

Review

Alleviation strategies of seed dormancy for conserving important medicinal plant diversity of western Himalaya

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Abstract

One of the major challenges in medicinal plant cultivation is seed dormancy which occurs more frequently in wild plants than crop plants. Seed dormancy enables seeds to endure periods that are unfavorable for seedling establishment. It may arise due to many factors like impermeable seed coat to water and oxygen, rudimentary and dormant embryo and germination inhibitors. Multiple mechanisms are known to be involved in the induction of dormancy and the transition from dormant to germination. Scarifications, stratification, chilling are some of the important ways to overcome different types of dormancy. *Saussurea costus*, *Inula racemosa*, *Sinopodophyllum hexandrum* and *Bunium persicum* are some of the important endangered medicinal plants of western Himalaya. These medicinal plants are in great demand in the pharmaceutical industry. These medicinal plants grow under diverse agro-climatic conditions of the western Himalayas' sub-temperate and dry temperate regions. Overharvesting of these medicinal plants from their natural habitat has resulted in the extinction of their population. Poor seed germination is one of the major limiting factors for their *ex-situ* conservation and large-scale cultivation. This review explores the mechanisms involved in the seed dormancy and their alleviation strategies. Considering the findings, these dormancy alleviation methods can be applied to cultivate these medicinal plants successfully in the western Himalayan region.

Keywords Conservation · Growth regulators · Scarification · Seed treatments · Stratification

1 Introduction

The Indian Himalayan region is known for its rich wealth of the most important medicinal plants [1]. There are over 460,000 medicinal plants in different climatic and geographical regions in the Himalayas [2]. These medicinal plants grow under diverse agro-climatic conditions viz. sub-temperate and dry temperate regions [3]. Various chemical constituents are obtained from medicinal plants used to cure many diseases and are essential in pharmaceutical activities like free radical scavengers, immunity boosters, cytotoxic drugs, low blood sugar and anti-cancerous [4]. According to the WHO (World Health Organization) the current demand for medicinal plants is expected to be around \$14 billion annually. The market for raw materials derived from medicinal plants is expected to rise by 25% per annum and approach \$5 trillion in revenue by 2050. In India, medicinal plant-related commerce is worth roughly \$1 billion annually [5]. The growing pharmaceutical industries' demand for medicinal plants worldwide has affected collection practices and overharvesting from the wild habitat has resulted in the extinction of their remaining population and loss of genetic variation. Further, the

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livelihoods of indigenous people who live in the forest margins have been severely impacted by the overuse and ongoing destruction of medicinal plants [6]. The natural regeneration of these important medicinal plants does not appear to open the door for mass multiplication covering broad areas or extending the planting area under unconventional crops. There is only 10% information on the standardised propagation protocols for the medicinal plants and less than 1% on improved agrotechnologies for their successful cultivation [7]. This pattern suggests that one of the main research areas should be developing agro-technology of medicinal plants for large-scale cultivation.

Seed is the foundation and initial input for transferring genetic characteristics during crop production. In nature, every plant's life cycle begins with germination, which is crucial for species survival and frequently influences population dynamics [8]. However, poor seed germination is a critical limiting factor for large-scale production and cultivation of endangered medicinal plant species [9]. Some Himalayan plant species have shown poor seed germination due to non-viable seeds [10], resulting in habitat depletion from their natural ecosystem [11]. Crop varieties including those of medicinal exhibit varying degrees of dormancy [12]. *Saussurea costus*, *Inula racemosa*, *Sinopodophyllum hexandrum* and *Bunium persicum* are some important medicinal plants of western Himalaya and cultivation of these species is very limited or no cultivation is done. Out of many factors seed dormancy is major and restricts farmers from domesticating these species for cultivation. Seed dormancy inhibits seeds from emerging in unfavourable conditions; it lowers the risk of seedling mortality and enables the regeneration of the seeds [13]. Seed dormancy may arise due to many factors viz. impermeable seed coat (physical dormancy), an immature embryo (morphological dormancy), a rudimentary embryo and inhibitors material (morphophysiological) [14]. The primary mechanisms involved in seed dormancy release are light temperature hormones and stratification [15, 16]. Exogenous hormone like GA₃ accelerates the percentage of final germination and alleviates seed dormancy [17]. The mechanism of GA₃ increases glyoxylate cycle metabolism and α -amylase hydrolysis in seeds, which boosts the embryo's capacity to expand and activates energy metabolism [18]. Furthermore, the cold/warm stratification treatment has been demonstrated to release physiological dormancy [19]. The hormone treatment stimulates and increases the seeds' metabolic processes, promoting cell division and radical elongation [16].

