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## The effect of high voltage electrical discharge (HVED) treatment on small seed plants

Lea Lojková<sup>1</sup>, Sanja Zec Zrinušić<sup>2</sup>, Helena Pluháčková<sup>1</sup>, Radim Cerkal<sup>1</sup>, Tihana Marček<sup>2\*</sup>

<sup>1</sup>Mendel University in Brno, Faculty of AgriSciences, Zemědělská 1665/1, 61300 Brno, Czech Republic

<sup>2</sup>Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology, Franje Kuhača 18, 31000 Osijek, Croatia

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### \*CORRESPONDENCE

Tihana Marček

✉ [tihana.marcek@ptfos.hr](mailto:tihana.marcek@ptfos.hr)

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### ABSTRACT

The High Voltage Electrical Discharge (HVED) is a physical hydropriming method which alters the physicochemical properties of water, creating the plasma activated water (PAW). The mixture of highly reactive ions and molecules can break down the seed dormancy and speed up the growing process. Previous studies presented the promotional role of HVED technology on the germination, growth and polyphenol content. The aim of this study was to explore the effect of HVED technology on germination ability and vitality of three types of seeds with low germination rate. Selected species included carrot (*Daucus carota* L.), melilot (*Melilotus albus* Medik.), and mallow (*Malva verticillata* L.). In carrot and mallow, HVED (20Hz/30s) significantly promoted germination percentage, whilst in melilot seeds, germination was unchanged. HVED treatment enhanced the growth of shoots and roots in both carrot and mallow. In melilot, shorter treatment time of exposure to high voltage (30Hz/10s) resulted in longer shoots and roots. Perspectives of HVED as a method for improvement of germination exist, especially in mallow and carrot, but it is necessary to optimize the process parameters for each type of seed separately.

### KEYWORDS

carrot; melilot; mallow; germination; high voltage electrical discharge

### KEY CONTRIBUTION

High voltage electrical discharge (HVED) treatment improved germination of mallow and carrot seeds and increased the growth rate of shoots and roots in all three small seed species. Applied technology could be useful in the promotion of the germination potential of seeds with low germination rate.



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## Introduction

Natural plant communities are complex systems of many different species, existing in long-term harmony or evolving very slowly with changing climate, but at the same time able to shift the balance according to actual vegetation year conditions. Survival strategies of some plants, essential for their prosperity in highly competitive biomes, are a source of considerable complications in human-regulated

cultivation systems. Uneven germination might be practical in the wild; all plants cannot be eaten, burned or killed by stress at the same time. From the agricultural viewpoint, the same phenological phase is required for optimal application of crop treatments and harvest can be troublesome due to shedding of seeds from overripe flowerheads and subsequent weeding of other crops in the next season (which is typical e.g., for milk thistle) (Vágnerová et al., 2017). In the nature, on the other hand, species that are able to form long-lived seed reserves are at an advantage; when dormancy is broken, various environmental factors cause seed germination to occur with proper timing, during certain part of the vegetation year (Baskin and Baskin, 2014).

