Review

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Seed Quality of Perennial Grasses and Forage Legumes in Relation to Dormancy, Storage, Presence of Pathogens and Possible Improvements

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Seed Quality of Perennial Grasses and Forage Legumes in Relation to Dormancy, Storage, Presence of Pathogens and Possible Improvements

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Abstract: The manuscript presents the review of the impact of dormancy, seed storage, and pathogens on seed quality of perennial grasses and forage legumes. The outcomes of earlier studies to enhance the germination of seeds from these species are listed. Numerous studies around the world show that seed dormancy, environmental conditions, storage time after seed harvest, and the presence of pathogens on seeds, affecting the reduction of seed quality of these two groups of plants. This article discusses the possibility to partially or fully controlling impact of different factors on seed quality. Tests and experience of the presence of pathogens (especially *Fusarium* spp.) indicates a small percentage of infected seed of perennial grasses and legumes, which would not justify the cost of fungicides during seed processing. By applying the optimal concentration of acid in the seed, in combination with the time of exposure, it is possible to improve seed germination for more than 25% in forage grasses and legumes. Similar effects on the increase seed germination of both groups of plants can be achieving by optimal temperature treatments. Mechanical damage to the seed coat on the seeds of these crops can potentially increase germination, but new approaches are also being investigated.

Keywords: fodder plants seed; dormancy; seed germination increase; pathogens; storage

1. Introduction

Since the beginning of plant cultivation, seeds have been the cornerstone of agriculture and human nourishment. The seed industry had a significant role in the tremendous advancements in agriculture and, every year, there is a greater requirement for high-quality seeds [1]. Excellent quality, starting with genetic purity, germination energy, and total germination [2], as well as that the seed is in good health and is not contaminated by pests or illnesses that harm plants [3], are the main requirements for effective seed production. Despite, there is a possibility that there will not be a high percentage of germination of seeds sown in the field due to unfavorable conditions for germination [4]. The causes of this occurrence might be brought on by outside factors [5], but in some species seed dormancy is one of the main reasons for non-germinating seeds after sowing [6]. Also, immature seeds may have less viability and germination is greatly influenced by the stage of seed development throughout the storage period [7].

In general, seed dormancy is a biological trait that allows species to germinate when environmental conditions are favorable [8]. Thanks to this trait many species survive in nature [9]. Although seed dormancy is one of the most studied traits, not all aspects of the complex mechanism of seed dormancy are known [10]. The seed engages in a number of physiological activities even if it is dormant prior to planting. This process is regulated by various aspects, and also, it seems to be a lot of variety in plants within the same species, both in terms of dormancy and germination as well as possible improvements in germination [11].

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Six broad categories of seed dormancy, and dormancy types are discussed in relation to seed species [12]. Dormancy is the result of a characteristic of seeds, called organic dormancy which can be classified as either endogenous or exogenous in general. Although the majority of seed biologists are unaware of the many types and combinations of dormancy and storage behavior in seeds of this family, seeds of legumes are typically thought to exhibit physical dormancy and to be orthodox [13]. Physical seed dormancy (PY) is determined by the seed coat, which is made of polyphenols, tannins and cutin present in the testa of seeds of different water permeability levels were determined. It has been hypothesized that the plasma membrane of the sensitive embryo contains similar or shared receptors for substances that break dormancy [14]. Physical dormancy, which prevents immediate seed germination and is therefore thought to be an adaptive characteristic in species of Mediterranean and tropical habitats where the rainy season is the best time for germination. Physical seed dormancy is determined by the seed coat, which is made of polyphenols, tannins and cutin present in the testa of seeds of different water permeability levels were determined [15]. But recent research, utilizing the dataset for *Fabaceae* (the largest family containing PY) obtained from the Global Biodiversity Information Facility (GBIF), has shown evidence to refute this assertion dormancy [16].

The typical components of a legume seed are an embryo, sometimes nutritive tissue (endosperm), and a protective seed coat (testa). Inner and exterior integuments combine to form the seed coat of legumes. While the exterior integument is in charge of creating the usual structure of a legume seed coat, the inner integument disappears throughout development. There is an area that divides the basal and terminal caps of cells (often known as the bright line) which creates a line that crosses the macrosclereid layer. The seed coat's mechanical strength is influenced by both the macrosclereid and osteosclereid layers. The layer of parenchyma cells functions as a tissue that supplies nutrients [17,18]. The outermost layer is made up of a single layer of densely packed macrosclereid epidermis (or palisade cell layer) which is typical for hard-seeded legumes [19]. Osteosclereids, or dead cells with strong walls and hourglass shapes that create significant intercellular air voids, make up the next cell layer. The final layer is parenchyma, which is made up of many layers of flattened cells.

The physiological basis for physical seed dormancy was discovered, along with an allele that causes variation in this feature [20]. The findings of this study offer compelling evidence for pectin acetylesterase 8's function in seed dormancy and suggest transgenic techniques for future research. Due to its effect on wild habitats, physical seed dormancy is a crucial characteristic in domesticating legumes. This resulted of the decreased production and quality, and it is typically regarded as an unfavorable feature in crops. Even when favorable conditions prevent the seed from germinating, the seed may become dormant as the seed coat thickness grows. The seed is made dormant by its thickness, which also shields it from harmful outside influences. The creation of secondary cell walls from the primary cell walls over time results in a thickened coat and makes the seed coat thicker [21].

Contrary to the dormant seeds of the wild progenitor, the domestication process of legumes has altered their dormancy, resulting in non-dormant seeds with a testa that is easily permeable for water, finally leading in fast and uniform germination [22]. However, in many forage species, there are no appreciable differences in seed dormancy between chosen varieties and wild populations of the same species [11,23]. When the testa has been treated and there is enough moisture to imbibe, physical seed dormancy is irreversible [24].

Variation in regenerative responses to environmental variables may be responsible for the variance in seed coat structure identified among the species tested [25]. In addition, it would be much better to conduct additional studies using molecular data, which can contribute to the active field of study to find genotypic differences and enable genetic control of seed dormancy.

Although a large portion of grasslands are natural meadows where specific plants are not required to be planted, grasslands are valuable mostly because of the space they occupy [26]. They are important for producing animal feed, but they are also crucial for horticulture, ecology, and the environment. Depending on the temperature and geography, grasslands make up a sizeable amount of the agricultural area in the following European countries: Iceland (73%), England (63%), Poland (21%), France (10.78%), Romania and Serbia (33%), and Greece (13.4%); [27].

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The *Poaceae* grass family and the genus *Trifolium* and *Medicago* (as well as, to a lesser extent *Lotus* and *Onobrychis*), are the major seed producing groups for perennial fodder plants in Europe (Table 1; Table 2).

Table 1. Genus, species and common name of the most important perennial grasses.

