Šerá B (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 2, Article number 13164
DOI:10.15835/nbha51213164
Note

Methodological contribution on seed germination and seedling initial growth tests in wild plants

Božena ŠERÁ*

Comenius University Bratislava, Faculty of Natural Sciences, Department of Environmental Ecology and Landscape Management, Mlynská dolina, Ilkovičova 6, Bratislava, Slovakia; bozena.sera@uniba.sk

Abstract

The text is intended for biologists and ecologists working with wild plant species. The article draws attention to possible methodological inaccuracies in determining seed germination and in various attempts to manipulate the seeds of wild plant species. The difference between seed germination in the laboratory and in the wild (in the field), basic principles of seed collection or selection, selection of test sample, basic parameters related to seed germination (seed germination, germination rate, mean germination time, germination index), and initial growth seedlings (seedling vitality index, R/S ratio) are described. The text aims to methodically clarify experiments related to seed germination and initial seedling growth, so different wild plant species can be better compared with each other.

Keywords: germination rate; germination index; mean germination time; R/S ratio; seed data analysis; seedling vitality index

Introduction

Seed germination is a biological process that depends on internal characteristics of the seed and external conditions such as water, temperature, light. Seed germination can be expressed as the percentage of seeds that germinate under suitable conditions in a certain time. This characteristic is one of the most frequently mentioned characteristics not only for agricultural crops and cultivated trees but also for wild plant species (Deno, 1993; Baskin and Baskin, 2014; Frischie *et al.*, 2020). A wild plant is any non-domesticated species of the botanical kingdom occurring in a natural or seminatural ecosystem. Biological and ecological researches of wild plants often work with the reproductive abilities of populations and use seed germination as one of the basic properties.

Certified determination of germination (e.g. from International Seed Testing Association, Association of Official Seed Analysts, United States Department of Agriculture, many national standards) carried out in specialized seed companies is given by the exact work procedure and the limit values or the given species or genera of cultivated crops, trees and flowers (Verma *et al.*, 2003). Requirements for a high rate of germination is associated with a future harvest, as a lower germination indicates a low the fitness of the seed and the uncertainty of the success of the future growth. Seed quality has an important bearing on the fate of the whole plant, and its development and lifespan. Seeds strongly influence the growth of the mature plants and

Received: 12 Apr 2023. Received in revised form: 25 Apr 2023. Accepted: 15 May 2023. Published online: 20 Jun 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

determines their survival under environmental stress conditions. That is why the fitness of the seeds is important from the observed point of view (Basra, 1995; Bewley *et al.*, 2013; Frischie *et al.*, 2020). Standard germination corresponds to the minimal quality attribute that seeds may have in order to reduce the risk for growers and ensure their possible profit.

The germination test is one of the most frequently and commonly measured characteristics of seeds. This property of the sample is "applicable" to the original seeds or to the plant population of interest. These data cannot always be generalized or even applied to plant species. The reason may be, for example, the characteristics of the seeds associated with their provenance and also the conditions under which the seeds were stored (Frischie *et al.*, 2020). The age of the seeds plays a significant role (Aniszewski *et al.*, 2012).

Seed germination, as a universal property, is a quantity that is difficult to compare in practice. Data on seed germination from different literature sources may vary depending on the situation on the used testing methodology, or on the used terminology that is not sufficiently explained. The reason is both the different technical parameters of the germination tests performed (amount of water, temperature, light regime, substrate etc.), but also the design of the tests performed (sample size, swelling of seeds before the test, use of stimulants, disruption of insemination etc.). Therefore, all these data, even if they seem banal, should be described in detail in the test methodology.

This methodological contribution will not deal with the discussion on the topic of certified methodologies for farmers and foresters, but will focus on tests performed in commonly equipped laboratories of various biological and ecological research facilities. There are no certified tests for wild plant species. We have available partially published methodologies of successful scientific teams. Due to the great diversity of data on the germination of wild species, it is most important that the process of determination of germination is clearly described. The methodology of the work should be based on correctly collected and preserved material (collection of seeds, transport of seeds from the habitat to the place of storage and processing) and must be sufficiently robust (statistical point of view) (Justice and Bass, 1978; Hendry and Grime, 1993; Meloun and Militký, 2006; Baalbaki *et al*., 2009). The following contribution will chronologically walk through the stages from sample collection to evaluation, highlight common testing mistakes, and explain how germination results can be affected. The aim of the contribution is to draw attention to possible errors in determining the germination of seeds (correct design of the experiment), especially in wild plant species.