On the other hand, a non-dormant seed (or another germination unit) will germinate under the broadest range of physical environmental conditions depending on genotype behaviour [20]. Therefore, there is a greater need to identify the type of germination and strategy to overcome dormancy and ideal germination conditions [10]. for further improvement in seed germination and cultivation of medicinal plants [21]. Otherwise, the success of seed-based conservation efforts would be limited, resulting in a loss of time and resources. This review discusses seed dormancy and its types and ways to alleviate seed dormancy of important medicinal plant species of the western Himalayas.

2 Induction of seed dormancy

One of the primary dormant seed states is induction dormancy, which develops when the conditions for seed germination (such as light water and temperature) are not achieved, resulting in no seed germination. The physical characteristics of the seed coat and environmental factors induces seed dormancy [22]. Seed dormancy is classified into two types, primary and secondary dormancy on the basis of the mode of seed origin. Out of both, primary dormancy is found in seeds maturing on the parent plant [23]. Once seeds are shed from the parent plant, they come out of primary dormancy; however, the phase of secondary dormancy initiates due to a lack of favorable environment. The secondary dormancy develops with seed dispersal [24] and the seed remains in secondary dormancy until the favourable conditions are achieved [25]. The seed coat is impermeable to water due to genetic and environmental variables; however, this characteristic is mostly inherited. Seeds have a hilum structure that prevents water from penetrating them. Further, suberin, lignin or cutin all combine to restrict water penetration into the seed [26, 27]. This dormancy can be broken only when the seed coat softens and allows gas or water exchange from the environment [10]. Permeability to oxygen increases at 4 °C while there are limits to oxygen absorption at 20 °C [28]. Mechanical resistance is combined with physiological dormancy, and it is due to the embryo, seed coat, or outer layer [29].

Poor seed germination is one of the main challenges for the large-scale cultivation of medicinal plants in cold desert environments, so identifying seed germination patterns is crucial. There was significant species-specificity in the way seeds responded to various treatments. The habitat in which they are collected and the length of time they are stored determine how different circumstances affect the dormant seeds of the same species [30]. In *I. racemosa*, *B. persicum*, *S. hexandrum* and *S. costus*, the highest germination percentage was achieved when seeds were held at – 20 °C for 30 days, followed by treatment with hot water at 80 °C for 20 min. These seeds also showed

the highest germination rate when treated with alcohol and acetone for 10 min. Different economic techniques like acid scarification, gamma rays, hormones (GA_3 or KNO_3) and hot water treatment have also been employed to alleviate seed dormancy in these crops. When compared to concentrated HCl and HNO_3 treatment, H_2SO_4 treatment showed a favourable impact on the seed germination of these medicinal plants [31]. However, the soaking duration resulted in a considerable drop in seed germination, which can be linked to damage to the embryo. *B. persicum* requires low temperature [32], while *I. racemosa* seemed to have GA_3 , replacing the need for a low temperature to break the seed dormancy. It is an indication of species distinctiveness. Seeds treated with low temperatures could be readily used to cultivate species. In comparison to the treatment, all species showed the best germination with H_2SO_4 for 5 min during a 30-day period.

3 Classes of seed dormancy

Seed ecologists have proposed many classification systems for different types of dormancy, but seed technologists require a widely recognized hierarchical system and the underlying mechanisms to create effective treatments for breaking dormancy in seeds. A modified form of Nikolaeva's (Russian seed physiologist) classification method for seed dormancy has been proposed by [10]. The revised system of seed dormancy classification includes three hierarchical categories: class, level and type, with five kinds of dormancy: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and combinational dormancy (PY + PD). The most comprehensive classification schemes have been identified in physical dormancy, which has three levels and five forms of dormancy at the non-deep level, whereas morphophysiological dormancy has eight levels and no distinction in their types. The most prevalent kind of dormancy in gymnosperms (gnetales and conifers) and all major clades of angiosperms is called physical dormancy (non-deep level) [10]. The new modified classification system have described the various types of seed dormancy along with classification [33, 34]. Biochemical molecular and genetic methods may have a wide range of applications in describing the fundamental mechanisms behind physiological dormancy in seeds [35]. The different types of dormancy in the Nikolaeva scheme of classification are based on the seed coat water permeability, the embryo's morphology (rudimentary versus fully developed) and the physiological responses of the seed to temperature or a range of temperatures [10].