Physical or exogenous dormancy belongs to unfavourable mechanisms that have been artificially suppressed in agricultural practice, and better methods have been investigated for over a century. In a short communication from 1927, references of three older studies are given (Berkeley, 1927). Seed coat cells, containing lignin, wax or other hydrophobic materials form hard, water-impermeable protective layer around the seed, often with specialized “water gap” structures (Ansari et al., 2017). Scarification, the most common form of treatment, was originally done using concentrated sulphuric acid. While the seeds “withstood the action of the concentrated acid for surprisingly long periods and germinated satisfactorily”, the chemical was highly corrosive, causing damage to instrumentation, as well as serious burns and tissue injuries to workers (Berkeley, 1927). Yet, chemical scarification was preferred in a study of little mallow (*Malva parviflora* L.) when mechanical scarification with a scalpel was found too time-demanding; boiling in water was tested as another alternative (Chauhan et al., 2006). Optimized scarification required 60 min exposition to 95% sulphuric acid, resulting in germination increase from 12% for untreated control to 59%. In wild plants, scarification occurs naturally due to random mechanical damage, pests (insects, fungi) and long-time degradation of protective layer due to sunlight, changing temperature and moisture conditions. Detailed overview of seed coat softening methods (mechanical or acid scarification, application of enzymes, organic solvents, percussion, high atmospheric pressure, wet or dry heating, sonication, cold stress and storage) is given in (Baskin and Baskin, 2014). High voltage electrical discharge (HVED) is environmentally friendly hydro-priming method known to increase germination ability of cereals, using nothing but water at extremely short treatment times (for wheat, 30 s exposition was optimized) (Marček et al., 2021; Marček et al., 2023). Primary effect of HVED is based on increased membrane transport by direct exposition to 30 kV at the frequency of 10–100 Hz. Electrical discharge causes chemical changes in water (formation of reactive oxygen species), as well as fluid turbulence, cavitation, high-pressure shock and emission of UV light (Boussetta et al., 2013; Gros et al., 2003). Combined with random collisions of individual seeds with each other, container walls and mechanical stirrer, scarification occurs as a secondary mechanism. Thus, the application of HVED for hard coat seeds combines two beneficial effects. Required scarification is provided at normal temperature without the need of toxic solvents, using low-cost medium and relatively labour-undemanding procedure. At the same time, freshly scarified seeds are exposed to HVED phenomena, targeted to suppress biochemical dormancy mechanisms.

Tall mallow (*Malva verticiliata* L.) is a very good model specie, being a troublesome weed, as well as medicinal plant used for human (digestive, dermatological, urological, respiratory) and veterinary medicine (colic, mastitis, constipation, infections and inflammations) (Batiha et al., 2023). Together with melilot (*Melilotus albus* Medik.), which is another hard-coat seed plant, mallow is used as a component of animal feed mixtures, which are not tilled crops and would take only limited scarification during the sowing (Chauhan et al., 2006).

Factors affecting seed dormancy of mallow have been reported in several studies, including storage time, seed drying, cold stress and scarification (Ansari et al., 2016), temperature, water potential (Ansari

et al., 2017) and osmotic stress (Ansari et al., 2018). Melilot, besides its use in animal feeds, is an important tool of phytorecultivation. It can be grown in dry, low-nutrient soils and gravels, with ameliorative effects. To break seed dormancy, scarification is required, or alternate exposition to high and low temperatures ( $\geq 30$  cycles) (Zhang et al., 2020). Studies of germination behaviour under drought stress are also available (Kintl et al., 2021), as well as the effect of cultivation parameters to the yield and nutrition characteristics (Sowa-Borowiec et al., 2022). Observations of germination behaviour of yellow sweet clover, *M. officinalis* L. (Ghaderi-Far et al., 2010) and *M. indicus* L. (Devi et al., 2013; Dhawan, 2009) have also been reported.

Seed that suffered hard coat damage before the hydro-priming, or scarification occurred at the start, would receive full HVED treatment, which should further increase their germination ability, but must not endanger the seed embryo. To be able to ascertain the effect of HVED independently, carrot seeds (*Daucus carota* L.) were selected for comparison.

Carrot plant forms up to 3000 achenes with 2 mericarps – true seeds – each. Germination ability of harvested seeds is low; in the wild, they are supposed to stay dormant in the soil during the winter. It is increased in spring of the next year, but the seeds can germinate unevenly during the period of 1-3 weeks and should be watered if the weather is dry. Without cultivation management, appearance of wild carrot seedlings is not confined to one part of the season (Dale and Harrison 1966). Treated seeds could grow more evenly and, at the same time, the hypothesis that no harm was done to the seeds at selected HVED conditions would be proved (or disproved). Carrot seeds germination has been reported to increase by hydro-electro hybrid priming. Proposed mechanism is given in (Zhao et al. 2022).

