

Review in English

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Review of the doctoral dissertation, **Mr. Msc. Wenping Liu** entitled: „The influence of selected factors on the yield and physicochemical parameters of flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee) [Wpływ wybranych czynników na plonowanie i wartość biologiczną kapusty chińskiej kwitnącej (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee).”

The basis for the review is the letter [10 July 2023] from prof. dr hab. Andrzej Bleharczyk, Chairman of the Scientific Council of the Discipline of Agriculture and Horticulture at the Poznań University of Life Sciences (UPP). It contained information about my appointment as a reviewer of this doctoral thesis.

Candidate data

Mr. Msc. Wenping Liu is a Chinese citizen. He completed his first degree studies (Bachelor degree in package engineering) at Heilongjiang Bayi Agricultural University in China.

He completed master's studies in the field of food technology and human nutrition at the Faculty of Food Sciences and Nutrition of the UPP. He graduated on 09-05-2017 with a final grade of good.

There is no information in the presented documents whether he has applied for a doctoral degree before, so I assume that this is his first doctoral dissertation. Currently, he is a doctoral student of paid English-language Doctoral Studies at UPP (PULS PhD Studies) - he completed the program of these studies and passed all the required exams.

The scientific achievements indicated in the documentation were related to participation in the PULS PhD Studies. He is a co-author of five original scientific publications, four of which contribute to the scientific achievement demonstrated in his doctoral dissertation. He participated in three scientific conferences.

Formal opinion on the doctoral thesis

Doctoral dissertation of **Mr. Msc. Wenping Liu** entitled: „The influence of selected factors on the yield and physicochemical parameters of flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee)” was prepared in the Plant Nutrition

Laboratory/Department of Plant Physiology, Faculty of Agriculture, Horticulture and Bioengineering of UPP under the supervision of:

- a) Supervisor: Prof. UPP dr hab. inż. Tomasz Kleiber; Department of Plant Physiology / Unit of Plant Nutrition, Faculty of Agriculture, Horticulture and Bioengineering, UPP,
- b) Auxiliary supervisor: Dr Małgorzata Muzolf-Panek; Department of Food Quality and Safety Management; Faculty of Food and Nutrition Sciences, UPP.

The self-report (evaluated doctoral thesis) constituting the culmination of the research work is prepared in an exemplary manner and mainly in English. It consists of 64 pages - not counting the pages of publications contributing to the scientific achievement.

The self-report contains the following chapters: a list of publications constituting a scientific achievement, a summary in Polish and English, an introduction, a review of the literature, the aim of the research and research hypotheses, material and methods, a concise description of the results obtained in individual publications, a summary of the research and a bibliography including nominal 111 items of literature (errors in the list of literature are presented further). The bibliography includes well selected and highly relevant works published mainly in English, only two references are in Polish. At the end of the self-report, four publications constituting the evaluated scientific achievement were included.

The reviewed doctoral dissertation has been positively verified by the JSA anti-plagiarism evaluation system.

Substantive opinion on the doctoral thesis

The doctoral thesis focuses on the determination of various factors related to the mineral nutrition of flowering Chinese cabbage. Nitrogen nutrition (i.e. different doses of nitrogen fertilizers) was mainly investigated in pot (with a mixture of loamy sand and peat as a substrate) and hydroponic experiments. The effect of foliar application of trace elements V, Li, Si and Se and exogenous salicylic acid (SA) - a selected plant phytohormone - was also studied. The yield and biological value of this species under the influence of tested factors were characterized.

Flowering Chinese cabbage belongs to *Brassica* vegetables and is a popular leafy vegetable (as the author of the dissertation wrote) originating from China and widespread in Southeast Asia. In Poland and Europe, this vegetable is little known - its cultivation in Europe is currently of marginal importance compared to other *Brassica* plants. This species has a short vegetation period and at the same time is rich in bioactive compounds characteristic to *Brassica* plants, e.g. vitamins and glucosinolates.

