

SABRAO Journal of Breeding and Genetics
 56 (5) 1982-1993, 2024
<http://doi.org/10.54910/sabrao2024.56.5.21>
<http://sabraojournal.org/>
 pISSN 1029-7073; eISSN 2224-8978



EFFICIENCY OF THE BIOSTIMULANT IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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SUMMARY

Pre-sowing treatment with plant growth regulators is one of the most efficient ways to improve seed germination and seedling tolerance to diverse environmental conditions. The presented study sought to evaluate the effects of polyprenols (emulsion concentrate) of the *Paulownia tomentosa* tree on the growth, physiological, and yield-related traits in winter wheat (*Triticum aestivum* L.). Biologically active substances (polyprenols) of the *Paulownia tomentosa* tree have stimulating and auxin-like activity, contributing to an increase in seed germination, plant height, spike length, grains per spike, grain weight per spike, 1000-grain weight, which increased the grain yield by 5.5–10.5 t/ha in winter wheat. According to the results, the Grom cultivars had the highest degree of drought resistance (94%). Local wheat cultivars Khisorak and Bunyodkor belonged to group IV (above average) and displayed 77.7% to 80.0% drought resistance. Polyprenols also positively affected the leaf chlorophyll a, b, and the total chlorophyll formation during the vegetation phase in winter wheat. Chlorophyll b increased by 0.23 mg/g for the cultivar Bunyodkor, 0.29 mg/g in Khisorak, and 0.07 mg/g in the Grom cultivar under the Republic's Southern Region conditions.

Keywords: Winter wheat (*Triticum aestivum* L.), cultivars, polyprenols, auxin-like activity, stimulating effect, drought resistance, growth and yield traits

Key findings: Biologically active compound polyprenols increased seed germination, plant height, spike length, grains per spike, grain weight per spike, 1000-grain weight, and grain yield in winter wheat (*T. aestivum* L.).

Communicating Editor: Prof. P.I. Prasanthi Perera

Manuscript received: March 02, 2024; Accepted: May 07, 2024.

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Citation: Turaeva S, Kurbanova E, Mamarozikov U, Nurmakhmadova P, Khidirova N, Juraev D, Shoymuradov A, Bakhramova N, Aynakulova Z (2024). Efficiency of the biostimulant in winter wheat (*Triticum aestivum* L.). *SABRAO J. Breed. Genet.* 56(5): 1982-1993. <http://doi.org/10.54910/sabrao2024.56.5.21>.

INTRODUCTION

Competitiveness in the global seed industry necessitates planning some strategies to improve the quality of seed material. It is imperative to enhance the grain crop yield to meet the food demand of the world's bulging population, including wheat (*Triticum aestivum* L.). Interstate seed trade also requires having high-quality products and crops that can withstand adverse impacts of abiotic stress factors and germinate in diverse field conditions, where promising drought-resistant spring soft wheat types are vital in food resource shortage (Fedorenko *et al.*, 2018). Drought stress severely affects food security by lessening crop yields and product quality. Drought resistance refers to a genotype's ability to maintain plant metabolic activities under unfavorable abiotic stress conditions (Juraev *et al.*, 2023).

The least-changing morphological variable under the influence of a selective agent is the stem and root length. Droughts are especially harmful during the vital phases of winter wheat plant growth and development, like stem elongation and ear formation (Alabushev *et al.*, 2019; Morgun *et al.*, 2019). In this regard, research is necessary to develop environment-friendly plant protection products and study their effect on germination, plant growth, and productivity. In addition, establishing effective methods for their use and selecting winter wheat drought-resistant cultivars are imperative for growing under varied conditions of the Republic.

In Uzbekistan in 2023, the sown area of all grain crops amounted to 1.546 million hectares (Statista, 2023), with the winter wheat commonly grown in the region (Knyazeva, 2013). In Southern Uzbekistan regions, especially in Kashkadarya and Surkhandarya, the climate is sharply continental, causing empty spikes and grain thinning, decreasing wheat yield (Danilov, 2017). Kashkadarya and Surkhandarya Oasis are constantly enriched areas with new winter wheat cultivars developed by local and world breeders. However, several abiotic stresses in the republic weaken the traditional and exotic wheat cultivars. A key strategy for resolving

the tension between Uzbekistan's limited water resources and wheat's high water requirement is using drought-resistant cultivars.

