and the polyprenols made up 14.4% of the sum of the extractive substances.

Thus, it was shown that in the leaves of an L-249 cotton plant the biosynthesis of important groups of SMs had been intensified.

To check growth-stimulating action we obtained the sum of the unsaponifiable substances (ΣSMs) from the L-249 line. It exhibited a positive growth-stimulating effect. Its influence on the activity of endogenous indolylacetic acid (IAA) was studied.

The IAA isolated from cotton leaves by the use of a mixture of solvents – *n*-butanol—glacial acetic acid–water (40:12:28) – with R_f 0.80-0.90 showed a nonuniform effect on the growth of wheat coleoptiles (Fig. 1): the IAA with R_f 0.80 (bound) did not exhibit a growth efect, while the IAA with R_f 0.90 showed a stimulating effect. Thus, the IAA isolated from control (untreated) plants stimulated the growth of the coleoptiles by 100-125%. Such increased growth was observed over 15 days. A high activity was exhibited by the IAA isolated from experimental plants treated with a 0.001% solution of ΣSMs from the L-249 line. The increase in the growth of the coleoptiles amounted to 150%.

The influence of this sum on the activity of endogenous IAA was prolonged for 30 days, after which the activity of the IAA fell, and the increase in the growth of the wheat coleoptiles was at the 50% level. The increase in the growth of the coleoptiles under the influence of 2% sucrose was at the 75-85% level. The shift in the activity of the endogenous IAA apparently comes about as the result of a change in the activity of enzyme systems forming and breaking down IAA.

We attempted to determine the activity of IAA oxidase after the action of the ΣSMs from L-249. Analyses showed that the activity of IAA oxidase in the control cotton plants ranged from 400 to 900 μg of IAA oxidized per 1 g of crude weight in 1 h. In a variant of the experiment with the ΣSMs from L-249 the activity of the IAA oxidase was considerably suppressed (Table 2). It is obvious that one of the reasons for the increase in the activity of the endogenous IAA in cotton leaves treated with the ΣSMs from L-249 must be ascribed to inhibition of the activity of IAA oxidase. Thus, the ΣSMs from L-249 possessed a stimulating activity. The moistening of the seeds in a solution (0.001%) of this sum from L-249 before sowing caused an

TABLE 2. Influence of the ΣSMs on the Activity of IAA Oxidase

Variant	Concentration of ΣSMs, %	Activity of IAA oxidase, μg/g in 1 h		Deviation, ±
		30 min	60 min	
Control	—	400.0	900.0	—
ΣSMs from L-249	0.001	410.0	880.0	—20

TABLE 3. Influence of the ΣSMs from L-249 on Some Biochemical Indices of Cotton Leaves

Variant	Concentration of ΣSMs, %	Sum of		
		sugars, mg/g	proteins, mg/g	NAs, mg-% P
3-4 true leaves				
Control	—	18.6	18.0	39.6
ΣSMs from L-249	0.001	20.7	13.0	49.7
Incipient flowering				
Control	—	19.7	14.5	29.0
ΣSMs from L-249	0.001	22.9	12.0	34.8
Incipient ripening				
Control	—	18.5	15.0	18.8
ΣSMs from L-249	0.001	16.5	27.0	22.6

increase in the level of green pigments in the cotton leaves. The maximum increase in the concentration of green pigments in cotton leaves under its action coincided with the period of entry of the cotton plant into the generative phase (budding, flowering). The increase in the chlorophyll content ranged from 15 to 38%.

It is known that an increase in the level of green pigments is usually accompanied by an increase in the activity of the photosynthetic apparatus of the cotton leaves. In actual fact, when potassium ferricyanide was added to a suspension of chloroplasts isolated from the leaves of the experimental plants, its reduction was accelerated. Thus, in the control the rate of reduction over an hour was 81 μmole/mg, and in the experiment 96.0 μmole/mg.

As a result of the increase in the activity of the photosynthetic apparatus of the leaves there was a change in some of biochemical indices (Table 3). For example, in the initial phase of growth and development of the cotton plant (3-4 true leaves) the total amount of sugars in the leaves had increased somewhat, but it subsequently decreased through outflow into other vitally important organs. So far as concerns proteins, the opposite pattern was observed; initially there was a fall and then a rise in their level, which may obviously be of interest for the use of the ΣSMs from L-249 in the cultivation of fodder crops such as alfalfa, maize, etc.

The ΣSMs from L-249 also exerted a positive influence on the biosynthesis of nucleic acids (NAs) both in the early and in the late periods of the vegetation of the cotton plant.

Thus, the participation of the ΣSMs in metabolic processes of plants can be characterized only from positive aspects: the activation of synthetic processes, an increase in the levels of green pigments determining the nature of the regulatory activity of the preparation and its interaction with endogenous phytohormones, which leads to an acceleration of the growth and development of the cotton plant and an increase in crop yield by 150 kg/ha.

EXPERIMENTAL

The total extractive substances (for L-249, L-15, L-598, and T-1) were obtained in similar way to the experiment of [4]. The yields of extractive substances amounted to 160, 180, 180, and 190 mg, respectively. The sum of the unsaponifiables for L-249 was obtained by a method decribed previously [5]. The yield amounted to 1 g from 10 g of dry plant. Biotesting was carried out by Boyarkin's method [6]. IAA oxidase activity was determined by Gamburg's method [7], the levels of sugars and proteins as in [8, 9], and SM content by the method of [3, 4].

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