

CHEMICAL COMPOSITION OF COTTON LEAVES EXTRACT VARIETY T-104 (*Gossypium hirsutum* L.) AND ITS INFLUENCE ON PLANTS' RESPONSE WHEN PRE- SOWING TREATMENT OF SEEDS

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Abstract

This study presents for the first time data on the composition of microelements and polyphenols in the extract of cotton leaves (*Gossypium hirsutum* L.) variety T-104 and the effect of presowing treatment with the extract on the responses of cultivated plants in the initial period of development. It has been established that, according to the quantitative composition of polyphenol homologues, the extract of the leaves of the cotton line T-104 differs from the previously developed drug Uchkun. In this extract, undecaprenol dominates with a content of 72.5%, and also decaprenol was detected in an amount of 8.8% and dodecaprenol 18.7%. It was revealed that the content of trace elements, such as manganese and copper in the leaves of cotton line T104 is 4-5 times higher than that of the 108-F variety. As a result of treatment with an extract of some cultural plants (wheat seeds, cucumber and radishes), it was observed that the growth of stems is more activated. It was found that the extract, as well as the drug uchkun, contributes to an increase in the content of cytokinin in wheat seedlings. The extract has been shown to have a high stress-protective activity. It stimulates plant growth and increases wet and dry mass when plants are grown under saline conditions.

Keywords: Gossypium hirsutum L., uchkun, polyphenols.

1. Introduction

In recent years, there has been a significant increase in the number of studies investigating the potential of secondary metabolites as growth regulators in agricultural practice. These metabolites exhibit high physiological activity, enhancing growth processes, increasing plant resistance to stress, and improving yield and quality [14].

The physiologically active substances that are widely used at present are polyphenols. In recent years, drugs based on polyphenols have been intensively developed due to their ultra-low toxicity. Great interest in polyphenols as potential agents that can be used to accelerate the process of wound healing is associated with their high regenerative activity [22]. In addition, much attention is paid to polyphenol-containing

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extracts isolated from various plant sources for use as biologically active additives, preparations for agriculture, cosmetology, etc. [3, 16, 24]. The drug, one of the active ingredients of which is polyprenols, is the *Verva biostimulant* [10, 21]. The natural compounds that make up the preparation increase the resistance of plants to extreme environmental influences. Triterpene acids contribute to the activation of biological and bioimmune processes in plants. Minor components such as monoterpenoids, polyprenols, fatty acids, flavonoids contained in the preparation determine its fungicidal activity [4, 5, 6]. An example of a growth regulator based on polyisoprenoids is Uchkun, developed at the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan [13]. The drug is the sum of biologically active substances of cotton leaves: tocopherol, polyisoprenoid alcohols, phytosterols and higher aliphatic alcohols, etc. At a consumption rate of 5–10 g/t of seeds, it increases the yield of many agricultural crops (cotton, wheat, cucumbers, tomato, etc.) and protects them from adverse conditions (water deficiency, saline soils) [26]. When crops infected with pests are sprayed with a bioregulator, the content of photosynthetic pigments in damaged leaves is restored [18, 25]. Plant growth regulators are able to cause various changes in the process of plant growth and development. Their regulatory function is carried out due to a change in the concentration of hormones and their distribution between plant organs under the influence of external influences [20].

The aim of the work was to study the elemental composition and content of polyprenols in the extract of cotton leaves of the T-104 variety and to establish its effect on the growth, protein content and hormonal balance of wheat seedlings during presowing seed treatment.

2. Material and methods

2.1 Method for determining the content of polyisoprenoids

In order to determine the content of polyisoprenoids, the amount of neutral substances (NB) was isolated according to the method [12], the composition of polyprenyl homologues was studied using high-performance current-layer chromatography - HPTLC (Camag, Switzerland), on SORBFIL HPTLC-AF-UV plates, size 10x10, solvent for washing the plate - distilled chloroform, drying in air at a temperature of 20-25°C, time 10 min., 1.0 mg of the solvent substance in 1 mL of the solvent, a sample of 5 µL was taken. The solvent system employed was toluene-ethyl acetate, with distance from start to finish 70.0 mm, a distance between tracks 7.7 mm and a wavelength of 200 nm [8].

