

# The Effect of Exogenous Copper-Quercetin Complex on Wheat (*Triticum aestivum* L.) Seedlings Growth in Drought Stress

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**Abstract:** Drought is a global problem in agriculture, which reduces the productivity of plants so is important environmental stress affecting plant metabolism and growth. Quercetin is a flavonoid with strong antioxidant properties and plays an important role in regulating the physiological processes in the plant. The study investigated the effect of the exogenous quercetin-copper complex (0.01, 0.05 and 0.1%) on wheat seedlings subjected to drought (30% f.w.c.). It was shown that drought stress had a negative effect on the photosynthesis process of plants. The application of spraying with a quercetin derivative caused an increase in the values of the parameters in wheat plants subjected to drought stress compared to the control, which was manifested by an increase in the values of chlorophyll fluorescence parameter, gas exchange and total antioxidant capacity. It was found that the highest dose of quercetin derivative tested (0.1%) had the best effect on plants subjected to drought stress, therefore it is necessary to conduct further research on the use of higher doses of this flavonoid.

**Keywords:** wheat (*Triticum aestivum* L.); drought stress; quercetin derivative; antioxidant activity; chlorophyll content; chlorophyll fluorescence; gas exchange

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## 1. Introduction

Wheat (*Triticum aestivum* L.) is a strategic species which, due to its significant role in the human diet, dominates cultivation all over the world [1] it is constantly exposed to environmental stresses that negatively affect the reduction of yields. Drought stress, classified as an abiotic stress, is one of the most important environmental stresses of particular importance in the era of global warming and changing climatic conditions [2]. Drought causes a decrease in water potential, water potential in leaves, closure of stomata and inhibition of growth, negatively affecting most of the processes occurring in plants, in particular photosynthesis, as a result of which there is a reduction in yield, contributing to a global decline in food security [3,4]. The effect of drought stress is damage to the phospholipid bilayer as a result of lipid peroxidation, leading to the production of reactive oxygen species (ROS) that cause oxidative stress. Plants, in order to minimize the negative effects of drought, developed various signaling pathways and cellular responses changing their growth pattern, up-regulation of antioxidants, accumulation of compatible solutes, and the production of stress proteins and chaperons [5]. Phenolic compounds belonging to non-enzymatic antioxidants play a significant role in maintaining the redox balance [6,7]. An example of such compounds is quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone), classified as a flavonoid, which, by forming a complex with copper ions, exhibits high antioxidant activity [8,9]. The study investigated the use of various solutions of the copper-quercetin complex on wheat seedlings subjected to drought stress, in particular,

the photosynthetic apparatus efficiency and antioxidant properties. Four grains of Artist cultivar winter wheat (breeder Deutsche Saatveredelung AG, Lippstadt, Germany) were sown in each pot.

## 2. Materials and Methods

### 2.1. Pot Experimental Design

The pot experiment was performed at the University of Rzeszów (Poland). In plastic pots (11 x 11 cm, 3 kg soil / pot) a soil with a clay sand grain size composition [10] and slightly acidic pH (pH KCl 6.35; H<sub>2</sub>O 6.52) was placed. The experiment was carried out in four replications in a growth chamber (Model GC-300/1000, JEIO Tech Co., Ltd., South Korea) at a temperature of 22 ± 2 ° C, humidity 60 ± 3% RH, photoperiod 16/8 h (L/D) and a maximum light intensity of about 300 µE m<sup>-2</sup> s<sup>-1</sup>. During the experiment in pots, soil moisture was maintained at the level of 70% (control sample) and 30% (under stress of drought) the soil moisture of the maximum water holding capacity (WHC). Plants were sprayed twice with a solution of copper-querctin at concentrations: 0.01% (Q1), 0.05% (Q2), 0.1% (Q3) with the use of a hand-sprayer. The quercetin derivative was diluted in ethanol (20 ml of solution for each pot).

### 2.2. Measurement of Physiological Parameters

Determination of selected physiological parameters was carried out four times on the first fully developed leaves of wheat: on the first and seventh day after each spraying. During the experiment, the following measurements were carried out: relative chlorophyll content (CCI), chlorophyll fluorescence (the performance index of PS II (PI)), and the gas exchange (net photosynthetic rate (PN)).

