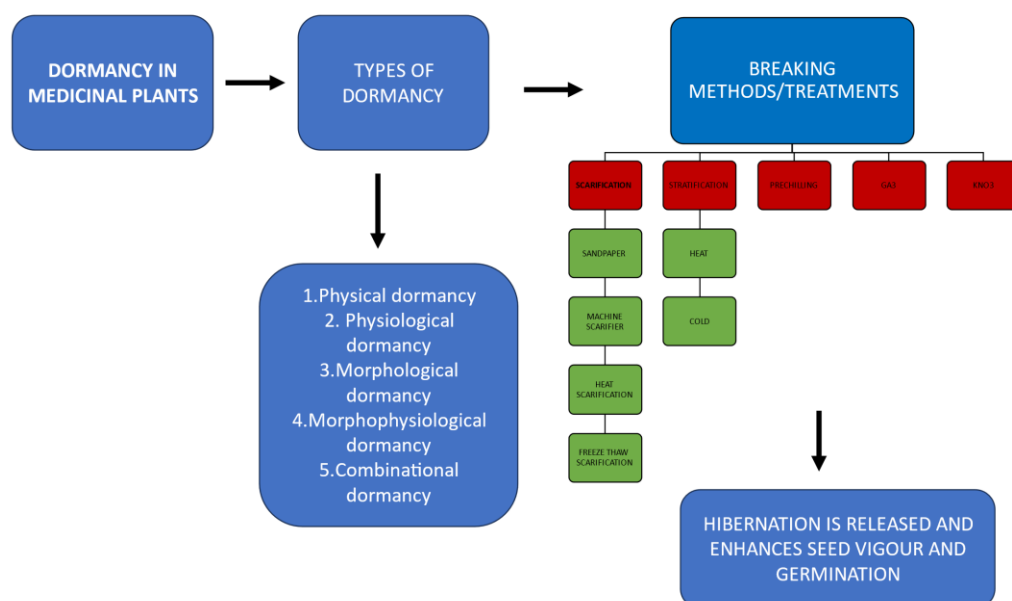


EXPLORING SEED DORMANCY FOCUSSING ON MEDICINAL PLANTS: A COMPREHENSIVE REVIEW

ABSTRACT

This review explores the complex field of studies on medicinal plants during dormancy, aiming to provide a comprehensive overview of research findings and methodologies. Dormancy, a crucial physiological phase in the growth stage of plants, plays a master role in regulating growth, development and survival strategies. In medicinal plants, understanding dormancy is of paramount importance due to its implications for cultivation, propagation and the production of some compounds **that are in high demand** for pharmaceutical industry. This review focuses on methodologies in dormancy research, elucidating the underlying mechanisms and exploring strategies to manipulate dormancy for enhanced medicinal plant cultivation and utilization. Furthermore, it discusses the challenges in dormancy studies, highlighting the potential for novel approaches and interdisciplinary collaborations to unravel the complexities of dormancy and harness its benefits in medicinal plant research and industry.

Graphical Abstract



KEYWORDS: Hormones, Physiology, Regulation, Scarification, Secondary Metabolites, Stratification

INTRODUCTION

Therapeutic plants are essential to both traditional and contemporary medicine because they are rich in bio active substances. Further to being used for medical intensions, medicinal plants can supply pharmaceutical businesses with raw ingredients [1]. Nevertheless, issues with seed quality, germination rates and genetic stability arise in the long-term production of therapeutic plants. To tackle these issues and promote the study culture, this analysis seeks to showcase the latest developments in seed technology. Medicinal plants constituting 4.4% of the total worldwide seed market, in which the United States makes up 27%, China 20%, France 8% and Brazil 6%. Medicinal plants are those that are often used to cure and prevent specific diseases and disorders [2]. Plants are the green factories of medication and a vital part of global health. Indigeneous health care systems are still commonly used in many contexts. The attention on utilizing plant materials as a source of pharmaceuticals for a diverse applications of human needs has increased as a result of infectious diseases. These include an insufficient supply of medicine due to population growth, excessive therapy costs, **unfavorable effects** of different synthetic drugs, and the establishment of drug resistance to frequently prescribed drugs [3].

These plants are used as a raw material for the pharmaceutical sector, which has led to their recent major relevance in agriculture, medicine and exports. The majority of medicinal plants **in the field have inconsistent seed quality, leading to problems with germination and stand establishment.** Techniques for improving seed germination and seedling establishment will improve the performance of medicinal plant production because of their significance in

the plant life cycle. Recently, the value of several plants for agriculture and medicine has been **retrieved** in an attempt to save people's livelihoods worldwide [4].

