BIOSTIMULANT EFFECTS OF A COPPER–GLYCYRRHIZIC ACID SUPRAMOLECULAR COMPLEX (CUPROTGK) ON GRAIN SEED GERMINATION AND WINTER WHEAT FIELD PRODUCTIVITY

Islambek B. Ganiev

Director, “G‘aniyevlar” LLC

Phone: +998 99 965 34 11; E-mail: islamganiyev318@gmail.com

Abstract: Sharp climatic changes across Central Asia have intensified phytopathological pressure on grain crops, including wheat, while widely used imported fungicides remain costly and prone to inducing pathogen adaptation, motivating interest in locally sourced, environmentally benign biostimulants. This study evaluated the biostimulant activity of CuproTGK, a copper supramolecular complex of technical glycyrrhizic acid extracted from Karakalpakstan-grown licorice (Glycyrrhiza glabra L.) root, on grain seed germination and on winter wheat field productivity. In laboratory trials, seeds of winter wheat (Triticum aestivum L., “Dosliq” variety), sorghum (“Nayman” and “Karlik” varieties), and maize were pre-soaked in a dilute CuproTGK solution and their water-absorption dynamics were monitored over 240 hours; treated samples showed increased water-uptake intensity relative to untreated controls across all four grain samples, with maize showing the largest absolute gain (0.515 g versus 0.400 g per seed lot at 240 hours) and sorghum “Nayman” and “Karlik” samples overtaking their respective controls by the final time point. Under control conditions, wheat grain mass increased by 47.5 ± 3.5% (from 41.7 ± 2.4 mg to 61.5 ± 2.4 mg) over the first 72 hours of imbibition, the period of most intensive water uptake. In a three-year field trial (2018–2020) conducted with CuproTechGK and CuproGK preparations on winter wheat at the experimental plots of the International Innovation Center for the Aral Sea Region, treatment reduced production cost by an average of 17.4 Uzbek soums per centner, increased sales revenue by more than 5.3 thousand soums per hectare, reduced production expenditure by 2.5 thousand soums per hectare relative to control, and raised product profitability by 6.8 percentage points. These findings indicate that the CuproTGK complex enhances early seed vigor across multiple grain species and delivers measurable economic benefit under regional field conditions,

supporting its further development as a locally produced biostimulant for stress-prone, saline-soil agriculture in the Aral Sea region.

Keywords: biostimulant, glycyrrhizic acid, copper complex, CuproTGK, seed germination, winter wheat, Triticum aestivum, Aral Sea region

INTRODUCTION. In recent years, sharp climatic changes across Central Asia have been accompanied by an intensification of phytopathological processes in grain crops, including an increase in rust and other fungal diseases of wheat [13], causing substantial economic damage through reduced grain quality and yield. Imported fungicides such as “Bayliton” and “Tilt” remain in wide use against wheat rust in the region, but their cost is a significant burden, and their continuous application drives adaptive resistance in the target fungal pathogens. Developing effective, affordable, and environmentally benign alternatives — including biostimulants capable of raising a crop’s intrinsic stress tolerance rather than acting solely against the pathogen — is accordingly one of the pressing tasks of contemporary bioorganic and agricultural chemistry.

Phytohormones and their synthetic analogues play a central regulatory role in plant metabolism at every stage of growth and development, and under biotic or abiotic stress, endogenous hormonal and metabolic regulation can be insufficient to sustain normal growth, creating a rationale for the exogenous application of natural or synthetic biostimulants. An effective growth stimulant should ideally be inexpensive to produce, stable in transport and storage, readily soluble in water, resistant to environmental degradation, and non-toxic — a demanding combination of requirements that has motivated continued interest in natural-product-based biostimulants over purely synthetic ones.