Genus	Species	Common name
Festuca	*F. pratensis* Huds.	meadow fescue
	F. arundinacea Schreb	tall fescue
	F. rubra L.	red fescue
Lolium	*L. perenne* L.	perennial ryegrass
	L. multiflorum L. *syn. L. italicum* A. Br.	Italian ryegrass
Poa	*P. pratensis*	meadow grass
	P. trivialis	ordinary meadow grass
Dactylis	*D. glomerata*	cocksfoot
Arrhenatherum	*A. elatius (L) Beauv.*	French ryegrass
Phleum	*Ph. Pretense L.*	Timothy

Table 2. Genus, species and common name of the most important perennial legumes.

Genus	Species	Common name
Medicago	*M. sativa* L -	blue alfalfa
Trifolium	*T. pratense*	red clover
Lotus	*L. corniculatus* L.	trefoil
Onobrychis	*O. sativa* L *syn. O. viciifolia Scop*.	sunfoin

The world's seed production of fodder crops is largely influenced by European regions, however seed quality and production vary due to the various levels of agricultural development in the various states. In Europe, perennial forage legumes provide a lot more feed than perennial forage grasses [28,29]. Therefore, seed quality is a factor in the production of fodder, seed yield, and the stability of seed production [30,31,32]. The demand for a specific kind of seed is determined by the needs of a specific agricultural culture as well as external variables, such as climate and soil type. In accordance with the capacities available (climatic conditions, experienced professionals, tradition, etc.) and keeping in mind that there are many opportunities for improving seed production, the volume of seed production, processing, and marketing on domestic and international markets could be significantly improved [33].

This article offers an overview of the most significant perennial forage plants, information on the variables influencing seed qualities, and recommendations for enhancing seed quality.

2. Changes in Seed Quality of Perennial Forage Species as a Result of Storage Conditions and Time

A seed is a biological system in which many activities may take place; how they do so primarily relies on the circumstances and time of storage [34]. In order to respond appropriately and promptly to minimize negative impacts, we should consider as many elements as possible that affect germination and understand about their methods of action. Apart from seed production, examining the effect of the length of seed storage on seed germination and seedling quality is also important for seed preservation in gene banks [35,36]. The length of a seed's dormancy period is influenced by the genetic and physiological potential of the species [37], as well as by the storage conditions and other interrelated environmental factors [38]. However, extended storage under conditions that encourage afterripening might cause seeds to undergo a second dormant stage or even lose viability [39]. Both

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post-harvest seed desiccation treatments and post-harvest drying of forage seeds may have an impact on germination [40,41].

Although it is well known that cold, dry environments help biological material last longer, each species has a different ideal range for temperature and moisture [42]. Through adaptation to the environment, seed dormancy as a barrier to germination has evolved differently among species, allowing germination to occur when conditions are likely to be favorable for generating a new plant generation [43]. Many species that are physically dormant, or have an impermeable seed coat, have orthodox seeds that may be kept in liquid nitrogen without losing viability. Preserving seed germination during aging greatly depends on the plant species, as well as storage conditions, especially humidity and air temperature in the warehouse[44]. It's less apparent, though, whether the dormancy should be broken before or after storage [13].

After a period of maturation, seed dormancy is released and achieved maximum germination (as agriculture goal), which is influenced by numerous factors [45,46]. Seed storage leads to physiological and biochemical processes that act on the seed aging and the reduction of seed germination [37]. Antioxidant activity varied between storage treatments and was not related to seed viability [38]. Depending on the temperature and moisture content of the storage, changes in the seed may also take place during storage; in the worst scenario, this could result in seed deterioration [47].

Numerous variables like a specialization of the species, the location of the seeds on the bloom, the weather during seed maturity, etc., affect the ratio of dormant and germinated seeds [48–50]. The seeds of perennial forage legumes have a significant proportion of hard (dormant-soaked) seeds immediately after harvest [51–53]. This is due to a robust seal that is waterproof and gas-tight [17]. Given that the market for grass and leguminous seed is established, seeds can germinate in the right circumstances, and boosting their potential can be utilized in different geographic latitudes [54,55].

In one of the most important forage legume - alfalfa the share of hard seeds after harvest is 23.3% [56]. After two years of storage, part of hard seeds decreased to 2.9%, and after 4 and 8 years of storage, hard seed is not registered. For the same period a dead seed is increased in proportion to the storage time, from 7.8 to 57.2%. Total seed germination after two years of storage was 85.8%, after four year was 73.7%, and after the eighth year germination was 42.8%. Cultivars have behaved variably according to the contents of the hard and germinated seed in relation to the length of storage [56]. Effect of long-term natural aging on germinability and biochemical characteristics in two different clover (*Trifolium repens* and *Trifolium pratense*) showed that only two types of the old seeds showed a discernible increase in peroxidase activity during the germination of the legume seeds, but catalase activity dropped [57]. Seed dormancy was found in 14 of the endemic species and 19 of the studied *Onobrychis* species, and germination rates ranged from 5.5% to 98.0% [58].

For perennial forage grasses, the presence of dormant (sleeping) seeds right after harvesting is also expressed [59], and significantly reduces germination in the time right after harvesting [55,60–62].

For example, in the classic storage conditions and maturing in the warehouse in Serbia, optimal use of Timothy seeds (maximum germination) is after three months, after nine months of Italian ryegrass seed, and after eleven months of cocksfoot seed. Commercial utilization period was slightly longer with Italian ryegrass 810 days (at germination 81%), cocksfoot up to 630 days and the same germination, and the Timothy up to 270 days and 73% germination [62].

The impact of type of packaging seed storage can have different effects on germination. Tall fescue seeds stored in plastic containers manifested by an average of 2.7% higher germination, than in paper and cloth packaging [34]. Seeds of meadow fescue were also similarly behaved [11]. Seed storage conditions on seedling emergence, seedling growth and dry matter production have been investigated of temperate forage grasses [63]. It is concluded that seedlings emerged at a slower rate and in less numbers from the refrigerator, than they did from the seed storage, while the linen bag was the least efficient way to store seeds in the refrigerator, too. The standards now in place for best seed storage do not take into account the physiological health of the seed, the chemical makeup of various seed species, or the physical status of water within the seed [64].

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3. Presence of Pathogens on Seeds and its Impact on the Seed Quality and Seedlings in the Initial Growth

Seeds can contain a wide range of fungi, which can cause significant problems in crop cultivation [65], as well as human and animal health when dealing with forage crops. The main harmful effects of fungi on seeds are related to transmission and spread of plant diseases, reduction of seed quality and seed longevity [66]. One of the major problems is that many seed-borne fungi can drastically reduce the germination of stored seed [67]. It is possible that the seed is a carrier of pathogens that can be transferred to growing plants when environmental conditions are suitable. Significant transmission and spread of seed pathogens has been associated with the growth of the seed industry and the global seed market worldwide [68].