Offering solutions to enhance seed germination and seedling establishment will contribute to improved concert in the cultivation of medicinal plants, since the life cycle of plant depends on these mechanisms. Globally, curative plants are a tremendous source of novel pharmaceuticals [5]. In the United States, over 118 of the top 150 prescription medications are extracted from natural sources, while over 1300 medicinal plants are explored in Europe, with 90% of them being gathered from wild resources (Shafi *et al.*, 2021)[6]. Moreover, approximately 25% of recommended medications in wealthy nations come from wild plant species, while up to 80% of individuals in underdeveloped nations completely rely on herbal remedies for their primary health needs [7] ; [8]. A conservative estimate suggests that the current rate of plant species loss is between 100 and 1000 times higher than the natural extinction rate and approximately one potential significant drug is lost every two years [9]. The International Union for Conservation of Nature and the World Wildlife Fund report that globally, there are between 50,000 and 80,000 flowering plant species used for medicinal purposes, with about 15,000 species endangered by expansion due to over harvesting and habitat destruction [10]. Despite awareness of this threat for decades, the accelerated loss of species and habitats globally poses an increased risk for the extinction of medicinal plants, especially in countries like Kenya [11], Nepal [12] India [13], Uganda [14], Tanzania [15] and China [5]. Over 50,000 plant species more than one-tenth of all plant species are utilized in medications and healthcare items [16]. However there are geographical differences in the global distribution of therapeutic plants [17]. With 11,146 and 7,500 species, respectively, China and India have the highest numbers of medicinal plants used. In **plant seeds**, dormancy signifies a temporary cessation of visible growth, serving as an adaptive mechanism to endure

adverse environmental conditions. This dormancy phase notably impacts the production of secondary metabolites, often reducing their synthesis. To improve germination in medicinal plants, knowledge on dormancy, types of dormancy and dormancy breaking treatments are essential. In this context, the information on above points are reviewed hereunder for the benefits of farmers, stakeholders and interested parties. Understanding dormancy patterns in medicinal plant seeds is essential for cultivators seeking to optimize germination success and crop yield. Tailoring germination protocols to address specific dormancy types can significantly enhance cultivation outcomes. Additionally, knowledge of dormancy can inform seed storage and handling practices to maintain seed viability over time. Understanding the germination behavior of medicinal plant seeds is fundamental for successful cultivation. One significant aspect to consider is seed dormancy, which can greatly influence germination rates and plant establishment.

DORMANCY – THE HIDDEN PULSE OF BOTANICAL VITALITY

Seed dormancy, characterized as the failure of a viable seed to complete germination under right circumstances, is influenced by environmental factors such as light, temperature and the duration of seed storage [18]. Seed dormancy is commonly understood as a hindrance to the complete germination of a viable seed even under favorable conditions. However, some literature has reported potential sources of confusion. Ecological studies, for instance, have encountered confusion between seed dormancy and soil persistence [19, 20] stemming from differing views on dormancy, particularly regarding the role of light in either terminating dormancy or inducing germination.

A formulated dormancy classification system acknowledging the interplay of both morphological and physiological features in seed dormancy was given by [21]. Expanding upon this framework, they introduced a comprehensive classification system comprising five

main classes of seed dormancy: Physical, Physiological, Morphological and Combinational dormancy. This hierarchical system, outlined below, further categorizes these classes into various levels and types. Dormancy in seeds is not just escaping the germination; rather, it refers to the seed's characteristic that determines the potential required for germination [20, 22]. Any environmental issue that alters the conditions which is necessary for germination is considered to be altering dormancy. Moreover, when the seed no longer requires specific environmental cues, it is considered to be nondormant. Researchers have shown that various environmental factors can influence dormancy. For instance, while some suggest that only temperature can alter physiological dormancy following dispersal [23] demonstrated the induction of secondary dormancy in *Nicotiana attenuata* seeds by naturally occurring chemical signals, such as abscisic acid (ABA) and other terpenes found in leachate from litter covering the seeds in their habitat. A seed that is entirely non dormant possesses the ability to germinate across the broadest range of normal physical environmental factors like temperature, water availability, nutrients that are feasible for its genotype [24].

EXPLORING DORMANCY PATTERNS IN MEDICINAL SEEDS

The exploration of seed germination in medicinal plant species has drawn particular attention from the scientific community, driven by the escalating demand for these plants in the pharmacological industry and the necessity to establish rational crops for herb production [4, 25]. In the domain of medicinal plant cultivation, recognizing and addressing seed dormancy is paramount for achieving successful germination and robust crop establishment. By elucidating the dormancy characteristics of various medicinal plant species, cultivators can refine their techniques and harness the full potential of these valuable botanical resources [26].

PHYSICAL DORMANCY

This type of hibernation arises from water-impermeable layers of palisade cells in the seed or fruit coat, which regulate water movement. Physical dormancy can be broken through mechanical or chemical scarification methods. Examples of species exhibiting this dormancy include *Melilotus* and *Trigonella* (Fabaceae) [27]. Some of the plants with this type of dormancy can be treated by scarification method in which it alters a seed coat to make it more permeable to water and gases, which helps improve germination rates and speed up water absorption. Physical dormancy and its breaking methods for few crops are listed in table 1.