The aim of this study was to investigate the possibilities of HVED treatment application to small seeds in a need of scarification due to physical dormancy or showing insufficient or uneven germination. *M. verticiliata* and *M. albus* were studied as representatives of hard coat seed species and *D. carota* as a plant with very tiny seeds. Germination was monitored and the seedlings were cultivated in controlled conditions for 10 days, when the effect of HVED treatment to the length of roots and shoots was evaluated.

## Materials and methods

Seeds of *M. verticiliata* and *M. albus* were obtained from the Research Institute of Fodder Crops, Ltd., Troubsko, Czech Republic; *D. carota* from Semo, a.s., Smržice, Czech Republic. For the high voltage electrical discharge (HVED) treatment 30 g of non-sterilized seeds was immersed into 800 mL of distilled water. HVED treatment was performed at 30 Hz for 30 s (*M. verticiliata*, *D. carota*), 30 Hz/10 s and 20 Hz/30 s (*M. albus*) (Marček et al., 2021).

At the same time, seeds soaked in distilled water represented control. All experiments were carried out at room temperature in HVED beaker with free water surface under constant stirring. After the treatment, seeds were exposed to activated water for 10 min. without further stirring. The seeds were put on the clean filter paper in a grid pattern in transparent plastic boxes (20×15×3 cm).

Each pot contained 50 (carrot) and 54 (melilot and mallow) seeds, and each treatment had three repetitions. After sowing seeds were left in the dark for 2 days and then exposed to 12 h light/12 h dark photoperiods under fluorescent lamp ( $80 \text{ mol m}^{-2} \text{ s}^{-1}$ ) and watered every two days with 10 mL of demineralized water. Germination data were collected daily, the length of roots and shoots was measured on the 10<sup>th</sup> day.

### Data analysis

For statistical analysis 3-10 repetitions were taken and one-way analysis of variance (ANOVA) was applied. Statistical analyses were done using Statistica 14.0.0.15 (TIBCO Software Inc., Palo Alto, CA, USA).

## Results and discussion

### Effect of HVED treatment on the germination

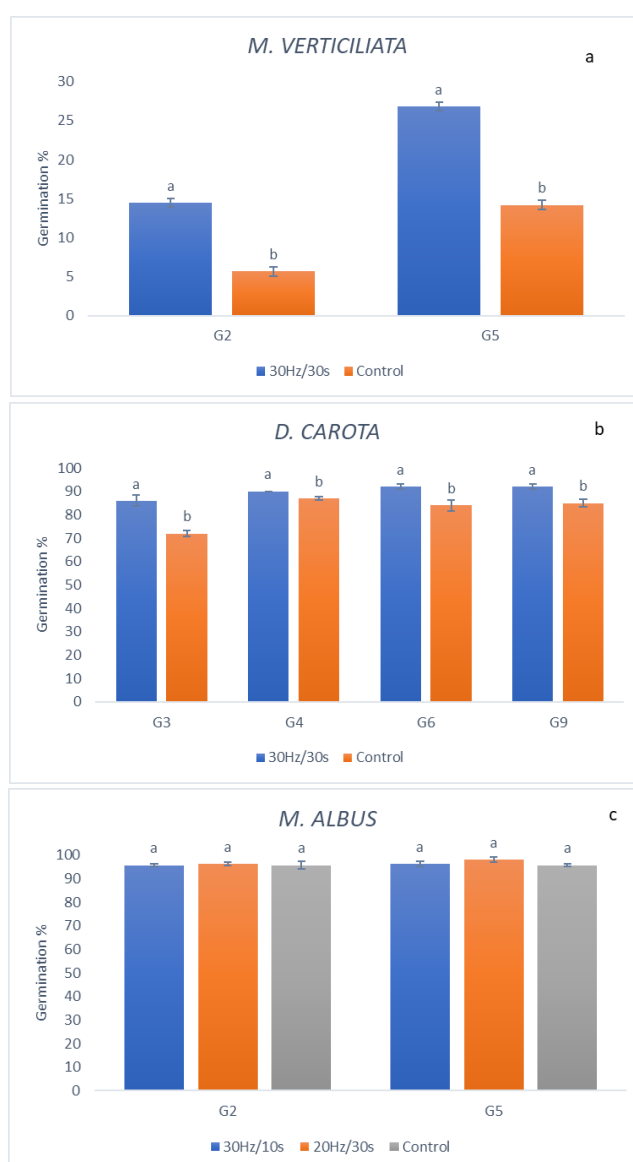
In previous studies, the beneficial effect of HVED treatment on the germination and growth of wheat under optimal, drought and stress conditions were presented (Marček et al., 2021; Marček et al., 2023). HVED treatment involves direct contact of water with high-voltage electricity which alters the physicochemical properties of water. In wheat seeds, HVED treatment increased water conductivity assuming the reactions of water dissociation and ionization (Marček et al., 2021).

