Mieczysław GRZESIK, Regina JANAS
Instytut Ogrodnictwa
ul. Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
e-mail: Mieczyslaw.Grzesik@inhort.pl

PHYSIOLOGICAL METHOD FOR IMPROVING SEED GERMINATION AND SEEDLING EMERGENCE OF ROOT PARSLEY IN ORGANIC SYSTEMS

Summary

The aim of the experiments was to determine the optimal parameters of hydroconditioning of root parsley 'Berlińska' seeds and an indication of how the moisture content and the time of incubation affect the dynamics of germination, physiological activity, emergence and growth of seedlings. Seeds were hydrated at 20°C, at different doses of distilled water, from the initial moisture content of 8% up to 30 , 35 and 45%. Then, they were incubated at 20°C, in airtight containers for 1, 3 and 6 days, with daily ventilating and assessing moisture content. After that, their sowing value was assessed on the basis of dynamics of germination at 20°C, mean time of germination, cytoplasm membrane permeability, total dehydrogenase activity, dynamics of emergence, the number of emerged seedlings and their height 45 days after sowing, as well as index of chlorophyll content and net photosynthesis in leaves. The results indicated that hydroconditioning greatly increased the dynamics and the percentage of seed germination and seedling emergence of parsley 'Berlińska'. Seeds hydrated up to about 45% and incubated at 20°C for a period of 3 or 6 days, and then dried down to the initial moisture content, were more vigorous, germinated more quickly and in higher percentage. They showed the higher enzyme activity and lower permeability of membranes. The obtained from them seedlings emerged and grew faster, contained more chlorophyll and exhibited the higher photosynthetic activity than seedlings from control.
***Key words**: parsley, hydroconditioning, seeds, germination, seedling emergence, growth*

FIZJOLOGICZNE METODY POPRAWY KIEŁKOWANIA NASION I WSCHODÓW SIEWEK PIETRUSZKI KORZENIOWEJ W SYSTEMACH EKOLOGICZNYCH

Streszczenie

Celem badań było określenie optymalnych parametrów hydrokondycjonowania nasion pietruszki korzeniowej "Berlińska" oraz wskazanie, jak stopień ich uwilgotnienia i czas inkubacji wpływa na dynamikę kiełkowania, aktywność fizjologiczną, wschody i wzrost roślin. Nasiona uwilgotniono w 20°C, w odpowiednich dawkach wody destylowanej, od wilgotności wyjściowej 8% do 30, 35 i 45%. Następnie inkubowano je w temperaturze 20°C, w hermetycznych pojemnikach przez 1, 3 i 6 dni, codziennie przewietrzając i oceniając ich wilgotność. W kolejnym etapie określono ich wartość siewną na podstawie dynamiki kiełkowania w temperaturze 20°C, średniego czasu kiełkowania, przepuszczalności błon cytoplazmatycznych, całkowitej aktywności dehydrogenaz i dynamiki wschodów siewek. Po 45 dniach od wysiewu oceniono wysokość siewek oraz indeks zawartości chlorofilu i aktywność fotosyntezy netto w liściach. Uzyskane wyniki wykazały, że hydrokondycjonowanie znacznie zwiększyło dynamikę i zdolność kiełkowania nasion oraz dynamikę wschodów i wzrost roślin pietruszki korzeniowej. Nasiona uwilgotnione do 45% i inkubowane w 20°C przez okres od 3 do 6 dni i następnie suszone do wilgotności wyjściowej, skiełkowały szybciej i w większym procencie. Charakteryzowały się one również wyższą aktywnością enzymatyczną i niższą przepuszczalnością błon cytoplazmatycznych. Uzyskane z nich siewki wschodziły i rosły szybciej oraz zawierały więcej chlorofilu. Aktywność fotosyntezy netto była w nich wyższa, niż w siewkach uzyskanych z nasion nie kondycjonowanych.
***Słowa kluczowe**: pietruszka korzeniowa, hydrokondycjonowanie, nasiona, kiełkowanie, siewki, wschody, wzrost*

1. Introduction

A reduction in the time and an increase in the uniformity of seed germination and seedling emergence is one of the overarching objectives of the seed industry in the world. The use of high-value seeds is necessary in all plant production and especially in organic farming, integrated plant cultivation and production of healthy food in adverse conditions of a changing climate. The accelerated and aligned seedling emergence causes the earlier and faster growth and development of crops than weeds. This enables and facilitates to make the necessary agricultural treatments in early spring which will result in higher yields of plants, especially under drought conditions and inadequate temperature.

