Mieczysław Grzesik
Research Institute of Horticulture, Konstytucji str. 3 Maja str. 1/3, 96-100 Skierniewice, Poland, *Mieczyslaw.Grzesik@inhort.pl*

Zdzisława Romanowska-Duda
Department of Ecophysiology and Plant Development, University of Lodz, Banacha str. 12/16, 90-131 Lodz, Poland, *romano@biol.uni.lodz.pl*

Regina Janas
Research Institute of Horticulture, Konstytucji str. 3 Maja str. 1/3, 96-100 Skierniewice, Poland, *Regina.Janas@inhort.pl*

QUALITY OF SEEDS AS A KEY TO COMMODITY CULTIVATION OF *LAVATERA THURINGIACA* L. – PLANTS WITH HIGH POTENTIAL FOR MULTI DIRECTIONAL USE

Abstract:

Lavatera thuringiaca 'Uleko' is a perennial plant little-known in Poland, although it has a very high potential for multi directional use in several areas of economy. However , very low seed germination harvested in the harvest ripening (maturity) phase (5-20%) makes commercial production of these plants difficult and reduces the economic efficiency of crops. The aim of this study was to develop effective methods of breaking seed dormancy using scarification. The study indicated the possibility of increasing the germination of seeds up to over 40% with mechanical scarification, and up to 85% after exposure to sulfuric acid. Application of these methods positively influenced the physiological activity, health and germination of seeds, as well as emergence and growth of plants, which can facilitate the production of this species on large acreages.

Key words:
Lavatera thuringiaca L. 'Uleko', seeds, germination, scarification, growth

Introduction

Thuringian mallow, synonyms: Lavatera, Tree lavatera (*Lavatera thuringiaca* L., family *Malvaceae*) is a future-oriented perennial plant in Poland (Photo 1, 2). Interest of its cultivation is growing due to the opportunities of multi-directional use in many areas of economy and its capability of growing under adverse environmental conditions. It can be cultivated as:

- a perennial plant which is suitable for production of high-performance energy biomass (15-25 t d.w. ha^{-1}), with a chemical composition similar to woody plants,
-a high value ornamental plant to be grown in gardens and parks on dry soils,
-a perennial plant , cultivated on wastelands and on uncultivated fields, to create pollen for bees to produce honey comparable to that produced on base of buckwheat,
- an ecological plant to be sown on roadsides and useful in recultivation of degraded land;
- raw material for the pulp and paper industry,
- raw material for the pharmaceutical industry, due to the high content of mucilage (rhamnose and arabinose) which is similar to hat in the black hollyhock (11%);
- as supplement for plant feed for ruminants, due to the high content of digestible protein and sugars in the forage . It can also be cultivated as a plant support for alfalfa and clover and to improve the ensiling of legumes [1, 2].

In view of its high relevance, especially in the production of biomass for energy needs, it is assumed that it will be cultivated on the large acreage in the near future in Poland [1, 2].

Photo 1. Plants of *Lavatera thuringiaca* L. and flowers pollinated by bees. *Author: M. Grzesik*

Photo 2. The seed stalk and plants of *Lavatera thuringiaca* 'Uleko' with maturing seed heads in autumn. *Author: M. Grzesik*

Commercial production of these plants is impeded by low germination of matured seeds [1, 2]. The ripening seeds in summer, having green or light brown color (in morphological or physiological ripening phase), poorly germinate after falling down on the soil and produce plants, which favors the renewal of plantation. These seeds turn black when storing, coats become hard and germination is greatly decreased. From practical point of view, for storage and sowing in the recommended spring period, the fully ripened seeds should be used. These seeds have black and hard seed coats and germinate very poorly (often 5-20%). Poor germination require higher amount of these seeds sown in spring, while emergence of seedlings is uneven, especially in adverse weather conditions. Thus the production of plant biomass on large areas for energy needs is hindered. The cause of very poor germination of *Lavatera thuringiaca* L. is likely to be connected with the impermeable seed coats for air and water and with embryo dormancy. To the best of our knowledge no high-efficiency technologies to improve of these seed sowing value were developed. In literature only limited information, mostly concerning mechanical and chemical scarification of seeds of deciduous trees can be found. The potential use of chemical scarification followed by stratification, was indicated e.g. for linden. It resulted in significant acceleration and increased number of germinated seeds [3]. In forestry, in addition to chemical scarification, mechanical scarifiers are also used, to damage thick seed coats but they are not always very effective [3, 4, 5, 6, 7, 8].