In individual climatic zones in China (from northern to southern areas), various forms of flowering Chinese cabbage are grown. However, despite the widespread cultivation of this species in China, there is a limited amount of English-language research on its mineral nutrition and the effect of fertilization on the size and quality of its yield.

The aim of the research in the dissertation was, among others, to evaluate the influence of the studied factors on the biological value of flowering Chinese cabbage. The term “biological value/quality” covers a very wide range of physicochemical, biochemical and microbiological characteristics of plants that determine its potential impact on metabolic processes and the health condition of the consumer. It is shaped, among others, by the content of: 1. nutrients; 2. compounds with a pro-health (prophylactic) effect, e.g. mineral salts (macro and micronutrients), vitamins and antioxidant compounds; 3. various types of anti-nutrient compounds and food contamination.

In experimental scientific works, the scope of research is usually narrowed down to the assessment of a few or a dozen biological/chemical quality parameters, which are tools to verify the formulated research hypothesis. That was also the case with the evaluated doctoral dissertation, in which research was carried out, among others, in order to analyse the mineral composition of flowering Chinese cabbage plants (nutrient status of plants) and their antioxidant potential. These parameters are important for widening the knowledge in the field of plant physiology and biochemistry as well as from the consumer's point of view.

The research studies carried out as a part of the doctoral thesis was fully justified. They are novel as well as current within the scientific discipline of agriculture and horticulture. The obtained results can be used to develop appropriate programs/recommendations for horticultural practice. Results of these studies can contribute to the improvement in the biological quality of the discussed crop, and finally on the health status of consumers of a very large percentage of the world's population. Summing up, the research results presented in the dissertation are of great importance for the development of basic research and at the same time have a great practical and implementation value for horticulture, especially in terms of the cultivation of flowering cabbage in soilless systems.

The dissertation has the form of a set of scientific publications. The preparation of this type of doctoral thesis is more time-consuming than classic monographic dissertation. This is due to the fact that, apart from carrying out research, the PhD student participates in the arduous process of writing and publishing original scientific publications. For this reason, I look with great appreciation at the presented dissertation and the involvement of **Mr. Msc. Wenping Liu** in the scientific work.

The dissertation contains four thematically related articles that were published in 2020, 2022 and 2023. **Mr. Msc. Wenping Liu** is the first author in each publication and had a large share in its preparation including: conducting of the research, analysis of the results and writing manuscripts. This is all the more important as the very labour-consuming research was carried out in a team of three people. The contribution of the doctoral student indicated in individual publications allows to include them to constitute his doctoral thesis. The reviewed dissertation consists of the following publications:

Publication 1.) Liu W., Liu Y., Kleiber T. 2021. A review of progress in current research on flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee). *J. Elem.*, 26(1): 149-162. DOI: 10.5601/jelem.2020.25.4.20

Publication 2.) Liu W., Muzolf-Panek M., Kleiber T. 2022. Effect of nitrogen nutrition and planting date on the yield and physicochemical parameters of flowering Chinese cabbage. *Agronomy*, 12, 2869. <https://doi.org/10.3390/agronomy1211286>

Publication 3.) Liu W., Muzolf-Panek M., Kleiber T. 2023. Effect of varied nitrogen sources and type of cultivation on the yield and physicochemical parameters of flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee). *Appl. Sci.*, 13, 5691. <https://doi.org/10.3390/app13095691>

Publication 4.) Liu W., Muzolf-Panek, M., Kleiber T. 2022. The effect of various foliar treatments and nitrogen nutrition levels on the yield and physicochemical parameters of flowering Chinese cabbage. *Agronomy*, 12, 737. <https://doi.org/10.3390/agronomy12030737>

The first publication is a literature review, the other three contain the results of original, thematically related scientific research. The common feature of these three publications was the nitrogen fertilization as the factor tested in various experimental systems.

The IFs of the 1-4 publications are, according to the year of publication: 0.923, 3.949, 2.838 and 3.949, respectively. The numbers of MEiN points for these publications are as follows: 70 points for the first and 100 points for each of the other three publications. The total score is 370 points, and the total IF = 11.659. In my opinion these are very good bibliographic indicators for the research conducted over several years.