The most common pathogens of forage grasses and legumes in the soil during germination are *Phythium* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. These pathogens are parasites of seedlings, causing their extinction, crop spacing and uneven growth of plants. From these seed pathogens *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp. are transmitted by seed [69]. These three types of pathogens were detected in a small percentage of the seeds of forage grasses and legumes. Additionally, very rarely, *Alternaria* species have been found on the seeds of some forage species. Based on morphological characteristics of *Alternaria* spp., it is assumed that the species *A. alternata* is widespread in nature but does not cause significant damage to forage crops [69,70]. At forage legumes and grasses is determined the presence of saprophytic fungi *Mucor* spp., but it does not adversely affect germination, since the infected seeds develop normal seedlings [71].

The pathogen which is much more prevalent in seeds of forage legumes than on seeds of forage grasses is *Fusarium* spp. [69,72]. Seeds infected with the fungus *Fusarium* spp. not germinate but, due to the small percentage of diseased seed, we do not think they can cause more economic damage to crops based on fodder grasses and legumes [73]. In regions with temperate and tropical climates, it is a cosmopolitan species. In addition to parasites and woody plants, citrus fruits, sugar cane, and carnations are also parasitized [74]. The seeds may indicate the occurrence of *F. oxysporum* f. sp. *medicaginis*, which causes root rot and wilting alfalfa plants. From morphological characteristics of *Fusarium* spp. originating from alfalfa, can be distinguished as follows: mycelium grows rapidly at 25°C, light white color with purple hues; conidia are slightly curved to almost straight with 3-5 transverse bulkheads; the length of the conidia order of 20 to 50 μm and a width of 2.5 to 4 μm. The pathogen is held in the soil and causes wilting of plants [72,73].

The following distinctions can be made between *Fusarium* species that are alfalfa-related: The conidia are slightly curved till almost finished with 3-5 transverse bulkheads; the lengths range from 20-50 μm and the breadth from 2.5-4 μm. Mycelium grows rapidly at 25°C, with whitish tints and purple undertones. The pathogen damages plant tissue and is kept in the soil. The pathogen *Fusarium poae*, which likewise grows quickly at 25°C and has a mycelium that is whitish pink and conidia that are 13–56 μm long with 2–5 transverse bulkheads, can also be detected on the seeds of fodder. When grain is used as animal feed, mycotoxins (secondary metabolites of fungi *Aspergillus, Fusarium, Alternaria and Penicillium*) could cause serious diseases in animals [75]. Seed treatment of forage legumes and grasses involves coating with fungicides, insecticides, bacterial preparations especially on alfalfa seed. Coating seeds with lime materials, combined with bacterial preparations, particularly on the seeds of fodder legumes is applied [76,77]. These findings and practical experience show that *Fusarium* spp. only affects a tiny fraction of the seed of fodder legumes and grasses, and the benefits of seed disinfection do not outweigh the costs associated with doing so.

On the seeds of fodder grasses also can be found the presence of the pathogen *Fusarium*. In this species, which is also developing rapidly at 25°C, mycelium is white, pink, conidia is sized from 13 to 56 μ, and with 2-5 transverse bulkheads in length. This is cosmopolitan species in areas of moderate and tropical climate, and in addition to plants from the family *Poaceae*, parasite woody plants, citrus, sugar cane, and carnation, too [73]. These results and practical experience indicate that *Fusarium* spp. occurs in a small percentage on the seeds of forage legumes and grasses, and the effects obtained by disinfecting seeds do not justify the investment created by applying fungicides for seed disinfection. Seeds infected with *Fusarium* spp. seeds do not swell, but due to the tiny number of diseased seeds,

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they cannot significantly harm leguminous and fodder crops economically. The seed of fodder grass is less likely to have this pathogen than the seed of fodder leguminous plants [69,72]. *Fusarium* species on the seed may signal the emergence of *F. oxysporum* var. *medicaginis*, which weakens alfalfa plants and causes root rot [72,73].

4. State of the Art on Seed Dormancy in Perennial Grasses and Forage Legumes, and Opportunities to Improve Germination

It is not necessary to overstate the value of fodder plants for agricultural productivity, because forage production and the problems caused by forage plant seed dormancy cut across national and geographic boundaries. In order to achieve a high yield of quality fodder, it is necessary to successfully establish fodder crops, for which a high seed quality is necessary, which is mostly reflected in seed germination [11]. But, the percentage of dormant seeds reduces seed germination.

Seed germination is a process that in the most general sense represents the extension of plant species, strongly influenced by temperature, soil moisture, air humidity, light, etc. [9], and mostly under the influence of seed dormancy [78]. Seed germination can be improved by applying different seed treatments [79,80], but treatments can also negatively affect germination [81]. New approaches to enhancing seed germination are being researched, too. The use of ultrasonic treatment could enhance seedling growth since it is quick, easy, inexpensive, and has favorable impacts on the germination of old seeds and seedling growth [82].

Seed dormancy can have a significant impact on seed production, as well as productivity and costs of forage production [83]. Legume and grass seeds differ from one another in terms of morphology, anatomy, chemical content, structure, and other factors. Reduced germination immediately following harvest or during the post-harvest storage phase, which is mostly conditioned with dormant seeds, is a characteristic of both kinds of seeds.

The properties of water sorption reflect the chemical composition of the biological components as well as the temperature at which hydration takes place [84]. Changes in sorption characteristics as a function of temperature and water content may be an indication that hydration has changed the structural makeup of cellular components [85]. A unifying theory of the structural and molecular reactions to drying within cells can be used to describe distinct types of seed physiology [86]. The biological features of each species should be investigated, because some seed pretreatments may cause subsequent seed damage [87]. Within and between populations, as well as for each species, there was significant variation in the most effective scarification technique [88], because there are many seed scarification methods and their use in forage legumes [89]. Mechanical scarification appears to be very effective, simple to use in the field, and within the farmer's capabilities, but it breaks seeds, therefore it can be replaced by liquid nitrogen treatment, which does not break seeds and quickly removes the seed coat [90]. One of the cutting-edge methods uses white light, which had no effect on non-dormant seeds but decreased the germination of dormant seeds. Endosperm is likely the main cause of the newly collected seeds' lower germination. [91]. The point at which seeds no longer tolerate desiccation is crucial for the entire seed growth process [92,93].

Immediately after harvest, germination of cocksfoot and tall fescue seeds increased by 24%, but only 13% in perennial ryegrass. Three months after harvest it was possible to increase germination by 20% (cocksfoot), 18% (tall fescue) and 6% (perennial ryegrass). Eight months after harvest it was still possible to increase seed germination of cocksfoot and tall fescue by 4-5% whereas, in ryegrass dormancy was completely lost after 8 months storage. [100].