One of the most widely used and effective methods for increasing seed germination and seedling resistance to various stresses is the pre-sowing treatment with plant growth regulators (PGR). The PGR are the physiologically active substances that can positively alter metabolic processes in plant ontogenesis, particularly photosynthesis, respiration, enzyme activity, biosynthesis of amino acids, nucleic acids, proteins, phytohormones, accumulation and distribution of nutrients, metabolism, formation of generative organs, productivity, and product quality (Zhemchuzhin, 2014; Abdurakhmanov *et al.*, 2019). Biologically active substances, including plant growth regulators, significantly affect the formation of the epidermis structure of plant leaves (Hrytsaienko and Karpenko, 2021). Low consumption rates of these compounds significantly change plants' growth, physiological, and morphogenesis processes. Applying growth stimulants in nurturing grain crops in economically developed countries could obtain about 20%–30% more crop products (Turaeva *et al.*, 2022, 2023a, b). The manifestation of their action in small doses allows for their wide use in crop production. Presently, their use is particularly relevant.

PGR use is especially crucial in the unfavorable region of light gray meadow soils of the Kashkadarya Region. The traditional intensive use of pesticides in agriculture has now come into conflict with the global problem of environmental protection (Mamarozikov *et al.*, 2019). Therefore, searching for environmentally acceptable compounds that do not harm humans and the environment is necessary. In recent years, practical work has progressed in the republic on developing and using innovative and environment-friendly PGR based on biologically active substances of plants to obtain high yields in grain crops, especially winter wheat (Amanov and Shoymuradov, 2019; Bakhramova *et al.*, 2023).

Plant polyphenols are essential in accelerating seed germination, regulating plant growth and development, and increasing plant

resistance to stress factors (Khidirova *et al.*, 2012, 2022). However, systematic studies to learn biologically active substances' participation in processes under various environmental conditions on seed germination and plant growth and development are limited. This work aimed to identify biologically active compounds obtained from the local flora that are environment-friendly and effective. Additionally, the study will examine the efficacy of these compounds in promoting winter wheat (*T. aestivum* L.) growth and development and provide evidence supporting their viability for grain crop cultivation.

MATERIALS AND METHODS

Physico-chemical properties of polyprenols

The extraction of dried and crushed leaves (1000 g) of the *Paulownia tomentosa* tree, collected in the Tashkent Region, transpired with petroleum ether three times, using the infusion method with a water ratio of 1:10. The organic solvent distillation on a rotary evaporator obtained a dry residue of 140.7 g. The resulting extract received fractionation on the column chromatography. The glass column was 3.0 cm × 100.0 cm in size, using silica gel 100–160 mesh as an adsorbent. For column chromatography, the process used systems of hexane and hexane-diethyl ether with a gradual increase in polarity (Khidirova *et al.*, 2012, 2022). The collected 100 ml of 85 fractions reached control by TLC, using silica gel on TLC Al foils plates, 10 cm × 20 cm with UF 254 nm (Sigma-Aldrich), with benzene-ethyl acetate system at 24:1, $R_f = 0.58$. The developer used KMnO₄ in sulfuric acid, an alcoholic solution of anisaldehyde and sulfuric acid, or iodine vapor. As a result, polyprenol fractions obtained had the amount of 16.88 g. Yield was 1.2% based on air-dry weight (ADW).

The qualitative analysis of polyprenols continued in high-performance thin-layer chromatography (HPTLC) in Camag, Switzerland. Removal conditions: SORBFIL

HPTLC-AF-UV plates, size 10 × 10, solvent for washing the plate - distilled chloroform, air drying at a temperature of 20 °C–25 °C, a time of 10 min, with 1.0 mg of the substance dissolved in 1 ml of the solvent, and a 5 µl sample taken. Solvent system of toluene-ethyl acetate ratio at 19:1, a start-to-finish distance of 70.0 mm, air drying at temperature 20 °C–25 °C, a time of 15 min, with a distance between tracks 7.7 mm, and wavelength 200 nm. Standard sample of polyprenols with isoprene units $n = 10-13$. IR spectra resulted from an FTIR - System 2000 spectrophotometer (Perkin-Elmer) in KBr (ν , cm^{-1}) tablets, using 100 µl Tween-80 as a solvent for 1.0 g of extract. Determination of the auxin activity of biostimulants proceeded on coleoptiles of wheat cultivar Bunyodkor (Kefeli *et al.*, 1975). Employing the split leaf discs method assessed the phytotoxic effect of the biostimulants. Symptoms (diameter of necrosis on plant leaf disks and length of necrosis on segments of wheat leaves) reached accounting after 72 h of incubation (Berestetskiy *et al.*, 2018).