In this study, the possibility of involvement of this technology in the stimulation of seeds with low germination abilities was tested. A promotive effect of HVED treatment on germination was visible in mallow and carrot, whereas in mallow, the effectiveness of the treatment was even more pronounced (Figs. 1a, 1b). Namely, HVED-exposed mallow seeds had 156% (2<sup>nd</sup> day) and 90% (5<sup>th</sup> day) higher germination percentage than untreated whilst HVED treated carrot seeds increased germination percentage by 19.4% (3<sup>rd</sup> day), 3.5% (4<sup>th</sup> day), 9.5% (6<sup>th</sup> day), and 8.2% (9<sup>th</sup> day), respectively. The study related to the impact of hydro-electro hybrid priming (HEHP) on carrot seed germination reveals beneficial effect towards classical hydropriming method (Zhao et al., 2022). The major findings of this study showed that HEHP treatment had ability to regulate the activity of differentially abundant proteins (DAPs) which control seed storage reserve and respiration metabolism. On the other hand, in melilot, HVED treatment did not stimulate the germination which could be connected with differences in seed anatomy (Fig. 1c). Physical dormancy in *Malvaceae* and *Fabaceae* seeds is determined by the presence of the specialized structures named malpighian cells situated in palisade layer(s). This mechanical tissue forms strong boundaries with hydrophobic compounds (lignin, suberin-cutin matrix, waxes) of seed, resulting in impermeability of coat and hardseededness (Ansari et al., 2016; Zhang et al., 2020). Leguminous seeds, like melilot, contain lens (strophile), which are the only available entrance for the water intake to the embryo (Karaki et al., 2012). Contrarily, seeds of *Malvaceae* family, such as mallow, possess chalazal cap or pore, which is often described as a deep-seated point of weakness of the coat (Serrato-Valenti et al., 1992; Tran and Cavanagh, 1984). From this aspect, it can be assumed that mechanical scarification caused by HVED treatment in mallow disturbed the stability of chalazal pores and triggered structural breaks between palisade cell layer and upper lignin and waxy structures, making it more permeable to water. Carrot seeds have oily structure, mallow endosperm may contain both oil and starch, while a melilot is a legume seed type, but its endosperm is unevenly distributed (Bevilacqua et al., 1990; Simpson, 2010). This observation led us to the conclusion that perhaps the seeds with a higher oil content in endosperm are more suitable material for HVED application. However, further analyses in that direction are needed.

The treatment of water with a high voltage current creates plasma-activated water (PAW), enriched with a mixture of short-living reactive species (OH, <sup>1</sup>O<sub>2</sub>) and long-lived species (O<sub>3</sub>, NO, H<sub>2</sub>O<sub>2</sub>) (Yan et al., 2022). PAW may also contain nutrient-important anions such NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> originating from the decomposition of short-living species ONOO<sup>-</sup> and ONOOH, respectively (Anderson et al., 2016). These ions could be responsible for the embryo dormancy-breaking events. The connection between reactive species and

induction of germination has been observed in numerous studies (Barba-Espin et al., 2010, Finkelstein et al., 2008, Puligundla et al., 2017).