One method of shortening the time and an increase in the uniformity of seedling emergence is a pre-sowing conditioning of seeds. It consists in the pre-sowing and controlled hydration of seeds and their subsequent incubation for a defined period of time, to initiate the metabolic processes prior to germination, but with the prevention of the penetration of the seed coat by the radicle [1-4, 7, 9, 11, 13, 16-18, 22, 23, 31]. This treatment initiates, among others, earlier and increased activation of several enzymes [12, 15, 17, 19, 25], mobilization of storage materials and transport of hydrolyzed compounds to embryo [7, 8, 17], DNA replication [7, 9, 17], intensification of oxygen phosphorylation in mitochondria [30]. These events, together with other initiated physiological processes, cause accelerated emergence and development of plants [14, 19]. However, this procedure, requires high precision, because the inappropriate moistening and incubation can lead to a decrease in the biological value of seeds.

World literature gives a lot of data on seed conditioning of horticultural plant species [1, 2, 3, 4, 5, 7, 9, 11, 13, 14, 16, 17, 18, 22, 23, 27, 29], among which a significant number contains only vague information, not suitable for a practical use in production. There are relatively little data concerning root parsley seeds, which are characterized by the slow germination and low percentage of the germinated seeds, what causes the weak and uneven emergence of seedlings, especially under adverse soil and climatic conditions. One of the methods to improve the health and germination of root parsley seeds are the 30 minute thermotherapy in hot water [10] or treatment with some biological compounds [21, 22]. Parsley seed germination was also improved by their treatment with GA_3 and osmopriming in KNO_3, polyethylene glycol (PEG 6000, PEG 8000) or hydrogel (Ekosorb Na), and matriconditioning in Micro-Cell E (MCE) [6, 7, 9, 11, 26, 30, 32] or soaking in water for 12-72 hours [11, 30]. As compared to non-primed seeds solid matrix priming (SMP) significantly increased the percentage and the speed of germination. SMP resulted in more significant increase of the germination ability after 10 and 28 days than soaking in water for 72 hours in not aerated containers. The subsequently used pelleting reduced the positive effect of priming on the germination ability without affecting the germination speed [30]. Other literature data indicated also that parsley seed conditioning can affect their infection by pathogenic microorganisms. However, it depended on the method of seed conditioning [21, 22, 30, 32]. In contrast, limited is information concerning hydroconditioning of the root parsley seeds in water (in the sense of hydration, subsequent incubation and drying of seeds), which is a procedure less complicated, cheaper and more useful in a commercial production than osmopriming in PEG or Ekosorb Na or matriconditioning in MCE. In addition, adequate hydration of seeds and subsequent incubation, cause the higher dynamics of germination and their uniformity than soaking alone without following incubation [16, 17].

The aim of the experiments was to investigate the effect of hydroconditioning on seed germination and seedling emergence of root parsley 'Berlińska', paying attention to application of this type of conditioning in organic seed production.

2. Material and methods

In order to develop the method of the proper hydration up to the desired moisture content, the root parsley 'Berlińska' seeds, own-produced, were hydrated in different doses of distilled water, ranging from 0 to 1280 ml of water · kg of seed $^{-1}$. After that, the moisture content was determined, according to ISTA rules [20]. The obtained data were the basis to plot the curve pointing to the moisture content depending on the doses of water added to seeds.

For further study, the seeds hydrated up to 30, 35 and 45% of moisture content were selected, as they were the most useful for the subsequent research, according to the preliminary experiments of authors. The moistened seeds were then incubated at 20°C in airtight containers for 1, 3 and 6 days, with daily ventilating and assessing their moisture content according to ISTA rules [20].

The conditioned seeds were then dried down in the laboratory conditions on filter paper, at 20°C and 50% RH, to the initial moisture content (8%) Their quality was evaluated on the basis of the measurements:

- dynamics and the percentage of germination, the mean time of germination at 20°C,
- electrolyte leakage which indicates the permeability of the cytoplasmic membranes,
- total activity of dehydrogenases,
- dynamics of emergence, the percentage of emerged seedlings and their height after 40 days from seed sowing under controlled conditions in the vegetation chamber,
- index of chlorophyll content in seedlings,
- net photosynthesis in the leaves of seedlings.