In the case of perennials, which include *Lavatera thuringiaca* L., there were also few attempts to interrupt seed dormancy by mechanical scarification and very rarely by chemical methods. Very few studies pointed to the attempts of improving germination of dormant seeds (having coats impermeable to water and air) by seed treatment with metabolic excretions of birds (penguin) or with enzymes degrading components of seed coats. These excretions damaged the seed coats, caused their increased permeability to water and gases and thus promoted higher germination [9, 10].

The aim of this study was to develop an effective method of breaking dormancy of *Lavatera thuringiaca* 'Uleko' seeds, using different methods of scarification.

Material and Methods

Storage and scarification of seeds

The seeds of *Lavatera thuringiaca* L. 'Uleko', harvested in the morphological (green color), physiological (light brown color) and harvest ripening (maturity) phases (black color) in the Research Institute of Horticulture, Skierniewice and in Seed Enterprise "ROLNAS", Bydgoszcz in Poland were used in experiments.

The harvested seeds, before the treatments, were stored up to January-April in next year in warehouse conditions recommended for orthodox seeds. After that they were germinated at 5-45^{o}C, in order to determine optimal, sub- and supra-optimal germination temperature. Then they were treated with five scarification methods which can presumably damage seed coats and increase their permeability to water and air, which is necessary for seed germination. According to literature they damaged the seed coats with different efficiencies depending on the species of perennials and deciduous trees.

The separate batch of seeds were scarified by:

- soaking at 20^{o}C in H_2SO_4 for 0, 5, 10, 15, 20, 25 and 30 minutes
- rubbing the seeds between sandpaper
- sudden freezing at -73^{o}C for 10 minutes
- soaking in metabolic excretions of birds for 2, 4, 8 days
- treating with suspensions of cellulase enzyme (0.4 mg l^{-1}) for 1, 2 and 4 days

Evaluation of seed quality

The number of germinated seeds, dynamics of germination, mean germination time, activity of acid (pH=6.0) and alkaline (pH=7.5) phosphatase (mU x g^{-1} f.w.) or RNase (mU x g^{-1} f.w.), electrolyte leakage from seeds (µS x $seed^{1}$ x ml^{-1}), contamination by pathogenic microflora, as well as dynamics of emergence and growth of seedlings were measured in order to assess the influence of the above treatments on germination, physiological activity, health and seedling growth. Seed quality was evaluated directly after scarification. Untreated seed were used as control.

Germination of seeds was evaluated at temperature of 20°C. For each treatment, 3 replicates of 100 seeds were sown to Petri dishes on filter paper moistened with distilled water and allowed to germinate in incubators. Germination (radical protrusion) was scored on a daily basis during 20 days since sowing and dynamics of germination and seed germination percentage were calculated using Seed Calculator Version 3.0, a computer program developed by Plant Research International B.V., Wageningen, the Netherlands. Seeds were counted as germinated when the radicals protruded through the seed coat [11, 12, 13, 14].

Activity of acid and alkaline phosphatase and RNase in leaves were examined according to methods described by Knypl and Kabzinska [15].

Electrolyte leakage was investigated at 20^{o}C after placing 30 seeds in test-tubes and adding 3 ml of distilled water. Electrolyte leakage was measured after 2 and 4 hours using microcomputer conductivity meter CC-551 Elmetron [16, 13]

Contamination of seed coat by pathogenic microflora was evaluated at 20°C. For each treatment, 10 replicates of 5 seeds were sown to Petri dishes on agar. After 5-7 days of incubation in incubators, the number of seeds contaminated by pathogenic micoflora was rated and species of fungi and bacteria colonizing the seed coats were identified.

Dynamics of emergence and growth of seedlings were evaluated at 20°C and 60% RH, under 8 hour dark/16 hour light cycle - SON-T AGRO 400 W, 100 µmol$m^{-2}s^{-1}$. For each treatment, 3 replicates of 50 seeds were sown to boxes filled with a mixture of sphagnum peat and sand (1:1=v:v) moistened with distilled water and then allowed to emergence. Emergence of seedlings was scored on a daily basis for 20 days and height of seedlings was measured 1 month after sowing [16, 17].

Collected data were analyzed using analysis of variance.