In agricultural species which breeding has been done intensively, seed dormancy is reduced. Forage grass seed dormancy is the result of an immature embryo or cell structures surrounding the embryo [24]. However, in forage grasses the percentage of dormant seeds of varieties and populations is still high and it make problems in seed production [99]. Also, with perennial grasses (fescues, ryegrass, meadow grass, orchard grass, timothy) immediately after harvesting is more exposed dormant seed [55] which causes a significant reduction in germination in the period immediately after harvest [11,33,41,45,46,50,55,60–62,99,100]. Dormancy and germination depend on numerous factors such as the specific type, location seed inflorescences, weather conditions during seed maturation and others [11,32–34,45,49,50,101].

For seed of fodder grasses (cocksfoot) and fodder-ornamental grasses (tall fescue, perennial ryegrass), acid (50% H_2SO_4 for 30 min.), or temperature treatments (80°C for a period of 90 min) immediately after harvesting can increase germination (cocksfoot seed to 21%, tall fescue to 24%, and perennial ryegrass by 13%). After three months, the effect of breaking dormancy and germination rate decreased. On the cocksfoot seeds increase of germination was up to 13%, the seeds of tall fescue up to 18%, and in English fescue up to 6%. After eight months, increasing seed germination in cocksfoot seed was possible for 5%, while the seeds of perennial ryegrass none treatment did not increase seed germination [100]. On the seeds of plants of the same genus is the high variability of dormancy, seed germination and possible improvement. For example, using the optimal chemical (H_2SO_4) and temperature treatment on seeds of red fescue is possible to improve the germination of 19%, 13% of sheep fescue, and up to 23% of meadow fescue [99]. It is also possible by selecting the temperature, in relation to the moisture content of seeds, affect the increase seed germination of cocksfoot [41], Table 3.

Table 3. The application of treatments to increase seed germination in most important forage species.

Forage species	Applied optimal treatment	References
M. sativa	Two years of storage in the classic conditions.	[56]
	Selection of varieties.	[54]
	Temperature of cold stratification treatment at (-80°C) for 2 hour plus sandpaper scarification.	[94]
Red clover	Heat stress and high humidity	[84]
	Exposure to a temperature of from -80°C; Immersion in hot water (90°C) for 5 minutes.	[95]
	Causing stress (cooling, heating, hot water, potassium nitrate, mechanical damage of the seed coat)	[96]
L. corniculatus	Cytokinines;	[97]
	Desiccant application before harvest.	[40]
O. sativa	Mechanical scarification with sandpaper.	[58]
	Chemical scarification (H_2SO_4, concentrations 90%, for 2 minutes).	[98]
F. pratensis	Chemical scarification (H_2SO_4, concentrations 75%, for 30 minutes) or temperature treatments (90°C, and 60 or 90 minutes).	[99]
F. arundinacea	Sixteen months storage in polyethylene packaging.	[34]
	Ultrasonication	[82]
F. rubra	Chemical scarification (H_2SO_4, concentrations 75%, for 20 minutes).	[99]
F. ovina	Chemical scarification (H_2SO_4, concentrations 75%, for 10 or 20 minutes) or temperatures treatments (80°C, and 60 minutes).	[99]
L. perenne	Seven months of storage.	[60]
L. italicum	Seven months of storage.	[60]
	Nine months of storage in the classic conditions.	[62]
D. glomerata	Temperatures treatments (40°C, and 90 minutes or 50°C, and 60 minutes).	[83,100]
A. elatius	Seven to ten months of storage in the classic conditions.	[45,61]

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Ph. pretense	One to three months of storage in the classic conditions.	[61,62]

The seeds of perennial forage legumes (alfalfa, red clover, sainfoin, birdsfoot trefoil) are characterized by the presence of a high percentage of hard (dormant) seed after harvesting [51–55,58,95]. The reason for this is a hard seed coat that is impermeable to water and gases [17]. The dormant seeds after planting can germinate later, when conditions become favorable (sufficient water, air, etc.). However, only emerged seedlings can't withstand competition with developed seedlings, which have sprung up immediately after sowing seeds [102].

It is best to avoid applying acidic treatments to seeds right away after harvesting (i.e., 50% H_2SO_4 for 30 minutes) alternatively, thermal treatments (80° C for 90 minutes). By doing this, the English ryegrass climbed to 13%, the high screw to 24%, and the germination of the scarring to 21% [60]. The benefit of breaking dormancy and boosting germination has worn off three months after harvest. As a result, it was feasible to boost germination to a maximum of 13% on seedlings, up to 18% on high heel seeds, and up to 6% on English ryegrass seeds.

While on the seed of the perennial ryegrass no treatment affected the increase in seed germination, it was feasible to boost seed germination by 5% eight months after harvest [100]. For instance, red fescue seeds can have their germination improved by 19%, sheep fescue by 13%, and meadow fescue by up to 23% with the application of the best chemical (H_2SO_4) and temperature treatment [11].

In agronomic practice, high germination allows immediate benefits, such as reduced seed density per unit area and cost-effective establishment of perennial forage crops. High germination has an indirect impact that is visible in the close and advantageous association between vigor and germination [34,50,83]. For the establishment of grass-legume combinations, the maximum amount of fodder grass seed is utilized, and the quality of the seeds is crucial for a good establishment [103].

After the creation of conditions that allow the passage of water and gases, dormant seeds usually germinate and provide normal hints, but they have little to no practical value for the establishment of fodder leguminous crops [103]. To increase the germination of alfalfa, [94], are recommended exposure seeds a temperature from -5°C for a period of 5 hours, or exposure to a temperature of -80°C for a period of 2 minutes, and/or damaging seed coat with grit. These treatments can significantly reduce the percentage of hard seeds and raise the level of germination energy and total germination. Heat stress in combination with high humidity also affects the increase in germination of alfalfa seed [104].

Cytokinins have a considerable impact on the birdsfoot trefoil seed's ability to increase germination in both young and old seeds [97]. The combination of high humidity and temperature stress causes a yellow star's germination rate to significantly rise [55]. By choosing the temperature in relation to the seed's moisture content during harvest, it is also feasible to affect the cocksfoot seeds' ability to germinate more readily [83].

The percentage of hard seeds of red clover is significantly reduced upon exposure to a temperature of from -80°C and results in an increase in germination [95]. A similar effect can be achieved after the immersion in hot water (90°C) for 5 minutes. The red clover can increase seed germination, causing stress (previous cooling, heating, hot water, using potassium nitrate and/or mechanical damage to the seed coats) [96]. Stress caused by the application of acid and damage the seed coats to become permeable to water and gases on the seeds of sainfoin also affects positively and significantly increases germination, especially on those seeds which are not in a pod [98].