2.2 Method for determining the content of macro- and microelements of the extract

Analysis of the determination of the content of macro- and microelements of ash and dry residue of the aqueous extract of cotton leaves of the T-104 line was carried out by flame atomic absorption spectrometry [1]. The T-104 line was created by employees of the Institute of Selection, Seed Production and Agricultural Technology of Cotton Growing. The breeding line T-104 (species *G. hirsutum* L.) was bred by repeated individual selection from the variety Avval-1. Early ripening, highly productive, resistant to pests and diseases with a combination of quality IV fiber (115-125 days of vegetation period). A distinctive feature of the variety was the anthocyanin color of all parts of the plant (stem, leaves, capsules, etc.). The high resistance of this line to pests and diseases was revealed [9].

2.3 Method for determining the content of phytohormones

The contents of auxin and cytokinin were determined in 10-day-old wheat seedlings. To do this, the seeds in the experimental variant were soaked for 18 hours in 0.0001% solutions of the extract from ten days old wheat seedlings and growth stimulator uchkun, in the control variant in water, after which they were planted on canvas and sprayed with water daily. In the plants grown in each type of experiments, the roots were separated from the stems and ground into fine pieces. Freshly crushed plant material was extracted with a solvent system of water: methanol at a ratio of 2:8 on a magnetic stirrer for 30 minutes. Next, the obtained extractive amount was centrifuged at room temperature at a speed of 12,000 g for 10

minutes. Final extract was filtered with a 0.45 µm filter and the filtrate was used to quantify the following phytohormones: Kinetin HIMEDIA EC No 208-382-2, 3-IAS SIGMA 12886-6G standard standards using HPLC.

HPLC LC 2030 C3D Plus (Shimadzu), Kolonka C18 250x4.6 mm 5 µm (Perkin Elmer). Absorption region 210 nm, thermostat temperature 40°C, 0.5% solution of H₂O/H₃C-CN/CH₃COOH was used as eluent at a ratio of 30/50/20, feed rate 0.5 mL/min. The sample volume for analysis was 10 µL. Analysis duration was 11 min.

3. Results and Discussion

Previously, we carried out a qualitative and quantitative analysis of the Uchkun preparation obtained from the Kelajak variety, and for the first time its detailed composition of polyisoprenoids was revealed. It was revealed that its main component was the sum of polyprenyl homologues with 10-12 isoprene units, the content of which reached 69.5%. Other components of the extract (phytosterols and tocopherols) accounted for 30.5% [25].

In this report, we present the results of studies on the composition of polyisoprenoids in the leaves of the industrial variety 108-F (medium ripe and 120-160 days of vegetation period) and the line T-104. The bush is pyramidal compressed. The height of the main stem was 100-120 cm, the boll was large, ovate-elongated, the leaf was large, and the shape of the middle lobe was triangular. Productivity, fiber yield and fiber type were 34-35 c/ha, 34-35% and good quality type V, respectively [11].

As a result of the analyzes, it was shown that the polyprenols (PP) of the leaves of the T-104 variety are presented with polyprenyl homologues with 10-12 isoprene units, where the content of decaprenol is 8.8%, undecaprenol 72.5% and dodecaprenol 18.7%, and in the case of 108-F mainly undeca (80.35 %) and dodecaprenol 19.55% of the total polyprenols (Table 1). PP from the leaves of *Rhus coriaria* was used as a standard sample of polyprenols [6].

Table 1. The content of polyprenols, in % of the total PP

No	Cotton variety	Exit HB, %	Content, PP		
			n=10	n=11	n=12
1	control	PP	42, 27±1	42,35±1.5	14,57±0,3
2	T-104	4,0	8,8±0.5	72,5±1.2	18,7±0.5
3	108-F		*ND	80.25	19.55±1

*:Not determined.

As a result of the GC/MS analysis of PP related substances, 9 compounds were identified, the major components of which were phytol (69.57%), caryophyllene (6.10%), 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-1H-benzocycloheptene (3.53%), the content of other components (geraniol, farnesylacetone, 1,5,9,9-tetramethyl-1,4,7-cycloundecatriene, 7,11,-dimethyl-3-methylene-1,6,10-dodecatriene, triacontan and butyl ester of phthalic acid) is only 20.8%.

The content of macro and micro elements of the leaves was examined. As a result, the yield of ash and dry residue was investigated and a yield of 4.0% and 4.6% of the dry raw material weight was obtained, respectively. Determination of the content of microelements in plant objects is of interest in connection with the high biological role of individual chemical elements. Plants contain almost all natural elements,

and their concentration is similar in content in the soil and may vary depending on the composition of the soil, moisture content, plant biology and other factors [2].