The set of published scientific articles constituting a monothematic doctoral dissertation does not raise my objections. They create a compact, logical arrangement of results and their interpretations. Scientific articles of the doctoral dissertation were positively reviewed by individual scientific publishing houses in which reviewers and editors were involved (peer-review). At this point, the evaluation of these publications for the purposes of the present assessment takes the form of a "super review". After studying all four publications, I admit that they have a very high scientific value and were prepared very reliably.

I do not find any substantial factual errors in them. The studies described in publications 2-4 were carried out correctly, with the use of acceptable research methods that the authors could have used. The discussion of the results in individual publications was consistent with the results and contained references to the relevant literature on the subject. It should be mentioned that the research covered by the dissertation was financed mainly by the Ministry of Science and Higher Education.

In his self-report, **Mr. Msc. Wenping Liu** described in details the subjects of the four publications. In this review, I will limit to stating that the information presented in the chapter V of the self-report [Summary of attached publications] correctly corresponds to the content of these publications. During the research process described in publications 2-4, a consistent and identical methodology of plant material analysis was used, i.e. measurement of plant colour, determination of the content of: macro- and micronutrients, photosynthetically active pigments, phenolic compounds, including flavonoids, as well as evaluation of the antioxidant activity with the use of DPPH, ABTS and FRAP methods. In the studies described in publications 2-4, the same variety of Chinese flowering cabbage with purple leaves was grown that was obtained from the Hubei Wuhan Hongshan Caitai Cultivation Center in China.

The thematic subject of individual publications constituting the evaluated scientific achievement is briefly provided below.

Publication 1 (Literature Review) describes the current research progress on Chinese flowering cabbage. It discusses the parameters of nutritional and biological value; determinants of mineral status of plants including nitrogen and other plant nutrients; the effect of cadmium, selenium and silicon on the nutritional value as well as determinants of yield and selected issues regarding the breeding of flowering Chinese cabbage.

Publication 2 presents the results of pot and hydroponic experiments with the cultivation of Chinese flowering cabbage in spring and autumn during which various levels of nitrogen fertilization (50, 70, 90, 110 and 130 mg N·dm⁻³) applied in the form of ammonium nitrate [NH₄NO₃] were tested.

Publication 3 presents the results of pot and hydroponic experiments with the cultivation of flowering Chinese cabbage, in which, apart from the type of cultivation (pot and hydroponic), various forms of nitrogen fertilizers were tested including: ammonium nitrate and urea, calcium nitrate, magnesium nitrate and sodium nitrate. Each of the nitrogen fertilizers in both types of cultivation was applied in doses of: 50, 70 and 90 mg N·dm⁻³.

Publication 4 presents the results of a pot and hydroponic experiment with the cultivation of flowering Chinese cabbage cultivated at two levels of nitrogen fertilization 70 and 90 mg N·dm⁻³ [applied in the form of ammonium nitrate] with the application of foliar spraying with trace element solutions (Se, Si, Li and V - used separately) as well as salicylic acid.

The scope of research presented in publications 2-4 was very wide but formed a logical arrangement of research. The results described in publication 2 allowed to present the yielding curve of flowering Chinese cabbage depending on the applied dose of nitrogen in pot and hydroponic cultivation. Yield increase was noted for doses ranging from 50 to 90 mg N·dm⁻³ and such doses were consequently applied in the experiments described in publication 3. The latter studies allowed to finally determine the most optimal N doses as 70 and 90 mg N·dm⁻³ that was further applied in the study described in the publication 4.