Seed germination was evaluated at 20°C. Three replicates of 50 seeds each, were sown in Petri-dishes on cotton wool, moistened with distilled water. Each seed was counted as germinated when its radical protruded through the seed coat. Germination (radical protrusion) was scored on a daily basis and seed germination percentage, mean time of germination were calculated using 'Seed Calculator Version 3.0, a computer program developed by CPRO - DLO, Wageningen, The Netherlands [12].

Electrolyte leakage, indicating the permeability of the cytoplasmic membranes, was tested at 20°C after placing of 1 gram of seeds in the tubes and the addition of 3 ml of distilled water. Electrolyte leakage was measured after 2 and 4 hours by using a conductivity meter Elmetron CC- 551 [12].

Total activity of dehydrogenases was evaluated after 24 hours of the seed moistening with distilled water on filter paper, at 25°C. Seeds were subsequently crushed and incubated at 25°C for 24 hours in sodium phosphate buffer (pH 7.2), containing 0.7% of 2,3,5 – trifenylochloride tetrazolium. After that, the samples were centrifuged and the pellet was extracted with acetone. Absorption of the solution was measured in comparison to pure acetone, at a wavelength of 510 nm. Dehydrogenase activity was determined using the standard curve prepared from known concentrations of formazan. Each determination was done in quadruplicate [12].

Dynamics of emergence and seedling growth were evaluated at 20°C. Three replicates of 50 seeds each, were sown in a standard horticultural bed soil and covered with 0.5 cm layer of sieved sand. Then they were placed in a growing chamber, at a constant temperature of 20°C and 16-hour circadian sodium light, emitted by the lamps SON-T AGRO 400. Photon flux density of photosynthetically active light was 100 micromol m^{-2} s^{-1}. Seedling emergence was scored on a daily basis and a number of emerged seedlings and mean time of emergence were calculated using Seed Calculator Version 3.0, a computer program developed by CPRO - DLO, Wageningen, The Netherlands. The height of seedlings was measured after 40 days from sowing time [12].

Index of chlorophyll content in seedling leaves were measured using a Minolta camera SPAD -502 (Konica Minolta, Japan) and expressed in SPAD units [28].

Net photosynthesis in the seedling leaves was measured with a gas analyzer TPS- 2 , PP Systems (USA) [24].

Non-conditioned seeds and seedlings derived from them were served as the control.

The study was performed in three series of experiments, and the presented results were the average of these sets and repetitions. The results were analyzed statistically by analysis of variance, followed by the separation of homogeneous groups, using Duncan's test at $p = 0.05$.

3. Results and Discussion

The obtained results indicate that in order to hydrate of one gram of the tested root parsley 'Berlińska' seeds from the starting 8.0% moisture content up to 30, 35 and 45%, was necessary to add to them 0,314; 0,415 and 0,672 ml of distilled water, respectively. All variants of the hydroconditioning of root parsley seeds favorably affected the germination and seedling emergence and their quality. The hydration of the tested seeds up to 45% moisture content and subsequent incubating them in airtight containers for a period of six days, followed by drying to an initial moisture, was the most effective method. It resulted in the most significant increase in seed germination percentage, dynamics of germination, their uniformity and emergence and growth of seedlings (fig. 1, 3).