#### 2.2.1. Relative Chlorophyll Content

Measurements were made using a hand-held Chlorophyll Content Meter CCM-200plus (Opti-Sciences, Hudson, NH, USA) calculating an index in CCI units based on absorbance at 650 and 940 nm. These measurements were made on full expanded wheat leaves. 5 leaves per pot were analyzed.

#### 2.2.2. Chlorophyll Fluorescence

Measurements of chlorophyll a fluorescence in leaves were performed with an apparatus (Pocket PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). The maximal available intensity was 3500 µmol which was applied for 1 s with light with a peak wavelength of 627 nm. The first fully developed leaves were dark adapted for a period of 30 min using leaf clips which were applied over adaxial leaf blades [11].

#### 2.2.3. Gas Exchange

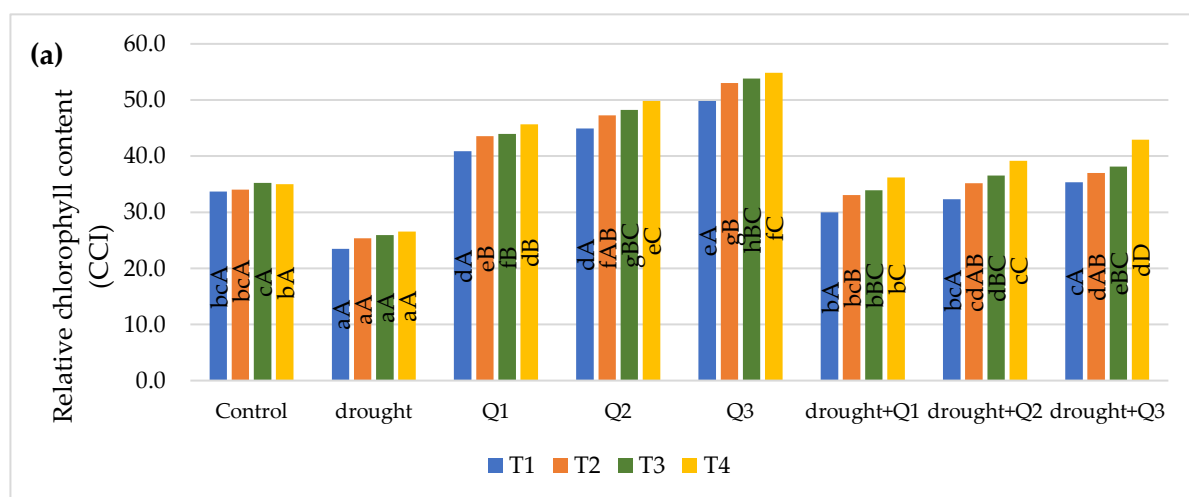
A Portable Photosynthesis Measurement System LCpro-SD (ADC BioScientific Ltd, Hoddesdon, UK) was used to determine the gas exchange parameter: net photosynthetic rate (PN). When taking measurements, light intensity was 300 µmol m<sup>-2</sup> s<sup>-1</sup> and the leaf chamber temperature was 22°C. Two leaves were analyzed for each pot.