Results and discussion

The germination of *Lavatera thuringiaca* L. seeds was very poor and depended on temperature, in each evaluated maturity phase, with 20-25^{o}C being optimal. These seeds also germinated at lower or highest temperatures, but less effectively (data not shown). Research have shown that *Lavatera thuringiaca* L. seeds have similar requirements for germination as most of horticultural and agricultural plants cultivated in climatic conditions in Poland [11, 12,13].

Scarification with an appropriate method greatly increased germination of matured *Lavatera thuringiaca* L. ' Uleko' seeds (harvested in harvest ripening phase) and their physiological activity, thus enhancing emergence and growth of plants (Fig. 1, 2, 3, 4, Tab. 1). However, these beneficial effects depended on the method of scarification. Treatment of seeds with metabolic excretions of birds, enzyme cellulase and sudden freezing at -73°C did not damage effectively the seed coats and thus they were not permeable enough to water and air. In consequence, germination did not change, as compared to the untreated seeds (Fig. 1). Mechanical scarification consisting in rubbing the seeds between sandpaper increased the germination up to over 40%. Chemical scarification with concentrated sulfuric acid (H_2SO_4) proved to be the most effective. It resulted in increased dynamics of germination and the number of germinated seeds up to over 80% (Fig. 1, 3). The obtained results are in line with the research of Suszka, Aliero, Fang et al., Lewak and Duczmal, Tucholska [3, 4, 5, 8, 18] who showed the positive effects of chemical scarification on seed germination of some woody plants and also with our previous studies, indicating increased germination of rose seeds after treatment with concentrated sulfuric acid [6].

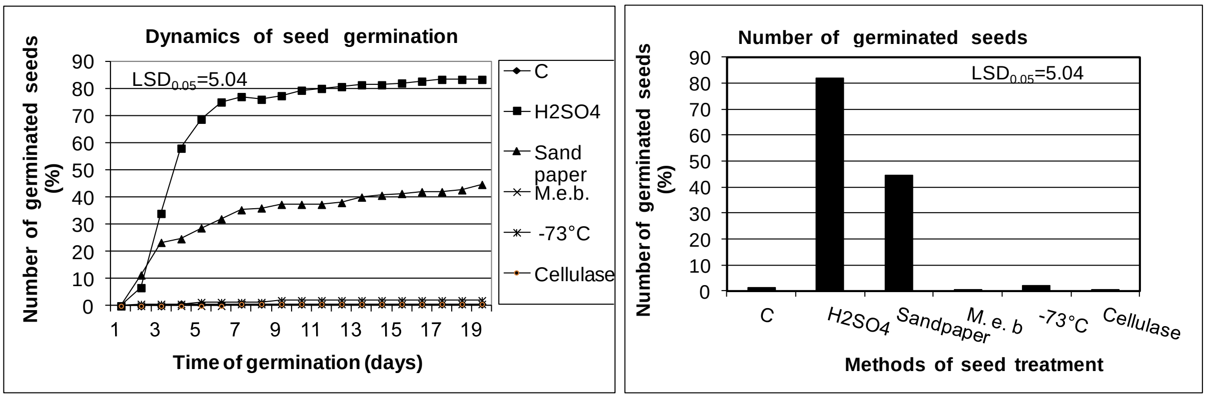


Fig. 1. Dynamics of germination and number of germinated seeds of *Lavatera thuringiaca* L. ' Uleko', harvested in harvest ripening phase and then scarified with different methods at 20°C. Untreated seeds, control (C) and these scarified: in H_2SO_4 (H2SO4), between sandpaper (Sandpaper), in metabolic extretions of birds (M. e.b.), by sudden freezing at -73°C (-73°C) and digested in cellulase suspensions (Cellulase).

The improved germination of matured seeds, caused by effective mechanical and chemical scarification, was associated with several physiological events, among others, with increased activity of acid (pH=6.0) phosphatase as well as alkaline (pH=7.5) phosphatase and RNase. The activities of these enzymes were higher after mechanical scarification of seeds and highest after their treatment with the concentrated sulfuric acid (for 10-25 minutes), as compared to the untreated seeds. The other used methods of scarification had no effect on enzyme activity, as it was also demonstrated in germination tests (Tab. 1).

Table 1. Effects of different scarification methods on the activity of selected enzymes in matured *Lavatera thuringiaca* L. 'Uleko' seeds.