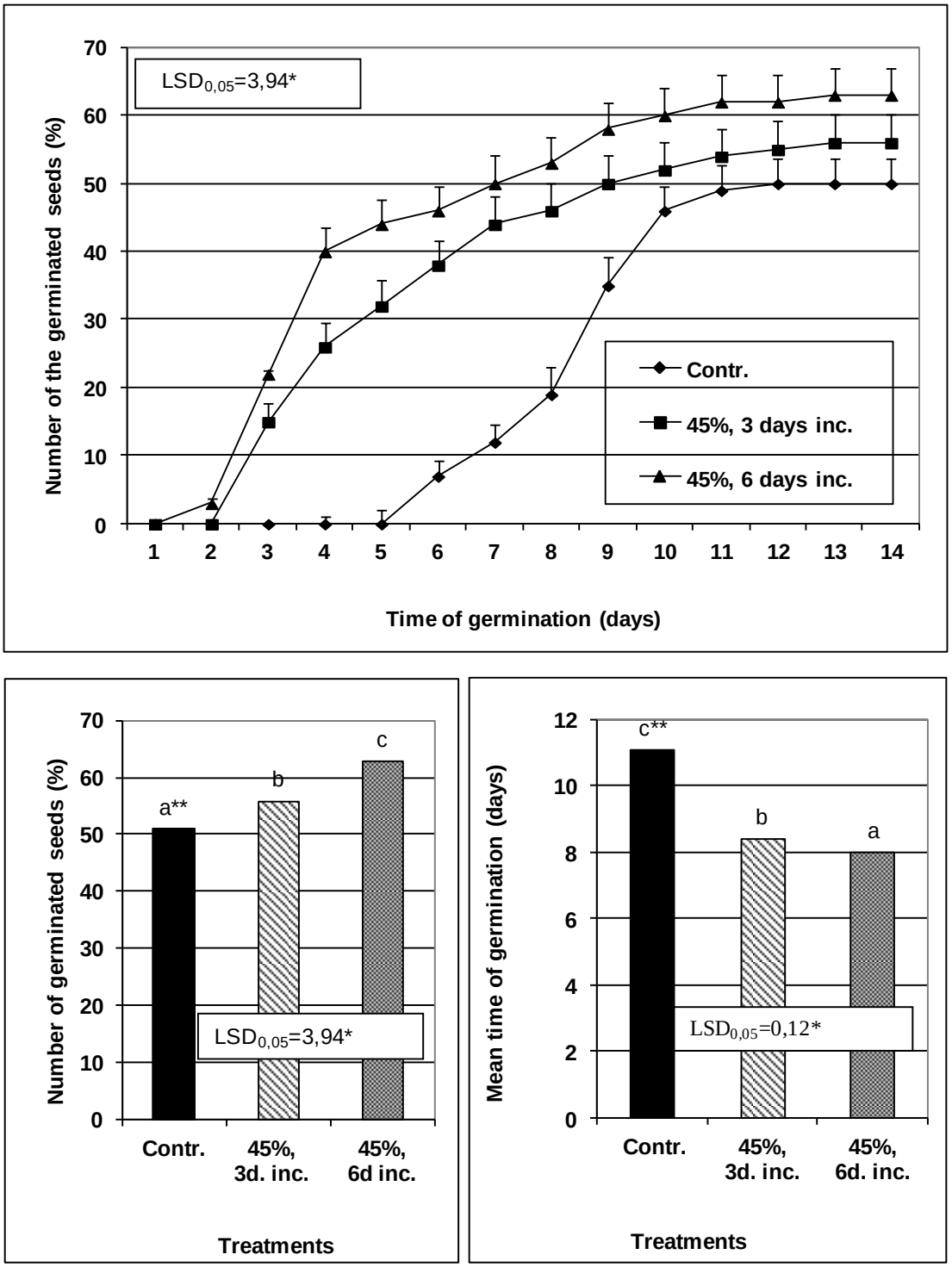

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Fig. 1. Dynamics and a number of germinated seeds and mean time of germination of root parsley 'Berlińska' seeds at 20°C, moistened up to 45% moisture content, subsequently incubated for 3 or 6 days and then dried to an initial moisture content in laboratory conditions. Vertical bars denote ±SD. *LSD at an alpha level of 0.05. **Means marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05

