Global J. Environ. Sci. Manage. 7(4): 555-564, Autumn 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Pre-sowing treatment of vetch hairy seeds, *vicia villosa* using ultraviolet irradiation

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ARTICLE INFO

Article History:
Received 11 February 2021
Revised 27 April 2021
Accepted 06 June 2021

Keywords:
Pre-sowing treatment
Germination
Hydration rate
Moisture content
Seed vigour
ultraviolet irradiation (UV-C radiation)

ABSTRACT

BACKGROUND AND OBJECTIVES: Aiming to increase crop yield the antimicrobial/bacterial or fungicidal pre-sowing seed treatment received more attention in modern agronomy. Ultraviolet-C irradiation of pre-sowing seeds is an environmentally friendly method that became of great importance in recent years. It is, hereafter, being shown that, along with known antimicrobial use, there is additional important advantage of Ultraviolet-C irradiation of pre-sowing seeds. It was revealed that Ultraviolet-C radiation on Vetch Hairy seeds stimulates seeds germination and vigour.
METHODS: Various doses of Ultraviolet-C irradiation of seeds were used. The main sowing qualities of seeds were determined: seed vigour and germination, as well as the content of photosynthetic pigments in plant leaves and the main parameters of the kinetic values of hydration−moisture and hydration rate.
FINDINGS: It was found that ultraviolet-C radiation has a positive effect on sowing qualities and content of photosynthetic pigments in plant leaves of Vetch vary. The most effective dose of ultraviolet irradiation applied to vetch hairy seeds; *vicia villosa* was 1000 J/m2. At this dose the seed vigour increases by 23.6%, germination by 15.1%, the mass of germinated seeds by 17.3%, the content of a- and b-chlorophyll by 12.4%, and 17.5%, respectively, the carotenoid content increased by 13.9%. The parameters of seeds hydration kinetics such as moisture content and hydration rate were determined. It was revealed that the hydration rate of seeds increased significantly in the first 100-minute time range. Later in time the hydration rate progressively decreased, achieving a saturated moisture content after 700 minutes. Additionally, it was found that Ultraviolet-C irradiation decreases the imbibition damage.
CONCLUSION: The results indicated that ultraviolet-C irradiation has a positive effect on sowing qualities of Vetch Hairy seeds, thus, could be proposed as a promising candidate for application in treatment pre-sowing agriculture seeds.

DOI: 10.22034/gjesm.2021.04.05

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
31	4	2

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Note: Discussion period for this manuscript open until January 1, 2022 on GJESM website at the "Show Article.