The research results presented in publications 2-4, and doctoral dissertation as a consequence, confirmed the formulated research hypotheses and make a significant contribution to the development of knowledge in the field of the mineral nutrition of flowering Chinese cabbage plants, as well as the assessment of its biological quality. **Results of the conducted research:**

- **indicated** that increasing the intensity of nitrogen nutrition had a positive effect on the yielding of flowering Chinese cabbage plants - it was noted both in the pot and hydroponic cultivation systems;

- **allowed** to observe the classic yielding curve of plants for nitrogen fertilization in the range from 50 to 90 mg N·dm⁻³. The optimal dose (for yield and quality) in soilless cultivation was found to be 90 mg N·dm⁻³;

- **indicated** that for plant cultivation in the substrate (in pot experiment), the N content in flowering Chinese cabbage increased with the growing intensity of nitrogen nutrition. Meanwhile, in hydroponic cultivation, the N content in the plant remained unaffected;

- **proved** that flowering Chinese cabbage plants grown in spring were characterized by higher values of antioxidant activity and phenol content than those from autumn season. In addition, plants from spring seasons had a higher biomass than those from the autumn seasons;

- **showed** that nitrogen fertilization in the form of magnesium nitrate had the most favourable effect on yield with the weakest effect exerted by calcium nitrate;

- **determined** that for pot cultivation in the substrate, fertilization with urea at a dose of 90 mg N·dm⁻³ had the most favourable effect on the content of photosynthetically active pigments, which correlated with the level of Mn in plants;

- **indicated** that plant spraying with salicylic acid improved crop quality in terms of antioxidant activity, chlorophyll and carotenoid content, brightness and leaf colour as well as nitrogen nutrition;

- **showed** that foliar application of Li and nitrogen fertilization at the dose 70 and 90 mg N·dm⁻³ had a beneficial effect on the yield of plants in pot cultivation;

- **determined** that foliar application of V reduced the yield of flowering Chinese cabbage plants in hydroponic cultivation;

- **presented** that foliar application of salicylic acid, Se, Si, Li or V variously affected the analyzed parameters of biological quality of plants grown in pot and hydroponic cultivation;

- **showed** that nitrogen fertilization and the season of cultivation affected the nutritional status of plants in N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu.

The self-report contains a very extensive chapter VI Conclusions. It covers as many as three pages of typescript and contains 5 conclusions with several sub-points. Conclusions are

drawn correctly but could be more concise. Basically, they are partly a replication of the conclusions/summaries of the results that were presented in the individual publications covering the doctoral thesis. The conclusions are followed by a one-paragraph summary of the research conducted.

After studying the self-report and articles constituting a scientific achievement, I have a few questions/issues that should be commented on by Mr. Msc. Wenping Liu during the public defence of the doctoral dissertation:

- 1. Chemically different nitrogen fertilizers were used in the study, namely physiologically acidic: ammonium nitrate and urea, as well as physiologically alkaline: calcium nitrate, magnesium nitrate and sodium nitrate. In the self-report and publications constituting a scientific achievement, the interpretation of the obtained results in terms of the physiological effect of nitrogen fertilizers was not included in the discussion. How can physiologically acidic and physiologically alkaline nitrogen fertilizers affect the physicochemical properties of the substrate and the biological quality of the crop?**
- 2. *Brassica* plants have high sulphur requirements. Why was ammonium sulphate not used in the study as compared to other types of nitrogen fertilizers?**
- 3. For foliar application of salicylic acid, V, Li, Se and Se, only one dose of these compounds were tested. Does the doctorate student see the need for further research on flowering Chinese cabbage evaluating the foliar application of several doses of salicylic acid, V, Li, Se and/or Se?**
- 4. In self-report and publications, I found the lack of meteorological data (external conditions) and/or of the microclimate conditions inside the cultivation facilities during the spring and autumn seasons. The self-report could have presented such data in order to discuss the obtained results in terms of differences between the spring and autumn seasons. Can climatic conditions modify the effect of nitrogen fertilization on the biological quality of plants?**
- 5. In the self-report and the review publication 1, it was stated that flowering Chinese cabbage can accumulate large amounts of selenium (work by Mo et al. 2006 was cited) but no detailed values were shown. What selenium content in plants is considered high? What plants can be selenium hyperaccumulators?**
- 6. In the publications, the indicated value of the salinity of the medium as the EC level was 2.0 mS/cm. I assume it was most probably the EC value of the base medium. Did, and to what extent, the increasing dose of nitrogen fertilizers increased the EC of the medium in hydroponic cultivation?**
- 7. Can the obtained level of sodium accumulation in the leaves of flowering Chinese cabbage be considered as physiological - especially for the fertilization with sodium nitrate?**

As a reviewer's duty, I present a list of editorial errors found in the doctoral dissertation:

- No reference to table 2 in the text of the self-report.
- Incorrect formatting of table 2 in the self-report.