Seed scarification using different methods and substations						$LSD_{0,05}$
Control	H_2SO_4	Sandpaper	Metabolic excretions of birds	Freezing at -73°C	Cellulase	
Activity of acid phosphatase (pH=6.0) (mU g^{-1} f.w.)						
0.18 a*	0.70 c	0.62 b	0.20 a	0.22 a	0.20 a	0.06
Activity of alkaline phosphatase (pH=7.5) (mU g^{-1} f.w.)						
0.04 a	0.41 c	0.30 b	0.05 a	0.06 a	0.06 a	0.02
Activity of RNase (mU g^{-1} f.w.)						
1,05 a	3.20 c	2.50 b	1.12 a	1.17 a	1,14 a	0.18

*The data marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

The electrolyte leakage from the seeds scarified mechanically and chemically was slightly higher than from the untreated ones, indicating the greater permeability of seed coats (Fig. 2). Tests measuring the activities of enzymes and electrolyte leakage from tissues are useful markers of seed quality and indicate vigour and physiological reactions of plants to treatments. The higher activity of enzymes, caused by the used mechanical and chemical scarification, indicated the increased seed vigour and permeability of seed coats, which in consequence led to improved germination, similarly as it was found in other research [11, 12, 13, 14, 17, 19, 20].

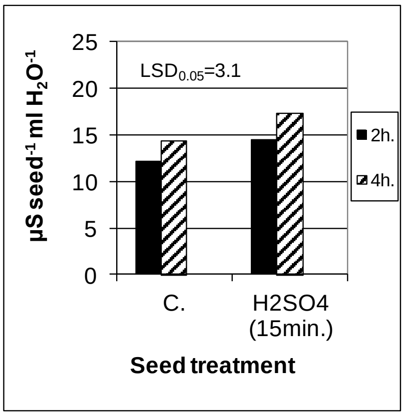
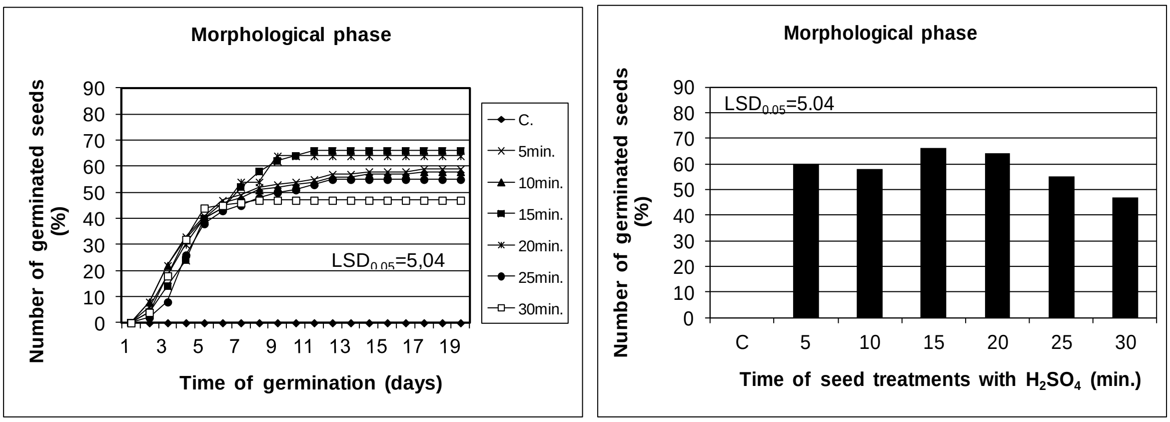


Fig. 2. Electrolyte leakage from the seeds of *Lavatera thuringiaca* L. 'Uleko' harvested in harvest ripening phase and then scarified in H_2SO_4 for 15 minutes at 20°C.

The influence of the chemical scarification on dormancy breaking and on total number of germinated *Lavatera thuringiaca* L. 'Uleko' seeds depended on seed ripening (maturity) phase, in which the seeds were harvested from plants (Fig. 3). Scarification of matured seeds for 15-25 minutes in concentrated sulfuric acid caused accelerated germination and increased the percentage of germinated seeds up to over 80%. The seeds harvested at physiological maturation phase, were less matured and their seed coats (in light brown color) were thinner and less woody. They sown at this time directly to the soil germinated at 40% (data not shown). However, during storage up to spring sowings their seed coats become black, hard and these seeds germinated very poorly after sowing. They germinated less effectively after chemical scarification (53-71%) than matured ones and damage of seed coats required a shorter time of treatment with sulfuric acid, not to injure the embryo. Extending of the sulfuric acid treatment period is not recommended, because it decreased germination. Seeds harvested in the morphological ripening phase also had thin and less woody seed coats (in green color) and not germinated after storage. Their scarification in sulfuric acid after storage improved germination up to 47-65%, however it was not high enough in comparison to germination of the matured seeds and subsequently scarified chemically (Fig. 3).