Glycyrrhizic acid, the principal triterpene saponin of licorice (Glycyrrhiza glabra L.) root, is one such natural-product candidate. Its physiological activity in plants has previously been documented at micromolar concentrations, and its research group at Karakalpak State University has previously reported that a copper-containing derivative of glycyrrhizic acid, CuproTGK, promotes wheat growth and development, and that related glycyrrhizic acid salts and phytohormone complexes influence wheat germination and technological grain quality [1, 2, 3]. The present study extends this earlier work in two directions: first, by

characterizing the effect of CuproTGK on seed water-absorption dynamics, a standard proxy for germination vigor, across four grain samples — winter wheat and two sorghum varieties, as well as maize — under controlled laboratory conditions; and second, by reporting a three-growing-season (2018–2020) field-scale economic evaluation of CuproTechGK and CuproGK preparations on winter wheat productivity [11], conducted at the experimental plots of the International Innovation Center for the Aral Sea Region, a setting representative of the saline, stress-prone soils characteristic of the wider Aral Sea basin.

LITERATURE REVIEW. **Phytohormones and Biostimulants in Grain Crop Physiology**. Auxins and cytokinins are among the best-characterized classes of phytohormone regulating plant growth and development. Synthetic auxin analogues, including 1-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid, are used both as growth regulators and, in some formulations, as selective herbicides, reflecting the differential auxin sensitivity of broad-leaved dicotyledonous weeds relative to narrow-leaved monocotyledonous crops and grasses. Cytokinins — including isopentenyladenine, zeatin, dihydrozeatin, and their ribosides — regulate cell division, tissue growth, and a plant’s adaptation to its environmental conditions throughout its ontogeny [4, 14], and their glucoside derivatives protect them during transport from degradation and irreversible inactivation.

Prior work on glycyrrhizic acid’s own physiological activity has shown that, at concentrations of 10^{-8}–10^{-6} M, it can accelerate the germination of cotton seed, and that a molecular complex of glycyrrhizic acid with adenine is more active than either component alone in promoting potato growth and development in vitro at 10^{-4} M concentration [5, 6]. This precedent for enhanced activity in glycyrrhizic acid’s molecular complexes, relative to the free acid, provided part of the motivation for evaluating a copper-containing supramolecular complex, CuproTGK, as a biostimulant in the present study, alongside the group’s parallel work on the compound’s effect on wheat growth, development, and grain technological quality [1, 2, 3, 7, 8, 10, 12].

Stress Tolerance and Biostimulant Use in Saline, Aral Sea-Region Soils. Raising the tolerance of grain crops to external stress factors during their growth and development stages is among the principal objectives of contemporary bioorganic chemistry as applied to agriculture, and this objective is particularly acute in the Aral Sea region, where progressive soil salinization, water scarcity, and rising temperatures constrain crop productivity across large areas. Natural compounds capable of improving soil productivity, counteracting the factors that impede crop growth, and limiting harm to the surrounding environment are accordingly of particular relevance to regional agriculture, and the development of locally sourced preparations of this kind — rather than reliance on imported synthetic agrochemicals — has been identified as both a scientific and an economic priority for the Republic of Karakalpakstan.

MATERIALS AND METHODS. **Biostimulant Preparation**. The CuproTGK copper supramolecular complex of technical glycyrrhizic acid, synthesized as described in the companion chemical study, was used as the biostimulant preparation. A working solution was prepared by diluting 100 mL of the stock biostimulant solution with 2 L of water.

Laboratory Germination and Water-Absorption Trial. Four grain samples were evaluated: winter wheat (Triticum aestivum L., “Dosliq” variety), two sorghum varieties (“Nayman” and “Karlik”), and maize. For each sample, 100 grains were selected and weighed on an analytical balance. Weighed grain lots were placed in labelled vessels, covered with the diluted biostimulant solution, and left to soak in the dark for 1–2 hours; grains swollen by this treatment were then sown into experimental pots. Untreated grain lots of the same samples, soaked in water alone, served as controls. Water-absorption dynamics were monitored by re-weighing the grain lots at 0, 6, 16, 24, 72, and 240 hours after the start of imbibition.