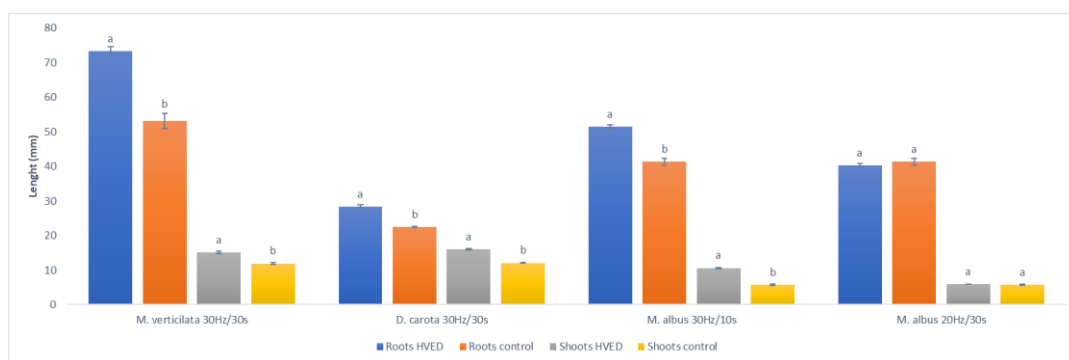
The promotional effect of HVED treatment on the germination has already been reported for oilseed rape (Li et al., 2015), *Nasturtium* sp. (Molina et al., 2018), wheat (Los et al., 2019, Meng et al., 2017), tomato (Zhou et al., 2011), carthamus (Dhayal et al., 2006), soybean (Li et al., 2014), poppy (Šerá et al., 2013), and *Brassicaceae* family (Ono and Hayashi, 2015). Plasma treatments reduce the contact angle which is the indicator of surface's wettability and also improve the surface tension making the seed surface more acceptable for water or some other biostimulant intake (Kriz et al., 2021). The reduction of contact angle as the consequence of different plasma-discharge treatments was noticed in *Erythrina velutina* (Alves et al., 2016), *Mimosa caesalpiniafoila* (da Silva et al., 2017), *Vigna radiate* (Sadhu et al., 2017), and *Leucaena* seeds (Guimarães et al., 2015).



**Figure 1.** Germination percentage (G) under control and high voltage electrical discharge (HVED) conditions in mallow (*M. verticiliata* L.) (a), carrot (*D. carota* L.) (b), and melilot (*M. albus* Medik.) (c) during time. Numbers behind "G"(G2-G9) denote days. Values are means of three repetitions  $\pm$  S.D. Different letters represent significant differences among the mean values between days at  $p < 0.05$  using LSD post hoc test.

### Effect of HVED treatment on growth

Generally, HVED treatment improved the growth in all small seed species (Fig. 2). After 10 days, HVED-treated mallow seeds developed longer shoots (by 27%) and roots (38%) than control, while in carrot, HVED-treated plants shoot and root length increased for 33.1% and 26%, compared to untreated group. Additionally, in mallow HVED treatment significantly stimulated the shoot appearance during the time, while for two other species this observation was not applicable (Table 1). According to Serrato-Vanenti et al. (1992) endotesta of *Malvaceae* family contains phenolic substances that play an important role in seed coat dormancy. Similarly, carrot tissue is also enriched with polyphenols, carotenes, and xanthophylls (Leja et al., 2013). Cells that have higher amount of protective metabolites and pigments possess a greater antioxidative activity and better balance between ROS/RNS creation and detoxification (Zhou et al., 2020). Previous study dealing with HVED impact on polyphenol and hormone profile in wheat showed a higher content of ferulic acid in 2-days-old roots, which was connected with root stress response to reactive particles in plasma activated water (Marček et al., 2023).