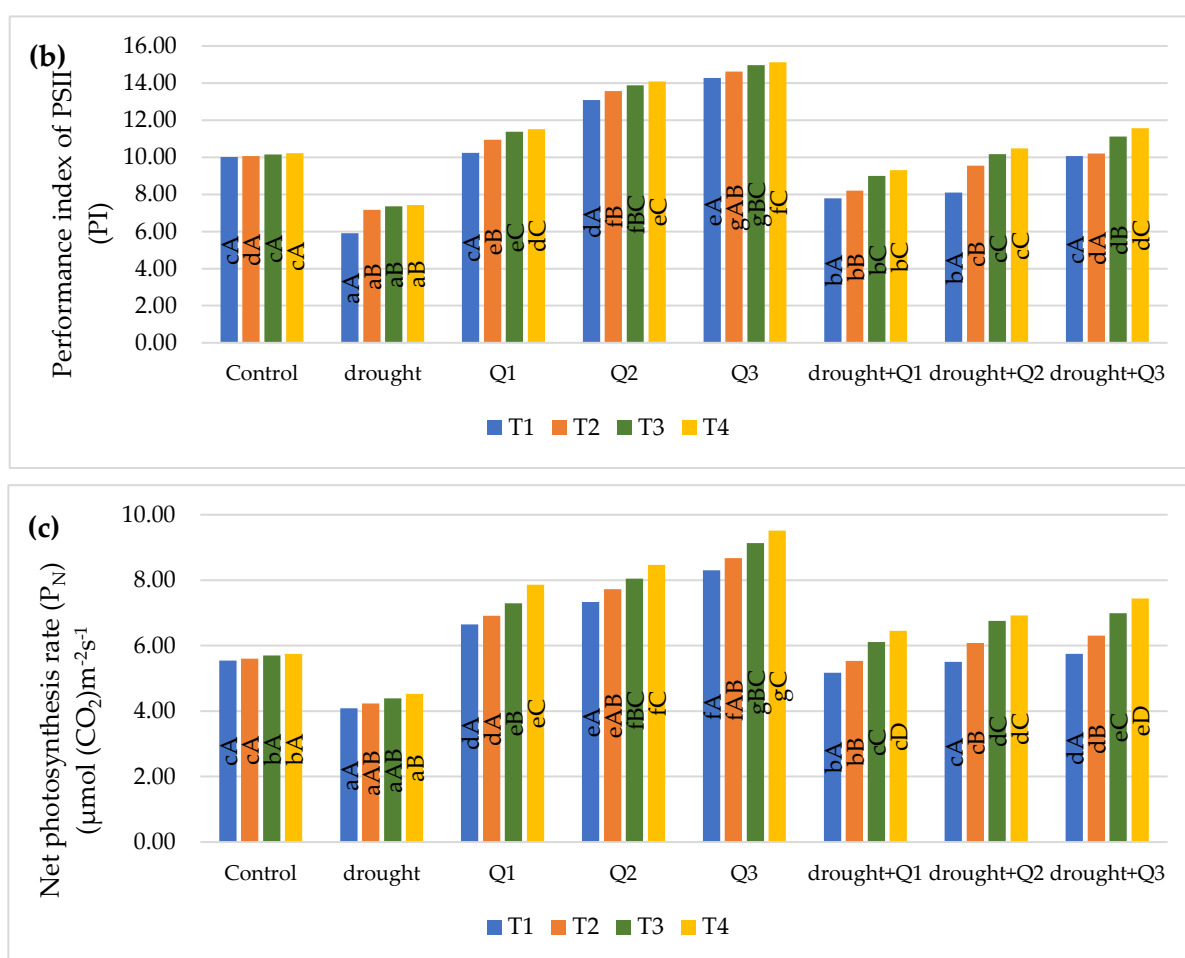
### 2.3. Determination of catalase (CAT) activity

To determine the CAT activity, 1 g of frozen tissue was homogenized with 4 ml of 0.9% NaCl solution containing 2% PVP, 0.05% Triton X-100 and a mixture of protease inhibitors. The homogenates were then centrifuged at 10,000g for 30 min (4° C) and the obtained supernatant was then collected for analysis. Catalase activity was determined by the method using ammonium metavanadate [12]. The enzymatic activity in the extracts was standardized to 1 mg of protein, the amount of which was determined by the Bradford method [13].