Seed germination and seedling vigor of birdsfoot trefoil after desiccation before harvest were significantly improved, using classical and accelerated methods for seed testing [99].

5. Summary and concluding remarks

Every year, at the time of sowing, the concern of whether there will be enough fodder legumes and grass seeds to meet demand emerges. This work would be made considerably simpler if a quick and affordable method to disrupt seed dormancy could be developed. The fact that various species

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have unique biological characteristics determined by genetic factors is another issue. Research on novel methods of breaking seed dormancy, including various approaches such as selection, physiological, chemical, and physical, is important to ensure enough quantity of seeds for growing fodder crops. Even though they are used on huge amounts of seeds for sowing, not all of the mechanisms of seed dormancy have been properly studied. If it were feasible to break dormancy of the harvested seeds without the need for storage period, the yield of forage crops would be considerably boosted as the security of fodder production would be increased.

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References

1. Copeland, L.O.; McDonald M.B. Seed production. In: Principles of seed science and technology. 4th ed. Kluwer Academic Publishers, Norwell, MA, USA, 231-252
2. Gibson-Roy, P.; Delpratt, J.; Moore, G. Restoring Western (Basalt) Plains grassland. 2. Field emergence, establishment and recruitment following direct seedlings. *Ecol. Manag. Restor*. **2007**, 8, 123-132.
3. Pellish, C.a.; Sherrard, M.E.; Leytem, P.A.; Jackson, L.L. Small vertebrate granivores reduce seedling emergence in native tallgrass prairie restoration. *Restor. Ecol*. **2018**, 26, 323-330.
4. Pedrini, S.; Lewandrowski, W.; Stevens, J.C.; Dixon, K.W. Optimizing seed processing techniques to improve germination and sowability of native grasses for ecological restoration. *Plant Biol*. **2019**, 21, 415-524.
5. Assaeed, A.M. Effect of temperature and water potential on germination of *Salsola villosa* Del. ex Roem. et Shult. *Assuit J. Agric. Sci.* **2001**, 32, 173-183.
6. Gao, R.; Zhao, R.; Huang, Z.; Yang, X.; Wei, X.; He, Z.; Walck, J.L. Soil temperature and moisture regulate seed dormancy cycling of a dune annual in a temperate desert. *Environm. Exp. Bot*. **2018**, 155, 688-694.
7. Gresta, F.; Avola, G.; Anastasi, U.; Miano, V. Effect of maturation stage, storage time and temperature on seed germination of *Medicago* species. *Seed Science and Technology* **2007**, 35(3), 698-708.
8. Klupczyńska, E.A.; Pawłowski, T.A. Regulation of seed dormancy and germination mechanisms in a changing environment. *International Journal of Molecular Sciences* **2021**, 22(3), 1357.
9. Soltani, E.; Baskin, C.C.; Gonzalez-Andujar, J.L. An Overview of Environmental Cues That Affect Germination of Nondormant Seeds. *Seeds* **2022**, 1(2), 146-151.
10. Baskin, J.M.; Baskin, C.C. A classification system for seed dormancy. *Seed Sci. Res*. **2004**, 14, 1–16.
11. Stanisavljević, R.; Đokić, D.; Milenković, J.; Terzić, D.; Djukanović, L. Development, status and possible improvement of yield and seed quality of forage grasses in Serbia. In proceedings of the International Conference on BioScience: Biotechnology and Biodiversity-Step in the Future The Fourth Joint UNS-PSU Conference, Novi Sad, Serbia, 18-20 June, 2012.
12. Baskin, J.M.; Baskin, C.C. The great diversity in kinds of seed dormancy: A revision of the Nikolaeva-Baskin classification system for primary seed dormancy. *Seed Sci. Res*. **2021**, 31, 249–277.
13. Jayasuriya, K.M.G.G.; Wijetunga, A.S.T.B.; Baskin, J.M.; Baskin, C.C. Seed dormancy and storage behavior in tropical Fabaceae: A study of 100 species from Sri Lanka. *Seed Sci. Res*. **2013**, 23, 257–269.
14. Baskin, C.C. and Baskin, J.M. Germination Ecology of Seeds with Physical Dormancy. In: Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. 2nd ed, Academic Press, San Diego, **2014**, 145-185.
15. Galussi, A.; Argüello, J.; Moya, M.; Zuriaga, F.; Zimmermann, L. Seed dormancy mechanism as a factor influencing seed physiological quality in alfalfa (*Medicago sativa*) cv. Baralfa 85. *Seed Science and Technology*. **2013**, 41(1), 50-59.
16. Jaganathan, G.K. Do Fabaceae species with physical dormancy occur mostly in the temperate ecosystems? A rebuttal to using global biodiversity information facility (GBIF) analysis. *Plant Sci. Today* **2020**, 7, 109–111.