3.1 Physical dormancy

Physical seed dormancy is due to water-impermeable seed coat and confers through tightly packed palisade cells impregnated with water-repellent substances [36]. It is believed that in nature, the repeated processes of thawing from many months to years under field seed bank conditions cause seed coverings to become water-permeable and overcome physical dormancy [33]. Physical dormancy is known within family: Fabaceae Convolvulaceae, Malvaceae, Rhamnaceae and Sapindaceae.

3.2 Physiological dormancy

This type of dormancy is the most prevalent category in dormant seeds and can be separated from physical dormancy by seed water permeability. It is caused by the mechanical restriction of surrounding tissues on embryo growth (e.g. endosperm, seed or fruit coat) and inhibitors present in the seed coat endosperm and embryo [37]. Enzymes that promote growth such as gibberellin may be inhibited by phytohormone action like abscisic acid [38]. These dormancy-caused compounds are water soluble further degraded into growth-promoting hormones and enable higher germination. Low temperature shifts the hormone balance and promotes the production of growth-promoting hormones and enzymes in the embryonal axis [39]. Winter temperature shifts the balance of promoters and inhibitors toward stimulating germination [14]. Example: Asteraceae, Betulaceae and Liliaceae.

3.2.1 Metabolic and osmotic inhibition

Inhibitor substances like cyanide affect seed respiration and inhibit germination [40]. Inhibitors hinder seeds' metabolic pathways and inhibit germination by affecting seed respiration. Seed respiration is greatly affected by less gibberellic growth hormone and more abscisic growth hormone within the seeds [23]. Growth hormones like gibberellic and cytokinin have the opposite action compared to inhibitors [41]. Moreover, phenolic compounds, coumarin and duromine are classified as natural germination inhibitors due to their substantial participation in the non-germination of seeds [42]. Osmotic inhibition is the ability of substances that produce a high osmotic pressure to inhibit germination. Salts and sugars can compete with seeds for water absorption, thus preventing seeds from absorbing water and inhibiting germination [27].

3.3 Morphological dormancy

Morphological seed dormancy is due to intrinsic seed properties, underdeveloped embryo and rudimentary embryo developed in the seed coat [43, 44]. Environmental factors available during seed development and maturity impact the length of genetic dormancy [26]. Embryo generally matures within a month of incubation after imbibition. Morphological dormancy is eliminated by physiological processes such as embryo maturation, growth regulators' response, temperature fluctuations and light exposure. Example: Apiaceae.

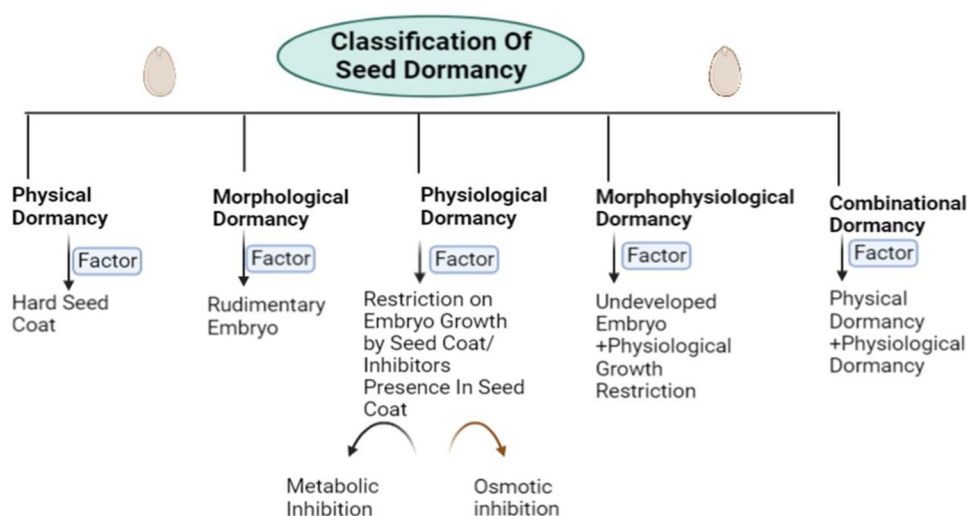
3.4 Morpho-physiological dormancy

This dormancy is a combination of physiological and morphological dormancy types [10, 45]. Therefore, the physiological part of morphophysiological dormant seeds requires exposure to appropriate conditions viz. warm and cold stratification, to allow embryo growth and maturation before seed germination [45]. Example: Podophyllaceae, Ranunculaceae, Dilleniaceae, Haemodoraceae and Stylidiaceae.

3.5 Combinational dormancy

This dormancy is a combination of two factors: physical dormancy and physiological dormancy [46]. Thus, the seed coat is water-impermeable and the embryo is physiologically inactive. The scarification process is performed to enable gaseous and water exchange in these dormant seeds [47]. Hence, seeds with this type of dormancy require a few weeks of cold stratification before germination [48]. Example: Fabaceae (Fig. 1).