Drought tolerance assessment in seeds

The assessment of drought resistance occurred on three selected winter wheat cultivars, viz., Bunyodkor, Khisorak, and Grom (Kozhushko, 1974). The study used the in situ method in laboratory conditions. Many osmotic agents helped imitate drought, including sodium chloride, sucrose, sorbitol, and mannitol (Gaze *et al.*, 2021). Healthy seeds of winter wheat cultivars incurred washing with formalin solution (3 ml of a 40% formalin solution per 1 liter of water) for 3-5 min before germination. After that, the seed washing continued under running water. Disinfected seeds in Petri dishes comprised 50 experimental units, with four replications, and the area for each option was 0.5 ha. Then, 5 ml of sucrose solution (14 atm) followed into Petri dishes, with 5 ml of distilled water added to the control. The sample-containing dishes proceeded into a thermostat of 20 °C–21 °C for five days, counting germinated seeds after. The average number of germinated seeds in the control credits is 100% (b). The average number of seeds

Table 1. Evaluation of the winter wheat plants resistance.

Groups by resistance	Resistance level, %	Classification
I	0-20	Nonresistant
II	21-40	weak resistant
III	41-60	average resistant
IV	61-80	above average resistant
V	81-100	highly resistant

germinated in the sucrose solution (a) was expressed as a percentage of the number of seeds germinated in the control (b).

$$P = \frac{a}{b} \times 100\%$$

The resistance level classification of wheat seeds in percentage appears in Table 1.

Field experiment

The field experiment happened at the Southern Research Institute of Agriculture, Kashkadarya Region, Uzbekistan. It followed the scheme of experimental cultivars: a) Control (without drug treatment), b) Uchkun 1.0% + pre-sowing seed treatment with 1L/t.s + spraying of plants at the tillering stage (3 g/ha), and c) Polyprenol of *P. tomentosa* 1.0% + pre-sowing seed treatment with 1L/t.s + spraying of plants in the tillering phase (3 g/ha).

The study's object was soft winter wheat cultivars Bunyodkor, Khisorak, and Grom. The field experiment commenced, probing the effects of biostimulants on seed germination, growth, the formation of generative organs, productivity, and product quality during the pre-sowing treatment of wheat seeds by moisturizing at a consumption rate of 0.001 g/L (Avdeenko *et al.*, 2018). Phenological and biometric observations on winter wheat followed the methods of field experiments (Dospikhov, 1979). The standard was the biostimulant Uchkun, registered in the list of pesticides approved for crop production in the Republic of Uzbekistan. Uchkun is a sum of polyprenols isolated from cotton leaves (Shakhidoyatov *et al.* 2012). Agrotechnics of cultivated crops were generally acceptable for this region. During the growing season, the

crops received two top dressings of nitrogen fertilizer, three cultivations, and three irrigations.

The quantitative content measurement of the chlorophyll employed the spectrophotometric method (A & Lab-900 UV/VIS Double Beam Spectrophotometer) (Tretyakov, 1990). The total leaf surface area estimation used a scanning method (Leaf area Meter RC-90) (Dmitriev and Khusnidinov, 2016).

Statistical analysis

The laboratory data analyses and phenological records on wheat yield underwent the mathematical method of analysis of variance on the computer program Origin Pro-6 and Excel 2016 using the Fisher criteria.

RESULTS AND DISCUSSION

The biostimulant obtained from the leaves of the *Paulownia tomentosa* tree is an oily liquid, dark yellow, with a polyprenol (PP) content of 70.2%. The extract yield was 14.7% of the air-dry mass of plants. The total yield of polyprenols from the extract was 2.2%. Column chromatography of the *P. tomentosa* leaf extract made it possible to isolate, in addition to polyprenols, bound and free phytosterols, and triterpenoids, which are of independent interest and could serve a separate purpose. Polyprenol identification emerged by comparing a standard (Uchkun) sample isolated from cotton leaves. Qualitative polyprenol analysis continued on high-performance thin-layer chromatography (HPTLC) in Camag (Figure 1). Figure 2 also shows the identified amounts of polyprenols.

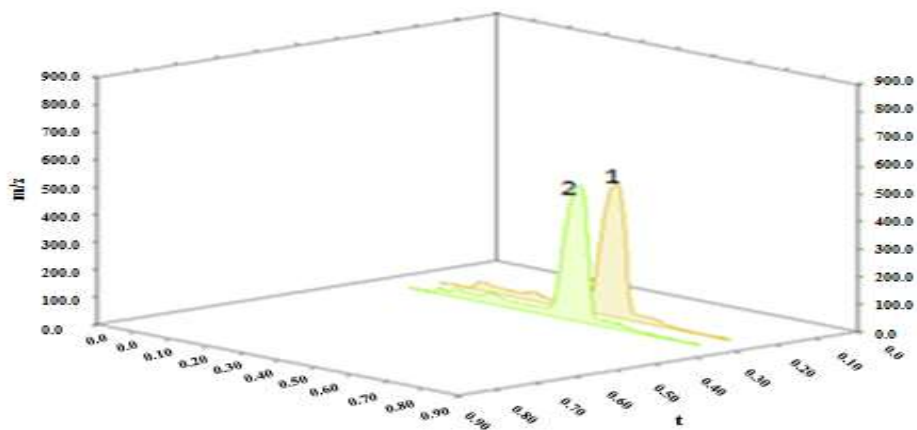


Figure 1. HPTLC of polyprenols (1 - standard sample of polyprenols from cotton leaves, 2 - polyprenols from leaves of *P. tomentosa*).