Seed Collection

Ideally, seeds should be collected in dry weather, from sufficiently large populations and from plants of average height. Selection of seeds from the stand should be carried out, for example, in such a way as to maintain the rule of 10 seeds per plant, i.e. 100 seeds from 10 different plants of the population (Knevel *et al.*, 2005). If the plant has a large number of small seeds, it is not a problem to follow this rule (or possibly increase it). If the plant has only a limited number of seeds, which is less than 10, then all seeds are collected. For small, rare populations, it is correct to use only healthy seeds (at the expense of numbers) rather than dogmatically sticking to sample sizes and including abnormally developed or damaged seeds in the test. The aim is to obtain a representative sample of the seeds of the given population.

For conifers, the number of tested seeds should depend on the size of the cones, and for deciduous trees, on the size of the seeds or fruits. When determining the germination of woody plants, it is important to pay more attention to the question of the optimal method of collecting a seed sample and, when publishing the results, to a detailed description of the collection, woody species and habitat. For example, if in a meadow we collect seeds from plant populations in an area of 10 x 10 m, then in a forest it can be an area of 250 x 250 m.

A direct influence on seed germination is their origin, its geographical origin – provenance (Gresta *et al.*, 2007). Climatic conditions during ripening also have a great influence on seed quality (Borg, 2005). The vitality of the future offspring, i.e. germination, is also determined by the overall habitus and health status of the parent plant (maternal effect, Schuttle *et al.*, 2008). It is important where and how long the seeds were stored (Vieira

et al., 2013). Generally, seeds stored in moist conditions lose their germination faster than seeds stored in dry conditions. It is also true that dry seeds tolerate higher and lower temperatures better than moist seeds (Hendry and Grime, 1993). In addition, the probability of the seeds being attacked by fungi increases in damp conditions. For longer term storage, it is recommended to dry the seeds and store them in the cold, or freeze them (Justice and Bass, 1978).

Seed Storage

An important factor in maintaining the viability of collected seeds is the method of their storage. Increasing seed age can reduce germination as the seed metabolic system begins to break down, resulting in seeds being slow or even unable to germinate, and poor seedling development and lower establishment for aged seeds that do germinate. Thus, effective seed storage relies on slowing down seeds' normal metabolism as much as possible without incurring damage (de Vitis *et al.*, 2020). Improper storage can fundamentally affect the vitality and germination of seeds, thus distorting (deteriorating) the obtained information on seed germination (Abdul-Baki and Anderson, 1972). In the case of commercial seeds, their moisture is monitored, which together with the storage temperature and relative air humidity determines the shelf life. In general, seeds become old and lose their vitality when they are stored.

We can distinguish orthodox and recalcitrant seeds. Orthodox seeds go through a drying stage (water content 5-20%, depending on the species), in contrast, recalcitrant seeds contain a high proportion of water (30-40%) at maturity. Orthodox seeds are long lived seeds and can be successfully dried to moisture contents as low as 5% without injury and are able to tolerate freezing. Orthodox seeds may be dried before storage (40 °C, 48 hours), on the other hand, recalcitrant seeds should be used for experiments without storage. Orthodox seeds should be dried and stored in sealed moisture proof containers that prohibit absorption of moisture from the atmosphere. On the other hand, recalcitrant seeds need a wet or humid environment (to avoid desiccation), temperatures between 5-17 °C, and good air movement during their short-term storage (de Vitis *et al.*, 2020).

If you know nothing about the species you are testing, then the following conditions can be recommended for seed storage: room temperature (but below 20 °C), very low relative humidity (below 14%), and storage in closed containers. The seeds and fruits of wild plants of the temperate zone should only be stored for a short time, therefore, the germination and initial growth tests should be carried out as soon as possible. Data obtained shortly after seed collection will be comparable (same age of seeds and fruits) (Ellis and Roberts, 1981; Basra, 1995; Fenner and Thompson, 2005).