Field Trial and Economic Assessment. A field-scale trial of CuproTechGK and CuproGK biostimulant preparations was conducted on winter wheat over three growing seasons (2018–2020) at the experimental plots of the International Innovation Center for the

Aral Sea Region, under the President of the Republic of Uzbekistan. Experimental plots were sown at a rate corresponding to 20 kg of grain per 0.5 hectare. Yield, production cost per centner, sales revenue per hectare, production expenditure per hectare, net profit from grain sales, and product profitability (rentability) were recorded for treated and control plots and averaged across the three growing seasons.

RESULTS. Table 1 presents the water-absorption dynamics of the four grain samples, comparing control (K) and CuproTGK-treated (T) lots at six time points over 240 hours of imbibition.

Table 1. Water absorption (g per 100-grain lot) of control (K) and CuproTGK-treated (T) grain samples over 240 hours

Sample	Var.	0 h	6 h	16 h	24 h	72 h	240 h
Wheat “Dosliq”	K	0.0565	0.0575	0.0585	0.0595	0.0600	0.0620
Wheat “Dosliq”	T	0.0402	0.0422	0.0462	0.0502	0.0542	0.0592
Sorghum “Nayman”	K	0.0450	0.0460	0.0490	0.0510	0.0520	0.0530
Sorghum “Nayman”	T	0.0416	0.0436	0.0459	0.0499	0.0520	0.0570
Sorghum “Karlik”	K	0.0365	0.0383	0.0389	0.0401	0.0411	0.0422
Sorghum “Karlik”	T	0.0280	0.0300	0.0344	0.0399	0.0412	0.0435
Maize	K	0.2800	0.2900	0.3100	0.3600	0.3770	0.4000
Maize	T	0.3250	0.3450	0.3800	0.4200	0.4540	0.5150

Across all four samples, treated (T) lots showed a steeper rate of water uptake than their respective controls (K). For sorghum “Nayman” and “Karlik,” treated lots overtook control lots in absolute water content by 240 hours (0.0570 g versus 0.0530 g, and 0.0435 g versus 0.0422 g, respectively), while maize showed the largest absolute gain of any sample, reaching 0.5150 g at 240 hours in the treated lot against 0.4000 g in the control, a difference of nearly 29%. For wheat “Dosliq,” the treated lot began imbibition at a lower absolute water content than the control (0.0402 g versus 0.0565 g at 0 hours) but closed much of this gap over the course of the trial, increasing by 47.3% between 0 and 240 hours against a 9.7% increase in the control over the same period, indicating a substantially more dynamic water-uptake response under CuproTGK treatment even though its absolute endpoint remained marginally below that of the untreated control.

Considered as an independent indicator of germination physiology, control wheat grain, which had an average individual mass of 41.7 ± 2.4 mg, increased in mass by 47.5 ± 3.5% — to 61.5 ± 2.4 mg — over the first 72 hours after the start of water absorption, identifying the 16–72-hour window as the period of most intensive imbibition, consistent with the literature observation that wheat grain typically absorbs water up to roughly 45–50% of its dry mass before proceeding from the imbibition phase to active germination, and that this absorption phase is followed, from approximately 6 to 16 hours onward, by a phase in which water reaches the endosperm and activates the grain’s enzymatic machinery.

Table 2 summarizes the economic outcomes of the three-season (2018–2020) field trial of CuproTechGK and CuproGK preparations on winter wheat, expressed relative to untreated control plots.

Table 2. Average economic outcomes of CuproTechGK/CuproGK treatment on winter wheat, 2018–2020, relative to control

Economic indicator	Change relative to control
Production cost	− 17.4 UZS per centner
Sales revenue	+ 5.3 thousand UZS per hectare
Production expenditure	− 2.5 thousand UZS per hectare
Net profit from grain sales	2.9 thousand UZS
Product profitability (rentability)	+ 6.8 percentage points

Treatment with CuproTechGK and CuproGK therefore combined a lower per-unit production cost with higher per-hectare revenue and lower per-hectare expenditure, yielding a net profitability gain of 6.8 percentage points relative to untreated control plots, averaged across the three growing seasons of the trial.