### 3. Results and Discussion

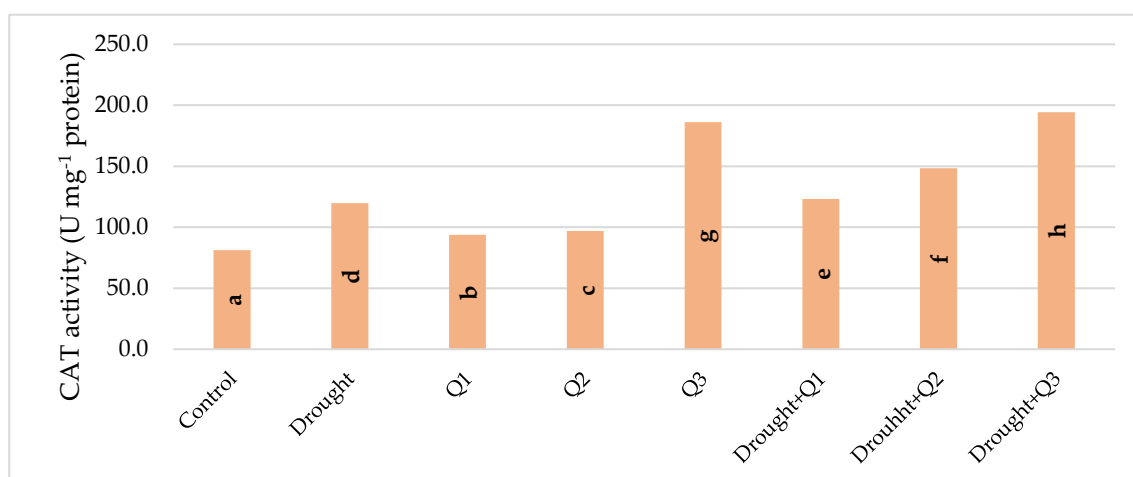
In comparison to the control, the plants treated with drought stress showed a significant decrease in the values of physiological parameters (Figure 1 a-c). The application of increasing concentrations of the quercetin derivative resulted in an improvement in the values of the tested parameters, except for the relative chlorophyll content, where no significant increase was found as a result of the application of the concentration of Q1 compared to Q2. Plants subjected to drought stress, in which the quercetin solution was sprayed, showed higher values compared to plants in which such treatment was not applied. It has been shown that the application of the highest concentration of quercetin (Q3) resulted in the highest values of all tested parameters, except for relative chlorophyll content at the time T2, where no significant differences were found between the concentrations of Q2 and Q3. An increase in the value of the tested parameters was demonstrated along with the successive dates of measurements, except for the control. The decrease in relative chlorophyll content due to drought stress is considered a typical symptom of pigment photo oxidation and chlorophyll degradation. The decrease in chlorophyll content in leaves can also be explained by damage to chloroplasts caused by ROS as a result of drought stress [14]. The water deficit also leads to a progressive inhibition of the photosynthesis process by disrupting the transport of electrons and limiting the penetration of CO<sub>2</sub> through the stomata, which in our research resulted in a decrease in the value of the P<sub>N</sub> parameter [6,15]. The improvement in the value of the PI parameter in connection with the application of quercetin can be explained by the fact that it participates in the light-dependent phase of photosynthesis, during which it improves the transport of electrons [16]. The stimulating effect of the application of exogenous quercetin on the content of chlorophyll in tomato plants not subjected to and exposed to abiotic stress was demonstrated by Parvin et al. [17]. As in the authors' own research, the application of a higher concentration of quercetin resulted in an increase in the content of chlorophyll in the leaves.





**Figure 1.** Effect of quercetin derivative concentrations, drought stress and terms of measurement on physiological parameters: (a) relative chlorophyll content; (b) performance index of PSII (PI) and (c) net photosynthesis rate ( $P_N$ ). (T1-first day after the first treatment, T2-seventh days after the first treatment, T3-first day after the second treatment, T4-seventh days after the second treatment). Capital letters indicate significant differences between the means at measurement terms for each quercetin derivative concentrations, lowercase letters indicate significant differences between the means at respective measurement terms according to ANOVA (followed by Tukey's HSD test,  $p = 0.05$ ).

The lowest CAT activity value was found in the control (Figure 2). A significant increase in CAT activity in plants treated with drought stress was demonstrated, as well as an increase in CAT activity along with an increase in the concentration of the quercetin derivative. The application of spraying this flavonoid to plants not subjected to stress caused the increase of the tested parameter, which in the concentration of Q3 was higher than in Q1 and Q2 by 98.6 and 92.2%, respectively. Also, spraying the plants treated with drought stress caused an increase in CAT activity compared to the control plants. This was probably due to the antioxidant properties of the copper-quercetin complex, which are well described by Bukhari et al. [8].



**Figure 2.** Effect of quercetin derivative concentrations and drought stress on CAT activity. Different letters indicate significant differences between each quercetin-Cu complex concentrations, according to ANOVA (followed by Tukey's HSD test,  $p = 0.05$ ).

#### 4. Conclusions

The conducted research showed that the drought stress had a negative effect on selected physiological indicators. The use of spraying with a solution of the copper-quercetin complex in both the control and the stressed drought samples resulted in the improvement of the values of these parameters and the highest CAT activity. The highest values of these parameters were found when the concentration of Q3 was used, which makes it necessary to conduct further research with higher doses of this flavonoid and may contribute to the introduction of sustainable agricultural practices in the future.

