

## **Pre-sowing treatments on seeds of forest tree species for overcome the germination problems**

### **Abstract**

Production of the quality planting material has get more emphasis under sub-mission on agroforestry (SMAF) with aiming to increase tree cover outside the forest area. Establishment of the tree species depends on the quality of the planting material, available soil, water for irrigation and the adopted protection measures. Most of the tree species get problem with germination of seed due to external and internal factors and causes seed dormancy. Different kind of pre-sowing treatments were tested and applied for the different kind of tree species by researchers of the forestry. Scarification (mechanical, acid), water soaking (hot/cold), the application of chemicals and plant growth regulators, or alternate wetting and drying prior to sowing effectively break seed dormancy and improve seed germination for producing required quality planting material. Auxins (IAA, IBA, 2-4D, 4-CPA), Gibberellic acid (GA3), Cytokinins (Kinetin, Zeatin, Benzyl adenine), Ethylene (Ethereal) and Abscisic acid (Dormins, Phaseic Acid) are plant growth regulators also used in different concentrations for improving tree seed germination.

**Keywords:** Pre-sowing treatment, seed dormancy, plant growth regulators, growth hormones, stratification, and scarification.

### **Introduction**

Plantation of the forest tree species has emerged as a crucial initiative in forestry sector to meet the increasing demand for timber, non-timber forest produce (NTFP), fodder, fuel wood etc. both from natural forests and plantation forestry. Establishment of plantations recognizing the current need for sustainable alternatives for depleting natural forests, and gained significance for industrial and domestic wood production on a large as well as small scale. The vital decision in initiating a plantation project is the selection of the suitable tree species. The availability of high-quality planting material stands as a significant determinant influencing the choice of species for large-scale afforestation. Therefore, the nursery practices of the suitable tree species is the key for successful establishment of plantations. A critical concern in this context is the insufficient awareness regarding suitable pre-sowing seed treatments for various tree species. This knowledge gap poses a significant obstacle to the mass production and conservation efforts of many species. Addressing this concern necessitates focused research into innovative seed technologies, considering the growing importance of this domain in the global crop protection markets, as emphasized by Sharma *et*

*al.* (2015). Thus, a profound understanding of seed science, encompassing aspects such as seed collection, storage, viability, and germination, becomes imperative for enhancing the success of restoration endeavours.

Seed germination constitutes the process through which the embryo evolves into a seedling under favourable conditions. It involves a series of physiological, biochemical, and morphological changes that collectively lead to germination. This intricate process can be delineated into three major phases: Phase I, characterized by rapid water imbibition; Phase II, dominated by the reactivation of metabolism, cell elongation, and chitting; and Phase III, marked by rapid cell division coinciding with radicle growth. Phase II, in particular, is pivotal, as it involves the reactivation of crucial physiological and biochemical processes such as the hydrolysis of food reserves, macromolecular biosynthesis, respiration, reorganization of subcellular structures, and cell elongation, all of which play a fundamental role in initiating germination (Bonsager *et al.*, 2010).

Seed germination can be classified into two types based on the outcome of the cotyledons:

1. **Epigeal Germination:** This process involves the cotyledon being pushed out of the soil due to the rapid growth and elongation of the hypocotyl, as seen in species like *Acacia*, *Adenantha*, *Albizia*, *Cassia*, *Dipteryx*, *Diphysa*, *Erythrina*, *Gliricidia*, *Haematoxylum*, *Hymenaea*, *Hymenolobium*, *Parkia*, *Parkinsonia*, *Pterocarpus*, *Samanea saman*, *Sclerolobium*, *Tamarindus*, *Casuarina*, *Annona*, *Cordia alliodora*, *Myrcia*, *Capparis*, *Cedrela*, *Melia*, *Zizyphus*, *Anacardium excelsum*, *Jacaranda*, *Ulmus*, *Ilex* and *Elaeocarpus*.
2. **Hypogeal Germination:** In this type, cotyledons remain below the soil due to the rapid elongation of the epicotyl. This predominantly occurs in monocotyledonous seeds, E.g. *Calophyllum*, *Swartzia*, *Syzygium*, *Swietenia*, *Prunus*, *Sapindus*, *Terminalia catappa* etc.