INTRODUCTION

The main centre of origin of the hairy vetch (vicia villosa) is Southwest Asia. However, this cultivar was found to be spread to the Mediterranean Sea and then to European countries, including England, Scandinavia and the Baltics. The widespread cultivation of Hairy Vetch in Ukraine makes it possible to obtain early spring forage during winter sowing and late summer forage during spring sowing. Hairy Vetch – a valuable legume, which on a number of biological and economic characteristics stands out among annual forage crops. There are twenty-two species of vetch in Ukraine, the most commonly used for the production of *vicia sativa* L., *Vicia villosa* Roth and the Pannonian vulture – Vicia pannonica Grantz. Hairy vetch is a source of vegetable protein, one of the best precursors for winter and spring cereals. It is part of most annual legume-cereal mixtures grown for green fodder, hay, silage, haylage. Biomass with a high protein content is well digestible and is a valuable feed for all farm animals. From the point of view of accelerating productivity in colder climes and obtaining the needed biomass yield (Wilke and Snapp, 2008; Campiglia *et al.,* 2012), Vetch seeds are unreliable and therefore there are not many studies regarding the requirements of this species (Aarssen *et al.,* 1986). At first stage of germination for seed multiplication Vetch species faces problems associated with low sowing qualities of seeds (Jacobsen *et al.,* 2010). The change of various parameters such as dormancy, temperature, humidity, as well as methods of pre-sowing stimulation of seeds may improve the sowing quality of seeds in various environmental conditions. Commercially available widespread chemical methods of pre-sowing seed treatment have environmental impact and only physical methods (i.e. use of electromagnetic radiation) are proved to be eco-friendly. In some studies, a limited number of chemicals were used, but majority of them were replaced with appropriate physical methods of processing, such as magnetic field, gamma radiation, electric field, laser radiation, ultrasound and optical irradiation (Govindaraj *et al.*, 2017; Thakur *et al.*, 2019; Ri *et al.*, 2019; Lazim and Ramadhan, 2020). The optical irradiation became of great interest in recent years that stimulate the growth and increase the tolerance of plants towards negative external factors therefore increasing the crops yield. Particularly, the pre-sowing treatment of agricultural seeds with ultraviolet radiation attracted significant attention (Neelamegam and Sutha 2015; Mariz-Ponte *et al.*, 2018). Thus, the study (Thomas and Puthur, 2017) described the effect of ultraviolet radiation on seeds during pre-sowing treatment to increase crop productivity, yield quality, as well as plant resistance to various biotic and abiotic stresses. Backer *et al.*, 2018 discussed the mechanisms of plant growth stimulation by inoculating plants with rhizobacteria that stimulate plant growth, or treating plants with microbial-to-plant signalling compounds. Other authors of publication (Brown *et al.*, 2001) described the treatment of cabbage seeds with Ultraviolet-C (UV-C) radiation at doses of 3.6 kJ/m^2, which ensured resistance to bacteriosis (black rot) and improved quality and growth of cabbage. In study (Kacharava *et al.*, 2009), an increase in the germination of seeds of black beans (Phaseolus vulgaris) under the influence of UV-C radiation was investigated. UV radiation at a dose of 460 mW/cm^2 for 60 minutes enhanced the growth and accumulation of biomass in beans. The application of UV-C radiation led to an increase in plant height by 11–39%. All these effects of pre-treatment of seeds with optical radiation in the UV-C range are associated with the changes in biological properties (Semenov *et al.*, 2017). The mechanisms of such effects themselves were investigated using various methods and models. However, no unambiguous explanation of these actions has been found until today. Temperature and moisture content (hydration) are the most important factors influencing seed germination. The water absorption kinetics during seeds hydration affects the biological properties during growth and the quality of the final product (Turhan *et al.*, 2002). The positive effect of various doses of pre-sowing UV-C irradiation on the sowing quality of seeds has been considered in many studies (Turhan *et al.*, 2002; Neelamegam and Sutha, 2015; Rocha *et al.*, 2015; Thomas and Puthur, 2017; Mariz-Ponte *et al.*, 2018; Sadeghianfar *et al.*, 2019; Korotkova *et al.*, 2020; Hernandez-Aguilar *et al.*, 2021). It was found that the optimal doses of irradiation of winter wheat (Semenov *et al.*, 2020), carrots (Semenov et *al.*, 2019) and rapeseed (Semenov *et al.*, 2018) are 400–600 J/m^2, 120–150 J/m^2 and 120 J/m^2, respectively. Reviewing literature in this area it is possible to note that despite an extensive study (Neelamegam and Sutha, 2015; Thomas and Puthur, 2017; Korotkova *et al.*, 2020), as it was understood, this study is dealing with pre-sowing treatment of vetch hairy seeds (Vicia villosa) by use of Ultraviolet-C radiation has not been reported yet. Aiming to fill this research gap the objective of this study was focused on determination of hairy seeds (Vicia villosa) sowing quality (i.e. vigour and germination) by pre-sowing treatment of seeds using different doses of ultraviolet radiation. In addition, the hydration rate was evaluated, for treated and not treated pre-sowing seeds by ultraviolet radiation. The study was performed in the scientific and technical centre of Poltava University of Economics and Trade, Ukraine in 2019–2020.