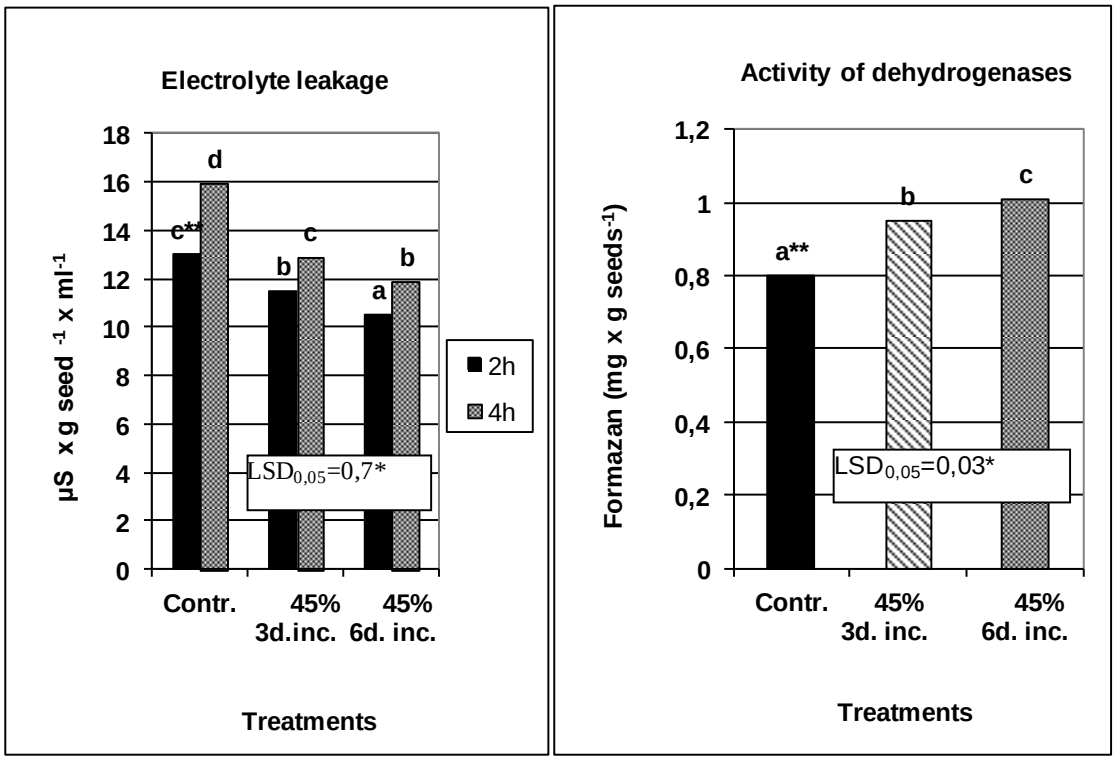

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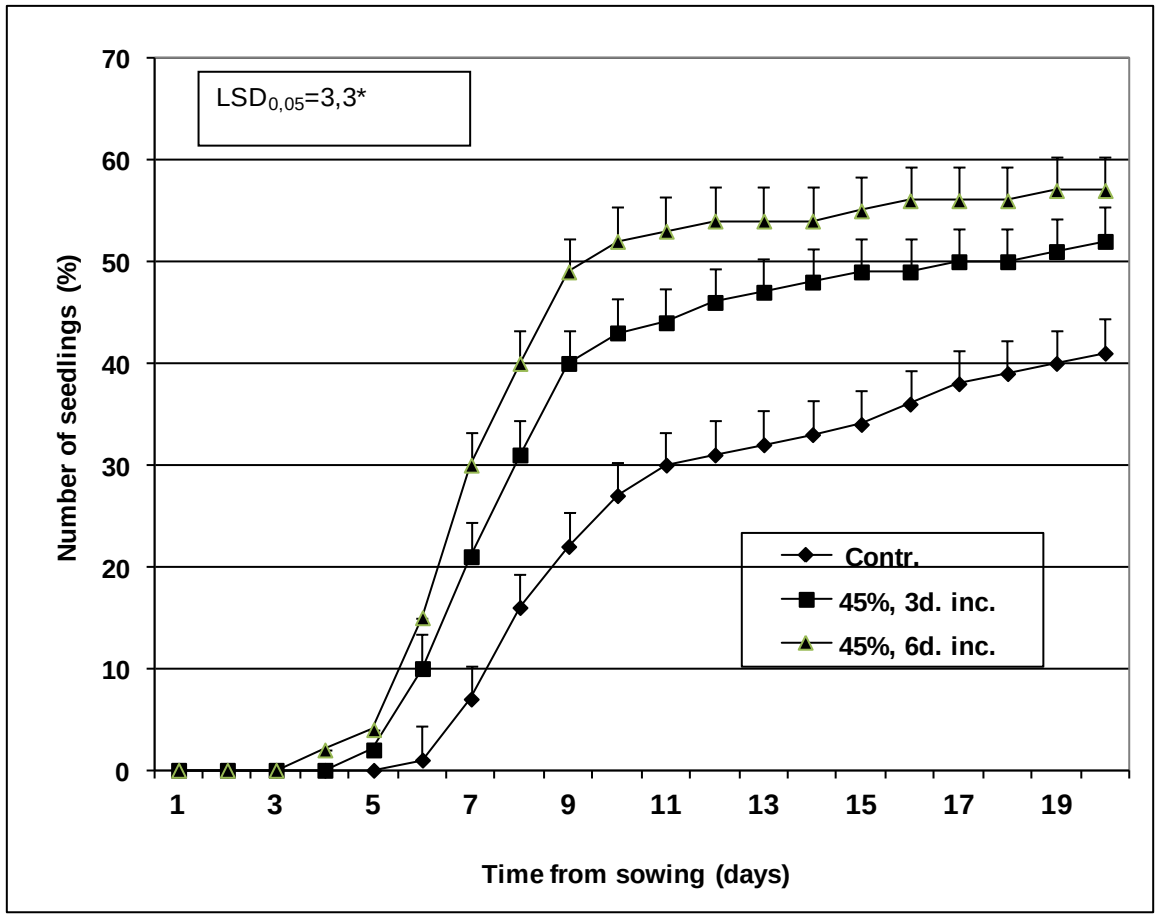
Fig. 2. Electrolyte leakage (after 2 and 4 hours) and total dehydrogenase activity in root parsley 'Berlińska' seeds at 20ºC, moistened up to 45%, subsequently incubated for 3 or 6 days and then dried to an initial moisture content in laboratory conditions. *LSD at alpha level of 0.05; **Means marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05