Seed germination and quality are influenced by both internal and external factors. Genetic makeup, seed viability and dormancy are internal factors inherited from parent plants, influencing germination potential and subsequent growth. Seed source variations among species can significantly affect seed quality and growth (Kumar *et al.*, 2022; Kumar *et al.*, 2021). External factors consider the growing environmental conditions, including temperature, moisture, light, and soil properties, also influence germination and seedling establishment (Penfield *et al.*, 2005; Srivastava, 2002). Additionally, external interventions such as seed priming, scarification, and chemical treatments can modify seed coat properties, break dormancy, or enhance seedling growth. The interaction between internal genetic factors and external environmental conditions is paramount in determining the success of seed germination and subsequent seedling establishment. This review specifically focuses on pre-sowing treatments for seeds of forest tree species to address germination challenges.

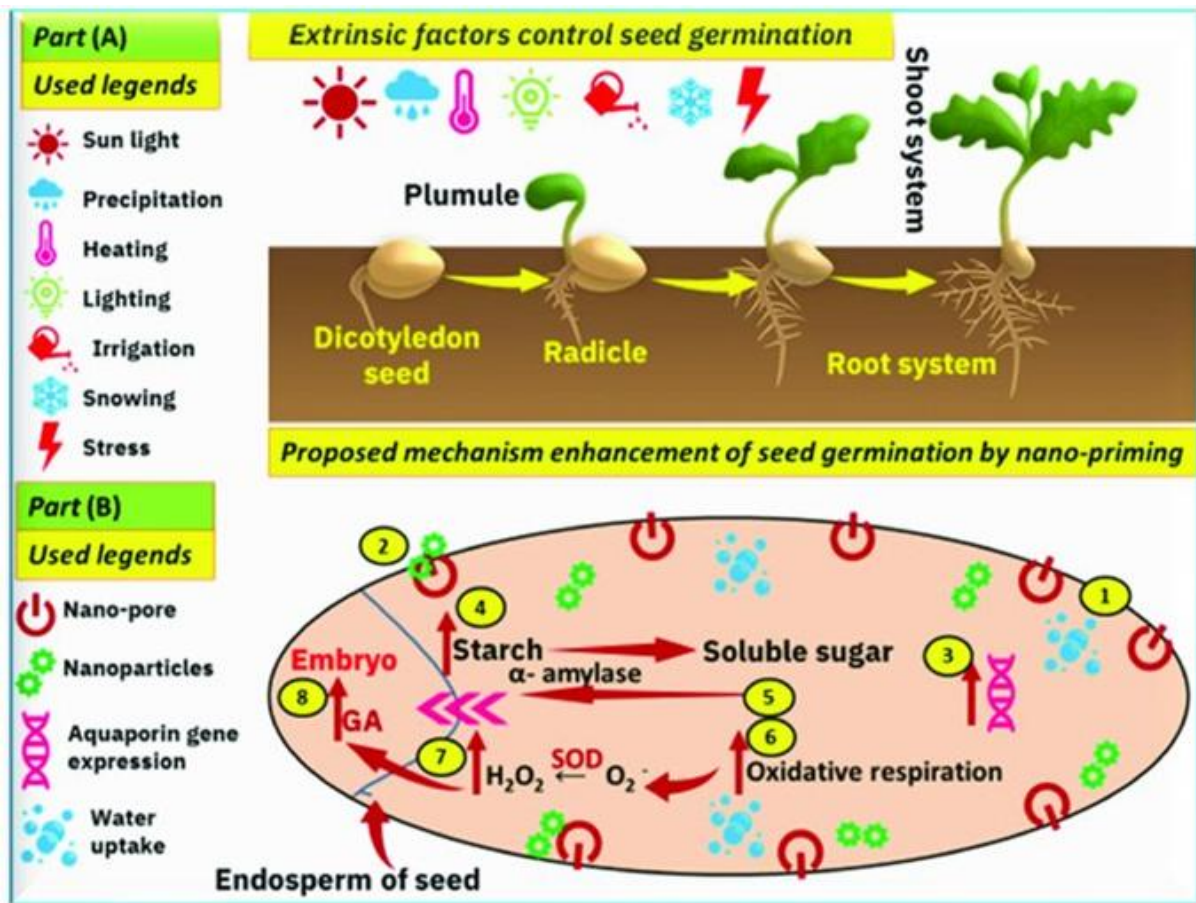
## **The Mechanism of Seed Germination**

Germination, a complex biological process, initiates with a crucial step known as imbibition, where the seed absorbs water. Following imbibition, activated enzymes commence the breakdown of starch into sugars, providing nourishment to the embryo. The initial sign of germination is the swelling of the radicle, marking the onset of growth. The duration for seedlings to emerge varies based on seed size. Species with large seeds possess substantial food reserves, enabling them to germinate at greater depths and extend their epicotyl to the soil surface. Conversely, seedlings of small-seeded species emerge more rapidly, typically closer to the soil surface.