It is well known that seed-borne plant pathogens can cause disease or death of plants, and thus they can distort the results of the germination tests. Seed disinfection (via antibacterial or fungicidal agent) is a common practice before testing of native seeds, and different products and methodologies are used. In many cases, the effects of these procedures in seed quality or performance (e.g., in their germination) are unknown or ignored. The seed surface should be decontaminated from bacteria and fungi. The disinfection treatment of the seed should remove viable germs from the surface of the seed and at the same time should not reduce the quality of the seed and their germination. From this point of view, a disinfectant on a smaller sample of seeds can be recommended first.

Numerous treatments have been proposed to minimize infection from seeds in agronomy and in forestry. The simplest means is disinfection with hot water, the most effective are chemical means (Martin-Garcia *et al*., 2019; Allen *et al*., 2004). However, few fungicides penetrate the seed coat and reduce internal seed contamination without a significant negative effect on seed germination (Agusti-Brisach *et al*., 2012). One of the available procedures is to use chlorine-based chemical disinfectant, as 0.6% sodium hypochlorite (dilute household bleach) (Gilbert *et al.*, 2023), for 10 minutes and then rinsed with distilled water. before germination test.

Seed Germination vs. Seedling Emergency

Laboratory germination tests are conducted with the aim of obtaining information on the absolute number of germinating seeds in the sample. Tests are established in given environment with adjustable factors to meet, if possible, optimal conditions for seed germination (Hendry and Grime, 1993). The advantage of laboratory tests is their repeatability and thus the reproducibility of data and comparability of results (Copeland and McDonald, 1995).

Seedling emergency, field germination, is a percentage expression of the amount of seeds that emerged under natural conditions from the total number of germinated seeds sown. The establishment of a plant from seeds is the result of the relationship between seed quality (vitality) and environmental conditions, which in practice may differ from the desired optimum (lack of soil moisture, soil crust etc.) (El-Keblawy *et al.*, 1997; Soares and Rodrigues, 2008). The physical, chemical, and biological properties of the soil and the microclimate are important. Important soil properties include moisture, temperature and structure (Kozlowski, 1999; Christofoletti *et al.*, 2013).

Determining or estimating field germination based on knowledge of seed germination determined in the laboratory is not a simple matter (Khan *et al.* 2010). This prediction is highly variable and the differences between these parameters are therefore unpredictable and can be large. Therefore, various laboratory tests are being introduced to determine the seed potential to emerge in the field (field germination). The seeds are subjected to various stress factors during incubation, or deteriorated seeds (aged: naturally or artificially) are used because they have lower vitality. Laboratory tests can then copy results from real habitats and substrates. Field germination is a parameter that essentially corresponds to the emergence of seedlings of botanical species tested in their natural environment (Belgacem *et al.*, 2006). For ecologists, field germination is a more important parameter than seed germination under controlled laboratory conditions.

Germination Test in Laboratory

For proper testing in the laboratory, we must first obtain a basic sample of seeds, which by its characteristics represents the seeds of our interest (without seeds of other species, fruiting remains, stalks, seeds that are insufficiently developed, damaged or attacked by fungi). It does not matter whether it is a seed lot or a sample of seeds characterizing a certain population of plants in the greenhouse, in the field, or in the wild. This basic laboratory sample should be sufficiently large (number of seeds, see below) and homogeneous in terms of size, color, hardness and shape of seeds (but only if testing for seed heterogeneity is not the goal). It is also worth remembering that the characteristic is a property of the sample. The properties of the base sample are the parameters. From a statistical point of view, a characteristic is an estimate of a parameter.

To establish a germination test, we need to randomly select at least 3 seed samples (preferably 5) from the basic seed sample. The randomness of the selection consists in fulfilling the condition that all seeds have the same probability (possibility) of being in the sample included. The recommended quantity for one sample set is 100 seeds (Knevel *et al.*, 2005). Scientific works usually follow this recommendation. A common problem when working with native seeds is that only a reduced number of seeds is available for evaluation. Minimum number of seeds per replication it is usually determined by the possibilities of a specific population in nature (e.g., a rare species with a limited number of seeds).