The conditioned seeds began to germinate at 20°C, as early as in the 2nd day after sowing, it is four days earlier than in the control variant. On day 6th after planting, conditioned seeds germinated in 46%, while the control only in 7%. In the final stage of this process, the germination in these variants was 63 and 50%, respectively, while the mean time of germination was 8.02 and 11.10 correspondingly. The obtained data indicate also the higher uniformity of germination, which shows the greater number of germinated seeds in a unit of time (fig. 1). These results are especially important in the field, where the lack of precipitation is observed and the soil become quickly dry and hard. The performed research and previous experience of authors indicate also that controlled seed hydration to particular moisture content in suitable doses of water is safer and more convenient than through the appointment of this degree of waterlogging by time of soaking.

Hydroconditioning of parsley seeds had also a positive effect on increasing of metabolic activity in the embryos. It was exhibited by the vigorous test of total dehydrogenase activity, which is one of the more useful in assessing the biological quality of the seeds (fig. 2). The activity of these enzymes was most intensive in the most hydrated seeds and subsequently incubated for 6 days, similarly as it was also exhibited by germination test. The performed treatment also caused a decrease in the permeability of the cytoplasmic membrane, as it was assessed by measurements of electrolyte leakage from cells (fig. 2). Reparation of the cytoplasmic membrane is one of the first processes during hydration and incubation of seeds. The primed seeds can have a higher potential to re-establish structural integrity and synthesize new compounds more rapidly during the early stages of hydration than non-primed seeds [29, 30] The obtained results indicate the usefulness of hydroconditioning procedure for improving of root parsley seed quality, as it was also stated in the case of other plant species [1-4, 11, 14, 16, 17, 19, 23, 27].

The consequences of the positive impact of hydroconditioning on dynamics and percentage of germination, mean time of germination and metabolic activity in seeds, were a significant decrease in the mean time of seedling emergence, the higher percentage of emerged seedlings, as well as their increased growth and physiological activity. Seedlings obtained from these seeds began to emerge 2 days earlier than in control. On day 9th after sowing, seedling obtained from the conditioned seeds emerged in 49%, while in control only in 22%. Finally, the number of emerged seedlings from conditioned seeds was higher by 18% and growth of seedlings was more accelerated than in control (fig. 3). Seedlings obtained from conditioned seeds were more stained, as it was showed by an index of chlorophyll content measurements. They also showed the significantly higher photosynthetic activity (fig. 4).

The obtained results indicate the possibility to improve germination, seedling emergence and plant growth of root parsley 'Berlińska' by the use of pre-sowing hydroconditioning of seeds, as it was also observed after the application of other seed treatments [7, 9 , 10, 11, 21 , 22, 24, 26]. Hydroconditioning is cheap and relatively easy to implement method of processing the seeds and therefore it can be preferable to its use in the production of parsley, especially grown in the organic system. Due to practical and economical aspects, it is more useful in plant production than osmoconditioning in PEG 6000, PEG 8000 and Ekosorb Na or matriconditioning in Micro-Cell E (MCE) [7, 9 , 26]. In addition, adequate hydration of seeds and subsequent incuba-