The germination process can be categorized into epigeous and hypogeous types. In epigeous germination, the hypocotyl elongates, and the cotyledons rise above ground. On the other hand, hypogeous germination involves the elongation of the epicotyl, with the cotyledon(s) remaining belowground. Epigeous species, like beans and onions, witness the cotyledons turning green and engaging in photosynthesis until they eventually detach. In contrast, hypogeous species, such as peas and corn, exhibit cotyledons that remain belowground throughout the germination process. This intricate interplay of imbibition, enzymatic activity, and seedling emergence underscores the diverse strategies employed by plant species during germination, influenced by factors like seed size and environmental conditions.

## **Factors Affecting Seed Germination**

Seed germination can be influenced by a combination of internal and external factors (Figure 1). Genetic characteristics, seed viability and dormancy are internal factors inherited from parent plants, impacting germination potential and subsequent growth. External factors encompass moisture, temperature, oxygen, light, and soil conditions. Conversely, internal factors influencing the germination process include the embryo maturity of the seed, seed viability, and seed dormancy. Understanding and manipulating these factors are crucial for optimizing the germination process in tree seedlings.



**Figure 1: Factors influencing seed germination and quality**

**Source:** adopted from El-Ramady *et al.* (2023)

## Seed Dormancy

Seed dormancy, the physiological state preventing a viable seed from germinating even under favourable conditions (Umarani and Vannangamudi, 2005; Willan 1985; Baskin and Baskin, 2004), exhibits considerable variation in storage duration among species. While some seeds can be stored for several months, others rapidly lose viability. Comprising an outer protective coat and an inner embryo developing into the plant, seeds initiate germination when water permeates the seed coat, resulting in swelling and subsequent plant growth.

## Types of Dormancies

1. *Physiological Dormancy:* Predominantly observed in species like Fagus, Pinus, and eucalyptus, physiological dormancy is initiated by either the embryo or surrounding endosperm tissues. Freshly matured seeds with non-deep physiological dormancy either cannot germinate at any temperature or only germinate over a narrow temperature range.
2. *Morphological Dormancy:* Seeds with underdeveloped embryos, requiring time for growth and germination (Umarani and Vannangamudi, 2005; Finch and Leubner 2006).
3. *Physical Dormancy:* Seed coats impermeable to water due to a layer of thick-walled, cutinized palisade-like macroscleroid cells. Various treatments, such as mechanical and

chemical scarification or soaking in boiling water, are employed to induce germination in water-impermeable seed coats. For water-permeable seed coats, cold stratification, treatment with gibberellic acid (GA<sub>3</sub>), or sodium hypochlorite, scarification, or heat treatment can be applied (Baskin and Baskin, 2020).

Various methods have been employed to induce germination in seeds with water-impermeable seed coats, including mechanical scarification, chemical scarification, and soaking in boiling or hot water. Conversely, for seeds with water-permeable coats, a range of treatments can be applied, such as cold stratification, treatment with gibberellic acid (GA<sub>3</sub>), or sodium hypochlorite, along with scarification or heat treatment (Baskin and Baskin, 2020). Some seeds naturally overcome dormancy over time, while others require specific pre-treatments. Seed dormancy poses a significant hurdle to germination, necessitating pre-sowing treatments. Techniques like scarification (mechanical, acid), water soaking (hot/cold), the application of chemicals and plant growth regulators, or alternate wetting and drying prior to sowing effectively break seed dormancy.

**1. Breaking Seed-Coat Dormancy:** Preferred procedures to overcome seed-coat dormancy include puncturing or scarifying the seed coat through methods like piercing, nicking, chipping, or filing with a knife, needle, or sandpaper.

**2. Breaking Embryo Dormancy:** Several recommended treatments exist to overcome embryo dormancy:

**a)Pre-chilling (Cold Stratification):** Seeds are placed in containers on a moistened germination substrate and kept at 3°-5°C in a refrigerator for 7-14 days. Seeds derived from cold-climate species, particularly perennials accustomed to prolonged snow cover and harsh winter conditions; necessitate significantly lower temperatures over extended periods for effective stratification. An illustrative case involves a perennial herb adapted to the extended cold winters of mountainous regions, demonstrating enhanced germination rates. Specifically, exposure to a low temperature of 32 °F (0 °C) for a duration of 7-months resulted in a notably higher germination rate, reaching 60% (Cuenca-Lombrana *et al.*, 2018).