The size of the sample (number of seeds) depends on the variability of the basic set. Variability is an indicator of the degree of heterogeneity (diversity) of a set. However, when planning the design of the germination test, especially for wild plants, we must take this variability into account (Meloun and Militký, 2006). How to estimate sample file size? It is possible to make a preliminary measurement of germination, calculate selected statistical characteristics (standard deviation, standard error of the mean) and determine the required number of seeds for the sample set (Milton, 1992). In general, the more seeds in the sample set, the more accurate the results. An excessively large sample of seeds will make the whole experiment longer and more

expensive. There should be at least three repetitions in the test, i.e., establish at least 3 x sample set of seeds (Milton, 1992; Meloun and Militký, 2006).

To determine germination in normal laboratory, some criteria need to be met (conditions we can usually use: temperature 20-25 °C, air humidity min. 80%, light/dark phase) (Hendry and Grime, 1993). The most botanical species germinate in the dark, so a light phase is not necessary. In any case, the light phase can be ensured by cold white fluorescent lamps, which provide physiologically suitable photosynthetically active radiation (e.g., photosynthetic photon flux density of 40-80 $\mu mol.m^{-2}s^{-1}$). The applicable light mode can be 8 h light with high temperature (e.g., 22 °C) + 16 h dark with low temperature (e. g. 15 °C), or 12 h light +12 h dark cycling with same temperature (20 °C) (Hampton and Tekrony, 1995; Baalbaki *et al*., 2009).

It is sufficient to test the seeds on sterilized Petri dishes with layers of filter paper. Because of the variability, it is statistically better to have fewer seeds planted in more Petri dishes. For example: 5 dishes of 30 seeds are better than 3 dishes of 50 seeds. It pays to think the design of the attempt well in advance. It is good to know which day (hour) the seeds will start germinating, how much time will be needed to read the data, how many people will be needed, etc. An overestimated experiment that is not handled personally can have fundamentally distorted results, because (for example) the data readings for the control samples take place in the morning and the readings of the tested samples seeds at different times in the late afternoon, etc. The most common evaluation is to count germinated seeds (radicle > 2 mm), and germinated seeds are removed from the dish. There are many working details that can significantly (as well as abiotic factors) affect the quality of the collected data.

Another possible influence on the result is connected with the placement of the seeds on the Petri dishes. The seeds should not touch and ideally should have a certain “seat order”. During seed germination, we can observe how individual seeds behave. Knowing the position of each seed will allow for “harder data”. Then, for example, it is possible to find out how the growth of the roots of individual seeds took place during the entire cultivation. Some types of plants show allelopathic effects, which can be manifested in influencing the germination of nearby seeds. Again, fewer seeds per dish with more dishes is better. This rule also applies from the point of view of possible seed infection, when we have to discard entire infected dishes from the experiment.

Statistics is said to be a precise tool that works with imprecise data (Meloun and Militký 2006). Therefore, the design of the experiment should be as free of interference as possible and therefore as "pure" as possible, so that the obtained data are as accurate as possible and can really answer the tested hypotheses. The above recommendations were selected based on seed germination studies of: Šerá *et al.*, 2009; 2021a; 2021b; Šerý *et al.*, 2020.

Short Guide to the Germination Test

The seed surface was decontaminated from bacteria and fungi (0.1% sodium chloride for 10 minutes) and then rinsed with distilled water. The seeds were placed on three layers of filter paper in disposable plastic Petri dishes. Moisten the filter paper with 6 ml of distilled water and keep it moist until the end of the experiment. The appropriate dish size, for small poppy seeds to larger oat seeds, is 9 cm (diameter). Petri dishes are placed into an incubator with cool white fluorescent lamps that produced a photosynthetic photon flux of 60 $\mu mol.m^{-2}s^{-1}$. Experiments should be carried out with a regime 16-hour light and 8-hour dark photoperiod at a room temperature of 22 °C. Seed germination is recorded daily and is considered complete when the root (or hypocotyl) protrudes about 2 mm in length.