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#### References

- Shewry, P.R.; Hey, S.J. The contribution of wheat to human diet and health. *Food Energy Secur.* **2015**, *4*, 178–202 DOI: 10.1002/fes3.64
- Anwaar, H.A.; Perveen, R.; Mansha, M.Z.; Abid, M.; Sarwar, Z.M.; Aatif, H.M.; Umar, U.U.D.; Sajid, M.; Aslam, H.M.U.; Alam, M.M.; Rizwan, M.; Ikram, R.M.; Alghanem, S.M.S.; Rashid, A.; Khan, K.A. Assessment of grain yield indices in response to drought stress in wheat (*Triticum aestivum* L.). *Saudi J Biol Sci.* **2020**, *27*, 7, 1818–1823 DOI: 10.1016/j.sjbs.2019.12.009.
- Anjum, S.; Xie, X.; Wang, L.; Saleem, M.; Man, C.; Lei, W. Morphological, physiological and Biochemical responses of plants to drought stress. *Afr. J. Agric. Res.* **2011**, *6*, 9, 2026–2032 DOI: 10.5897/AJAR10.027
- Seleiman, M.F.; Al-Suhaibani, N.; Ali, N.; Akmal, M.; Alotaibi, M.; Refay, Y.; Dindaroglu, T.; Abdul-Wajid, H.H.; Battaglia, M.L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants.* **2021**, *10*, 259 DOI: 10.3390/plants10020259

5. Kaur, G.; Asthir, B. Molecular responses to drought stress in plants. *Biol. Plant.* **2017**, *61*, 2, 201-209 DOI: 10.1007/s10535-016-0700-9 1
6. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A.: Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.* **2009**, *29*, 185-212 DOI: 10.1051/agro:2008021 2
7. Sallam, A.; Alqudah, A. M.; Dawood M. F. A.; Baenziger P. S.; Börner A. 2019. Drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. *Int. J. Mol. Sci.* **2019**, *20*, 3137 DOI:10.3390/ijms20133137 3
8. Bukhari, S.B.; Memon, S.; Mahroof-Tahir, M.; Bhanger, M.I. Synthesis, characterization and antioxidant activity copper-quercetin complex. *Spectrochim Acta A Mol Biomol Spectrosc.* **2009**, *71*, 5, 1901-6. DOI: 10.1016/j.saa.2008.07.030. 4
9. Peşkal, A.; Biesaga, M.; Pyrzynska, K. Interaction of quercetin with copper ions: complexation, oxidation and reactivity towards radicals. *Biomaterials.* **2011**, *24*, 41–49 DOI 10.1007/s10534-010-9372-7 5
10. IUSS Working Group WRB. International soil classification system for naming soils and creating legends for soil maps. In Word Reference Base for Soil Resources 2014, Update 2015; Word Soil Resources Reports No. 106; FAO: Rome, Italy, **2015**; pp. 172–173. 6
11. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence-A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668 DOI: 10.1093/jexbot/51.345.659 7
12. Hadwan, M.H.; Ali, S.K. New spectrophotometric assay for assessments of catalase activity in biological samples. *Anal. Biochem.* **2018**, *542*, 29-33 DOI: 10.1016/j.ab.2017.11.013 8
13. Kruger, N.J. The Bradford Method For Protein Quantitation. In: Walker J.M. (eds) The Protein Protocols Handbook. **2009**. Springer Protocols Handbooks. Humana Press, Totowa, NJ. DOI: 10.1007/978-1-59745-198-7\_4 9
14. Ashraf, M.; Harris, P.J.C.: Photosynthesis under stressful environments: an overview. *Photosynthetica.* **2013**, *51*, 163-190 DOI: 10.1007/s11099-013-0021-6 10
15. Obidiegwu, J.E.; Bryan, G.J.; Jones, H.G.; Prashar, A. Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Front. Plant Sci.* **2015**, *6*, 542 DOI: 10.3389/fpls.2015.00542 11
16. Mastrangelo, S.; Tomassetti, M.; Caratu, M. Quercetin reduces chromosome aberrations induced by atrazine in the *Allium cepa* test. *Environ. Mol. Mutagen.* **2006**, *47*, 254–259 DOI: 10.1002/em.20199 12
17. Parvin, K.; Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Mohsin, S.M.; Fujita, M. Quercetin mediated salt tolerance in tomato through the enhancement of plant antioxidant defense and glyoxalase systems. *Plants.* **2019**, *8*, 247. DOI: 10.3390/plants8080247. 13