**b)Treatment with Hormones:** Dormancy can be broken by directly applying the plant hormones: abscisic acids (ABA), gibberellin (GA<sub>3</sub>), ethylene, and jasmonic acid (JAS). Gibberellin (GA<sub>3</sub>) is the most effective in inducing germination (Shu *et al.*, 2016). To assess germination, germination test paper is dampened with a 0.05% gibberellic acid (GA<sub>3</sub>) solution, prepared by dissolving 500 mg of GA<sub>3</sub> in 1 litre of water. The mechanism involves softening the endosperm, promoting embryo growth, and facilitating seedling emergence by breaking through the seed coat.

**c)Chemical Scarification:** Seed impermeability, governed by the seed coat, can be addressed through chemical scarification. The impermeability of seed coats to water poses a challenge

for the germination of seeds from tree species in dry and semi-arid regions (Kheloufi *et al.*, 2018). Dormancy can be overcome by soaking seeds in chemicals such as acidic or basic solutions, organic solvents, alcohol, disinfectants, or boiling water. Common acids employed for scarifying seeds include phosphoric acid (25 to 75% concentration depending on the seed type), potassium nitrate, nitric acid, and sulphuric acid (96%).

For potassium nitrate treatment, a 0.2% solution is prepared by dissolving 2 g of potassium nitrate (KNO<sub>3</sub>) in 1 litre of water. This solution is used to moisten the germination paper at the initiation of the test. This specific treatment aids in seed scarification, facilitating water absorption and breaking dormancy barriers for improved germination outcomes.

**d)Light Treatment:** Exposing seeds to strong cool-white and infrared light mimics the natural environment during spring and summer. The light source, close to the seeds, should be on for at least 8 hours daily in a moist environment under low temperature. After light treatment, seeds should be incubated in the dark until germination. Positive results have been observed with both cool-white and red light, while germination rates are lower in blue and green light (Penfield *et al.*, 2005; Liand Khan, 2005).

The role of light varies across different seed sizes. Generally, medium and large-sized seeds exhibit a neutral photoblastic nature, allowing them to germinate in both light and darkness. The germination process for these seeds is primarily governed by factors such as soil moisture and temperature, with light having minimal influence. Conversely, some seeds, termed negatively photoblastic, necessitate darkness for successful germination. Notably, sizable seeds, approximately 1 inch (2.5 cm) in length, fall into this category, demonstrating a preference for germination in the absence of light. For this species, exposure to light impedes germination, attributed to the presence of phytochrome A in the seeds. Phytochrome A plays a crucial role by diminishing the production of germination-stimulating enzymes upon exposure to light (Goggin *et al.*, 2011).

On the other end of the spectrum, tiny seeds, measuring less than ¼ inch (6 mm), present distinct light-dependent germination requirements. These little seeds typically mandate a minimum of 8 hours of light exposure to initiate germination. This need arises from their limited food reserves, requiring efficient utilization for soil penetration and light access. Notably, certain vegetable and herb seeds fall into this category, relying on light to break seed dormancy. The mechanism involves photoreceptors, specifically phytochrome B, which responds to light stimuli. Phytochrome B acts by suppressing the production of abscisic acid, a key dormancy-maintaining factor, and simultaneously promoting the synthesis of gibberellins hormone (Leite and Takaki, 2001; Socolowski and Takak, 2004; Attri *et al.*, 2015), thereby facilitating germination in these small-sized seeds.

e) **Hot or Cold Water:** This is an economical and farmer-friendly practice was identified for enhancing germination. This treatment, applied to most medium-sized dry seeds, improves germination when seeds absorb adequate water and soften the seed coat. Heat treatment is moderately effective in breaking dormancy. The study suggests 1-30 minutes of acid scarification and one-hour (55-77°C) heat treatment for optimizing germination based on species (Koutouan-Kontchoi *et al.*, 2020). Seeds require soaking in water for 24-48 hours, serving to soften hard seeds and leach out chemical inhibitors (Umarani and Vanangamudi, 2005).

f) **Stratification method (warm and/or cold)** - Stratification is the process of breaking down the hard outer coating of a seed to promote germination. This process involves exposing the seed to a moist environment, which softens the outer shell, making it easier for the seed to sprout. There are two types of stratification: cold stratification and warm stratification (D'Esteet *et al.*, 2019).

g) **Scarification**- Scarification is the process of breaking or weakening the hard outer coating of a seed to promote germination. This process involves physically damaging the seed coat by scratching, nicking, or rubbing it with sandpaper or a file. Scarification is commonly used for seeds that have a hard outer coating that prevents water and air from reaching the embryo (D'Esteet *et al.*, 2019).