Macro and microelements play a certain role in metabolism, affect many physiological processes that occur in plants: development, growth, respiration, photosynthesis, etc. Some microelements activate enzyme systems and play an important role in plastic processes and the formation of body tissues [15, 23]. For this reason, analysis flame atomic absorption spectrometry was used to determine some elements and the obtained data are shown in Table 2.

From the given data it can be seen that in the ashes there are no such trace elements as titanium, chromium, zinc, nickel and gallium, which are present in the dry residue, but in this case there was chromium with a content of 0.0001%. In addition, the content of microelements, for example manganese and copper, in the T104 line was 4-5 times higher than the 108-F variety.

In order to determine the optimal concentration of the extract, wheat and radish seeds were soaked in the dose range from 0.001% to 0.00001%. It should be noted that the extract had a greater effect on the growth of the aerial part of the seedlings of the two cultures under study. For wheat, the optimal concentration was 0.0001%, if the length of the roots (5.6 cm) exceeded the control (3.6 cm) by 55.5%, the length of the stems (3.2 cm) was higher than the control by 28.0% (Table 1).

These figures were higher than the variant with the use of the drug Uchkun by 3.7% and 10.3%, respectively. On the culture of radish, 0.0001% and 0.00001% concentrations showed high activity, the length of the roots in these variants was 3.9 cm and exceeded the control by 34.5%, the stems by 25.0% and 37.5%, respectively, when seeds were treated with Uchkun, the length of roots (3.6 cm) and stems (1.7 cm) was higher than the control by 24.1% and 6.2%, respectively.

Table 2. Comparative analysis of macro and microelements of ash and dry residue of an aqueous extract of cotton leaves of the T-104 line

No	Elements: cotton varieties	%	No	Elements	%
Dry residue					
1	Potassium		10	Barium	
	T-104	6,0		T-104	0,05
	108-F			108-F	
2	Calcium		11	Strontium	
	T-104	20,0		T-104	0.05
	108-F			108-F	0.08
3	Magnesium		12	Titanium	
	T-104	6,0		T-104	0.008
	108-F			108-F	0.008

4	Phosphorus	2,0	13	Chromium	0.001
	T-104			T-104	
	108-F			108-F	
5	Marganese		14	Cuprum	0,010
	T-104	0,08		T-104	
	108-F	0,02		108-F	
6	Silicium		15	Zinc	0.008
	T-104	10,0		T-104	
	108-F			108-F	
7	Aluminum	4,0	16	Nickel	0,002
	T-104			T-104	
	108-F			108-F	
8	Sodium	4,0	17	Molybdenum	0.0007
	T-104			T-104	
	108-F			108-F	
9	Ferrum		18	Gallium	0.0005
	T-104	1,0		T-104	
	108-F			108-F	
Ash					
1	Potassium	1,0	11	Strontium	0.03
2	Calcium	25,0	12	Titanium	-
3	Magnesium	4,0	13	Chromium	-
4	Phosphorus	0,6	14	cuprum	0,0007
5	Marganese	0,07	15	Zinc	-
6	Silicium	0.6	16	Nickel	-

7	Aluminum	0.8	17	Molybdenum	0.0006
8	Sodium	1,0	18	Gallium	-
9	Ferrum	0,002	19	Argentum	0.0001
				T-104	0.0004
				108-F	
10	Barium	0,02			

Table 3. Growth-stimulating activity of buttermilk extract T-104 on wheat and radish seeds

No	Variant	Concentratio	Wheat		Radish	
			Root length	Height of stems	Root length	Stem height
1	Control		3,6±2,0	2,5±1,43	2,9±1,	1,6±1,02
			2		16	
2	Uchkun	0,0001	5,4±0,9	2,9±0,39	3,6±1,	1,7±0,53
			2		01	
3	T-104	0,001	5,4±0,4	3,0±0,53	3,8±1,	1,9±0,81
			8		48	
4		0,0001	5,6±0,3	3,2±0,51	3,9±0,	2,0±0,90
			6		48	
5		0,00001	5,5±0,6	2,9±0,49	3,9±2,	2,2±1,37
			2		34	

In the system for evaluating the action of various synthetic and natural growth stimulants appropriate include indicators of the content of phytohormones in the tissues of seedlings, the ratio of their in roots and shoots. Studies have shown that the kinetin and indoleacetic acid (IAA) content of 10-day-old wheat seedlings increased (Fig. 1).