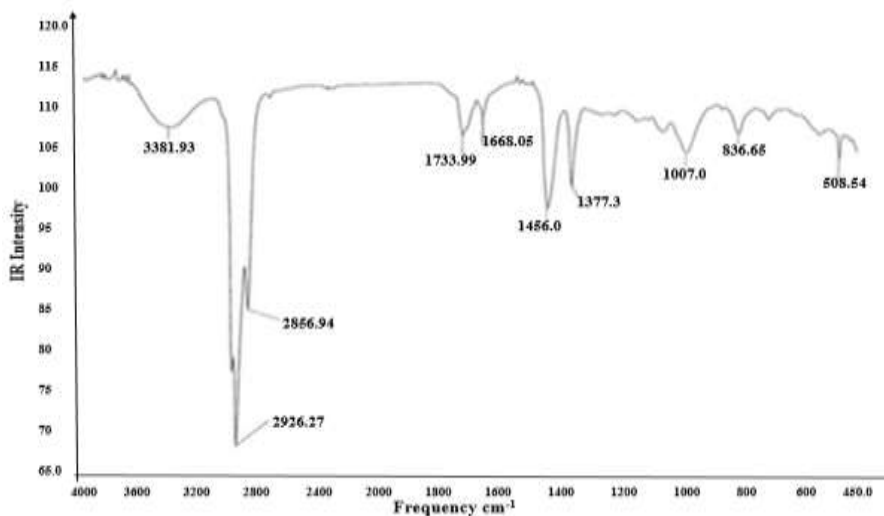


Figure 2. IR spectrum of polyprenols.

In the IR spectrum of polyprenols appeared absorption bands at 836.65 cm^{-1} , characteristic of C-H bending vibrations of trisubstituted olefins ($\text{CH}_2\text{-C}[\text{CH}_3] = \text{CH-CH}_2$), C-O) and vibrations of allyl primary alcohol ($\text{CH}=\text{CH-CH}_2\text{-OH}$) at 1007 cm^{-1} . The bending (C-H) vibrations of the CH_3 group have an absorption band at 1377 cm^{-1} , and the CH_3 and CH_2 groups were distinct at 1456 cm^{-1} . The stretching vibrations of the C = C-bond in the isoprenoid residue appear at 1668 cm^{-1} , and at 2856 cm^{-1} are absorption bands characteristic of the -C-H vibrations of the CH_2 fragment. At 2926 cm^{-1} were C-H vibrations of the CH_2 and CH_3 groups, and at 2962 cm^{-1} -C-H vibrations of the CH_3 groups. The free hydroxyl group has an absorption band at 3605 cm^{-1} . In the region of 3381 cm^{-1} , absorption bands of polymer associates surfaced (Figure 2). The results of the chemical analysis of the study of plant polyprenols (IK, mass-spectrum) correspond to the data of the study of polyprenols from *Vitis vinifera* leaves (Zokirova et al., 2015; Ali et al., 2023; Turganbayev et al., 2023).