### **Example of Seed Pre-Treatment for Improved Germination**

**Soaking in Cold Water:** Soaking seeds in cold water for 24 to 48 hours prior to sowing has been explored as an effective pre-treatment method. According to Hossain *et al.* (2005), *Terminalia chebula* seeds exhibited the highest germination percentage (66.7%) with a 48-hour cold-water treatment, further increasing to 73% when de-pulped before the cold-water treatment. A study by Viswanath *et al.*, 2021, on *Terminalia paniculata*, also supported the positive impact of soaking seeds in water for 24 hours. Soaking *Jatropha* seeds in water overnight proved effective for enhancing germination (Sunil *et al.*, 2016).

**Soaking in Hot Water:** For seeds with hard coatings, soaking in boiling hot water has proven to be an advantageous method. Commonly applied to seeds with robust shells like *Albizia*, *Acacia*, *Cassia*, *Poplars* and *Leucaena leucocephala*, this treatment involves soaking seeds in hot water for 2-48 hours, depending on the species. Compared to traditional methods like sulphuric acid, hot water treatment is considered a better, safer, and more cost-effective alternative. Notably, *Subabul* seeds soaked in hot water for 12 hours exhibited a significantly increased germination percentage (42.22%) (Hamad and Anwer 2021). Similarly, *Sapindus mukorssi* seeds soaked in hot water for 10 seconds achieved the highest germination rate (72%). For *Acacia mearnsii*, immersion in hot water at 80°C, either for 5 minutes or simply

at 80°C, was found to be the most optimum treatment, resulting in total germination rates of 83 % (São José 2019) and for *Acacia auriculoformis* (Azad *et al.*, 2011), respectively.

**Alternate Wetting and Drying:** This method proves effective for seeds with thicker seed coats (Dwivedi, 1993). Gupta and Pathanath (1975) reported that teak seeds from certain localities require no treatment, while others got benefit from alternate wetting and drying procedure. Similarly, Pamei *et al.* (2017) found that *Tectonagrandis* seeds subjected to alternate wetting (12 hrs) and drying (12 hrs) for 8 days exhibited the highest germination percentage (43.3%), along with optimal seedling height and root length. For *Buchanania lanzan* seeds, the highest germination of 86.7% was achieved with alternate wetting (24 hrs) and drying (12 hrs) (Thounaojam and Dhaduk 2020).

**Knuckling and Sand Paper Scarification (Mechanical):** Mechanical treatments play a crucial role in breaking the impermeable and hard seed coats of species such as *Acacia catechu*, *A. nilotica*, *Albizia spp.*, *Cassia fistula* etc. Research indicates that knuckling the distal ends of *Gliricidia sepium* seeds enhances germination without causing damage to the embryo (Alamgir and Hossain, 2005; Chichaghare *et al.*, 2020). The scarification of seeds from *Melia azedarach* and *Acacia auriculoformis* with sandpaper resulted in impressive germination rates of 80% and 78%, respectively (Azad *et al.*, 2010 & 2011). *Tamarindus indica* recorded a germination percentage of 82.3% following scarification (Azad *et al.*, 2013), while *Parkia biglobosa* demonstrated a remarkable 91.7% germination rate (Okunlola *et al.*, 2011). Additionally, *Schleichera oleosa* seeds, when rubbed with sandpaper at the distal end, exhibited a 42% germination rate (Tanjina *et al.*, 2014). Nail clipping one side of *Albizia saman* seeds at the distal end resulted in the highest germination rate of 50% (Alamgir and Hossain, 2005). Manual cutting of seeds also proved effective, with *Prosopis juliflora* and *Dalbergia sissoo* achieving 100% and 90.4% germination, respectively (Asif *et al.*, 2020).

**Nitrogenous Compounds:** The impact of nitrate compounds on seed germination, in conjunction with other factors like temperature or light, is well-established (Eremrena and Mensah, 2016). Potassium nitrate (KNO<sub>3</sub>) stands out as a widely used chemical to release seed dormancy and stimulate germination. Recommended solutions of 0.1–0.2% KNO<sub>3</sub> have proven effective in optimizing germination for species exhibiting low or shallow dormancy patterns (Gashi *et al.*, 2012). KNO<sub>3</sub> is also a frequently applied priming chemical, known to enhance seed germination by improving both uniformity and speed of germination (Tapfumaneyiet *al.*, 2023; Thongtip *et al.*, 2022). Moreover, the application of KNO<sub>3</sub> has shown utility in mitigating the adverse effects of salt on seed germination, seedling growth, mitotic activity, and chromosomal aberrations (Çavuşoğlu *et al.*, 2017) and also effective in improving emergence and seedling growth under drought (Ali *et al.*, 2020).