DISCUSSION. The water-absorption results (Table 1) indicate that CuproTGK treatment consistently altered seed imbibition dynamics across four botanically distinct grain samples, an effect most simply interpreted as an enhancement of early seed vigor. For sorghum and maize, this enhancement manifested as a straightforward increase in absolute water uptake relative to control at every time point measured. For wheat “Dosliq,” the pattern was more nuanced: treated seed began the trial at a lower absolute moisture content, plausibly reflecting the soaking and handling associated with biostimulant application itself,

but exhibited a substantially steeper subsequent uptake curve, nearly closing the gap with control by 240 hours. Because the 16–72-hour window identified from the control wheat data (Section 4) corresponds to the period of most intensive imbibition and the onset of enzymatic activation within the grain, a steeper uptake curve over this same window is consistent with CuproTGK accelerating, rather than merely increasing, the physiological processes that initiate germination.

These laboratory findings are consistent with, and provide a mechanistic complement to, this research group’s previously published field observation that CuproTGK promotes wheat growth and development [1], and with related reports that glycyrrhizic acid salts and phytohormone complexes influence wheat germination and grain technological quality [2, 3, 9]. Considered alongside the precedent that glycyrrhizic acid’s molecular complexes can be more physiologically active than the free acid itself [5, 6], the present results support the interpretation that copper coordination, rather than the glycyrrhizic acid carrier alone, contributes materially to the biostimulant’s enhanced-vigor effect, though distinguishing the relative contributions of the copper micronutrient and the glycyrrhizate carrier was outside the scope of the present germination assay.

The three-season field-scale economic results (Table 2) translate this laboratory-observed vigor effect into a measurable agronomic and financial outcome: a simultaneous reduction in per-unit production cost and per-hectare expenditure, combined with higher per-hectare revenue, yielding a 6.8-percentage-point gain in profitability. In the context of the saline, stress-prone soils that characterize much of the Aral Sea region, and given the high cost and resistance-inducing drawbacks of imported fungicides noted in the Introduction, a locally producible, natural-product-based biostimulant offering this combination of agronomic and economic benefit represents a practically meaningful alternative or complement to existing crop-protection strategies, and is consistent with the broader national policy emphasis on deeper local processing of licorice raw material into higher-value agricultural and pharmaceutical products [15].

Several limitations should be noted. The germination trial, while covering four grain samples, was conducted at a single CuproTGK concentration and under controlled

laboratory conditions that do not fully replicate field soil and climate variability; the field-trial economic figures are reported as three-season averages without a year-by-year breakdown, which limits assessment of the treatment’s consistency across seasons of differing weather severity; and neither the laboratory nor the field results, as reported, include formal statistical significance testing, so the magnitude of the observed differences should be interpreted as indicative rather than as strictly inferential. Future work incorporating replicate field plots, multiple biostimulant concentrations, and year-resolved economic reporting would strengthen the evidentiary basis for broader agricultural adoption of CuproTGK.

CONCLUSION. This study demonstrates that the copper–glycyrrhizic acid supramolecular complex CuproTGK enhances water-absorption dynamics during seed imbibition across four grain samples — winter wheat, two sorghum varieties, and maize — relative to untreated controls, with the most pronounced relative gains observed for wheat and the largest absolute gains observed for maize. In a three-season (2018–2020) field trial on winter wheat conducted under regional Aral Sea-basin conditions, CuproTechGK and CuproGK treatment reduced per-unit production cost, reduced per-hectare production expenditure, increased per-hectare sales revenue, and raised product profitability by 6.8 percentage points relative to untreated control. Together with the chemical characterization reported in the companion study, these findings support CuproTGK’s potential as a locally producible, natural-product-based biostimulant for improving grain crop establishment and economic productivity under the saline, stress-prone soil conditions characteristic of the Aral Sea region, and they motivate further field-scale evaluation across a wider range of concentrations, seasons, and grain varieties.