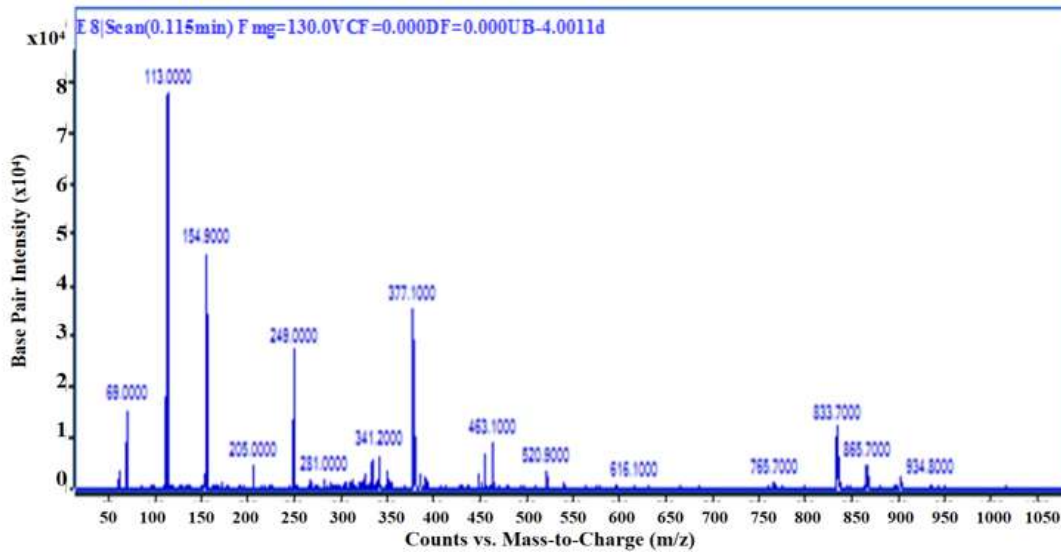


Figure 3. Mass spectrum of polyprenols.

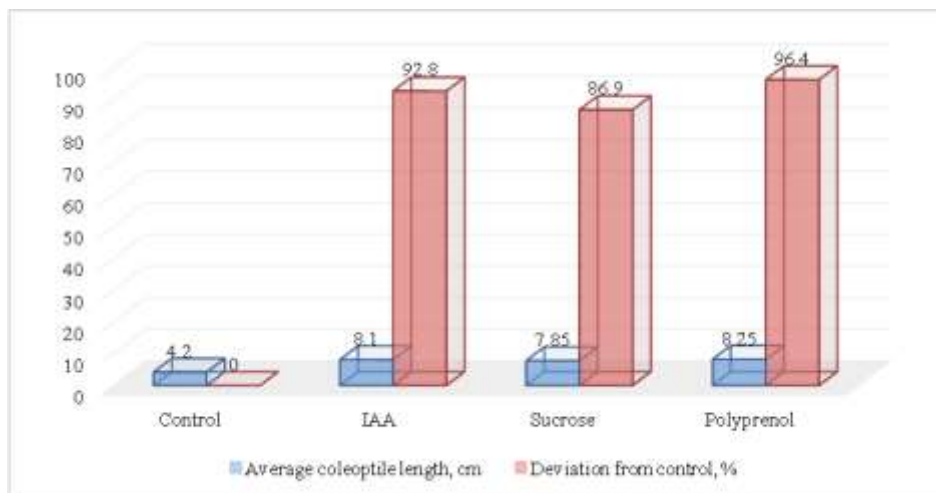


Figure 4. Auxin activity of polyprenols (0.005 mg/MI) on the winter wheat coleoptiles.

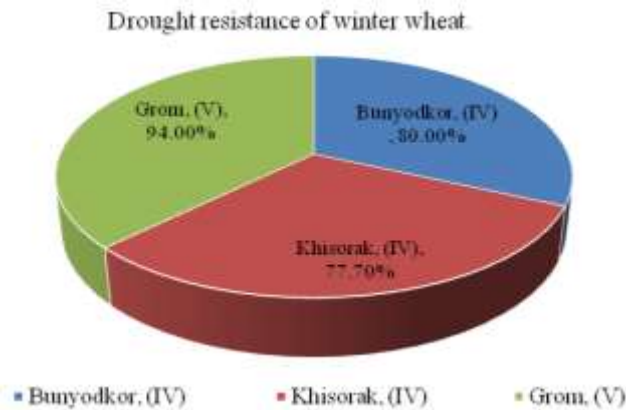
In the mass spectrum of polyprenols from the leaves of *P. Tomentosa* arose low-intensity peaks of molecular ions with m/z 902 ($C_{65}H_{106}O$ $n=13$), (834 [$C_{60}H_{98}O$] $n=12$) related to dodecaprenol and 766 to undecaprenol ($C_{55}H_{90}O$ $n=11$). The fragment 69 was consistent with the fragmentation of polyprenols described in the literature from other sources (Figure 3). The 1.0% aqueous emulsion preparation with surfactants was for agricultural use. The resulting stable aqueous emulsion meets the requirements of GOST-

6243-75 (SCSCMU, 1976), with density at 200 °C - 0.996 g/cm³. Biotests proceeded in a laboratory to determine the auxin activity of polyprenols. As shown in Figure 4, using polyprenols of *P. tomentosa* at a concentration of 0.005mg/mL resulted in an increased length of wheat coleoptiles (96.4%) compared with the control treatment. In the variant with sucrose, the said increase was 86.9%, while with IAA (Indole-3-acetic acid), the obtained percentage increase was 92.8%.