TABLE 1. PHYSICAL DORMANCY OF MEDICINAL PLANTS AND THEIR TREATMENTS

SN	NAME OF THE MEDICINAL PLANT	BREAKING METHODS	REFERENCE
1	Common wormwood (<i>Artemisia absinthium</i>)	Pre soaking in distilled water for 8 h	[28]
2	Amaranthus (<i>Amaranthus annus</i>)	Pre soaking of seeds in distilled water for 6 h	[29].
3	Cornflower (<i>Centaurea cyanus</i>)	Mechanical scarification until the seed coat is damaged.	[30].
4	Saw Palmetto (<i>Serenoa repens</i>)	Scarification by sand paper	[31].
5	St.John's Wort (<i>Hypericum perforatum</i>)	Soaking in KNO ₃ at 2g/lit for 3h	[32]
6	Andrographis(or King of Bitters) (<i>Andrographis paniculata</i>)	Acid scarification using H ₂ SO ₄ for 4min.	[33]
7	Greater Burdock (<i>Arctium lappa</i>)	Leaching in fresh water	[34]
8	Chinese Lantern (<i>Physalis alkekengi</i>)	Soaking in GA ₃ 250ppm for 6h	[35]
9	Summer Savory (<i>Satureja hortensis</i>)	Soaking in KNO ₃ at 2g/lit for 5h	[36]
10	Securigera	Leaching in fresh water	[36]

	(<i>Securigera securidaca</i>)		
11	liquorice (<i>Glycyrrhiza glabra</i>)	Acid scarification for 5 min using HCl	[36]
12	Tanacetum (<i>Tanacetum sp</i>)	Soaking in KNO ₃ at 2g/lit for 3h	[36]
13	Valerian (<i>Valeriana officinalis</i>)	Cold stratification at 5 °C for 8 days	[37]

PHYSIOLOGICAL DORMANCY

It is the most common type of hibernation found in seeds of gymnosperms and all major groups of angiosperms. It is especially prevalent in seeds from temperate regions [38]. It can be divided into three groups based on the depth or intensity of dormancy deep, intermediate and nondeep. Deep dormancy requires more prolonged exposure to cold stratification or other treatments to break dormancy, while nondeep physiological dormancy can be broken with shorter periods of stratification. The varying depths of this dormancy allow plants to stagger germination and protect themselves against unforeseen conditions. Its nuanced nature, with differing depths of dormancy, provides an adaptive advantage for plants in temperate climates with seasonal variations in climate [39]. Physiological dormancy has many reasons and background to occur and differs from one to on. Some of them are narrated below in table 2.

TABLE 2. PHYSIOLOGICAL DORMANCY AND THEIR BREAKING TREATMENTS

SN	NAME OF THE MEDICINAL PLANT	BREAKING METHODS	REFERENCE
1	Dill (<i>Anethum graveolens</i>)	Pre chilling at 5 °C for 6 days	[40].
2	Colocynth (<i>Citrulus colocynthis</i>)	Fluctuating temperatures	[41].
3	Wild sweet William (<i>Saponaria officinalis</i>)	Warm stratification	[42]
4	Ammoniacum	Cold stratification at 5 °C	[43]

	<i>(Dorema ammoniacum)</i>	for 6 days	
5	Purple cone flower <i>(Echinacea purpurea)</i>	Prechilling at 5 °C for 6 days	[44]
6	Galbanum <i>(Ferula gummosa)</i>	Prechilling at 5 °C for 6 days	[45]
7	Hyssop <i>(Hyssopus officinalis)</i>	Prechilling at 4 °C for 4 weeks	[46].
8	Tree Mallow <i>(Malva dendromorpha)</i>	Stratification (alternating temperatures)	[47]
9	High Mallow <i>(Malva silvestrys)</i>	Prechilling at 5 °C for 6 days	[48]
10	White Horehound <i>(Marrubium vulgare)</i>	Stratification (alternating temperatures)	[49].
11	Lemon Balm <i>(Melissa officinalis)</i>	Prechilling at 5 °C for 6 days	[50].
12	Oregano <i>(Origanum vulgare)</i>	Warm stratification	[51].
13	Flea Plant <i>(Plantago psyllium)</i>	Prechilling at 5 °C for 6 days	[36]
14	Black-eyes Susan <i>(Rudbeckia hirta)</i>	Prechilling at 5 °C for 6 days	[36]
15	Tobacco Sage <i>(Salvia dorrii)</i>	Cold stratification at 3-5 °C	[36]
16	Common Sage <i>(Salvia officinalis)</i>	Pre chilling at 5 °C for 6 days	[36].
17	Ginseng <i>(Panaxginseng)</i>	cold stratification	[36]
18	Echinacea <i>(Echinacea purpurea)</i>	Stratification and Scarification	[36]
19	St. John's Wort <i>(Hypericum perforatum)</i>	Moist stratification at 4 °C	[36].
20	Chamomile <i>(Matricari chamomilla)</i>	Stratification followed by pre soaking for 4h	[36]
21	Feverfew <i>(Tanacetum parthenium)</i>	Warm stratification	[52]
22	Peppermint <i>(Mentha piperita)</i>	Stratification and prechilling treatment	[53]

23	AloeVera (<i>Aloebarbadensis</i>)	Chemical soaking in KNO ₃ for 6h	[54]
24	Arnica (<i>Arnica montana</i>)	Warm stratification	[55]
25	Catnip (<i>Nepeta cataria</i>)	Cold stratification at 5 °C for 5 days.	[56]
26	Perwinkle (<i>Vinca rosea</i>)	Chemical soaking followed by warm stratification	[57]

MORPHOLOGICAL DORMANCY

It is characterized by seeds possessing underdeveloped embryos in terms of size, yet these embryos are differentiated into structures such as cotyledons and hypocotyl-radical. Seeds exhibiting this kind of dormancy are not physiologically dormant but rather require time for growth and germination. For instance, celery (*Apium graveolens*) exemplifies this dormancy [58]. The assertion that underdeveloped embryos represent the ancestral state of dormancy in seed plants suggests that this form of dormancy predates other forms such as physiological dormancy, morphophysiological dormancy and physical dormancy [59]. Seeds exhibiting morphological dormancy have evolved strategies to persist in the soil seed bank until all the requirements are fit for germination and seedling establishment. This dormancy mechanism allows plants to optimize their reproductive success by timing germination with favorable environmental conditions, thereby increasing the likelihood of seedling survival and establishment. Stratification methods also helps to overcome this type of dormancy.