- Chapter 4 "Nitrogen fertiliser" should have a different title as it describes the importance of Na, Ca and Mg for plants.
- On page 21. Citation errors: all authors are listed - should be Wachtel-Galor et al. 2008; Q. He et al. (2016) not (2016b). Lee et al. (2018) is not listed in the bibliography.
- Table 2 - citation error: supposed to be Deng et al. (2013) not (2012); should be Wiczowski et al. (2013), Ciska et al. (2005); the Zhang and Zhang (2016) is not included in the reference list.
- On page 25 should be Elrys et al. (2018)
- On page 26 should be Fariduddin et al. (2003).
- On page 35 should be Xiahui et al. (2006), and G. Li et al. (2009)
- References in the self-report - there is an entry of Olszowy and Dawidowicz (2018), but it was not cited in the text.
- In publication 2, I found minor discrepancies in the calculation of the percentage change in phosphorus content in plants - see table 1, average values from hydroponic cultivation in the spring and autumn seasons.
- In publication 2, in tables 3, 4 and 5, there are no average values for both growing seasons; such data were in tables 1 and 2 in that publication.
- In publication 3 on page 3 in the chapter "Material and methods" there is an inaccuracy regarding the substrate in the pot experiment. Once it said "The pot experiment was performed with limed peat moss" in the next paragraph it said "The pot experiment was conducted ... in a medium that was a mixture of mineral soil ... and peat (v/v/1/1).

I would like also to present a general critical remark regarding the entire research carried out. The nitrate(V) (NO_3^-) accumulation is one of the most important parameters for assessing the biological quality of vegetable yield and the study lacked that part. However, even without these analyses, the dissertation contains a significant amount of valuable results.

Final conclusion from the review

The doctoral dissertation of **Mr. Msc. Wenping Liu** entitled: „The influence of selected factors on the yield and physicochemical parameters of flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee) [Wpływ wybranych czynników na plonowanie i wartość biologiczną kapusty chińskiej kwitnącej (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee).” is an original solution to a scientific problem. It presents the candidate's general theoretical knowledge in the discipline of agriculture and horticulture. The assessed doctoral thesis confirms his ability to conduct scientific work. The obtained results allows to provide a very good assessment of this dissertation due to: the choice of unique, niche and current research issues as well as the applied methodology of carrying out vegetation experiments as well as chemical and biochemical analyses of the obtained plant material. Being a part of a very good research team, the doctoral student obtained a large number of original results of great importance both in the field of basic research as well as allowing to be implemented for horticultural practice.

The conducted research is original and constitutes an important solution not only for the discipline of agriculture and horticulture, but also for the scientific discipline of food technology and nutrition. The scope of documented research results indicates their universal and interdisciplinary nature.

I positively evaluate the doctoral dissertation of Mr. Msc. Wenping Liu and confirm that it fulfils the conditions set out in Art. 187 of the Act of 20 July 2018 Law on Higher Education and Science (consolidated text, Dz. U. z 2023r. poz. 742).

It is an original solution to a research problem in the discipline of agriculture and horticulture. I am applying to the High Scientific Council of the Discipline of Agriculture and Horticulture of UPP to admit Mr. Wenping Liu to further stages of the doctoral thesis.

At the same time, I am applying to the Scientific Council of the Discipline of Agriculture and Horticulture of UPP to grant Mr. Msc. Wenping Liu with an appropriate award due to the large cognitive scope of the team's scientific articles constituting a doctoral dissertation. It is worth emphasizing that the doctoral student was involved in the implementation of time-consuming and labour-intensive vegetation research in soilless crops on a little-known species of vegetable plant, i.e. Chinese flowering cabbage. For this reason, I consider the application for the award of a doctorate to be justified.

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