Basic Parameters of Seed Germination

During seed germination, we can observe how individual seeds behave. In the following paragraphs, the list of measurable quality parameters that indicate the germination of seeds and initial growth of seedling will be deal and are useable in wild plant ecology. The primary feature is seed germination. On the one hand, the

term seed germination is used in a broader sense (sensu lato), when it generally refers to the germination properties of the tested seed samples. In a broader sense, seed germination is used by the less informed community. It is good to note that germination can refer not only to seeds, but also to spores or pollen grains.

The following list of parameters is related to the number of germinating seeds and the very early development of seedlings. These very frequently used parameters are easy to use and can serve well outside the seed community of farmers and foresters (Table 1).

Table 1. Overview of basic parameters

Parameters	Unit	Symbol	Formula
Seed germination	%	SG	100 x (number of germinated seeds / total number of seeds)
Germination rate*	%	GR	100 x ($n_1 + n_2 + ... + n_i$) x (total number of seeds)$^{-1}$
Mean germination time**	day	MGT	$\sum(n \times d) / N$
Germination index	1	GI	(number of germinated seeds on 1st count) / (day of the 1st count) + ... + (number of germinated seeds on final count) / (day of the final count)
Seedling vitality index I	mm	SVI I	SG x seedling length
Seedling vitality index II	mg	SVI II	SG x fresh seedling weight
Seedling vitality index III	mg	SVI III	SG x dry seedling weight
Root:shoot ratio I	1	R/S I	root length / shoot length
Root:shoot ratio II	1	R/S II	fresh root weight / fresh shoot weight
Root:shoot ratio III	1	R/S III	dry root weight / dry shoot weight

* n_i - number of seeds germinate on i-day, ** n - number of seeds germinated on each day, d - number of days from the beginning of the test, N - total number of seeds germinated at the termination of the experiment

In the narrower sense of the word (sensu stricto), seed germination (SG) is an estimate of the viability of a population of seeds. Seed germination is based on the number of germinated seeds, which germinate within (usually) 5-21 days. The equation to calculate seed germination is in Equation 1:

$$SG\ (\%) = 100 \text{ x (number of germinated seeds/total number of seeds)} \quad (1)$$

Many works in this field use seed germination (synonym: germination percentage) to present results, and if different treatments are applied, the germination percentage of each treatment is given. Sometimes seed germination is presented as the number of germinated seeds, other times it is given as the percentage of germinated seeds in a test seed sample (e.g., treated with a stimulator) compared to a control sample (no treatment, 100%). In this case, more than 100% germination can be obtained. For this reason, it is good to state the method of calculating seed germination in the methodology, as it is easy to get it wrong. Among the parameters with which seed germination is confused is also germination rate or maximum germination. This is understandable, since they relate to the same principle, but express different time periods of the germination test. A good parameter supporting the expression of seed vitality is the germination rate (GR). It is the germination of seeds expressed for individual days (Equation 2):

$$GR\ (\%) = 100 \times (n1 + n2 + ... + ni) \times (\text{total number of seeds})^{-1} \quad (2)$$

where ni is number of seeds germinate on i-day.

In the graphical representation of the germination rate during seed cultivation, a dynamic graph can be obtained showing the progress of seed germination over time. The values of seed germination and germination rate for the last day of seed cultivation are identical.

Germination in targetbred species tends to be balanced, that is, the seeds germinate in approximately one wave of time. If this time wave is loose, it is useful to know something about germination energy (GE) and mean germination time (MGT) (Ellis and Roberts, 1981). GE expresses the temporal uniformity of germination and indicates the intensity and evenness of germination (Hampton, 1993). It can be expressed as the proportion of germinated seeds at the time of the first day of counting (the day according to the specialized methodology) in relation to the total achieved germination at the end of the test. Outside of agricultural research, this parameter is unnecessary.

MGT is a measure of the time it takes for the seed to germinate, focusing on the day on which most seeds have germinated. MGT is an accurate measure of the time taken for a lot to germinate, but does not correlate this well with the time spread or uniformity of germination (Kader, 2005). MGT is calculated by using the equation in Equation 3:

$$MGT\ (day) = \sum(n \times d) / N \quad (3)$$

where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment.