Table 2. Phytotoxicity of the polyprenols.

Options	Necrotic spot length (mm)		
	Wheat	Tomato	Cucumber
Control	i/a*	i/a	i/a
Polyprenols (10.0 mg/ml)	i/a	i/a	i/a
Polyprenols (100.0 mg/ml)	i/a	i/a	i/a
Polyprenols (1000.0 mg/ml)	i/a	i/a	i/a

* i/a – inactive, length of necrotic spot on segments of wheat leaves and on disks of tomato and cucumber leaves, mm.

**Figure 5.** Drought resistance of different winter wheat cultivars.

One of the primary requirements for pesticides is the lack of phytocidal action on the protected plant, manifested as necrosis (Berestetskiy *et al.*, 2020). The screening results revealed that polyprenols do not have phytotoxic activity against the test plants, and necrotic patches were not visible in the leaves. Many plant extracts and their secondary metabolites, including polyprenols, are not phytotoxic (Turaeva *et al.*, 2021). Thus, the study results showed that the polyprenols of *P. tomentos*a were not hazardous to the cultivated plants (Table 2).

Abiotic stress tolerance has become a long-desired characteristic in winter wheat, particularly in areas with low humidity during the growing season (Nazarenko *et al.*, 2022). In the presented study, all the samples split into highly resistant (group V – 81%–100%) and above-average resistant (group IV - 61%–80%) categories (Table 1). The results of assessing the drought resistance of winter wheat indicated no unstable samples with a germination rate between 0% and 40% were notable. According to screening results, in the

wheat cultivars Khisorak and Bunyodkor, drought resistance degree ranged from 77.7% to 80.0%, placing them in group IV (above average). However, the wheat cultivar Grom had the highest drought resilience (94%) (Figure 5).

Field studies

Productive tillering, grains per spike, and 1000-grain weight, although interacting with the environment, are crucial in managing grain yield (Juraev *et al.*, 2020). The laid-out field experiments had successive replications in a randomized arrangement of options. Phenological observations showed that the experimental variants surpassed the control and the standard variants in plant height, spike length, grain number, and grain weight per spike, with the 1000-grain weight enhancing the grain yield of winter wheat. Polyprenols provide defense capabilities against environmental stress conditions and pathogen attacks (Satish *et al.*, 2020).

The field experiment results showed that by treating winter wheat cultivars Bunyodkor and Khisorak seeds with polyphenols, germination energy increased by 22.3% to 22.5%, and the field germination by 17.2% to 20.1% compared with the control variant. Correspondingly, a high percentage of field germination was evident in the seeds treated with Uchkun (92.1%–96.5%), which surpassed the control by 12.7% to 20.0% (Table 3). Modern cultivars with high crop potential, grain quality, and disease resistance adapted well to the existing environmental conditions (Morgounov *et al.*, 2005).

The findings further revealed that pre-sowing treatment and foliar application of biostimulants positively affected the yield-contributing traits compared with the control. The number of productive tillers increased in the variant with the pre-sowing treatment of polyphenols and exceeded the control by 9.7 to 9.5 pcs/m² (Table 4). Polyphenol (3 g/ha) foliar application during vegetation had a noticeable effect on the number of productive tillers, the grains per spike, grain weight per spike, and 1000-grain weight, which are the primary prerequisites for high grain yield formation. A similar pattern was also notable in sowing other winter wheat cultivars (Khisorak and Grom) (Table 4). According to a past study on developing the plant growth regulator, polyphenol facilitates plant growth, resulting in faster germination and higher grain yield (Kwon, 2004). The plant growth regulator promotes crop germination with uniform development, allowing for easier mechanical

harvesting. Furthermore, it can enhance crop output in cereals, vegetables, and fruits, helping crop development (Kwon, 2004).

Pre-sowing treatment and polyphenol foliar application increased the number of grains per spike by 4.2 pieces, while with Uchkun, the said parameter increased by 4.0 compared with the control (Table 4). Foliar treatment of winter wheat cultivar Bunyodkor with biostimulants revealed an upsurge in grain weight per spike, which also exceeded the control by 13.7% and 7.8%, as well as 1000-grain weight by 12.2% and 6.2%, respectively. Positive results also prevailed for the other studied winter wheat cultivars. The presented research authenticates using polyphenols in winter wheat crops increases the yield by more than 10.5 t/ha (Bunyodkor), 7.7 t/ha (Khisorak), and 5.5 t/ha (Grom) (Table 4). The highest economic yield of 8.61 t/ha emerged for the cultivar Bunyodkor with biostimulant use. Winter wheat cultivar Khisorak, treated with Uchkun, gave an economic yield of 8.26 t/ha. In the experiments, the market yield of the cultivar Grom was lower than that of all other varieties, which amounted to 4.3–4.53 t/ha (Table 4).