MORPHO PHYSIOLOGICAL DORMANCY

It has a lot in common with morphological dormancy because it involves seeds that have immature embryos. Nonetheless, seeds exhibiting this also have a physiological aspect to their dormancy, which calls for a dormancy-breaking intervention such as the application of

gibberellic acid or warm and/or cold stratification. There are eight recognized levels. *Trollius* (Ranunculaceae) and *Fraxinus excelsior* (Oleaceae) species are two examples [55].

COMBINATIONAL DORMANCY

It is characterized by seeds possessing water-impermeable coats, as seen in physical dormancy, along with physiological embryo dormancy. Examples of species displaying dormancy include *Geranium* and *Trifolium* [24]. Medicinal plants often have specific requirements for germination, which may include exposure to particular temperature regimes, light conditions, or even chemical cues. Combinational dormancy in medicinal plant seeds may arise as an adaptive strategy to ensure that germination occurs under favorable conditions for seedling establishment and survival [60]. For example, seeds may exhibit both physiological dormancy, where internal physiological processes inhibit germination until certain conditions are met, as well as physical dormancy, where impermeable seed coats prevent water uptake and germination until they are sufficiently scarified or broken down (TABLE 3).

TABLE 3. LIST OF COMBINATIONAL DORMANCY AND THEIR BREAKING METHODS

SN	MEDICINAL PLANT	TYPE OF DORMANCY	BREAKING METHODS	REFERENCE
1	Anise (<i>Pimpinella anisum</i>)	Combinational dormancy	Scarification by sand and stratification at 20 °C	[36]
2	Rue (<i>Ruta graveolens</i>)	Combinational dormancy	Stratification and scarification in mechanical scarifier for 12 min	[36]
3	Milk Thistle (<i>Silybum marianum</i>)	Combinational dormancy	Scarification and stratification at 3°C for 48 days.	[61]

CONNECTING THE DOTS - DORMANCY AND QUIESCENCE IN BIOLOGICAL ADAPTATION

A condition of dormancy during which seeds are not actively growing or germination but are still viable is known as the quiescence period in seeds. At this time, seeds are essentially in a "resting" state, with little metabolic activity and little cellular activity. Quiescence is the character of seeds to survive for long periods of time in unfavourable environments until they are ready for germination. Quiescence is distinct from other types of dormancy in seeds, such as embryo dormancy or hibernation imposed by the seed coat, in that it is not always brought on by environmental cues like light or temperature. Rather, it is a natural characteristic of some seeds and is frequently genetically programmable. Seeds can emerge from their quiescent state and resume active growth and germination when the proper circumstances are met [62].

PLANT HORMONES AND THEIR MULTIFACETED ROLES IN SEED DORMANCY REGULATION

The opposing forces ABA and Gibberellic acid (GA) control seed dormancy. While mutants lacking GA biosynthesis or signaling have more dormancy, mutants lacking ABA biosynthesis or signaling often have less dormancy in seeds. This suggests that whilst GA promotes germination, ABA serves to induce dormancy. GA is hypothesized to have two effects on dormancy. It does this by first causing the expression of enzymes that weaken the endosperm and make room for the embryo to grow. Secondly, it directly increases the embryo's capacity for growth. In an experiment, [63] found that ABA may control GA responses in the endosperm, where GA operates predominantly to stimulate the break of dormancy. There is strong evidence that the ABA and GA signaling pathways interact in the embryo. The degree of dormancy in an embryo may be influenced by the balance of ABA and GA sensitivity, as mutations that damage one pathway might partially attenuate abnormalities in the other [64] his partial suppression, however, suggests that distinct ABA and GA pathways exist in parallel.

Thus, ABA and GA have opposing and complementary effects on endosperm and embryo in the intricate hormonal regulation of seed dormancy [65]. Genetic regulatory networks of great complexity are involved in the crosstalk between ABA and GA₃. While DELLA (aspartic acid–glutamic acid–leucine–leucine–alanine) proteins are important in GA signaling pathways, transcription factors like ABI3 (ABA-INSENSITIVE 3) and ABI5 are crucial in mediating the effects of ABA on dormancy. In the promoter regions of target genes, interactions between ABA-responsive elements (ABREs) and GA-responsive elements (GAREs) coordinate the transcriptional responses to ABA and GA₃ during dormancy regulation [66]. Known for its role in senescence in plants and fruit ripening, ethylene has been found to be essential in a variety of physiological processes, such as the escape of dormancy in seeds, the development of root hairs, and reactions to viruses and wounds, among other stimuli [66]. Many plant species, including peanuts, apples, redroot pigweed, cocklebur, lamb's quarters, *Amaranthus retroflexus*, and sunflowers, have been discovered to break their dormancy when exposed to ethylene. Notably, research with the *Arabidopsis* ga-1 mutant has demonstrated that, in light settings, ethylene gas can cure germination problems; however, it will cause the seedlings to exhibit the distinctive triple response (Curved apical hook, swollen nodes and horizontal growth). This implies that certain ethylene signaling components are involved in the process of germination. The exact genetic processes by which ethylene affects seed germination, however, are still unknown [67].