Therefore, the results indicated, that from the practical point of view seeds of *Lavatera thuringiaca* L should be harvested in the harvest maturity phase and then scarified for 10-25 minutes in concentrated sulfuric acid. After this treatment they should be rinsed under running water to remove the residues from the seed coats. This treatment is easy to perform and results show significant increase in germination of seeds [6].



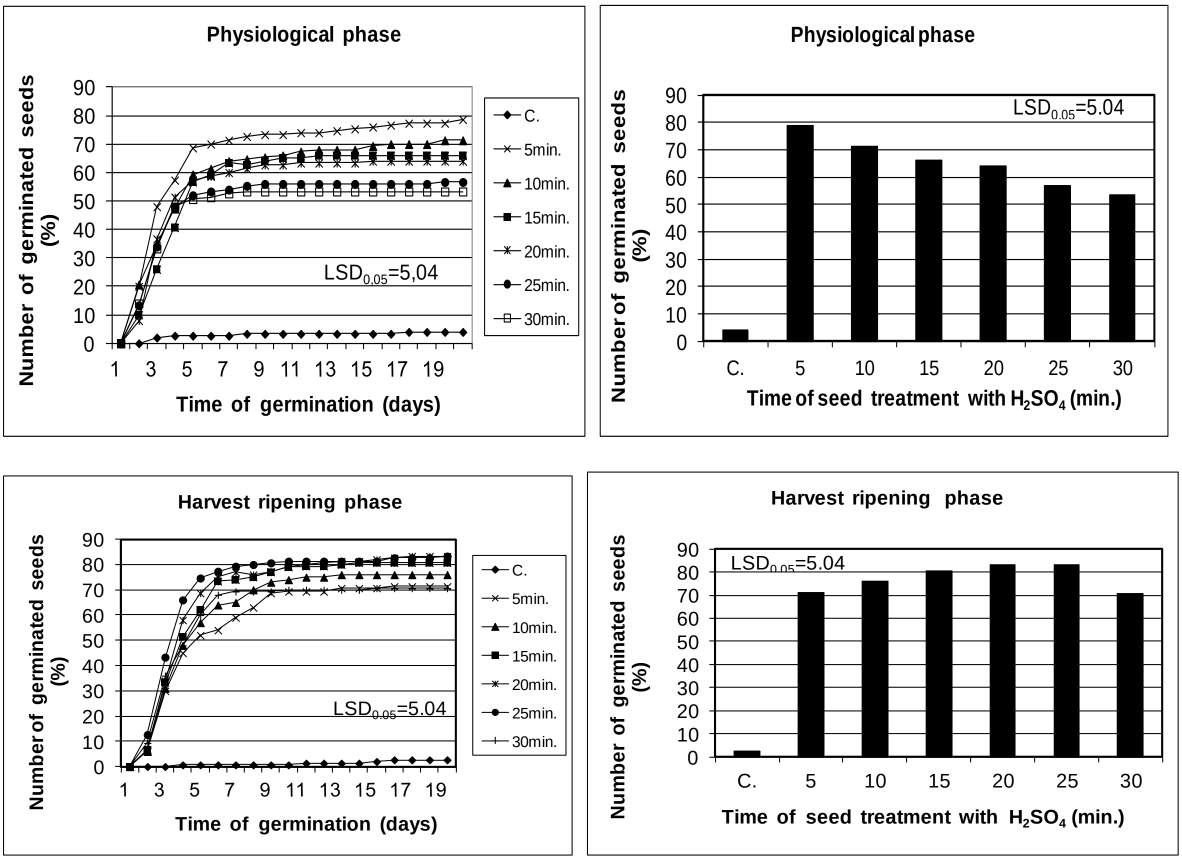


Fig. 3. Dynamics of germination and percentage of germinated seeds of *Lavatera thuringiaca* L. ‚Uleko' harvested in morphological, physiological and harvest maturity (ripening) phase, subsequently stored and then scarified in H_2SO_4 for 5, 10, 15, 20, 25 and 30 minutes at 20°C.