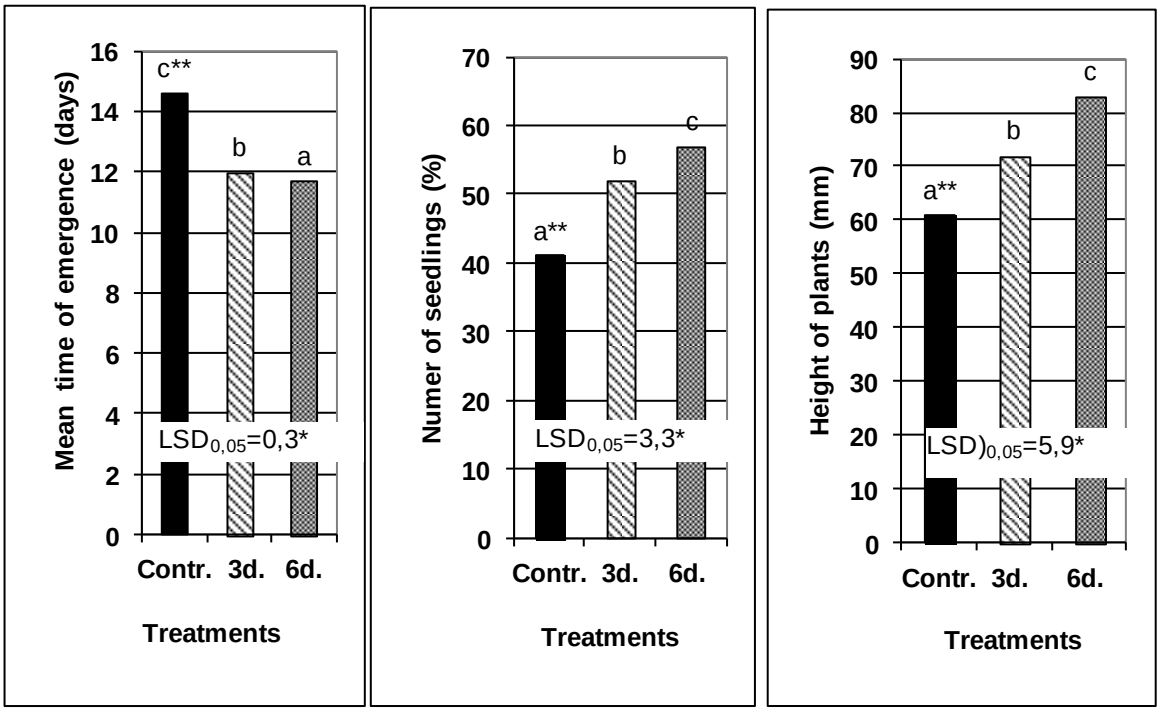


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Fig. 3. Dynamics of emergence at 20°C,, mean time of emergence and number and height of emerged plants, obtained from root parsley 'Berlińska' seeds moistened up to 45% and subsequently incubated for 3 or 6 days and then dried to an initial moisture content in laboratory conditions. Vertical bars denote ±SD. *LSD at alpha level of 0.05. **Means marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05