**Acid Scarification:** Soaking seeds in concentrated sulphuric acid is a common method for softening the seed coat. Sulphuric acid is believed to disrupt the seed coat and expose the lumens of the macrosclereids cells, facilitating water imbibition (Nikoleave, 1977). *Semicarpus anacardium* seeds treated with 98% concentrated H<sub>2</sub>SO<sub>4</sub> for 1 minute recorded the highest germination percentage at 74.67%. Olatunjii *et al.* (2013) reported that *Acacia auriculiformis* seeds treated with concentrated H<sub>2</sub>SO<sub>4</sub> for 5-10 minutes exhibited the highest germination percentage of 92-96%. Oxidation of phenolic compounds through hypochlorite or hydrogen peroxide, or decreased polyphenol oxidase activity, can enhance seed germination when seed coats are impermeable (Gendreau and Corbineau, 2009).

A study on breaking the dormancy of *Prunus yedoensis* seeds also found the best germination rate (40%) when the seeds were chemically treated, washed, and stratified for 9 weeks (Kim, 2019).

**Seed Priming Treatments:** Seed priming, a preparatory treatment before sowing, which involves hydrating seeds sufficiently to initiate metabolic processes before germination without allowing radicle emergence (Rashid *et al.*, 2004; Rehman *et al.*, 2011). Natural priming has proven beneficial for on germination of tropical trees such as *Albizia saman*, *Cedrela odorata*, and *Swietenia macrophylla*, while hydropriming exhibited positive effects on *Enterolobium cyclocarpum* seeds (Peraza *et al.*, 2018). Inoculation of *Acacia senegal* (L.) Willd. seeds with rhizobium also exhibited positive effects on the germination process (Singh *et al.*, 2011).

### **Hormonal Regulation in Seed Germination**

Plant growth regulators play a pivotal role in seed germination, with hormones such as GA, ABA, auxin, and ethylene (table 1) demonstrating significant influence (Han and Yang, 2015). Ethylene might be crucial in regulating dormancy through interaction with GA metabolism. Seed treatments with GA solutions have been found to enhance germination parameters, improving seed vigour and uniform germination (Maharana *et al.*, 2018). Additionally, applying plant growth regulators like GA<sub>3</sub>, IAA, and IBA, along with cold stratification, has been effective in breaking dormancy and enhancing germination in various species (Dhiman *et al.*, 2015; Iralu *et al.*, 2019).

### **Plant Growth Regulators:**

**a. Abscisic Acid (ABA):** ABA inhibits the germination of non-dormant seeds and is a crucial factor inducing dormancy, accumulating in maturing seeds. ABA biosynthesis genes directly influence seed germination and respond to abiotic stresses, with varying ABA levels at different developmental stages regulating processes like seed maturation and germination

(Vishal and Kumar, 2018; Martínez-Andújar *et al.*, 2011). ABA is synthesized at radical, blocks embryo growth, and maintains dormancy in the seed (Yang *et al.*, 2022).

**b. Gibberellin (GA3):** GA stimulates seed germination in many species, particularly overcoming dormancies with chilling and light requirements. The effectiveness of GA3 treatments depends on species, concentrations used, and the timing of application (Cornea-Cipcigan *et al.*, 2020). Gibberellins are multifunctional phytohormones influencing germination, flowering, sex determination, and seed and fruit development (Cornea-Cipcigan *et al.*, 2020). GA3 has shown significant effects on seed germination and seedling growth in various species, showcasing its potential in overcoming seed dormancy (Hemalatha and Chaudhari, 2021; Dilip *et al.*, 2017). For example, pre-soaking Anola seeds in a GA3 solution at 200 ppm resulted in the highest germination at 88 percentages (Chiranjeevi *et al.*, 2017). Maximum germination percentage (34.66%) was recorded in the sandalwood seeds treated with 300ppm GA3 (Hemalatha and Chaudhari 2021). Maharana *et al.* (2018) studied seed treatments of *Gmelina arborea* Roxb with GA solution and recorded enhanced germination percent and other seed germination parameters and thus improved seed vigour and uniform germination. The application of GA3 at 80 ppm for a duration of 12 hours significantly enhances both seed germination and seedling growth in Kagzi Lime, offering a viable recommendation for improved growth and yield (Dilip *et al.*, 2017). Furthermore, *Jatropha curcas* seeds exhibited a notably high germination percentage when soaked in GA3 solutions, particularly at 300 ppm for 8 hours and 400 ppm for 10 hours (Pawar *et al.*, 2010).