The increased dynamics of germination, total number of germinated seeds and their physiological activity, caused by scarification in sulfuric acid, resulted in the enhanced dynamics of seedling emergence and the number of plants obtained from the treated seeds. The greatest increase in emergence of seedlings and growth of the plants was caused by scarification of *Lavatera thuringiaca* L seeds in sulfuric acid for 15-25 minutes (Fig. 4), similarly as in the case of rose seeds [6]. The scarified seeds in the winter can be stored up to spring sowings.

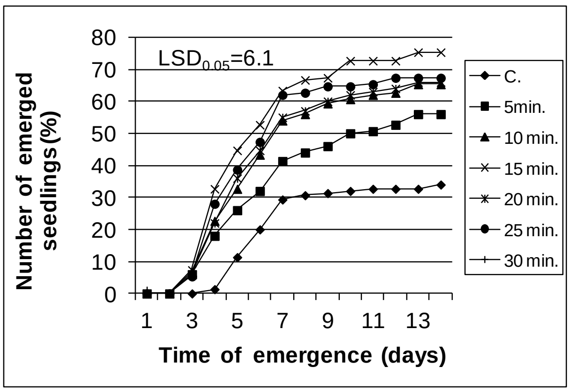


Fig. 4. Dynamics of seedling emergence from the seeds of *Lavatera thuringiaca* L. 'Uleko' harvested in the harvest maturity phase and then scarified in H_2SO_4 for 5, 10, 15, 20, 25 and 30 minutes at 20°C.

The research also indicated that the scarified seeds exhibited better health status than the untreated ones. Mechanical and chemical scarification removed all pathogens from the seed coats making the seeds healtier (data not shown) and well germinated (Fig. 1, 3).

The results broader the previous theoretical and practical knowledge concerning the improvement of germination of *Lavatera thuringiaca* L. and seeds of other species which are in a similar state of dormancy and thus their sowing value is low. Implementation of the developed methods to practice will allow to reduce the amount of sown seeds of *Lavatera thuringiaca* L. and will result in higher yield after using precision equipment. Moreover, it will accelerate germination in favorable and unfavorable weather conditions and will improve the economic efficiency of these crops. Thus these methods will allow the cultivation of *Lavatera thuringiaca* L. plants on large acreages [1, 2].

Conclusion

Commercial production of *Lavatera thuringiaca* L. plants is impeded by low germination of seeds harvested in harvest maturity phase, which should be stored up to spring sowings. Treatment of these seeds with metabolic excretions of birds, enzyme cellulase and sudden freezing at -73^{o}C did not damage effectively the seed coats and thus germination is not improved. Mechanical scarification consisting in rubbing the seeds between sandpaper increased the germination up to over 40%. Chemical scarification with concentrated sulfuric acid (H_2SO_4) increased dynamics of germination and the number of germinated seeds up to over 80% and positively affected physiological activity, emergence and growth of plants.

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JAKOŚĆ NASION JAKO KLUCZ DO POWSZECHNEJ UPRAWY ŚLAZÓWKI TURYNGSKIEJ - ROŚLINY O DUŻYM POTENCJALNYM WIELOKIERUNKOWYM WYKORZYSTANIU

Streszczenie
Lavatera thuringiaca 'Uleko' to wieloletnia, mało znana w Polsce roślina, mimo że ma bardzo duży potencjał wykorzystania w wielu dziedzinach gospodarki. Problemem w jej uprawie jest bardzo niska zdolność kiełkowania nasion zebranych w fazie dojrzałości zbiorczej (5-20%), która utrudnia komercyjną produkcję tych roślin i zmniejsza opłacalność upraw. Celem pracy było opracowanie skutecznych metod przełamywania spoczynku nasion za pomocą różnych metod skaryfikacji. Badania wskazały na możliwość zwiększenia kiełkowania dojrzałych nasion do ponad 40% poprzez zastosowanie skaryfikacji mechanicznej i do 85% pod wpływem traktowania kwasem siarkowym. Zastosowanie tych metod pozytywnie wpłynęło na aktywność fizjologiczną, zdrowotność i kiełkowanie nasion oraz wschody i rozwój roślin, co może ułatwić produkcję tego gatunku na dużych areałach.
Słowa kluczowe: *Lavatera thuringiaca* L. ' ULEKO', nasiona, kiełkowanie, skaryfikacja, wzrost