17. Smýkal, P.; Vernoud, V.; Blair, M.W.; Soukup, A.; Thompson, R.D. The role of the testa during development and in establishment of dormancy of the legume seed. *Frontiers in plant science.* **2014**, 5, 351.
18. Janská, A.; Pecková, E.; Sczepaniak, B.; Smýkal, P.; Soukup, A. The role of the testa during the establishment of physical dormancy in the pea seed. *Ann Bot*. **2019**, 123(5), 815-829.
19. Carruggio, F.; Onofri, A.; Impelluso, C.; Giusso del Galdo, G.; Scopece, G.; Cristaudo, A. Seed dormancy breaking and germination in *Bituminaria basaltica* and *B. bituminosa* (*Fabaceae*). *Plants*. **2020**, 9(9), 1110.
20. Soltani, A.; Walter, K.A.; Wiersma, A.T.; Santiago, J.P.; Quiqley, M.; Chitwood, D, Porch, T.G.; Miklas, P.; McClean, P.E., Osorno J.M.; Lowry D.B. The genetics and physiology of seed dormancy, a crucial trait in common bean domestication. *BMC Plant Biol*. **2021**, 21, 1-17. 10.1186/s12870-021-02837-6.
21. Islavath, N.S.; Deshpande, K.V. Seed coat dormancy: An overview in legumes. *The Pharma Innovation Journal*. **2021**, 10(11), 620-624.
22. Sedláková, V.; Hanáček, P.; Grulichová, M. Zablatzká L, Smýkal P. Evaluation of seed dormancy, one of the key domestication traits in chickpea. *Agronomy*. **2021**, 11(11), 2292.
23. Adkins, S.W.; Bellairs, S.M.; Loch, D.S. Seed dormancy mechanisms in warm season grass species. *Euphytica*. **2002**, 126, 13-20.
24. Van Klinken, R.D.; Lukitsch, B.; Cook, C. Interaction between seed dormancy-release mechanism, environment and seed bank strategy for a widely distributed perennial legume, *Parkinsonia aculeata* (*Caesalpinaceae*). *Ann Bot.* **2008**, 102(2), 255-264.
25. Yousif, M.A.I., Wang, Y.R.; Dali, C. Seed dormancy overcoming and seed coat structure change in *Leucaena leucocephala* and *Acacia nilotica*. F*orest Science and Technology*. **2020**, 16(1), 18-25.
26. Stošić, M.; Lazarević, D. Country Pasture/ Forage Resource Profiles: Serbia and Montenegro. 2010. www.fao.org/ag/agp/agpc/doc/counprof/.../serbia.pdf.
27. Peeters, A. Importance, evolution, environmental impact and future challenges of grasslands and grassland-based systems in Europe. *Grassland science* **2009**, 55(3), 113-125.
28. Tomić, Z.; Lugić, Z.; Radović, J.; Sokolović, D.; Nešić, Z.; Krnjaja, V. Perennial legumes and grasses stable source of quality livestock fodder feed. *Biotechnology in Animal Husbandry* **2007**, 23(5-6-1), 559-572.
29. Moisuc, A.; Djukić, D. Cultura pajiştilor şi a plantelor furajere. In: (Ed.): Lonel, A. Cultura pajistilor si a plantelor furajere: indrumator de lucrari practice. University of Banat, Faculty of Agronomical Sciences and Veterinary Medicine Timisoara, Romania. 2002, 100-130.
30. Đokić, D.; Terzić, D.; Milenković, J.; Dinić, B.; Anđelković, B.; Stanisavljević, R.; Barać, S. Importance and condition of forage crops seed production in agriculture of the Republic of Serbia. *Selekcija i semenarstvo* **2013**, 19, 11-25.
31. Poštić, D.; Momirović, N.; Stanisavljević, R.; Štrbanović, R.; Gavrilović, V.; Aleksić, G.; Đukanović, L. Seed quality of perennial ryegrass, Italian ryegrass and red fescue. *Zaštita bilja* **2014**, 65(2), 70-76.
32. Stanisavljević, R.; Simić, A.; Sokolović, D. Seed production of perennial forage grasses in Serbia. *Biotechnology in Animal Husbandry* **2010**, 26, 159-172.
33. Stanisavljević, R.; Beković, D.; Djukić, D.; Stevović, V.; Terzić, D.; Milenković, J. Djokić, D. Influence of plant density on yield components, yield and quality of seed and forage yields of alfalfa varieties. *Romanian Agricultural Research*. **2012**, 29, 245-254.
34. Stanisavljević, R.; Đokić, D.; Milenković, J.; Terzić, D.; Đukanović, L.; Vuga-Janjatov, V. Effect of storage time and type of packaging on seed quality of tall fescue. *Journal on processing and energy in agriculture* **2012**, 16(1), 33-35.
35. Ellis, R.H.; Nasehzadeh, M.; Hanson, J.; Ndiwa, N.; Woldemariam, Y. Medium-term seed storage of diverse genera of forage grasses, evidence-based genebank monitoring intervals, and regeneration standards. *Genet Resour Crop Evol*. **2019**, 66, 723–734. https://doi.org/10.1007/s10722-019-00748-y.
36. Martin, I.; De la Cuadra, C. Evaluation of different scarification methods to remove hardseededness in *Trifolium subterraneum* and *Medicago polymorpha* accessions of the Spanish base genebank. Seed Sci. Technol. 2004, 32, 671–681.
37. Bewley J.D. Seed Germination and Dormancy. *The Plant Cell* **1997**, 9, 1055-1066.
38. Merritt, D.J.; Senaratna, T.; Touchell, D.H.; ,Dixon, K.W.; Sivasithamparam, K. Seed ageing of four Western Australian species in relation to storage environment and seed antioxidant activity. *Seed Sci. Res*. **2007**, 13/2, 155-165. https://doi:10.1079/SSR2003133.
39. Baskin, C.C.; Baskin, J.M. Breaking Seed Dormancy during Dry Storage: A Useful Tool or Major Problem for Successful Restoration via Direct Seeding? *Plants* ***2020***, 5, 636. https://doi.org/10.3390/plants9050636.