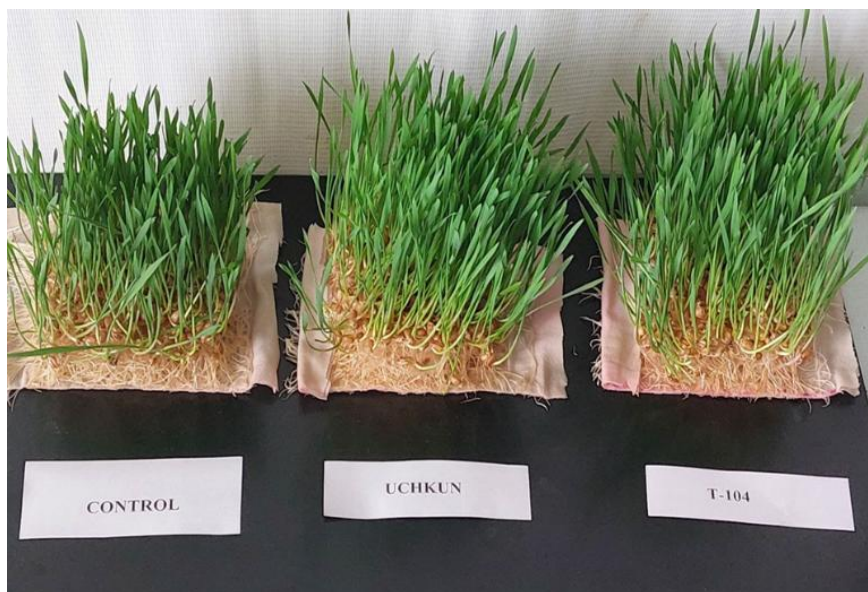


Fig 1. Wheat seedlings grown for chemical analysis for the content of phytohormones

Seed treatment with Uchkun extract and preparation contributed to the accumulation of cytokinin in the roots of wheat seedlings, its content in the experimental variants using the extract and Uchkun preparation was at the same level 0.046 mg / ml and significantly exceeded the control variant (seedlings obtained by soaking seeds in water) by 24.3% (Fig. 2).

The content of kinetin in the stems obtained by treatment with the extract (0.016 mg/mL) was lower than the control by 78.4%, in the variant of the experiment with the use of the drug Uchkun in the aerial parts (0.079 mg/L) exceeded the control by 6.8%.

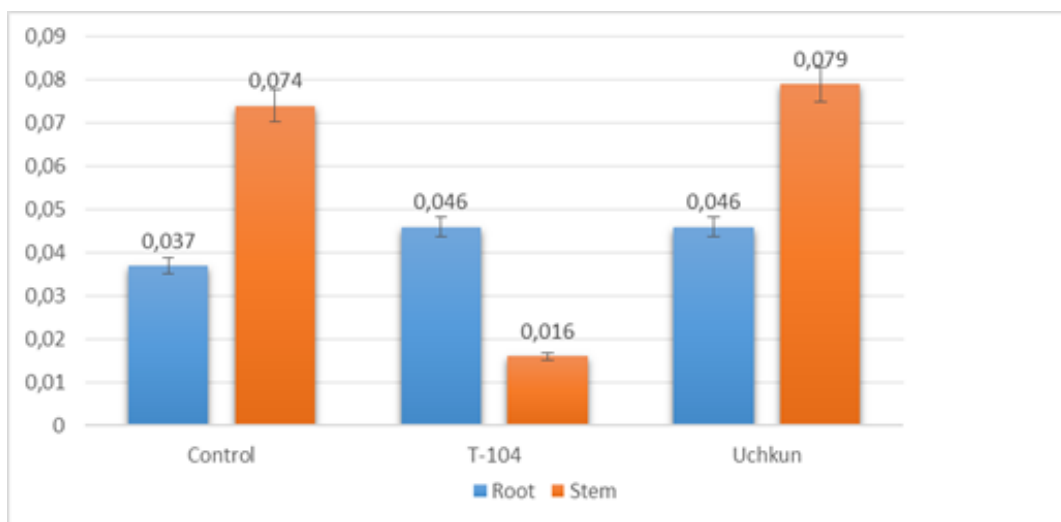


Fig 2. Level of kinetin content in roots and shoots of 10-day-old wheat seedlings after pre-sowing treatment of cotton leaf extract, variety T-104

The content of auxin (0.001 mg/mL) in the roots of the experimental variants was 50% lower than the control one, while in the stems in the experiment using the extract it was lower by 1.1%, with the use of Uchkun at the control level (Fig. 3.).