Fig. 1 Schematic diagram of seed dormancy classification



4 Techniques to overcome different classes of dormancy

4.1 Physical seed dormancy

An imbibition test is used to distinguish between physically dormant and non-dormant seeds. Scarification is one of the important ways to overcome physical dormancy [36]. Further, there are three methods of scarification viz. physical, mechanical and chemical scarification. Scarification is a process that breaks seed dormancy by reducing the restrictions imposed by the seed coat [49]. Nicking can create tiny holes (e.g. using a knife) in the seed coat and make water movement inside and outside [50]. In normal conditions, soil heating and chilling microorganism activity, natural forest fires and other events can break primary seed dormancy [10]. To overcome the effect of an impermeable seed coat, mechanical scarification can be performed by rubbing seeds between two pieces of sandpaper, abrasive sand or with severe shaking [51]. Cooling, heating, extreme temperature changes, piercing the coat of seeds with a needle, dipping seeds in boiling water for a short time and exposure of seeds to intermittent wavelengths are important techniques to make the seed permeable to air and water [52]. Chemical compounds are also used to remove the hard seed coat. Acid treatments are done at a specific temperature and period to remove thick, impermeable seed coat [53]. Depending on the species, acid should be used at room temperature for a few minutes to several hours [49]. Thus, seeds should only be immersed in acid for a short time, as excessive stirring generates a higher amount of heat and blackens them. Enzymes like cellulase and pectinase are present in the seed coat and play a significant role in the breakdown of physical seed dormancy [27].

4.2 Physiological and morpho-physiological dormancy

This type of dormancy can be overcome by hot or cold temperature treatments individually or in combination with stratification and drying after ripening [10, 32]. Presowing seed treatments with gibberellaic acid, moist stratification for weeks and months at warm temperatures of 15 °C or cooler temperatures of 0 to 10 °C can overcome this dormancy [43, 54]. Seeds from arid and Mediterranean climate zones require an after-ripening period and warm stratification. Warm stratification altered the ratios between growth promoters and inhibitors, allowing germination and performed in dry soil during the hot temperatures of late spring and summer. Growth promoters are present within the embryonic axis and growth inhibitors are found in the cotyledons endosperm or other food-storing tissues. Many of the seed germination inhibitor compounds are water-soluble and therefore can be leached from seeds by water soaking [55]. Winter temperatures (between 5 and 10 °C) regulate the shift between the inhibitors and growth promoters [56]. Example: Dasypogonaceae, Iridaceae and Pittosporaceae. The following seed treatments are used to overcome seed dormancy.

4.2.1 After ripening and precision nicking

An after-ripening period is required to alleviate physical dormancy [57]. Seeds are incubated at different warm temperatures for germination at regular intervals [58]. Morphophysiological dormant seeds are often stored at 20–30 °C and at higher temperatures of 40–80 °C for faster germination with controlled seed moisture content [30]. Examples: Apiaceae, Asteraceae, Brassicaceae, Haemodoraceae, Poaceae, Solanaceae and Stylidiaceae.

4.2.2 Precision nicking

Seeds with these dormancy types can be overcome by nicking and piercing with a needle. Thus, precision nicking removes the mechanical barrier imposed on embryo growth and the nearby tissues. The location and depth of nicking are species-specific [59]. Generally, the testa is breached, and the nicking is undertaken at the radicle end of the seed without damaging the embryo [60]. This technique also effectively removes combinational dormancy, making seeds water-permeable and softening seed coats for germination [43] (Fig. 2).