40. Stanisavljević, R.; Đokić, D.; Milenković, J.; Terzić, D.; Koprivica, R.; Stevović, V.; Tomić, D. Seed germination and seedling vigor of birdsfoot trefoil after desiccation before harvest: Using classical and accelerated methods for seed testing. In: Proceedings of the 3rdInternational conferences sustainable postharvest and food technologies-INOTEP 2013 and 25thand National conference processing and energy in agriculture–PTEP. Vrnjačka Banja, Serbia. 21-26 April, 2013.
41. Stanisavljević, R.; Đokić, D.; Milenković, J.; Terzić, D.; Stevović, V.; Tomić, D.; Dodig, D. Drying of forage grass seed harvested at different maturity and its utility value in autumn and spring sowing time. *Zemdirbyste-Agriculture* **2014**, 101(2), 169-176.
42. Vertucci, C.W.; Leopold, A.C. Water binding in legume seeds. *Plant Physiol* **1987**, 85, 224–231.
43. Nonogaki H. Seed dormancy and germination—emerging mechanisms and new hypotheses. *Front Plant Sci*. 2014, 5, 233.
44. Walters, C. Orthodoxy, recalcitrance and in-between: Describing variation in seed storage characteristics using threshold responses to water loss. Planta 2015, 242, 397–406.
45. Stanisavljević, R.; Djokić, D.; Milenković, J.; Terzić, D.; Djukanović, L.; Stevović, V.; Dodig, D. Desiccation, postharvest maturity and seed aging of tall oat-grass. *Pesquisa Agropecuária Brasileira*. **2010**, 45, 1297-1302.
46. Stanisavljević, R.; Đokić, D.; Milenković, J.; Terzić, D.; Đukanović, L.; Vuga-Janjatov, V. The influence of seed storage on germination of tall fescue during after-ripening period. *Journal on Processing and Energy in Agriculture*. **2011**, 15(2), 106-108.
47. Bewley, J.D.; Black, M. Seeds. In: Seeds. Springer, Boston, MA, USA, 1994. https://doi.org/10.1007/978-1-4899-1002-8_1.
48. Boyce, K.; Cole, D.; Chilcote, D. Effect of Temparature and Dormancy on Germination of Tall Fescue 1. Crop Sci. 1976, 16(1), 15-18.
49. Stanisavljević, R.; Sokolović, D.; Đokić, D:; Milenković, J.; Gajić, T.; Terzić, D., Simić, A. Panicle branches position influence on meadow fescue (*Festuca pratensis* Huds.) and tall fescue (*Festuca arundinacea* Schreb.) seed yield and quality. *Selekcija i semenarstvo*. **2007**, 13(1-2), 17-22.
50. Stanisavljević, R.; Dragičević, V.; Milenković, J.; Đukanović, L.; Đokić, D.; Terzić, D.; Dodig, D. Effects of the duration of after-ripening period on seed germinations and seedling size in three fescue species. *Spanish Journal of Agricultural Research* **2010**, 8, 454-459.
51. Hall, J.W.; Stout, D.G.; Brooke, B.M. Alfalfa seed germination tests and stand establishment: The role of hard (water impermeable) seed. *Canadian Journal of Plant Science* **1998**, 78(2), 295-300.
52. Fairey, D.; Lefkovitch, L. Hard-seed content of alfalfa grown in Canada. *Canadian Journal of Plant Science* 1991, 71(2), 437-444.
53. Souza, F.H.; Marcos-Filho, J. The seed coat as a modulator of seed-environment relationships in *Fabaceae*. *Brazilian Journal of Botany* **2001**, 24, 365-375.
54. Hakl, J.; Mášková, K.; Šantrůček, J.; Fer, M. Seed emergence of lucerne varieties under different soil conditions. Czech Journal of Genetics and Plant Breeding. 2012, 48(2), 93-97.
55. Stanisavljević, R.; Milenković, J.; Đokić, D.; Terzić, D.; Petrović, M.; Đukanović, L.; Dodig, D. Drying of meadow fescue seeds of different moisture contents: Changes in dormancy and germination. *Plant Soil and Environment* **2013**, 59(1), 37-43.
56. Čupić, T.; Popović, S.; Grljušić, S.; Tucak, M.; Andrić, L.; Šimic, B. Influence of storage time on the quality of alfalfa seed. *Journal of Central European Agriculture* **2005**, 6(1), 65-68.
57. Turgay, C.; Ökkeş, A.; Güleray, A. The natural aging related biochemical changes in the seeds of two legume varieties stored for 40 years. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science* 2010, 60/4, 353-360, https://doi: 10.1080/09064710903005690
58. Avci, S.; Kaya, M.D. Seed and germination characteristics of wild Onobrychis taxa in Turkey. Turkish Journal of Agriculture and Forestry. 2013, 37(5), 555-560.
59. Simpson, G.M. Seed Dormancy in Grasses. New York: Cambridge University Press. 1990. 1-60.
60. Kon, K.; Follas, G.; James, D. Seed dormancy and germination phenology of grass weeds and implications for their control in cereals. *New Zealand Plant Protection* **2007**, 60, 174-182.
61. Stanisavljević, R.; Sokolović, D.; Milenković, J.; Terzić, D.; Djokić, D.; Simić, A. Quality changes of Timothy (*Phleum pratense* L.) and French reygrass (*Arrhenatherum elatius* L.) during ripening and correlative dependence of their significant characteristics. *Selekcija i semenarstvo*. **2008**, 14, 39-43.

62. Stanisavljevic, R.; Đjokic, D.; Milenkovic, J.; Đukanovic, L.; Stevovic, V.; Simic, A. Dodig, D. Seed germination and seedling vigour of Italian ryegrass, cocksfoot and timothy following harvest and storage. *Ciencia e agrotecnologia*. **2011**, 35, 1141-1148.
63. Marshall, A.H.; Lewis, D. Influence of seed storage conditions on seedling emergence, seedling growth and dry matter production of temperate forage grasses. *Seed Science and Technology* **2004**, 32(2), 493-501.
64. Vertucci, C.W.; Ross, E.E. Theoretical Basis of Protocols for Seed Storage. *Plant Physiol*. **1990**, 94(3), 1019–1023.
65. Khan, A.M.; Khan, M.; Salman, H.M.; Ghazali, H.M.Z.U.; Ali, R.I.; Hussain, M.; Yousaf, M.; Hafeez, Z.; Khawja, M.S.; Alharbi, S.A.; Alfarraj, S.; Arif, M.; Nabeel, M.. Detection of seed-borne fungal pathogens associated with wheat (*Triticum aestivum* L.) seeds collected from farmer fields and grain market. *Journal of King Saud University-Science* **2023**, 35(4), 102590.
66. Neergaard, P. Storage fungi. In *Seed pathology*. Red Globe Press London, UK. 1977; Volume 1-2, 147-269.
67. Magan, N.; Sanchis, V.; Aldred, D. Role of spoilage fungi in seed deterioration. *Fungal biotechnology in agricultural, food and environmental applications* **2004**, 311-323.
68. Martín, I.; Gálvez, L.; Guasch, L.; Palmero, D. Fungal Pathogens and Seed Storage in the Dry State. *Plants*. **2022**, 11(22), 3167.
69. Štrbanović, R.; Gavrilović, V.; Stanisavljević, R.; Poštić, D.; Marković, J.; Trkulja, N.; Dolovac, N. Health testing different genotypes alfalfa seeds. *Zaštita bilja* **2013**, 64(4), 212-217.
70. Blagojević, J.; Oro, V.; Nikolić, I.; Popović, T.; Aleksić, G.; Gavrilović, V.; Ivanović, Z. Morpho-physiological study of *Alternaria* spp. isolates from celery. *Zaštita bilja* **2014**, 65(1), 15-26.
71. Martin, I.; Gálvez, L.; Palmero, D. Fungal Pathogens and Seed Storage in the Dry State. *Plants* 2022, *11*(22), 3167; https://doi.org/10.3390/plants11223167
72. Krnjaja, V.; Lević, J.; Ivanović. M.; Tomić, Z. *Fusarium* species associated with seeds of alfalfa cultivars. *Czech Journal of Genetics and Plant Breeding* **2003**, 39 (Special issue), 275-278.
73. Levic, J. Species of the genus *Fusarium* in the field of agriculture, veterinary and human medicine Institut za kukuruz" Zemun Polje" i Drustvo geneticara Srbije (Ed.). Cicero, Beograd. 2008.
74. Krnjaja, V.; Lević, J.; Ivanović, M.; Tomić, Z. Virulence of *Fusarium* species to alfalfa seedlings. *Zbornik Matice srpske za prirodne nauke* **2005**, 109, 65-72.
75. Agriopoulou, S.; Stamatelopoulou, E.; Varzakas, T. Advances in occurrence, importance, and mycotoxin control strategies: Prevention and detoxification in foods *Foods*. **2020**, 9(2), 137.
76. Hill, M.; Hampton, J.; Hill, K. Seed quality of grasses and legumes. Forage seed production, *Sci. agric. Piracicaba* 5 (Número Especial), **1998**, 110-115.
77. Sulc R. Factors affecting forage stand establishment. *Scientia Agricola* **1998**, 55, 110-115.
78. Kim, H.M.; Kim, J.H.; Lee, D.H.; Jung, Y.H.; Park, C.Y.; Lee, M.H.; Kim, K.M.; Lee, J.H.; Na, C.S. Non-deep simple morphophysiological dormancy and germination characteristics of *Gentiana triflora* var. *japonica* (Kusn.) H. Hara (*Gentianaceae*), a rare perennial herb in Korea. *Plants* **2021**, 10(10), 1979. https://doi.org/10.3390/plants10101979.
79. Corbineau, F.; Taskiran-Özbingöl, N.; El-Maarouf-Bouteau, H. Improvement of Seed Quality by Priming: Concept and Biological Basis. *Seeds* **2023**, 2(1), 101-115. https://doi.org/10.3390/seeds2010008.
80. Terzić, D.; Tabaković, M.; Oro, V.; Poštić, D.; Štrbanović, R.; Filipović, V.; Stanisavljević, R. Impact of essential oils on seed quality and seed-borne pathogens of *Althea officinalis* seeds of different ages. *Chem. Biol. Technol. In Agric*. **2023**, 10(1), 33. https://doi.org/10.1186/s40538-023-00405-8.
81. Chenyin, P.; Yu, W.; Fenghou, S.; Yongbao, S. Review of the Current Research Progress of Seed Germination Inhibitors. *Horticulturae* **2023**, 9(4), 462. https://doi.org/10.3390/horticulturae9040462.
82. Liu, J.; Wang, Q.; Karagić, Đ.; Liu, X.; Cui, J.; Gui, J.; Gu, M.; Gao, W. Effects of ultrasonication on increased germination and improved seedling growth of aged grass seeds of tall fescue and Russian wildrye. *Scientific reports* **2016**, 6(1), 22403.
83. Stanisavljević, R.; Đokić, D.; Milenković, J.; Terzić, D.; Beković, D.; Štrbanović, R.; Poštić, D. Influence of the air drying temperature on germination and dormancy of cocksfoot seeds (*Dactylis glomerata* L.). *Journal on Processing and Energy in Agriculture* **2014**, 18(4), 147-150.
84. Rutar, R.; Stjepanović, M.; Popović, S.; Bukvić, Z.; Pacek, D. Effect of temperature on germination and hard alfalfa seed. Ciheam 2001, 2, 137-139.
85. Vertucci, C.W.; Leopold, A.C. The relationship between water binding and desiccation tolerance in tissues. *Plant Physiol*. **1987,** 85(1), 232-238. https://doi: 10.1104/pp.85.1.232