It may become clearer whether ethylene promotes germination by modifying ABA or GA levels or sensitivity, or whether it works through different signaling components, by investigating its interactions with important regulators of ABA and GA production and signaling pathways in more detail. There might also be linkage with other signaling channels that are not yet known to exist according to traditional ethylene response mutant screening [68].

IMPACT OF DIFFERENT DORMANCY BREAKING TREATMENTS

Seed germination is a multifaceted physiological process shaped by environmental cues like water availability, light exposure, and other variables. The challenge of limited seed germination significantly hampers the cultivation of threatened medicinal plants, especially in harsh cold desert environments. Natural inhibitors derived from compounds like benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic acid, and abscisic acid (ABA) play pivotal roles in regulating germination [65]. Certain temperate species experience a warm phase that is followed by a cold one, which breaks their dormancy division into groups. The most common correlation between this reaction and morpho-physiological dormancy is that seeds that exhibit this response have embryos that are not fully formed [69].

To expedite this method further, it can be enhanced through the integration of additional treatments such as chemical applications or mechanical seed coat removal [70]. Various researchers have explored the impact of exogenous growth regulators on seed germination. For instance, Gibberellins have been found to eliminate the chilling requirements of peach and apple seeds, thereby enhancing their germination rates [70]. Recent investigations have indicated that cold stratification directly influences the production of gibberellins (GAs) in seeds of *Arabidopsis thaliana* [71] externally applied GA has been shown to overcome seed dormancy in multiple species [4] and to stimulate germination in species that typically necessitate cold stratification, light exposure, or after-ripening [72] standard procedures for enhancing the germination of dormant seeds involve pre-chilling, scarification, and treatments with gibberellic acid (GA₃) or potassium nitrate (KNO₃).

Various methods, including scarification, using plant growth regulators as a pretreatment (PGRs), and temperature shocks, are employed to break dormancy, depending on the plant species and dormancy type [73, 74]. Seed germination is influenced by both intrinsic and extrinsic factors, with certain PGRs playing a crucial role. Abscisic acid (ABA) is involved

in germination inhibition, while gibberellins (GAs) participate in terminating seed dormancy [75, 76]. PGRs commonly enhance seed germination capacity, increase biomass yield, and confer resistance to diseases and adverse growth conditions (Papadopoulos *et al.*, 2006). GAs, synthesized by seeds, are believed to hydrolyze storage nutrients and directly impact embryo growth. External application of PGRs, including auxins like IAA, IBA and NAA, has been found to break seed dormancy and enhance seedling establishment in various aromatic and medicinal plants [72, 77, 78].

The study on *Ochradenus arabicus* seeds revealed GA₃ as the most effective in overcoming dormancy, resulting in 80-89% germination when treated with 100µM GA₃ and stored for up to 12 months. Increasing GA₃ concentration showed an inhibitory effect on seed germination, and seeds treated with different chemicals exhibited asynchronous germination. These findings suggest that GA₃ is the most effective in promoting seed germination in *O. arabicus*, emphasizing the necessity for hormone application to break dormancy, as documented by [80]. The dormant nature of *O. arabicus* seeds may be attributed to inhibitors, a hard seed coat, low internal hormones, or underdeveloped embryos, aligning with reports on the application of gibberellic acid to alleviate innate and environment-induced dormancy [81]. The seeds of glory lily soaked in boiled water (100 °C) for 40 minutes recorded the highest germination of 62 per cent accompanied with faster rate of germination. This is because the seed coat gets weakened by the hot water and eventually the lignins and pectins present in the epidermal layer of seed gets dissolved [82]. Hence there are numerous ways to break down the dormancy present in seeds according to its nature.

SCARIFICATION

Scarification techniques have been evolved during time and has become practically feasible for all the crops. Some of the notable methods of scarification include heat, freeze-thaw, mechanical and chemical. Heat scarification uses high temperature to break the hard seed

coat whereas freeze thaw breaks the seed coat by alternating temperatures to high and low. Mechanical scarification breaks the seed coat or makes an injury over it in order to accelerate the seed's rate of imbibition [83] .

HEAT SCARIFICATION

Heat scarification is way more easier method due its easy application. Two main devices that are being applied to scarify are oven and hot water bath. Its efficacy depends on various factors like treatment, time, temperature and seed used. It differs from seed to seed like some may break at low temperature and some may be subjected to very high temperatures [84]. The primary purpose of heat scarification is to break seed dormancy. For some species, this dormancy is due to a hard seed coat that inhibits water absorption. Heat scarification helps to overcome this barrier, allowing water to penetrate the seed coat and initiate germination. The temperature and duration of heat exposure vary based on the species of seed being treated. Generally, temperatures range from 100 °C to 120 °C (212°F to 248°F), and exposure times can vary from a few minutes to several hours. It's crucial to research the specific necessities for the seeds you are treating, as excessive heat can damage or kill the seeds [85] .