Other useful parameters expressing the vitality of the seeds include the germination index (GI). This dimensionless parameter (index) can be successfully applied for botanical plant species as well (Hafez *et al.*, 2021). The GI appears to be the most comprehensive measurement parameter combining both seed germination and time of germination (speed) (Kader, 2005). Therefore, the faster the batch of seeds germinated, the higher the germination index. Here is the equation for the calculation (Equation 4):

GI = (number of germinated seeds on 1st count) / (day of the 1st count) + ... + (number of germinated seeds on final count) / (day of the final count). (4)

Seedling vitality index (SVI) is a relatively good parameter that is based on seed vitality (seed germination) and the quality of young seedlings (Uddin *et al.*, 2021). A higher vitality index will indicate a more vital set of seeds within the same species with different types of treatments or from different populations. In common practice, three modifications of SVI can be used, depending on which parameter you use from the seed. At the end of seed cultivation, seedling lengths or live biomass or dried biomass weights can be measured. SVI I. is calculated by multiplying seed germination (%) and seedling length (mm). For calculation of SVI II. and SVI III. fresh and dry weights (mg) of seedlings are used. The equations to calculate parameters of SVI I.-III. are in Equations 5-7:

$$SVI\ I.\ (mm) = SG \times \text{seedling length} \quad (5)$$
$$SVI\ II.\ (mg) = SG \times \text{fresh seedling weight} \quad (6)$$
$$SVI\ III.\ (mg) = SG \times \text{dry seedling weight} \quad (7)$$

The last of the presented parameters is the root:shoot ratio (R/S), which is not related to SG, but it is derived only from seedling growth. In general, the ratio between the underground (root, R) and aboveground (shoot, S) part of the plant must be in a balanced harmony in terms of stability, water intake and solar energy. The parameter R/S ratio is specific for different life forms and is an important parameter at the species level. In general, the value of the R/S parameter increases from annuals, through herbaceous perennials to shrub species.

This ratio has wide application (as does SVI) within a plant species when comparing different growth conditions or different seed treatments. Similar to SVI, it can be calculated with the lengths of R and S (R/S I.), with their fresh weight (R/S II.) or with the dry weight (R/S III.). The R/S ratio is dimensionless parameter.

Here, a basic overview of seed germination and initial growth parameters that could be useful in the study of germination and vitality of wild plants has been selected. More parameters can be found in the

professional literature (Orchard, 1977; Ellis and Roberts, 1981; Powell *et al.*, 1984; Hampton and Coolbear, 1990; Hampton, 1993; Kim *et al.*, 1994; Hampton and Tekrony, 1995; Fenner and Thompson, 2005; Ranal *et al.*, 2006; Baalbaki *et al.*, 2009).

Conclusions

The germination test and the initial growth of the seeds are important information about the fitness of the plant. For cultivated species, there are precise standards for determining many parameters that describe germination and initial growth. Plant ecologists very often play catch up with the germination of seeds of monitored plant populations. This information from different literature sources may differ depending on the testing methodology used or different terminology that may not be sufficiently explained. The reason may be different properties of specific seeds related to their origin, collection, age and storage. This article discusses the methodological principles involved in wild plant seed testing. It defines seed germination and many other parameters that are useable in the field of biology and ecology of wild plants.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was partly supported by the grant projects VEGA No. 1/0217/23 and 1/0007/21 from the Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdul-Baki AA, Anderson JD (1972). Physiological and biochemical deterioration of seeds. In: Kozlowski TT (Ed). Seed Biology. New York, Academic Press, pp 283-315.

Agusti-Brisach C, Perez-Sierra A, Armengol J, Garcia-Jimenez J, Berbegal M (2012). Efficacy of hot water treatment to reduce the incidence of *Fusarium circinatum* on *Pinus radiata* seeds. Forestry 85:629-635. *https://doi.org/10.1093/forestry/cps074*

Allen T, Enebak S, Carey W (2004). Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. Crop Protection 23:979-982. *https://doi.org/10.1016/j.cropro.2004.02.010*

Aniszewski T, Haikonen J, Helwig B, Konert G, Oleksinska Z, Stenman A, Ylinampa T (2012). Vigor, vitality and seed dormancy of *Avena sativa* cultivars in a long-term experiment. Journal of Applied Botany and Food Quality 85:150-158.

Baalbaki R, Elias S, Marcos-Filho J, McDonald MB (2009). Seed vigour testing handbook. Association of Official Seed Analysts, Ithaca, NY, USA.