**c. Auxin:** Auxin, like IAA, IBA, NAA, and 2, 4-D, plays a role in the later stages of embryo expansion during seed germination. These auxins are commonly used in seed soaking to enhance germination and break dormancy, with NAA and IBA reported to stimulate germination (Attri *et al.*, 215; Dhiman *et al.*, 2015).

**Table 1: Various Plant Growth Regulators (PGR) and their classes along with functions**

| PGR         | Classes  | Functions  | Site of production  |
|-------------|--|--|---|
| Auxin       | IAA (Indole-3-acetic acid),<br>NAA (1-Naphthaleneacetic acid),<br>IBA (Indole-3-butyric acid),<br>2-4D (2,4-Dichlorophenoxyacetic acid),<br>4-CPA (4-Chlorophenoxyacetic acid) | (a) Involved in Apical dominance<br>(b) stimulates Cell division and enlargement<br>(c) Shoot and root growth<br>(d) Plant growth movement<br>(e) Parthenocarpy<br>(f) Abscission (g) root induction | Meristem of apical buds, embryo of seed, young expanding leaves |
| Gibberellin | Gibberellic acid (GA3)   | (a) Prevent genetical dwarfism<br>(b) Regulation in bolting  | Immature seeds  |

|                |                                 |   |  |
|----------------|---------------------------------|---|--|
|                |                                 | and flowering<br>(c) Production of parthenocarpic fruit<br>(d) Germination<br>(e) Increase flower and fruit size                                  |  |
| Cytokinins     | Kinetin, Zeatin, Benzyl adenine | (a) Cell and organ enlargement<br>(b) Seed germination<br>(c) Development of bud and shoot growth<br>(d) Flower induction<br>(e) delay senescence | Root apex, endosperm of seeds, young fruits                                    |
| Ethylene       | Ethanal                         | (a) Ripening of fruit<br>(b) Seedling growth and emergence<br>(c) Abscission of leaf.   | Ripe fruits, flowers and leaves and nodes of stem                              |
| Abscissic acid | Dormins, Phaseic Acid           | (a) Abscission<br>(b) Maintaining Dormancy<br>(c) Inhibit seed germination and development<br>(d) stimulate stomatal closure                      | In the roots of the plant as well as the terminal buds at the top of the plant |

### **Mechanistic Insights into Growth Hormone Influence on Seedling Germination:**

Plant growth regulators are the chemical substances, which govern all the factors of development and growth within plants (Srivastava, 2002). Seed coats impermeable to oxygen pose a challenge to germination, but hormonal treatments have proven effective in overcoming this barrier. A crucial balance between Abscissic Acid (ABA) and Gibberellic Acid (GA) regulates dormancy, with ABA synthesized at the radical to block embryo growth and maintain dormancy (Chenet *al.*, 2020; Sabaghet *al.*, 2021). Gibberellins, notably gibberellic acid (GA<sub>3</sub>), are pivotal hormones widely employed to stimulate seed germination by breaking dormancy. GA<sub>3</sub>, a common form utilized in seed treatments, initiates germination by promoting enzyme production and cell elongation in the embryo. It operates by overcoming seed dormancy and stimulating various enzyme production, which is helpful playing a crucial role in seed germination.

Gibberellins reduce abscissic acid levels, a hormone associated with seed dormancy. Further, to overcoming seed dormancy, signalling the seed that the conditions are conducive for germination. Simultaneously, they enhance enzyme production required for germination, promoting cell elongation and division, crucial for embryo growth and seed coat penetration. Gibberellins induce the synthesis of enzymes like amylases and proteases as stimulating enzyme. Amylases break down stored starch into sugars, providing energy for the growing embryo, while proteases assist in breaking down proteins into essential amino acids

for seedling development. The promotive effect of GA<sub>3</sub> on seed germination might be due to its participation in the activity of alpha-amylase which catalyses the starch conversion into simple carbohydrates and chemical energy is liberated which is used in the activation of embryo (Chenet *et al.*, 2020; Sabaghet *et al.*, 2021).