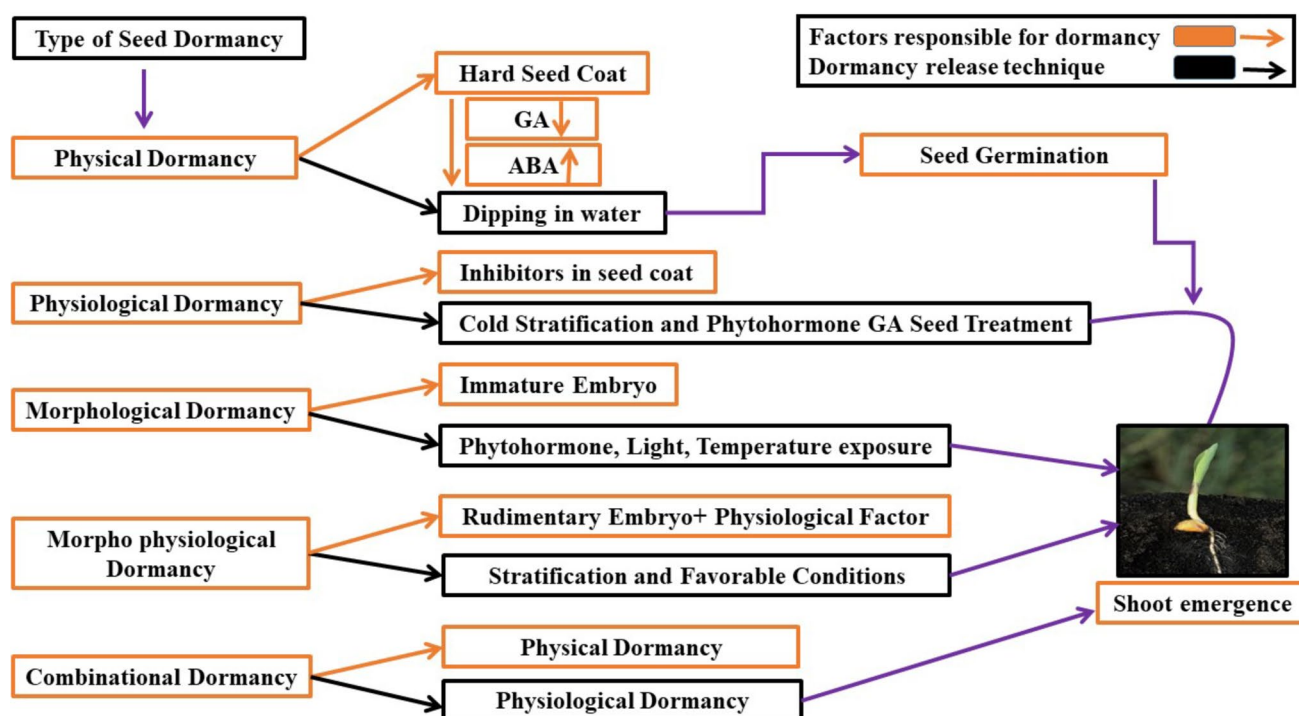


Fig. 2 Seed dormancy: types, causes and methods of alleviation

5 Physiology and contribution of hormone in seed dormancy

Seed dormancy and germination are greatly affected by hormones [61]. In an antagonistic relationship gibberellins and abscisic acid (ABA) are important regulators for seed germination or dormancy and chemical equilibrium between these two acts differently in diverse environments during the seed development phase [62]. Seed dormancy is caused by an increased abscisic content during the seedling growth phase [63]. Gibberellic hormone biosynthesis mimics the effects of exogenous abscisic in various crops throughout seed development [42]. There is a distinct transference toward increased GA biosynthesis in addition to abscisic deprivation, which reduces the ratio of abscisic to gibberellic [64]. Increase in abscisic: gibberellic fractions enhance dormancy preservation, while increased gibberellic biosynthesis indicates a direct transference towards seed dormancy release. ABA depletion leads to a decline in the abscisic: gibberellic content ratio due to transcription and less protein synthesis ability. Establishing a functional hormonal balance and catabolism ensures the stability of the ABA and gibberellic ratio, triggering seed development. The lower abscisic and higher gibberellic levels are required to break seed dormancy. On the other hand, seed tissues influence germination in different ways. Abscisic is synthesized in the endosperm tissue of seeds that have coat-imposed dormancy. The transporters signal in seeds export abscisic from the endosperm into the embryo. When gibberellic enters the aleurone layer of the endosperm it causes the breakdown and mobilization of endospermic carbon reserves and that reserve consumed by embryo during germination in seeds. Metabolic activation vacuolization and reserve mobilization of endosperm cells are the initial visible indications that dormant seed turns into germination. The embryo cells grow rupturing the testa endosperm and emerge as radical [65].

6 Important RET (rare, endangered, and threatened) medicinal plants of western Himalaya and their seed dormancy behavior

6.1 *Saussurea costus*

6.1.1 Importance and distribution

Saussurea costus (Falc.) Lipsch. Syn. *Saussurea lappa* (Decne.) Sch. Bip. commonly known as Kuth and belongs to the family Asteraceae. It is an endangered medicinal herb [66] native to the western Himalayas and grows on steep slopes between 2600 and 4000 m (amsl). The species is most commonly used for treating rheumatism, leprosy, stomachaches toothaches, colds and coughs [67]. Its roots are used as an antiseptic to treat bronchial asthma [68] and root oil is used in high-end hair oils and perfumes [69]. It is distributed from eastern Afghanistan (north-western Himalayas) to Nepal (central Himalayas) [70]. The genus *Saussurea* comprises 410 species, which are found in Eurasia, Australia and America. Its maximum species are found in India [71]. *S. costus* is under cultivation in the Lahaul valley of Lahaul and Spiti, few other locations viz. Kinnaur, Chamba and Kullu districts of Himachal Pradesh (2600–3300 m amsl) and Nanda Devi Biosphere Reserve of Uttarakhand (2000–3500 m amsl).