**Figure 2.** Root and shoot growth under control and high voltage electrical discharge (HVED) conditions in mallow (*M. verticiliata* L.), carrot (*D. carota* L.), and melilot (*M. albus* Medik.) after 10<sup>th</sup> day. Values are means of ten repetitions  $\pm$  S.D. The different letters represent significances among the means between treatments separately for root and shoot at  $p < 0.05$  using the LSD post hoc test.

**Table 1.** The shoot appearance (SA) in small seed species under control and high voltage electrical discharge (HVED) treatment during time. Numbers behind "SA"(SA4-SA9) denote days. Values are means of three repetitions  $\pm$  S.D. The different letters represent significances among the means between treatments within one time point at  $p < 0.05$  using the LSD post hoc test.

Mallow ( <i>M. verticiliata</i> L.)			
Treatment	SA5	SA6	SA9
30Hz/30s	13.3 $\pm$ 0.3 <sup>a</sup>	14.7 $\pm$ 0.3 <sup>a</sup>	17.3 $\pm$ 0.8 <sup>a</sup>
Control	6.7 $\pm$ 0.3 <sup>b</sup>	7.3 $\pm$ 0.3 <sup>b</sup>	8 $\pm$ 0.1 <sup>b</sup>
Carrot ( <i>D. carota</i> L.)			
Treatment	SA4	SA6	SA9
30Hz/30s	41 $\pm$ 1.2 <sup>a</sup>	42 $\pm$ 1.1 <sup>a</sup>	44.3 $\pm$ 1.2 <sup>a</sup>
Control	39.3 $\pm$ 1.6 <sup>a</sup>	41.3 $\pm$ 1.7 <sup>a</sup>	42.3 $\pm$ 1.4 <sup>a</sup>
Melilot ( <i>M. albus</i> Medik.)			
Treatment	SA5		
30Hz/30s	51.3 $\pm$ 0.3 <sup>a</sup>		
20Hz/30s	51.7 $\pm$ 0.3 <sup>a</sup>		
Control	51.3 $\pm$ 0.3 <sup>a</sup>		

In this case, it is possible that HVED triggers the stress signal which speeds up the growth of mallow and carrot, but the damaging effect is diminished due to natural presence of phenolic compounds. The elongation of stem and roots after exposition to various types of cold technologies has been reported. For instance, an oxygen radio-frequency plasma (RF) treatment increased the shoots in radish (Kitazaki et al., 2012). In maize, low-temperature plasma application increased the fresh and dry biomass (Henselová et al., 2012), and the growth rate of cold plasma (CP) treated alfalfa was higher under drought condition (Feng et al., 2018). All these studies confirmed the important role of plasma treatments in the regulation of water balance signalling routes.

In melilot under 30Hz/10s treatment conditions, shoot length increased for 24.7%, whilst roots were 82.8% longer than in control group (Fig. 2).

Moreover, a higher frequency and shorter treatment time (30Hz/10s) had a beneficial impact on the shoot and root growth compared to control and lower frequency treatment with longer exposure time (20Hz/30s) indicating that prolonged time exposure (more than 10s) of seeds to high voltage dosage could be harmful for the embryo. Similar findings were reported for the spinach, where a high voltage nanosecond pulsed plasma reduced seed germination and growth in plants treated with more than 10 shots (Ji et al., 2016).

## Conclusions

The results provided in this study indicate that HVED treatment can notably improve the germination abilities of mallow and carrot, but not melilot seeds. Other growth parameters, such as root and shoot length, were improved in all tested small seed species. For melilot, longer HVED exposure time (30s) was not as effective as shorter treatment (10s). Our results show that the success of HVED treatment depends on the treatment optimization, seed topography, species type and developmental stage of the plant.

**Author Contributions:** L.L.: Methodology, Investigation, Writing - original draft. S. Z. Z.: Methodology, Investigation. H. P.: Resources, Writing - review & editing. R. C.: Resources, Supervision, Writing - review & editing. T. M.: Conceptualisation, Data Curation, Formal analysis, Methodology, Investigation, Supervision, Writing - original draft, review & editing

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**Conflicts of Interest:** "The authors declare no conflict of interest."

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