MATERIALS AND METHODS

For conducting research, Hairy Vetch (*Vicia villosa*) of Poltava-77 variety was used, which is determined by the national standard for the conditions of the Forest-Steppe and Steppe of Ukraine. During pre-sowing treatment, Hairy Vetch seeds were irradiated using low-pressure ZW20D15W type ultraviolet lamps with a power of 20 W at an emission spectrum the C region: 200–280 nm. Irradiation was carried out with the following doses of 50, 120, 500, 1000 and 3000 J/m^2 and controlled using a Tensor-31 radiometer. Seed vigour and germination of seeds of control (without irradiation) and irradiated samples were determined by germinating 4 samples of 50 seeds each in Petri dishes on moistened filter paper in accordance with the International Safe Transit Association (ISTA) standard. The content of photosynthetic pigments in vetch leaves in the phase of three true leaves was established using a spectrophotometer, determining the optical density of the chlorophyll and carotene extract at 665, 649, and 440 nm (absorption maxima of chlorophyll a (Ca), chlorophyll b (Cb), and carotenoids (Ck), respectively). The concentration of pigments was calculated using Eqs. 1 to 3 (Porra, 2002).

$$C_a = 11{,}63 D_{665} - 2.39\, D_{649},\ \text{mg/L} \tag{1}$$

$$C_b = 20{,}11\, D_{649} - 5{,}18\, D_{665},\ \text{mg/L} \tag{2}$$

$$C_к = 4{,}695\, D_{440} - 0{,}268\, C_{a+b},\ \text{mg/L} \tag{3}$$

To determine the moisture content (hydration), samples of Hairy Vetch seeds in an amount of 10 ± 0.5 g were soaked in 200 ml of distilled water at each dose of UV-C radiation. The soaked samples were removed from the water at intervals of half an hour and an hour. The samples were placed on absorbent paper to remove excess water, and then weighed using a precision analytical balance, model WLC 0.2, TM Radwag, Poland with an accuracy of 0.001 g. Eqs. 4 to 5 were used for determination of such parameters as moisture content (MC) and hydration rate (HR) (Shafaei *et al.*, 2016):

$$MC = \left(\frac{W_f - W_i}{W_i} \right) \times 100\% \ \text{(d. b. \%)} \tag{4}$$

$$HR = \frac{MC_{(t+\Delta t)} - MC_{(t)}}{\Delta t} \ \text{(d. b. \%/min)} \tag{5}$$

At each value of the UV-C radiation dose, a hydration procedure was carried out, which lasted 12 hours. At this stage, the change in weight and, accordingly, the content of saturated moisture in the sample were determined. In order to reduce the measurement error of hydration indicators, the studies were carried out in triplicates. The appropriate statistical processing was used for interpretation of obtained experimental data.

RESULTS AND DISCUSSION

Determination of seed vigour and germination

Analysis of the seed vigour and germination of Hairy Vetch indicates the dependence of its sowing qualities on the energy dose of ultraviolet radiation (Table 1). At an irradiation dose of 500 J/m^2, the following results were obtained: in comparison with the control samples, the seed vigour was increased by 10.8% and the germination was increased by 6.6%. The maximal changes were observed at irradiation doses of 1000 J/m^2: the seed vigour was increased by 23.6% and the germination was increased by 15.1%.

Less significant effect was observed at an irradiation dose of 3000 J/m^2: in comparison with the control samples, the seed vigour was increased by 18.2% and the germination was increased by 9.6%. The obtained regularities of the pre-sowing effect on biological indicators (seed vigour and germination) are in good agreement consistent with the results of study (Kalsa and Abebie, 2012), where the effect of hydro- and osmotic priming in pre-sowing treatment on Hairy Vetch seeds was investigated.

Determination of biometric indicators (mass of germinated seeds)

The positive effect of UV-C irradiation on Hairy Vetch's growth processes was also noted. An effective change in their biometric parameters was observed at high doses of UV-C irradiation of seeds - 1000 J/m^2 and 3000 J/m^2 (Table 2).

The maximum stimulating effect of UV-C irradiation on growth processes was obtained with a dose of 1000 J/m^2: in comparison with the control samples the mass of germinated seeds increased by 17.3% (Table 2). At the same time, the root length and seedling length were increased by 18.1%, and 6.1%, respectively. At an irradiation dose of 3000 J/m^2: the mass of germinated seeds was 5% less than the maximum value obtained, and the root length and seedling length were decreased by 5.1% and 1.3%, respectively. At doses up to 500 J/m^2, changes in the biometric parameters of Hairy Vetch seedlings were insignificant and varied within the experimental error.