6.1.2 Morphology

It is a perennial erect pubescence herb that can grow to a height of 2 m. Basal leaves are long triangular and stalked. Top leaves are bigger hanged soft with a tiny hair and long petioles and have a pavilion base [72]. The inflorescence is the head type with purple sessile axillary and terminal flower clusters. Its bract is involucre oval-lanceolate and acuminate [73]. Fruit is achene compressed, curled upward and greyish in the shade. Seeds are 2.85 mm broad and 9.01 mm long [69].

6.1.3 Seed dormancy and germination

S. costus exhibits physiological dormancy as the dormancy is overcome by chilling [74]. The techniques like application of stratification light or after-ripening in seeds overcome physiological dormancy and induce seed germination [75]. Seed treatments like vernalization phytohormone and mechanical scarification enhance dormant seed germination [76–78]. For better germination and to prevent seed viability, *Saussurea* seeds should be stored at low temperatures and sown in field conditions within 18 months of collection [75]. The stored seeds' viability significantly influences the germination rate [79, 80]. The seeds of *S. costus* generally have good viability, and no losses occur for eight months [71]. The decrease in germination may be due to seed deterioration with age or the onset of secondary dormancy [81, 82]. *S. costus* needs a temperature near 5 °C to the resting stage [26, 33]. All seed quality parameters viz., plant growth and root production are enhanced when seeds are collected during September and October [75].

6.2 *Inula racemosa*

6.2.1 Importance and distribution

Inula racemosa Hook.f. syn. *Inula royleana* C.B. Clarke commonly known as pushkarmool or manu belongs to the Asteraceae family. It is a critically endangered species due to its habitat destruction and exploitation. It is used as a rejuvenator, expectorant and immunological modulator and to cure rheumatism, antispasmodic, hypotensive, cardiac, asthma, liver problems, lung problems, antiseptic, heart, derma and stomach problems [83, 84]. Its roots are used to lower blood sugar and cholesterol [85]. It is distributed in China, Europe and India [86]. In India, from temperate to alpine western Himalayas Kashmir to Kumaon and Afghanistan to Nepal [2]. It grows at an elevation from 1500 to 4200 m. It has three types of species viz., *I. racemosa*, *I. obtusifolia* and *I. angustifolia* [87]. Among all three spp., only *I. racemosa* is cultivated at a high altitude in the western Himalayas from 2400 to 3600 m amsl.

6.2.2 Morphology

I. racemosa is a 2 m tall perennial upright, vigorous, pubescent herb. All the vegetative parts are scabrid and tomentose, and the stem is herbaceous, thick, rough and grooved. Basal leaves are broader and lower leaves are narrower. Leaves are long basal stalked, triangular rough and hairy. Upper leaves are lanceolate and clasp the stem [88]. Radical leaves are broad, elliptical-shaped and stalked. Cauline leaves are smaller, oblong and semi-amplexicaul in shape. The abaxial laminal face is densely tomentose. Flowers are compact head-type hermaphrodite sessile and purple. The inflorescence type is a terminal raceme with yellow flowers with purple centres [89]. Outer bracts have broad triangular tips bent back and inner bracts are linear and sharp-pointed. These are on an apical spike-like cluster [71, 90]. The stem leaves are oblong half-clinging and usually slightly curved at the base. The fruit is cylinder, hairless, and circular, with an 8 mm long reddish pappus. The roots are fusiform, irregular yellow on the inside, and dull brownish outside with a sweet and camphoraceous odour and a bitter taste [91].

6.2.3 Seed dormancy and germination

Uniform seed germination is the primary cultivation practice to design production methods and rebuild natural populations of *I. racemosa* [88]. The seeds of *I. racemosa* have physiological dormancy [92]. It requires illumination for seed germination as there was no germination in the dullness. Pre-sowing treatments help enhance seed germination and shorten the mean germination time [93]. Phytohormones ensured the highest germination [32]. Gibberellins are primarily used to improve the germination percentage of plant seeds with low-temperature requirements. The application of GA₃ breaks the seed dormancy with low chilling requirements and attains higher germination [94]. Chilling alone was also recorded to enhance germination compared to the application of phytohormones [95]. After harvesting seed should be dried in the sun and stored in an airtight container to maintain seed viability. The maximum germination was recorded when sowing was done in June at 25 °C while low seed germination was recorded at 20 °C and found that seed germination occurs after 13 days of incubation at 25 °C [96, 97].