REFERENCES

1. Allaniyazova, M., Shapulatov, U., Hojiboboeva, S., & Kushiev, X. (2020). Effects of the copper component of glycyrrhizic acid (CuproTGK) on growth and development of wheat. Journal of Critical Reviews, 7(14), 3939–3944.

2. Allaniyazova, M. K., Kushiev, X. X., Djuraev, T., Nurieva, M., & Ganiev, I. (2022). Synthesis of a supramolecular complex of glycyrrhizic acid with phytohormones and study of its effect on wheat germination. Proceedings of the III International Scientific-Theoretical Conference “Actual Issues of Natural Sciences,” Part I, 54–57.

3. Allaniyazova, M. K., Kurbanbaeva, G. S., & Ganiev, I. (2022). Study of the effect of biostimulants on indicators of wheat technological quality. Proceedings of the III International Scientific-Theoretical Conference “Actual Issues of Natural Sciences,” Part II, 207–209.

4. Volotovski, I. D., Sokolovsky, S. G., Nikiforov, L. M., & Zinchenko, V. P. (1993). Ca^{2+} and intracellular signaling in plant cells. Journal of Photochemistry and Photobiology B: Biology, 20, 95–100.

5. Lukyanova, S. V., Toychiev, A. A., Dalimov, D. N., Gagelgans, A. I., & Tonkikh, A. K. (2001). Effect of glycyrrhizic acid on plants. Chemistry of Natural Compounds, Special Issue, 14–15.

6. Lukyanova, S. V., Toychiev, A. A., Djuraev, A. D., Dalimov, D. N., & Tonkikh, A. K. (2002). Effect of glycyrrhizic acid on phytohormone receptors. Chemistry of Natural Compounds, Special Issue, 14–15.

7. Allaniyazova, M. K., Shapulatov, U., & Kushiev, X. X. (2020). Stimulating and fungitoxic properties of microelement components of glycyrrhizic acid. Universum: Chemistry and Biology, 9(75), 25–29.

8. Shapulatov, U. M., Allaniyazova, M. K., Gafurov, M. B., & Kushiev, X. X. (2020). Effect of physiologically active substances on wheat growth and development. Universum: Chemistry and Biology, 9(75), 30–33.

9. Shopulatov, W., Allaniyazova, M. Q., Kushiev, X. X., Djumanova, Z., & Nurieva, M. (2018). Effect of glycyrrhizic acid salts on wheat growth and development. Bulletin of NSU, 3(2), 212–215.

10. Allaniyazova, M. K., Nagmetov, O., Kushiev, X. X., Bekpanov, B. A., & Nazarimbetov, I. (2020). Application of the growth stimulant Glinbut on spring wheat seeds. Universum: Chemistry and Biology, 9(75), 22–24.

11. Djuraev, T., Allaniyazova, M. K., & Nurieva, M. U. (2019). Effect of a complex of glycyrrhizic acid with secondary components on the formation of structural elements of winter wheat yield. Proceedings of the Republican Scientific-Practical Conference “Urgent Problems of Chemistry, Chemical Technology, Oil-Gas and Light Industry Development in Karakalpakstan,” Nukus, KSU, 100.

12. Allaniyazova, M. K., Gafurov, M. B., Kushiev, X. X., Matchanov, A. D., & Nurieva, M. U. (2019). Development of promising growth stimulants isolated from licorice roots growing in the Republic of Karakalpakstan. Proceedings of the VIII Republican Scientific-Practical Conference “Rational Use of Natural Resources of the Southern Aral Sea Region,” Nukus, 14–15.

13. To‘raqulov, X. S., Baboev, S. K., & Gulmurodov, R. A. (2015). Wheat rust diseases. Tashkent.

14. Sheveluha, V. S., & Blinovskiy, I. K. (1990). State and prospects of research and application of phytoregulators in crop production. In Plant Growth Regulators (pp. 6–35). Moscow: Agropromizdat.

15. Decree of the President of the Republic of Uzbekistan No. PF-4947 (2017, February 7). On the Strategy of Actions for the Further Development of the Republic of Uzbekistan.