Seeds can be exposed to dry heat using methods such as placing them in an oven, using hot sand, or applying heat from a heat gun. Dry heat methods are suitable for seeds that are tolerant of low moisture conditions. Seeds are subjected to high temperatures in a moist environment, such as steam or hot water. Moist heat scarification is often preferred for seeds that require higher moisture levels for germination. When using heat scarification methods, it's essential to take proper safety precautions to avoid injury or damage to the seeds. This includes using heat-resistant containers or equipment, wearing protective gear when handling hot materials, and closely monitoring the temperature to prevent overheating (Priyadharshini & Lekha, 2021).

After heat scarification, seeds should be cooled and then planted promptly to take advantage of the weakened seed coat and initiate germination. It's important to provide optimal growing conditions, including proper moisture, light and temperature, to support seedling development. Heat scarification can be highly effective for breaking dormancy in certain species of seeds, particularly with hard seed coats or seeds from arid environments (Priyadarshini & Lekha, 2021). However, not all seeds require scarification, so it's essential to research the specific germination requirements of the seeds **we are working with**. Overall, heat scarification is a valuable technique for promoting germination in seeds that have dormancy mechanisms related to hard seed coats. When done correctly, it can significantly improve germination rates and support successful seedling establishment. Scarification duration differs from crop to crop.

TABLE 4. SCARIFICATION TREATMENTS

SN	CROP	TREATMENT	EFFECT	REFERENCES
1	Saskoon berry seeds (<i>Amelanchier alnifolia</i>)	Scarification at 110°C with heat for 30 min	Radicle protrusion was earlier	[84].
2	Burclover (<i>Medicago polymorpha</i>)	Sandpaper scarification for 10-40min	Breakage of hard seed coat	[86].
3	Barrel Medic (<i>Medicago truncatula</i>)	20 min sandpaper scarification followed by mechanical scarifier	Imbibition of water takes place immediately after soaking	[87]
4	Arrow leaf (<i>Myriopteris intertexta</i>)	Mechanical scarifier for 15 min	Breakage of hard seedcoat	[88]
5	Loofah (<i>Luffa aegyptiaca</i>)	Mechanical scarifier at 100rpm for 11 min	Increases seed germination from 67 to 75%	[89]

FREEZE-THAW SCARIFICATION

The hidden theory behind this method is to make tiny scars on the seed coat and to make it **weaken** so that germination takes place. This scar making method depends on the seed size, shape, water content, intensity, duration etc. In this freeze thaw technology freezer, carbon

dioxide, dry ice, snow, liquid air, acetone, liquid nitrogen are combined and used [90]. It has to be noted that temperature below -80 °C or lower, may be more effective than higher temperatures (above -80 °C). Cryo scarification of seeds is a method that involves using liquid nitrogen and sulfuric acid to overcome the hardness of seeds' endocarps and seed coats. Some medicinal plants exposed to freeze thaw are listed below in Table 5.

TABLE 5. FREEZE THAW TREATMENTS

SN	NAME OF MEDICINAL PLANT	TREATMENT	REFERENCES
1	Alfalfa (<i>Medicago sativa</i>)	Exposing the seeds in -15 for 36h so that the seed coat becomes brittle	[90]
2	Ashwagandha (<i>Withania somnifera</i>)	Exposing the seeds at -22 °C for 180 days followed by placing the seeds in dry ice at -20 °C.	[91]
3	Pepper (<i>Piper nigrum</i>)	Placing the seeds in liquid nitrogen for 10 min and soaking the seeds in hot water bath of 100 °C for 10 min.	[92]
4	Brahmi (<i>Bacopa monnieri</i>)	Placing of seeds in liquid nitrogen for 180 days.	[93]

PRECHILLING TREATMENT

The pre-chilling treatment proved an efficient in overcoming hibernation for *Calendula officinalis*, *Saponaria officinalis*, *Echinacea purpurea*, *Malva silvestris*, *Melissa officinalis*, *Plantago psyllium*, and *Rudbeckia hirta*, resulting in increased germination among the 15 species subjected to pre-chilling. The most best results were noticed in *Plantago psyllium*, with germination increasing from 40 to 99%, and in *Saponaria officinalis*, from 0 to 30% [36].

Stratification improved germination in five out of six commercial *E. purpurea* seed lots, with the most significant improvement occurring at 10 °C for 10 days. Highest germination of *Echinacea angustifolia* in response to a combination of 250 ppm GA₃ and 4 weeks of pre-chilling was reported by [34]. Probert *et al.* 2009 also found that pre-chilling treatment was effective in enhancing *Saponaria officinalis* germination.

An increase in the germination of *Ferula gummosa* with pre-chilling at 5 °C was noticed by [94]. [77] observed the highest germination of *Dorema ammoniacum* in 30 days at 3 - 4 °C. It has been investigated that pre-chilling is effective in breaking dormancy in *Salvia sp.* The pre-chilling was not effective in breaking dormancy for *Calendula officinalis* has been reported by [34]. In the case of *Ferula gummosa* and *Dorema ammoniacum*, seeds did not germinate, and pre-chilling had no effect on breaking their dormancy. Nevertheless, both the application of GA₃ and pre-chilling at 5 °C stimulated both the rate and final germination of *Ferula gummosa* [94]. Physiological dormancy in some species can be broken by relatively short periods of cold stratification. Thus, species for which pre-chilling was effective in breaking dormancy might possess physiological dormancy. A range of the prechilling treatments for medicinal plants are mentioned below in table 6.