Determination of Chlorophyll

The intensity of photosynthesis is determined by the amount of plant pigments – chlorophylls and carotenoids, and an increase in these parameters lead to an increase in the vegetative mass of plants. Pre-sowing UV-C irradiation of seeds affects the content of photosynthetic pigments in Hairy Vetch leaves (phase of three true leaves) (Fig. 1).

The concentrations of chlorophyll a and chlorophyll b in the leaves of the shaggy branch maximally increase (by 12.4% and 17.5%, respectively, compared with the control samples) under UV-C irradiation of seeds with doses of 1000 J/m^2. The content of carotenoids also increased by 13.9%. The obtained results agree with a number of works (Salama *et al.*, 2011; Geeta *et al.*, 2014; Li, *et al.* 2020). In (Li *et al.*, 2020), the amount of chlorophyll in the leaves of Hairy Vetch was determined. It was found that, depending on the year of sowing, fluctuations in the amount of chlorophyll can reach 10% or more. It follows from (Geeta *et al.*, 2014) that the content of total chlorophyll and chlorophyll a and b increased upon UV-B irradiation of germinated seeds for 1 h by 7.31%, 4.52%, and 14.92%, respectively. At the same time, the chlorophyll content decreased by 5.85%, 3.29%, and 11.19% compared to the control samples after an additional 3 hours of irradiation. A decrease in the content of chlorophylls was observed in a study (Salama *et al.*, 2011), where, after irradiation with 254 nm, the content of chlorophyll a and b

Table 1: Influence of UV-C irradiation on the sowing quality of vetch hairy

Radiation dose (J/m^2)	Seed vigour, (%)	Germination (%)
Control samples (without irradiation)	74, 0	83,0
50	75,0	83,0
120	76,5	85,0
500	82,0	88,5
1000	91,5	95,5
3000	87,5	91,0
LSD_{05}	3,8	4,1

Table 2: Biometric indicators of seedlings of sowing Hairy Vetch after 14 days

Radiation dose (J/m^2)	Root length (cm)	Shoot length (cm)	Weight 50 pcs Germinated seeds (g)
Control samples (without irradiation)	3,97	6,43	4,90
50	4,00	6,35	4,86
120	4,02	6,50	4,98
500	4,15	6,63	5,16
1000	4,69	6,82	5,75
3000	4,45	6,73	5,46
LSD_{05}	0,21	0,30	0,28

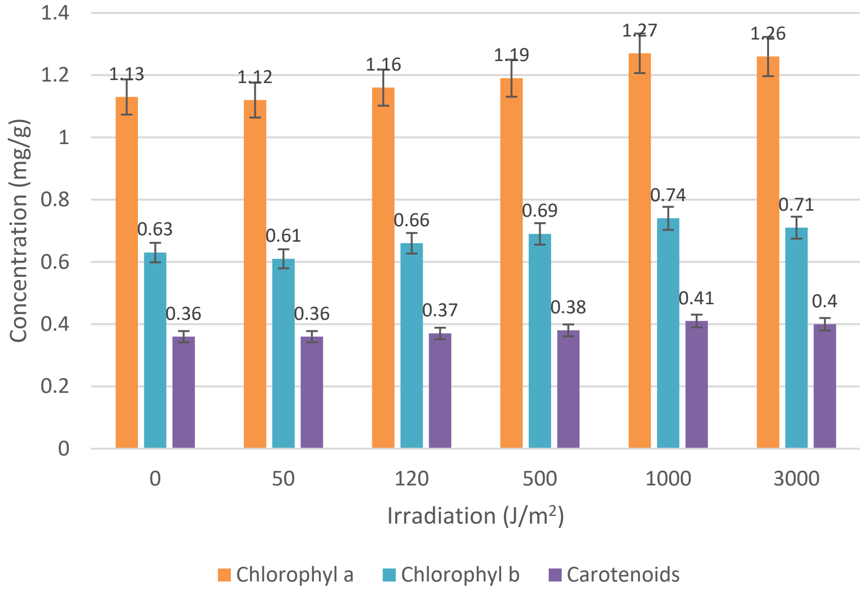

Fig. 1: Content of chlorophylls a, b and carotenoids in hairy vetch leaves (phase of three true leaves)