86. Walters, C.; Wheeler, L.; Stanwood, P.C. Longevity of cryogenically stored seeds. *Cryobiology* **2004**, 48(3), 229-44.
87. Pritchard, H.W.; Manger, K.R.; Prendergast, F.G. Changes in Trifolium arvense seed quality following alternating temperature treatment using liquid nitrogen. *Ann. Bot*. **1988**, 62, 1–11.
88. Mira, S.; Schnadelbach, A.; Correa, E.C.; Pérez-García, F.; González-Benito, M.E. Variability of physical dormancy in relation to seed mechanical properties of three legume species. *Seed Sci. Technol.* **2017**, 45, 540–556.
89. Kimura, E.; Islam, M.A. Seed scarification methods and their use in forage legumes. *Res. J. Seed Sci*. **2012**, 5, 38–50.
90. Farida, A.; Rabeha, C.; Aissa, A. Effect of different scarification techniques on the germination of some endemic Medicago species in Algeria. *J. Ponte* **2020**, 76, 62–74.
91. Bolingue, W.; Ly, V.B.; Leprince, O.; Buitink, J. Characterization of dormancy behaviour in seeds of the model legume Medicago truncatula. *Seed Sci. Res.* **2010**, 20, 97–107.
92. Smolikova, G.; Leonova, T.; Vashurina, N.; Frolov, A.; Medvedev, S. Desiccation Tolerance as the Basis of Long-Term Seed Viability. *Int. J. Mol. Sci*. **2021**, 22, 101.
93. González-Benito, M.E.; Salinas, P.; Amigo, P. Effect of seed moisture content and cooling rate in liquid nitrogen on legume seed germination and seedling vigor. *Seed Sci*. Technol. **2003**, 31, 423–434.
94. Kandil, A.; Sharief, A.; Odam, A. Dormancy overcoming of some alfalfa varieties. *Research Journal of Seed Science* **2012**, 5(1), 19-31.
95. Tiryaki, I.; Topu, M. A novel method to overcome coat-imposed seed dormancy in *Lupinus albus* L. and *Trifolium pratense* L. *Journal of Botany*. **2014**, 647469. http://dx.doi.org/10.1155/2014/647469.
96. Ates, E. Influence of Some Hardseededness-breaking Treatments on Germination in Persian Clover (*Trifolium resupanatum* ssp. *typicum* Fiori Et Paol.) Seeds. *Rom Agric Res*. **2011**, 28, 2067-5720.
97. Nikolić, R.; Mitić, N.; Živković, S.; Grubišić, D.; Nešković, M. Cytokinins and urea derivatives stimulate seed germination in *Lotus corniculatus* L. *Archives of Biological Sciences* 2007, 59(2), 125-128.
98. Majidi, M.; Barati, M. Methods for breaking seed dormancy in one cultivated and two wild *Onobrychis* species. *Seed Science and Technology* **2011**, 39(1), 44-53.
99. Stanisavljevic, R.; Vuckovic, S.; Simic, A.; Markovic, J.; Lakic, Z.; Terzic, D.; Đokić, D. Acid and temperature treatments result in increased germination of seeds of three fescue species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **2012**, 40(2), 220-226.
100. Stanisavljević, R.; Vučković, S.; Štrbanović, R.; Poštić, D.; Trkulja, N.; Radić, V.; Dodig, D. Enhancement of seed germination in three grass species using chemical and temperature treatments. *Range Management and Agroforestry* **2015**, 36(2), 115-121.
101. Boyce, K.G.; Cole, D.F.; Chilcote, D.O. Effect of temperature and dormancy on germination of tall fescue. *Crop Science* **1976**, 16, 15–18.
102. Bass, L.; Gunn, C.; Hesterman, O.; Roos, E. Seed physiology, seedling performance, and seed sprouting. *Alfalfa and alfalfa improvement* **1988**, 29, 961-983.
103. Stošić, M.; Lazarević, D. Results of grassland research in Serbia. *Zbornik radova Instituta za ratarstvo i povrtarstvo* **2007**, 44(1), 333-46.
104. Štrbanović, R.; Stanisavljević, R.; Đukanović, L.; Poštić, D.; Marković, J.; Gavrilović, V.; Dolovac N. Application of accelerated aging test on alfalfa seed. Proceedings of the 4th international conference sustainable postharvest and food technologies INOPTEP and 27th national conference processing and energy in agriculture PTEP. 19-24 April, 2015.