TABLE 6. LIST OF MEDICINAL HERBS AND THEIR PRECHILLING TREATMENTS

SN	MEDICINAL PLANT	PRECHILLING TREATMENT	EFFECT	REFERENCES
1	Corn Poppy (<i>Papaver rhoeash</i>)	Wet pre chilling	Improved germination rate	[95]
2	Long-headed Poppy (<i>Papaver dubium</i>)	Wet pre chilling	Increased seed germination rate from 46% to 90%	[96]
3	Alfalfa (<i>Medicago sativa</i>)	Prechilling followed by soaking of seeds in GA ₃ at 250ppm for 1 week	Seed germination was easy as the hard seed coat gets softened	[97]

4	California Burclover (<i>Medicago polymorpha</i>)	Prechilling at 20 °C for a week	Seedcoat was permeable	[98]
5	Yellow Sweet Clover (<i>Melilotus officinalis</i>)	Prechilling at 4°C followed by soaking in GA3 at 750ppm	Physical dormancy was broken	[45].
6	Yellow Sweet Clover (<i>Melilotus oficinalis</i>)	Prechilling at 4°C	Maturity of embryo was quick	[96]
7	Douglas Fir (<i>Psedostuga menziesii</i>)	Prechilling at 15°C for 128 weeks	Protrusion of embryo	[99]
8	Asafoetida (<i>Ferula assafoetida</i>)	Prechilling at 9°C for 90 days	Increased germination percentage	[100]
9	Galbanum (<i>Ferula gummosa</i>)	Prechilling at 5°C and soaking of seeds in GA3 at 400ppm	Sprouting of seeds	[101]
10	Colocynth (<i>Citrulus colocynthis</i>)	Prechilling at 4°C for 50 days	Dormancy was broken and germination at earlier stage.	[102]

GIBBERELLIC ACID (GA₃)

The significant improvement in sprouting of seeds with GA₃ treatment in *Plantago psyllium*, *Rudbeckia hirta* and *Satureja hortensis* compared to untreated seeds were studied , **inconclusive** results were obtained in *Opuntia tomentosa* [103]. Plant growth regulators like GA₃ and KNO₃ seem to play a role in breaking dormancy by establishing hormonal balance and reducing germination inhibitor substances. The results were compatible with [104]

observation of an enhanced germination rate in *Cuminum cyminum* with GA₃ application. [105] also reported increased germination of *Hyssopus officinalis* seeds with GA₃ pre-treatment. Loquat (*Eriobotrya japonica* L.) being a prime fruit crop that has potential of curative properties is mainly cultivated through seeds. The exogenous growth regulators can modify the digestibility of seed coat and if treated with GA₃ 250 mg.L⁻¹ soaking duration is 36h [77].

POTASSIUM NITRATE (KNO₃)

One of the major issue in cultivating medicinal herbs in a commercial way is they readily germinate in natural or native environment but when they are mandatory to test for germination under laboratory conditions they get failed. Dormancy is not a single phenomenon and it contributes together with various aspects [106] KNO₃ was found to be effective in breaking seed dormancy for *Calendula officinalis*, *Cynara scolymus*, *Ocimum basilicum*, *Physalis alkekengi*, and *Satureja hortensis* compared to their germination in H₂O. Similar instances were noted in studies on *Calendula officinalis* and *Echinacea purpurea* [34] As mentioned earlier, KNO₃, acting as a plant growth regulator, plays a role in breaking dormancy by establishing hormonal balance and reducing germination inhibitor substances. Galbanum seeds when stratified with perlite medium and then subjected to various concentrations of KNO₃ (0, 0.1, 0.2 and 0.3% (v/v)), after 7 weeks of stratification the seeds soaked in 0.3 percent KNO₃ the germination percentile shoot up to 86 % [98].

STRATIFICATION OF SEEDS

Exposure to cold temperatures during stratification mimics the natural winter conditions that many seeds would experience in their native habitats. This cold treatment is particularly effective for seeds of plants adapted to temperate climates [107]. The cold

temperatures initiate physiological changes within the seed that break dormancy and prepare it for germination when conditions become favorable. In moist stratification, seeds soak up moisture from their environment. This hydration softens the outer covering and triggers biochemical processes within the seed [108]. The cold and sometimes moist conditions of stratification can soften the seed coat, which is often impermeable to water and gases. Softening of the seed coat allows for enhanced water uptake and gas exchange, facilitating metabolic activity within the seed.