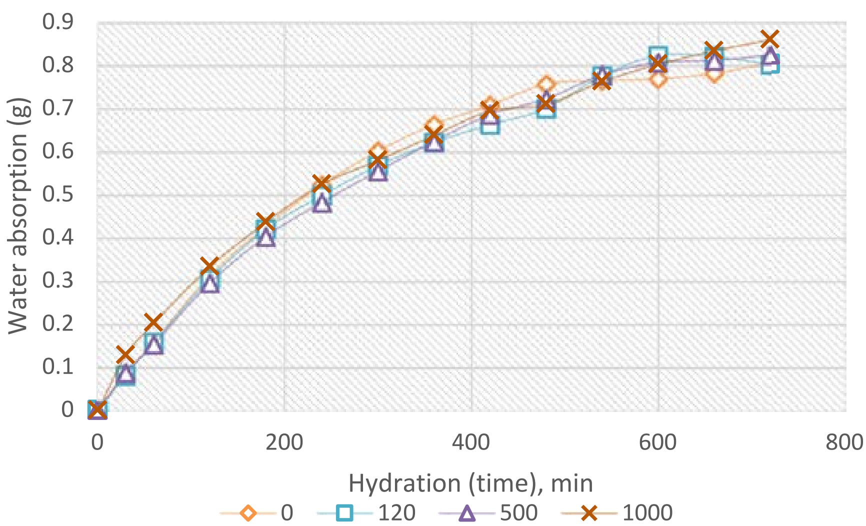

Fig. 2: Dependence of hydration on time at radiation doses: 0; 120; 500 and 1000 J/m[2]

and total chlorophyll decreased by 79, 43, and 74%, while the content of carotenoids increased by 91%. The results showed that the content of chlorophyll a, chlorophyll b and total chlorophyll decreased with UV-C irradiation.

Determination of hydration kinetics

Besides, the hydration characteristics that affect the quality indicators of production were studied (Turhan *et al.*, 2002). According to the literature (Kumar *et al.*, 2021; Turhan *et al.*, 2002; Shafaei *et*

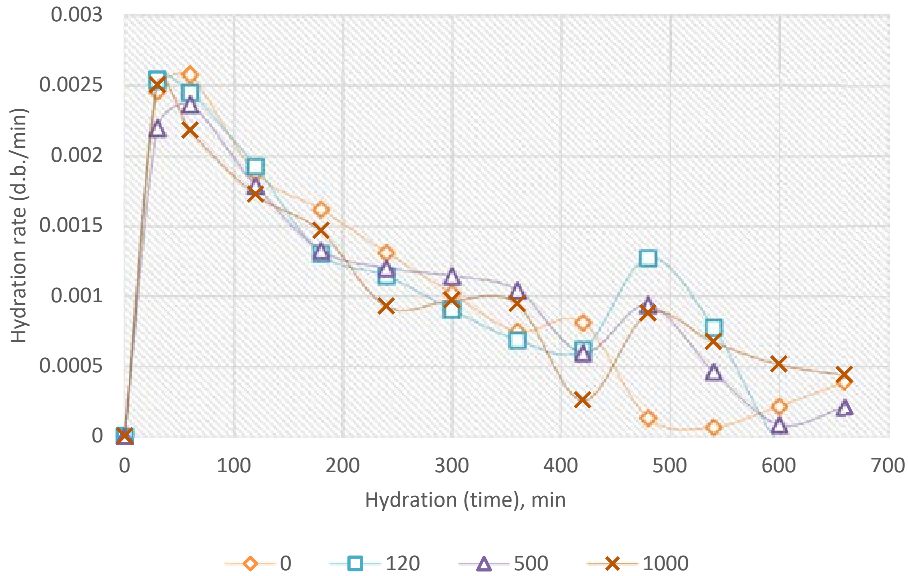

Fig. 3: The dependence of the change in the rate of water absorption on time at doses: 0; 120; 500 and 1000 J/m[2]