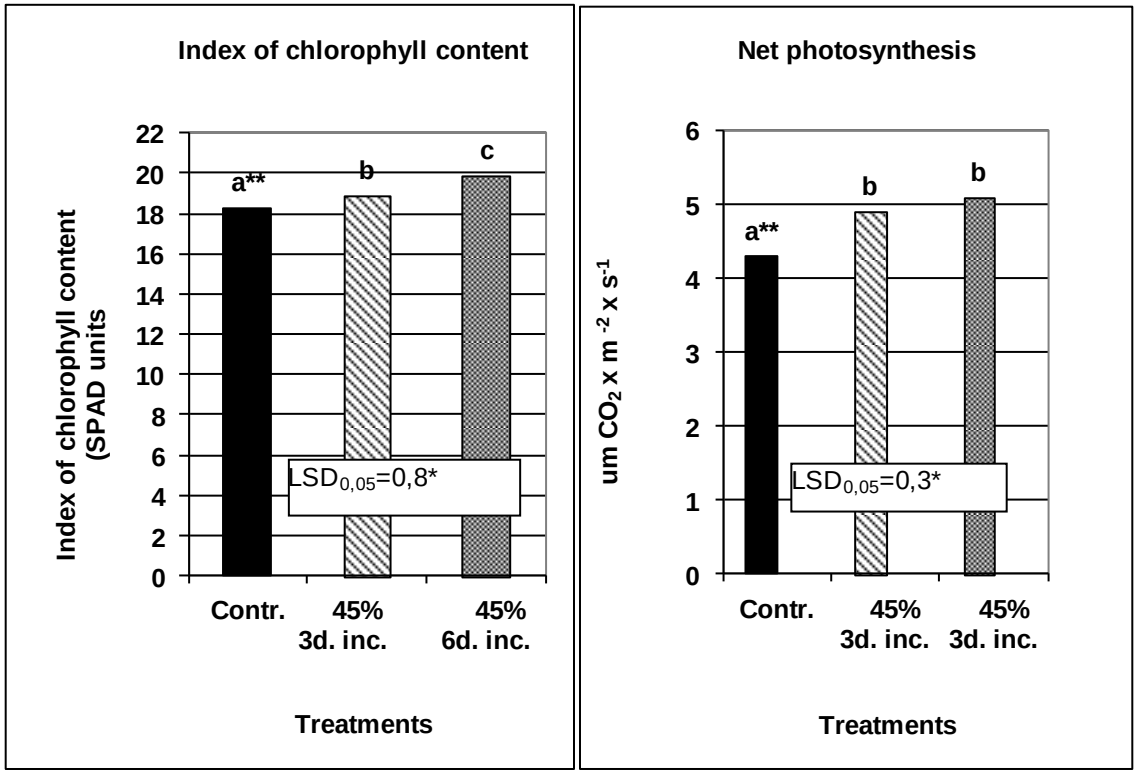

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Fig. 4. Index of chlorophyll content and net photosynthesis in seedlings obtained from root parsley 'Berlińska seeds, moistened up to 45%, subsequently incubated for 3 or 6 days and then dried to an initial moisture content in laboratory conditions. *LSD at alpha level of 0.05. **Means marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05

tion cause the higher dynamics and uniformity of germination than their soaking alone without following incubation [11, 17]. In the performed experiments the increased seed vigor and higher germination (affected by conditioning) caused the accelerated seedling emergence. Finally, it resulted in accelerated growth of the higher quality seedlings. This phenomenon is advantageous in the plant production, enabling the use of agro-technical procedures in the early spring, and promoting increased yielding of plants. The performed research also indicate that the elaborated seeds with a moisture content of about 8% can be hydrated without causing damages of their cytoplasmic structures by water quickly penetrating the embryo. This claim is based on the results of the performed test of electrolyte leakage, which showed the lower permeability of cytoplasm membranes in seeds subjected to hydropriming. Similar situation can be observed in practice, where seeds packaged in hermetic bags (dried down to 7% moisture content) germinate after sowing to the wet soil. However, Podlaski et al. [30], in studies performed on root parsley, observed the cracked surfaces of seeds subjected to matriconditioning and more after soaking in water for 72 hours. The number of seed cracked surfaces differed according to the priming method used. They pointed that it was connected with the imbibitional damage associated with rapid water absorption.

In order to optimize the elaborated procedure, further studies are needed to improve the method of hydroconditioning of seeds, which are produced under different environmental conditions. The indicated incubation period can be changed in case of conditioning of seeds of other varieties of parsley, a differently cleaned or ripening in other environmental conditions, particularly in excessive drought or heavy rainfall. This is consistent with the research of Podlaski et al. [30], whose research has shown that the effectiveness of seed priming of two root parsley varieties, 'Cukrowa' and 'Berlińska' differed. In their studies, moistening of seeds of cv. Cukrowa in water for 72 hours and matripriming were significantly more effective after 28 days of germination, compared to control seeds. In the case of cv. Berlińska only matriconditioning significantly improved the percentage of germination ability. Podlaski and coauthors [30] also cite other authors who conclude that response to a given priming treatment can vary not only between cultivars, but even between seed lots of the same cultivar. An important factor in determining the effects of the elaborated treatment is also temperature used during processing. It is conceivable that a different temperature than that used in the present study (20°C), eg. 15 or 25°C would also be beneficial. The explanation of this problem, as well as identification possible conditions for drying and storage capabilities of conditioned seeds, require a separate study.

4. Conclusion

1. Hydroconditioning of root parsley 'Berlińska' seeds reduces the mean time of germination, increases the number of germinated seeds, improves their metabolic activity and accelerates the emergence and growth of seedlings.
2. Effectiveness of hydroconditioning depends on the moisture content and the subsequent incubation period of parsley 'Berlińska' seeds. Hydration of them up to 45% moisture content, and subsequent incubation in airtight containers for a period of six days, followed by drying to an initial moisture is the most effective method, resulting in the most significant increase in germination, dynamics of germination, seedling emergence and growth.

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