TABLE 7. STRATIFICATION TREATMENTS

SN	MEDICINAL PLANT	TREATMENT	REFERENCES
1	Fennel <i>Foeniculum vulgare</i>	Exposing the seeds at 10°C for about weeks	[109]
2	Echinacea <i>Echinacea sp</i>	Exposing the seeds at 4°C for 10 days	[110]
3	Kalajira <i>Bunium persium</i>	Moist stratification at 4°C for 5 days	[111]
4	Loddon lilly <i>Leucojum aestivum</i>	Warm stratification at 30°C for 6 weeks	[112]

SECONDARY METABOLITES (SM) - METABOLIC MAESTROS OF DORMANCY AND LONGEVITY

The duration of dormancy is critical for the secondary metabolites. When seeds remain dormant, they protect as a defensive mechanism against a distinct combination of biotic and abiotic stressors. Certain secondary metabolites, like as phenolic compounds, have antioxidant properties that shield them from oxidative damage. As a result, they provide protection throughout the dormant phase, raising the likelihood of successful germination in the event that favorable conditions continue.

SMs found in medicinal plants are essential components that underpin their clinical curative effects and serve as crucial indicators for evaluating the quality of medicinal materials. The

synthesis and accumulation of SMs are intricate processes influenced by both internal developmental genetic circuits (such as regulated genes and enzymes) and external environmental factors (including light, temperature, water, and salinity). While existing literature extensively explores the impact of environmental factors on SM synthesis and accumulation, there is a gap that requires a systematic classification and summary of the effects of developmental growth and genetic factors on SMs. The biosynthesis of SMs initiates from basic pathways such as glycolysis or shikimic acid pathways, with subsequent diversification depending on cell type, developmental stage, and environmental cues [113]. Distinct cells, tissues and organs of medicinal plants may exhibit different medicinal properties at various developmental stages due to the influence of developmental factors on cellular structures involved in SM biosynthesis and storage [52]. Plant growth and development, influenced or impeded by different environmental conditions, significantly impact the accumulation of secondary metabolites [114]. Ashwagandha being an important medicinal plant of Indian origin has numerous secondary metabolites in which Withanoloid increases during saline stress is evident in experimental study and all the secondary metabolites behave in the same way to express their origin during external stress condition [115]. Environmental variations strongly affect SM pathways and their regulation, as the expression of genes involved in SM pathways undergoes alterations in response to different stresses [116]. Secondary metabolites have a heavy interaction with external environment in plants and are initially produced by primary metabolites in higher plants [117]

The plant kingdom boasts approximately 100,000 secondary metabolites, classified into three major groups based on bio synthetic pathways: nitrogen-containing compounds (cyanogenic glycosides, alkaloids, and glucosinolates), phenolic compounds (flavonoids and phenylpropanoids), and terpenes (isoprenoids) [118]. Despite progress in SM biosynthesis and accumulation research, reports on how developmental and environmental factors stimulate the

synthesis and accumulation of secondary metabolites in medicinal plants are limited. The stimulation of metabolites biosynthesis in medicinal plants by controlling and optimizing external and internal factors holds promise for advancing bio technologies aimed at high-quality drug production [119].

Secondary metabolite synthesis, accumulation, and distribution patterns in medicinal plants categorize medicinal parts into four main types: roots and stems, leaves, flowers, and fruits and seeds. The complexity and diversity of these metabolites in different parts of medicinal plants end up to the synthesis of different SMs through specific regulatory pathways and transport routes in certain organs, tissues, and cells [42]. Thus, SM biosynthesis and accumulation exhibit organ or tissue specificity.

Fruits and seeds of medicinal materials in many plants, are influenced by their developmental stages, impacting the content and composition of components. For instance, in Citrus fruits, volatile oil content, the main active ingredient, is highest when the fruit is light yellow, serving as a morphological index for harvesting [120]. Essential oil yields in *Citrus medica L. var. sarcodactylis* significantly increase during maturation, and the content of specific compounds varies during maturation stages [121]. Similarly, capsules of *Papaver somniferum L.* reach the maximum morphine content at maturity [122]. Therefore, the developmental stages of plants significantly affect the content and composition of SMs. The synthesis and accumulation of SMs are also closely associated with the developmental stage of medicinal plant seeds. For example, in coffee quinic acid content remains relatively stable, but dicoffee quinic acid content decreases noticeably with seed development. The content of quinic acid, a precursor substance for chlorogenic acid synthesis, is high in the early developmental stage of seeds and decreases significantly later on, related to the expression characteristics of the HQT gene, a key enzyme regulating phenolic acid biosynthesis [123].

CONCLUSION

This comprehensive review of medicinal plant seeds and their dormancy mechanisms highlights the critical importance of seed biology in the conservation and cultivation of valuable medicinal species. The diverse dormancy types observed reflect complex evolutionary adaptations, with physiological dormancy being prevalent among many medicinal plants. Environmental factors such as temperature and light play significant roles in regulating dormancy breaking and germination processes. The insights gained from this review can inform more effective strategies for medicinal plant propagation, conservation, and sustainable use, addressing the rising global demand for natural medicines. Since prehistoric times therapeutic plants have many distinct purposes. Protection and Defense activity is one of the significant act of medicinal herbs. Seed market is expected to be on top with hybrid varieties developed indigenously for domestic markets and commercial farmer. Studies on these medicinal plant remains to be a growing part for further research. Dormancy pertaining to the seeds must be considered as in advantage or disadvantage way according to the various external factors. Sometimes this bane helps in seeds preservation and in other case it is considered as serious issue for germination. Since the availability of seeds itself is difficult these days, considering these dormancy issues can lead to a big advantage in pharmacological industry for availing drugs at earlier and cost efficient way.

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