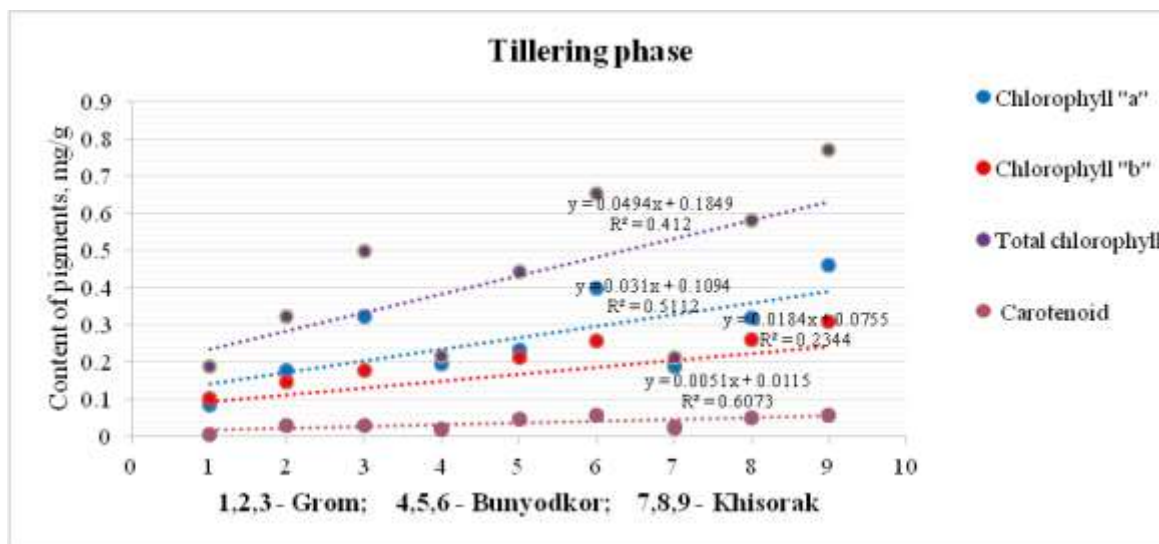
The polyphenol application enhances the plant leaf area and the grain quality indicators of the Republic’s central territory conditions (Kurbanova *et al.*, 2023). Previous studies also established the effect of polyphenols on hormone-like activity and growth stimulation of crops like cotton, tomato, and cucumber (Mamatkulova *et al.*, 2016). Chlorophyll is a unique compound that

Table 3. Influence of polyphenols on the germination energy of seeds, field germination, and tillers length in winter wheat.

Cultivars	Options	Germination energy of seeds (%)	Field germination (%)	Plant height (cm)
Bunyodkor	Control	68.3	79.4	9.1±0.6
	Uchkun 1.0%, 1L/t.s	80.4	92.1	14.3±0.7
	Polyphenols 1.0%, 1L/t.s	90.6	96.6	15.4±0.5
Khisorak	Control	75.0	80.5	9.3±0.7
	Uchkun 1.0%, 1L/t.s	90.0	96.5	13.7±0.5
	Polyphenols 1.0%, 1L/t.s	91.0	96.6	16.1±0.5
Grom	Control	76.9	83.5	7.6±0.6
	Uchkun 1.0%, 1L/t.s	88.5	96.5	11.2±0.7
	Polyphenols 1.0%, 1L/t.s	89.0	93.3	12.1±0.3

Table 4. Influence of polyprenols on the productivity and its contributing traits in winter wheat.

Options	Dose (L/t.s.+ g/ha)	Productive tillers plant ⁻¹ (pcs/m ²)	Grains spike ⁻¹ (pcs)	Grain weight spike ⁻¹ (g)	1000-grain weight (g)	Grain yield (t/ha)	Economic yield (t/ha)	
Bunyodkor	Control	411.5±0.4	48.2±0.3	2.55±0.7	45.82	5.60	7.34	
	Uchkun	1L/t.s.+3g/ha	417.5±0.5	52.2±0.4	2.75±0.6	48.68	6.30	8.00
	Polyprenols	1L/t.s.+3g/ha	421.2±0.4	52.4±0.7	2.9±0.2	51.42	5.95	8.61
	LSD _{0.5} = 3.48	S _x = 1.13						
Khisorak	Control	414.0±0.8	40.7±0.2	1.63±0.6	46.60	6.00	4.72	
	Uchkun	1L/t.s.+3g/ha	420.1±0.5	46.2±0.6	2.81±0.5	50.50	6.85	8.26
	Polyprenols	1L/t.s.+3g/ha	422.4±0.6	45.9±0.3	2.35±0.5	51.50	6.77	6.95
	LSD _{0.5} = 1.72	S _x = 0.56						
Grom	Control	352.7±0.5	40.2±0.4	1.57±0.2	39.02	4.27	3.93	
	Uchkun	1L/t.s.+3g/ha	365.1±0.2	38.1±0.4	1.72±0.5	41.78	4.37	4.30
	Polyprenols	1L/t.s.+3g/ha	362.2±0.6	38±0.7	1.79±0.2	42.02	4.25	4.53
	LSD _{0.5} = 2.10	S _x = 0.68						