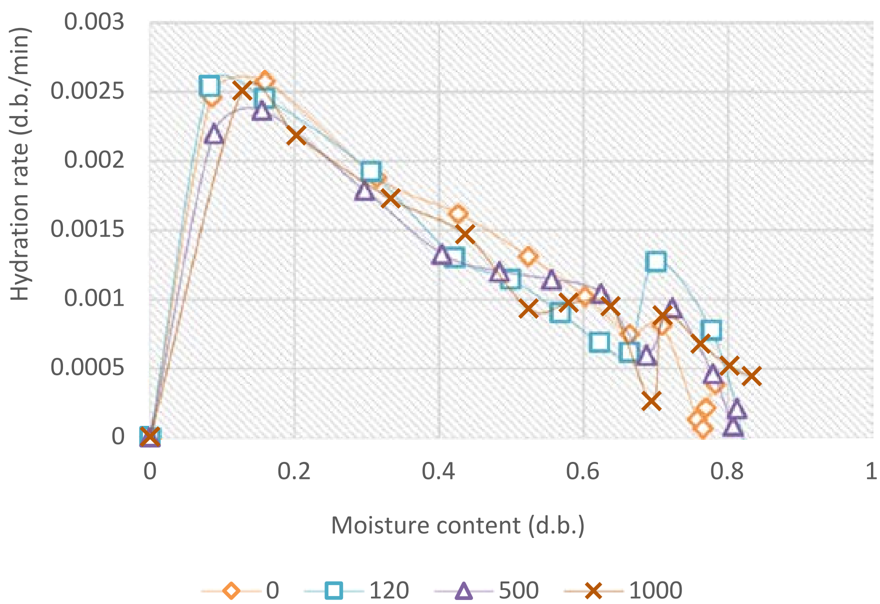

Fig. 4: Dependence of the change in the rate of water absorption on humidity at irradiation doses: 0, 120, 500 and 1000 J/m[2]

al., 2016, 2021), it can be concluded that a better understanding of hydration kinetics is achieved through accurate modelling. It helped to initiate the development of several mathematical models for describing hydration characteristics (Shafaei *et al.*, 2016). The dependence of the kinetics of swelling of Hairy Vetch seeds on the dose of UV-C irradiation limits the process of their awakening and germination, and therefore, germination and yield. The time dependence of water absorption at various irradiation doses is shown in Fig. 2.

When hydrated, water slowly diffuses into the granular structure of the grain and after a certain period of time reaches a constant level. It is understood that as the hydration time increases, so does the moisture content of the Hairy Vetch grain. It was also found that water absorption decreases over time with increasing hydration time and radiation dose. Moreover, this dependence is broken after 9 hours of hydration. The study of the dependence of the rate of hydration is associated with the fact that often too rapid water absorption occurs due to a high gradient of water potential, which can lead to a decrease in seed germination after soaking (Semenov *et al.*, 2021). The results are presented based on the forecast of an artificial neural network (ANN) model, where the input parameters are the radiation dose and the hydration time, and the output parameters are the moisture content and the rate of hydration. Figs. 3 and 4. Irradiation dose and hydration time were used as input parameters, and moisture content and hydration rates were used as output parameters.

The forecast of the ANN model shows (Figs. 3 and 4) that in the initial phase of hydration the rate sharply increased and gradually and slowly decreased in the middle and until the saturated moisture content was reached. As can be seen from Figs. 3 and 4, the rate of hydration decreases with increasing radiation dose under the same experimental conditions.

Pre-sowing irradiation with various doses of UV-C leads to a decrease in water absorption, which reduces damage to the seed coat during rapid water absorption (damage by imbibition) and ultimately leads to an increase in germination energy and seed germination. Thus, UV-C irradiation in most cases reduces the rate of water uptake by the seeds as the dose is increased, which prevents damage to the seed membrane during rapid water uptake and thus allows a higher percentage of Hairy Vetch seeds to germinate.