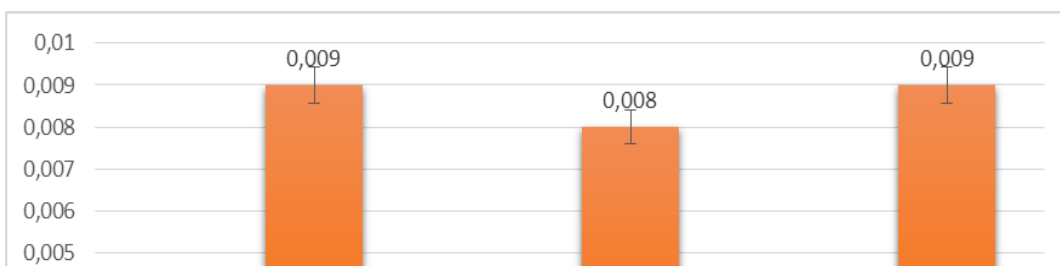


Fig 3. The level of auxin IAA in the roots and shoots of 10-day-old wheat seedlings after presowing treatment with an extract of cotton leaves of the T-104 variety

It is known that stress factors, such as exposure to elevated temperature, affect the influx of cytokinins from the roots. Thus, a change in the delivery of cytokinins from wheat roots under this treatment was shown [17, 19].

We have studied the activity of the extract of cotton leaves of the T104 variety during presowing treatment of seeds of wheat, cucumbers and radishes when these crops are grown under saline conditions. Studies have shown that the extract promotes active growth of wheat when exposed to salt stress, the length of the roots (6.4 cm) was higher than the control variant by 33.3%, stems (6.3 cm) by 75%, fresh weight of seedlings (1.0 g) by 41.9%, dry (by 35.0%).

The extract also showed a high stimulating activity on dicotyledonous plants. When processing cucumber seeds, the length of roots (3.3 cm) was higher than the control by 83.3%, stems (2.5 cm) by 38.8%. Fresh weight (2.17 g) exceeded the control by 35.6%, dry by 38.0%. The length of the radish roots (1.3 cm) was 18% higher, the length of the stems was at the control level, raw (0.39 g) exceeded the control by 44.4%, dry (0.055 g) by 37.5%.

Table 4. Effect of presowing treatment of seeds of wheat, cucumbers, and radishes with an extract of cotton leaves of the T-104 line on the growth and weight of seedlings when grown under salt stress

No	Option name	Concentration (%)	Root length	Stem height	Wet weight (g)	Dry weight (g)
Wheat						
1	Control	-	4,8±1,92	4,6±0,56	0,775±0,045	0,100±0,003
2	Uchkun	0,0001	5,0±1,60	5,2±0,74	1,075±0,005	0,120±0,005
3	T-104	0,0001	6,4±1,10	6,3±0,88	1,100±0,056	0,135±0,003
Cucumbers						
1	Control	-	2,5±0,62	1,8±1,23	1,605±0,003	0,250±0,005
2	Uchkun	0,0001	3,0±1,20	2,3±0,98	1,875±0,003	0,305±0,003
3	T-104	0,0001	3,3±1,13	2,5±1,14	2,170±0,004	0,345±0,005
Radish						

1	Control	-	1,1±0,83	2,2±1,56	0,270±0,004	0,040±0,002
2	Uchkun	0,0001	1,2±0,63	2,4±1,46	0,380±0,003	0,055±0,002
3	T-104	0,0001	1,3±0,99	2,5±1,41	0,390±0,003	0,055±0,003

4. Conclusions

Thus, it was found that in terms of the quantitative composition of polyprenol homologues, the extract of the leaves of the cotton line T 104 differs, where undecaprenol dominates with a content of 72.5%, decaprenol content is 8.8%, and dodecaprenol content is 18.7%. In addition, the content of trace elements in the T104 line, for example manganese and copper, is 4-5 times higher than the 108-F variety.

It was found that on wheat and radish crops, the extract activates the growth of stems to a greater extent. This is explained by the fact that the treatment of seeds with the extract and preparation of uchkun increases the content of cytokinin in wheat seedlings.

The extract exhibited high stress-protective activity; when plants were grown under saline conditions, stimulation of plant growth and accumulation of their wet and dry mass were observed.

Author Contributions

Z.R.P., N.P., M.N.M., K.M., M.K., E.B., K.N.K., S.I.V., A.B. and E.E.O. wrote the manuscript; Z.R.P., N.P., M.N.M., K.M., M.K., E.B., K.N.K. and S.I.V. were performed laboratorial work; M.K. and S.I.V. carried out analyses for 108F and E.E.O. critically revised the manuscript. All authors approved the final version of the manuscript.

Conflicts of interest

The authors declare no competing interests.

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