**Figure 6.** Effect of polyprenols on the chlorophyll content during the tillering phase in winter wheat.

determines photosynthesis intensity, distinguishing green plants' viability, yield, and nutritional values (Zhu-Xin *et al.*, 2010; Hamblin *et al.*, 2014).

Polyprenols also caused a considerable increase in chlorophyll a at the tillering phase of all winter wheat cultivars (Figure 6). Cultivar Bunyodkor increased by 0.2 mg/g, Khisorak by 0.27 mg/g, and Grom by 0.24 mg/g, compared with the control. Chlorophyll b rose by 0.23 mg/g for the cultivar Bunyodkor, by 0.29 mg/g in Khisorak, and by 0.07 mg/g in the cultivar

Grom under Kashkadarya conditions, Uzbekistan. Total chlorophyll and carotenoid contents varied significantly among different cultivars, ranging from 0.3–0.56 to 0.02–0.56 mg/g after seven days of foliar treatment. However, chlorophylls escalated during the wheat's tubing phase (Figure 7). Promising results were also analogous to past findings, which revealed that polyprenols could influence plant photosynthetic properties by modifying the dynamics of the thylakoid membrane (Akhtar *et al.*, 2017).

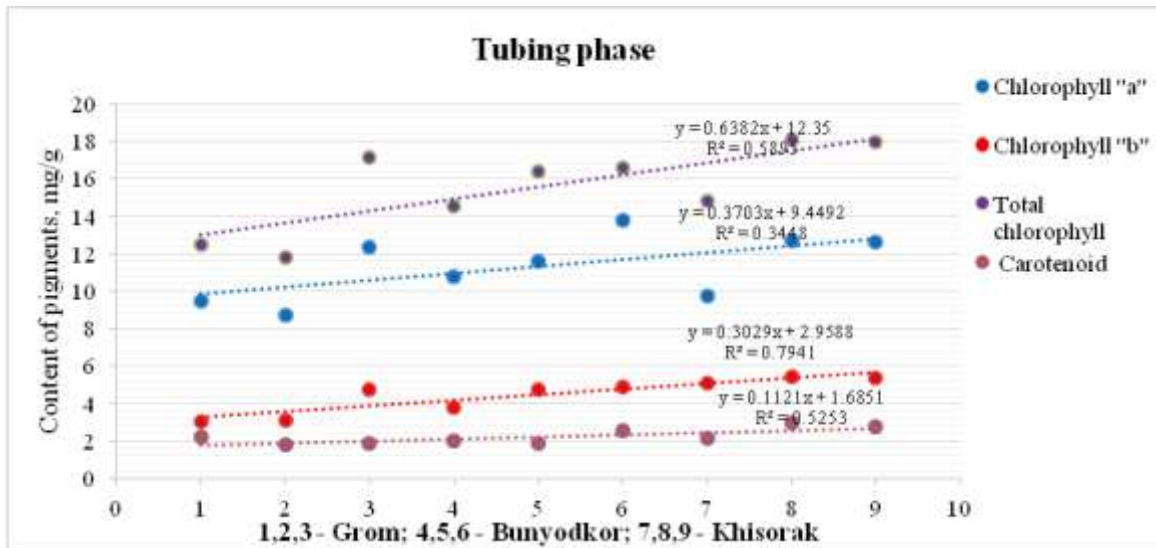


Figure 7. Effect of polyprenols on the chlorophyll content during the tubing phase in winter wheat.

CONCLUSIONS

Using polyprenol (0.01 g/L) developed better conditions for the growth and development of winter wheat (*T. aestivum* L.), ultimately leading to an increased grain yield. The biological tests convincingly indicated the stimulating activity of biologically active substances of *P. tomentosum*, which favorably affects seed germination, tillering, grain weight, and grain yield.

ACKNOWLEDGMENTS

The authors express deep gratitude to Prof. Amanov O.A. for his help in conducting field experiments in research.

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