Baskin CC, Baskin JM (2014). Seeds: ecology, biogeography and evolution of dormancy and germination. 2nd ed., Academic Press, New York, NY, USA.

Basra AS (1995). Seed Quality: Basic Mechanisms and Agricultural Implications. Haworth Press, N.Y.

Belgacem AO, Neffati M, Papanastasis VP, Chaieb M (2006). Effects of seed age and seeding depth on growth of *Stipa lagascae* R. & Sch seedlings. Journal of Arid Environment 65:682-687. *https://doi.org/10.1016/j.jaridenv.2005.10.001*

Bewley JD, Bradford K, Hilhorst H, Nonogaki H (2013). Physiology of development, germination and dormancy. 3rd Ed., Springer.

Borg SJ (2005). Dormancy and germination of six *Rhinanthus* species in relation to climate. Folia Geobotanica 40:243-260. *https://doi.org/10.1007/BF02803238*

Christofoletti CA, Escher JP, Correia JE, Marinho JFU, Fontanetti CS (2013). Sugarcane vinasse: Environmental implications of its use. Waste Management 33:2752-2761. *https://doi.org/10.1016/j.wasman.2013.09.005*

Copeland LO, McDonald MB (1995). Principles of Seed Science and Technology. Seed Enhancements. Chapman and Hall, New York.

Deno NC (1993). Seed germination theory and practice. 2nd ed., Norman C. Deno, State College, USA.

de Vitis M, Hay FR, Dickie JB, Trivedi C, Choi J, Fiegener R (2020). Seed storage: Maintaining seed viability and vigor for restoration use. Restoration Ecology 28:S249-S255. *https://doi.org/10.1111/rec.13174*

El-Keblawy A, Shaltout KH, Lovett-Doust J, Ramadan A (1997). Population dynamics of an Egyptian desert shrub, *Thyme-laea hirsute*. Canadian Journal of Botany 75:2027-2037.

Ellis RH, Roberts EH (1981). The quantification of ageing and survival in orthodox seeds. Seed Science and Technology 9:373-409.

Fenner M, Thompson K (2005). The ecology of seeds. Cambridge University Press, Cambridge.

Frischie S, Miller AL, Pedrini S, Kildisheva OA (2020). Ensuring seed quality in ecological restoration: native seed cleaning and testing. Restauration Ecology 28:239-248. *https://doi.org/10.1111/rec.13217*

Gilbert GS, Diaz A, Bregoff HA (2023). Seed disinfestation practices to control seed-borne fungi and bacteria in home production of sprouts. Foods 12:747. *https://doi.org/10.3390/foods12040747*

Gresta F, Avola G, Abbate V (2007). Germination ecology of *Scorpiurus subvillosus* L. seeds: the role of temperature and storage time. Plant Ecology 190:123-130. *https://doi.org/10.1007/s11258-006-9195-3*

Hafez M, Popov AI, Rashad M (2021): Integrated use of bio-organic fertilizers for enhancing soil fertility–439 plant nutrition, germination status and initial growth of corn (*Zea mays* L.). Environmental Technology and Innovation 21:101329. *https://doi.org/10.1016/j.eti.2020.101329*

Hampton J (1993). The ISTA perspective of seed vigor testing. Journal of Seed Technology 17:105-109.

Hampton JG, Coolbear P (1990). Potential versus actual seed performance - can vigour testing provide an answer? Seed Science Technology 18:215-228.

Hampton JH, Tekrony DM (1995). Handbook of Vigor Test Methods. 3rd ed. International Seed Testing Association, Zurich.

Hendry GAF, Grime JP (1993). Methods in Comparative Plant Ecology. A laboratory manual. Chapman and Hall, Sheffield.

Justice OL, Bass LN (1978). Principles and Practices of Seed Storage. U.S. Government Printing Office, Washington, D.C.

Kader MA (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceedings of the Royal Society NSW 138:65-75.

Khan AZ, Shah P, Mohd F, Khan H, Amanullah PS, Nigar S, Khalil SK, Zubair M (2010). Vigor tests used to rank seed lot quality and predict field emergence in beat. Pakistan Journal of Botany 42:3147-3155.