CONCLUSIONS

Pre-sowing treatment of seeds with UV-C radiation has a positive effect on sowing qualities and biometric indicators. UV-irradiated seeds germinate 1.5-2.0 times faster than non-irradiated seeds. In addition, UV irradiation promotes seed germination both under optimal conditions and under stress. The most effective dose of UV radiation of Vicky seeds is 1000 J/m^2. At this dose vigour and laboratory germination increased by 23.6% and 15.1%, respectively. The weight of germinated seeds increased by 17.3% while the content of chlorophyll, chlorophyll b and carotenoids increased by 12.4%, 17.5% and 13.9%respectively. The kinetic parameters of hydration of irradiated seeds were defined by moisture content and hydration rate. This in turn, causing faster water absorption and thus more efficient seed hydration, positively affecting sowing qualities and other agronomic traits. The results of the analysis showed that the rate of seed hydration increased significantly in the initial phase and gradually and slowly decreased in the middle and end of the hydration procedure. Water absorption increased significantly with increasing hydration time from 0 to 700 minutes, following increase of irradiation dose, water absorption decreased, limiting this pattern after 9 hours. Improved water absorption after UV-C irradiation during germination cannot be considered as the only factor responsible for enhanced germination. It is established that one of the reasons for the rapid germination of seeds is the initiation of processes associated with germination during pre-hydration. The increase in germination energy during UV-C irradiation of seeds is the result of many mechanisms caused by irradiation, including efficient water absorption. Rapid absorption of water at an early stage can lead to the so-called seeds imbibition, which is manifested in the disruption of structural integrity of the membranes. In addition, the results of the study showed that pre-sowing treatment of seeds with UV irradiation reduces the rate of hydration thereby suspending seeds imbibition during rapid water absorption. However, at higher doses of UV irradiation the germination potential decreases. These studies demonstrated that UV-C radiation of pre-sowing seeds increases germination not only by UV degradation of pathogens originally present on seeds surface but also explains the nature of seeds hydration rate that changes with time of treatment. Since the report shows clear evidence that UV treated seeds at given dose do not promote seeds biodegradation while contrarily, increase germination, it may potentially lead to expansion of this research to other cultivars.

AUTHOR CONTRIBUTIONS

A. Semenov developed experimental design of equipment of ultraviolet action, conducted experiments, analysed and interpreted the data. T.

Sakhno performed the literature review, participated in experiments and translated. O. Hordieieva performed some of the remained experiments, compiled data. Y. Sakhno prepared the manuscript text, and manuscript edition.

ACKNOWLEDGMENTS

Part of this work was partially supported by a research grant from the U.S. Department of Agriculture [NIFA award 2018-67021-27965].

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

%	Percent
Δt	Hydration time min
ANN	Artificial neural network
Ca	Chlorophyll a
Cb	Chlorophyll b
Ck	Carotenoids
cm	centimetre
D	Optical density or absorbance at three wavelengths: 440, 649, 665 nm
d.b.	Content dry basis
Eqs.	Equations
et.al	others
Fig.	Figure
Figs.	Figures
g	Gram
h	Hour
HR	Hydration time (d. b./min)
J	Joules
J/cm^2	Joules per Square Centimetre
J/m^2	Joules per Square meters
kJ/m^2	Kilojoule per Square meters
L	litre
LSD_{05}	Least Significant Difference
m^2	Square meters
MC	Moisture content
MC(t)	Moisture content at time (t) (d. b. %)
MC(t+Δt)	Moisture content at time (t + Δt) (d. b. %)
mg	Milligram
mg/L	Milligram per litre
min	Minute
MR	Moisture ratio
mW/cm^2	Mill watts per centimetre squared
nm	Manometer
pcs	Pieces
UV-C	Ultraviolet C irradiances are defined as the wavelength range of (100 u λ < 280) nm.
W	Watts
Wf	Mass of wet seed of Hairy Vetch (grams)
Wi	Initial mass seed of Hairy Vetch (grams)

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HOW TO CITE THIS ARTICLE

Semenov, A.; Sakhno, T.; Hordieieva, O.; Sakhno, Y., (2021). Pre-sowing treatment of vetch hairy seeds, vicia villosa using ultraviolet irradiation. Global J. Environ. Sci. Manage., 7(4): 555-564.

DOI: 10.22034/gjesm.2021.04.05

url: https://www.gjesm.net/article_244274.html