6.3 Sinopodophyllum hexandrum

6.3.1 Importance and distribution

Sinopodophyllum hexandrum (Royle) T.S. Ying syn. *Podophyllum hexandrum*, commonly known as Mayapple, is an endangered perennial shade-loving rhizomatous herbaceous plant. It belongs to the Podophyllaceae lineage besides originates from lower elevations of the Himalayan region [98, 99]. *S. hexandrum* is endangered because of the whims of weather, poor seed germination, low seedling survival, poor seed setting and unethical medicine industry exploitation [100]. The traditional Ayurvedic and Chinese medical systems refer *S. hexandrum* as a divine drug. It is used to cure several disorders like warts and skin tumorigenesis [101, 102]. Its roots and rhizomes are highly prized for their anticancer, anti-mitotic and immuno-stimulatory characteristics because they are rich in podophyllotoxins [103]. It also exhibits free radical scavenger provocative, antimycotic, immunosuppressive and harmful radiation shielders [104]. It is spread out across the lower elevations of the Himalayas between 2000 and 4500 m amsl including Bhutan, Pakistan, Afghanistan, Nepal, Taiwan, China, Virginia and North Carolina as well as the Central United States [105, 106]. In India, it is grown in highly restricted areas of inner Himalayan, greater Himalayan and trans-Himalayan and the Himalayan regions of Sikkim and Arunachal Pradesh [100, 106]. *S. hexandrum* grows in association with other plants, often in humus-rich soil, scrub woods and alpine meadows [87]. The plant has been extensively exploited to extract crude drugs [106]. India's National Medicinal Plant Board has started working to conserve these highly valuable rare, endangered and vulnerable medicinal plant nationwide [107].

6.3.2 Morphology

S. hexandrum is an upright perennial plant 15–40 cm tall. It has a low-growing glossy-green grand node rhizome with several adventitious roots. Branches are stiff and leaves are dark green with brown splotches and umbrella-shaped [108]. In spring, plump round red berries accompany white or light pink and 6-petal blooms at the tips of sturdy stalks [98].

Inflorescence comprised of hexapetalous and triadelphous giving its species the name “*hexandrum*” which means “six stamens”. The average seed weighs 20.0 g [109]. Fruits are of berry type with many seeds embedded in the pulp [101]. It can be multiplied by sexual or asexual reproduction (underground stem division) [98, 102].

6.3.3 Seed dormancy and seed germination

The endosperm or seed coat imposed the dormancy in *S. hexandrum* [110] and the seed germination rate is slow and inconsistent. Seeds mature and germinate during the favourable warm season (20–25 °C), taking about 53 days to complete germination [111]. *S. hexandrum* exhibits an inferior progeny generation and more remarkable quiescence for 2 years [97]. Cold treatment may increase germination by enhancing gibberellic acid sensitivity or stimulating its production [97]. Chilling has a stimulating impact on seed germination-promoting substances and a decrease in the abscisic acid breakdown [95, 112]. Similarly, hydropriming significantly improved seed germination by softening the thick, hard seed coat and facilitating the leaching of germination inhibitors. Treatment of fresh seeds with GA₃ breaks down the physiological dormancy and results in the highest germination after 80 days [113]. Osmo conditioning has been shown to improve seed germination by promoting fast seedling emergence [114].

Hypocotyl dormancy is another major problem that inhibits seedling growth and establishment [113]. However, hypocotyl dormancy helps this plant to survive in the Himalayan alpine environment [97, 115]. Manual removal of cotyledonary leaves can overcome hypocotyl dormancy, but this practice requires more time and labour. Therefore, the treatment of cotyledonary leaves with GA₃ @ 200 ppm easily overcomes the hypocotyl dormancy in *S. hexandrum* [115]. Hypocotyl dormancy of seedlings can also be broken at 10 °C temperature [111]. Similarly, acid scarification and GA₃ achieved the greatest germination percentage after 25 days of seed treatment [102]. Pre-sowing freezing water dipping and synthetic sterilization with benzene dichloromethane or liquid bleach may improve germination percentage [116]. In field conditions, maximum seedling emergence and survival occurred when seeds were sown in a combination of soil FYM and coco peat (2:1:1) [117]. Harvested seeds should be packed in a sealed container and maintained under ambient temperature to preserve sprouting capacity and viability [118]. The germination of seeds decreases with time and storage [119] due to morphological, biochemical and genetic changes [120]. *S. hexandrum* loses its strength and germination capacity in 1 year [6, 120].