Kim SH, Choe ZR, Kang JH, Copeland LO, Elias SG (1994). Multiple seed vigour indices to predict field emergence and performance of barley. Seed Science and Technology 22:29-38.

Knevel IC, Bekker RM, Kunzmann D, Stadler M, Thompson K (2005). The LEDA Traitbase. Collecting and Measuring Standards of Life-history Trans of the Northwest European Flora. Scholma Druk B.V., Bedum, Netherlands.

Kozlowski TT (1999). Soil compaction and growth of woody plants. Scandinavian Journal of Forest Research 14:596-619. *https://doi.org/10.1080/02827589908540825*

Martin-Garcia J, Zas R, Solla A, Woodward S, Hantula J, Vainio EJ, ... Diez JJ (2019). Environmentally friendly methods for controlling pine pitch canker. Plant Pathology 68:843-860. *https://doi.org/10.1111/ppa.13009*

Meloun M, Militký J (2006). Kompendium statistického zpracování dat. Metody a řešené úkoly. Academia, Praha.

Milton JS (1992). Statistical Methods in the Biological and Health Science. 2nd ed., Mc Graw Hill, New York.

Orchard T (1977). Estimating the parameters of plant seedling emergence. Seed Science and Technology 5:61-69.

Powell AA, Haigh R, Phillips G, Tonkin HGB, Wheaton OE (1984). Assessment of repeatability of the controlled deterioration vigour test both within and between laboratories. Seed Science and Technology 12:421-427.

Ranal MA, de Santana DG (2006). How and why to measure the germination process? Brazilian Journal of Botany 29:1-11. *https://doi.org/10.1590/S0100-84042006000100002*

Schutte BJ, Regnier EE, Harrison SK (2008). The association between seed size and seed longevity among maternal families in *Ambrosia trifida* L. populations. Seed Science Research 18(4):201-211. *https://doi.org/10.1017/S0960258508082974*

Šerá B, Kraus K, Hnilička F, Medvecká V, Zahoranová A, Šerý M (2021a). Effect of atmospheric non-thermal plasma treatment by DCSBD apparatus on sugar beet seeds. Romanian Reports in Physics 73:602.

Šerá B, Šerý M, Straňák V, Špatenka P, Tichý M (2009). Does cold plasma affect breaking dormancy and seed germination? A studyon seeds of Lamb's Quarters (*Chenopodium album* agg.). Plasma Science and Technology 11:750-754.

Šerá B, Šerý M, Zahoranová A, Tomeková J (2021b). Germination improvement of three pine species (*Pinus*) after diffuse coplanar surface barrier discharge plasma treatment. Plasma Chemistry and Plasma Processing 41:211-226. *https://doi.org/10.1007/s11090-020-10128-5*

Šerý M, Zaharonová A, Kerdík A, Šerá B (2020). Seed germination of black pine (*Pinus nigra* Arnold) after diffuse coplanar surface barrier discharge plasma treatment. IEEE Transactions on Plasma Science 48:939-945. *https://doi.org/10.1109/TPS.2020.2981600*

Soares PG, Rodrigues RR (2008). Direct seeding of forest leguminous trees: rhizobia inoculation effect on plantlet emergence and early growth in field conditions. Scientific Forestry 36:115-121. *https://doi.org/10.21750/REFOR.4.07.46*

Uddin S, Ullah S, Nafees M (2021). Effect of seed priming on growth and performance of *Vigna radiata* L. under induced drought stress. Journal of Agriculture and Food Research 4:100140. *https://doi.org/10.1016/j.jafr.2021.100140*

Vieira BGTL, Barbosa GF, Barbosa RM, Vieira RD (2013). Structural changes in soybean seed coat due to harvest time and storage. Journal of Food, Agriculture and Environment 11:625-628.

Verma SS, Verma U, Tomer RPS (2003). Studies on seed quality parameters in deteriorating seeds in *Brassica* (*Brassica campestris*). Seed Science and Technology 31:389-396. *https://doi.org/10.15258/sst.2003.31.2.15*

The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.

License - Articles published in ***Notulae Botanicae Horti Agrobotanici Cluj-Napoca*** are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.

➢ Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.