6.4 *Bunium persicum*

6.4.1 Importance and distribution

Bunium persicum (Boiss.) B. Fedtsch. also known as kala zeera or shahi zeera, is a valuable herbaceous spice belonging to the Apiaceae family. Plant parts are used in cooking, blooming fragrances and flatulence relievers. It contains an essential oil high in terpenoids and phototoxic furanocoumarins [121]. It can potentially be used as a natural antioxidant and preservative in various food systems and in novel food applications such as the formulation of bioactive films/coatings for extending food product shelf life [122]. It can potentially be employed as a free radical absorber and stabilizer in various dietary processes and unique industrial purposes, such as producing organic substances to increase the longevity of food products [122]. It is cultivated naturally and obtained from middle Asiatic Persia, Egypt, Asian countries and the northern Himalayan region [123]. It grows naturally under alpine and subalpine habitats in the north-western Himalayan grove pastoral plains and lesser quantity can be obtained from low altitudes regions, especially Lahaul & Spiti, Uttarakhand and Srinagar at 1800 to 3500 m amsl [121].

In general, *B. persicum* is propagated through seed and takes 2–3 years for first flowering [124]. The long juvenile period of *B. persicum* and the effects of seasonal fluctuations pose the most difficult challenges in propagation [121]. Seeds initiate the germination process after the end of the winter season and produce two cotyledonary leaves during the last fortnight of March and the first fortnight of April [125–127]. The first year's growth is limited to forming small bulbs after which the cotyledonous leaves wither or dry out [128]. This bulb grows over time to produce larger bulbs and the flowering stage begins in the third year [129].

6.4.2 Morphology

The plants are small to tall (about 80 cm), thickly covered with a canopy, bulbous and everlasting [130]. The leaves are filiform and freely pinnate [2, 3]. Inflorescence is conical stalked and emerging from the same disc floret. Fruits

are indehiscent one-seeded carpel [121]. Shizocarp comprises several seed carpels. These seed carpels are adhered to terminal spikes and include spherical or oblong secretory channels. The internodal region of the stem is often hollow with ducts containing gums and flavonoids [131]. The flowers are small, white, and present in compact umbels with readily symmetrical small sepals, petals and stamens [121]. Bracts are linear, sometimes divided and lack bracteoles. The gynoecium comprises two pistils with syncarpous and meso ovary [132]. The test weight of seeds is approximately 2 g [133].

6.4.3 Seed dormancy and germination

B. persicum seeds have physiological type dormancy due to the presence of a dormant embryo and are overcome by stratification and accumulation of growth promoters and shifting the hormonal balance in seeds [134]. Cold stratification for approximately eight weeks at 39.2 °F using or not using phytohormone is required to generate significant sprouting [135]. Cooling temperature strategies have played a vital role in the germination of dormant seeds [136]. Cold stratification of seeds for three weeks at 37.4 °F leads to seed germination and higher seed germination in one and a half months [137]. Judd et al. [131] discovered that kinetin or benzyl adenine (10^{-5} mM) and a chilling temperature of 39.2 °F for 30 days improved seed germination. Phytohormones like KNO_3 carbamide GA_3 cytokinin and chilling temperature resulted in significant sprouting [138]. Nitrogenous compounds increased the activity of GA_3 ethylene and cytokinins and enhanced seed germination [139]. Therefore, stratification and phytohormones seed treatment plays an important role in the germination of *B. persicum*.

7 Conclusion

S. costus, *I. racemosa*, *S. hexandrum* and *B. persicum* are important endangered medicinal plants of the high-altitude region of western Himalayas. These crops have a great demand in the pharmaceutical industries. Seed dormancy is one of the major problems in cultivating these crops in the farmers fields. Seed dormancy enables seeds to survive in the unfavorable environment. Dormancy is regulated at various developmental stages through interactions with environmental factors. Several processes have been identified as being involved in the induction of dormancy and the transition from the dormant to the germinating state of seeds. The above discussion concludes that most high-altitude medicinal plants have physiological, morpho-physiological and hypocotyl dormancy. Chilling is essential to release inhibitors and break the dormancy of high-altitude crop species. Unlike chilling treatment, dormancy can be overcome by shifting the balance between growth inhibitors and growth-promoting hormones. Stratification is one of the important techniques used to overcome the dormancy of these plants. This review may help in the successful cultivation and conservation of these medicinal plants in the western Himalayan region.

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