Cytokinins are primarily found in growing tissues like roots, embryos, and fruits, where cell division occurs. They regulate various functions including root development, shoot meristem formation and maintenance, organ development, seed germination, fruit development, senescence delay, and stress response (Sharma *et al.*, 2022). As a class of phytohormones, cytokinins participate in regulating plant growth, physiological activities, and yield, playing a crucial role in responding to abiotic stresses like drought, salt, and temperature variations (Li *et al.*, 2021; Prasad, 2022). Cytokinins delay leaf senescence, promote mitosis, and stimulate meristem differentiation in shoots and roots. They often work in conjunction with auxin or other hormones, such as gibberellins, to regulate plant development. For instance, they contribute to balancing apical dominance by inhibiting lateral buds while promoting bushier growth. Cytokinins, along with auxins, are key phytohormones that regulate plant growth and development from the cellular to the whole-plant level (Schaller *et al.*, 2015). Their diverse functions and interactions are best described as 'complementary' (Schaller *et al.*, 2015; Großkinsky and Petrášek, 2019).

IAA (Indole-3-acetic acid) is a ubiquitous endogenous auxin in plants, vital for root growth and seedling establishment post-germination. Auxin specifically stimulates the development of the radicle (embryonic root) and the primary root system. It plays a central role in almost every facet of root development, controlling transitions between cell division, growth, and differentiation, and establishing the root apical meristem. (Zhang *et al.*, 2022; Roychoudhry and Kepinski, 2022).

The role of ethylene in regulating seed germination and seedling growth varies depending on its interaction with other internal and external signals. Ethylene plays a crucial role in breaking seed dormancy and regulating the germination process. It contributes significantly to promoting seed germination effectively in certain species. It counteracts the effects of ABA by regulating ABA metabolism and signalling pathways. Ethylene's role in dormancy release is significant in numerous species, with effective concentrations for germination of dormant seeds ranging from 0.1 to 200  $\mu\text{L L}^{-1}$  (Corbineau *et al.*, 2014). In a complex signalling network, ethylene interacts with ABA and GAs, both of which are essential regulators of germination and dormancy. Further, ethylene promotes seed germination through antagonistic interactions with ABA and synergistic actions with GAs (Ahammed *et al.*, 2020).

**Why we need Seed Pre-treatments?**

Efficient nursery management involves strategic pre-treatment of seeds, particularly those with a longer germination period exceeding one week. This proactive approach not only conserves time but also minimizes resource allocation. Conducting germination tests is essential to determine the optimal seed orientation for sowing, especially when dealing with species prone to twisted shoots or roots upon emergence, such as Mahogany and Mango. Emphasizing the pre-treatment of seeds with prolonged germination periods contributes to accelerated production timelines in the nursery. This not only reduces nursery costs but also facilitates optimal planting schedules for farmers, aligning with the most favourable planting times. Some important tree species and their pre-sowing treatments listed in Table 2.

Additionally, a prudent nursery practice involves germination rate testing for seeds stored for more than one month. Sowing 100 seeds in a shaded germination bed, followed by regular watering, allows for an accurate assessment of germination rates. The outcome guides the determination of the number of seeds required per container to ensure a single plant per pot. For instance, if only 25 out of 100 seeds germinate, adjusting the sowing strategy to include four seeds per container aligns with effective resource utilization (Alamgir and Hossain, 2005; Azad *et al.*, 2010 & 2011; Tanjina *et al.*, 2014; Statwick, 2016).

### **Controlling germination**

Creating optimal conditions for germination is paramount to successful seed development. The key factor in this process is maintaining consistent humidity around the seed. To achieve this, various coverings, such as sieved soil, sand, rice hulls, or pine needles, can be utilized to keep the seeds moist. It is crucial, however, not to add supplementary fertilizer to the substrate, as this could elevate the risk of diseases like damping-off (Sarvade *et al.*, 2014a & 2014b; Lamichhane *et al.*, 2017; Raphael *et al.*, 2023).

Germinating seedlings typically draw all the necessary nutrients from the cotyledons or, in the case of palms, from the first leaf developed inside the seed. Consequently, a substrate devoid of fertilizer, like sand, is generally deemed an ideal germinating medium. Small seedlings are particularly prone to damping-off, a condition caused by fungi present on the seed surface or in the substrate (Sarvade *et al.*, 2014a & 2014b; Lamichhane *et al.*, 2017; Raphael *et al.*, 2023).

To counteract damping-off and other potential contaminants, seed sterilization becomes imperative. One effective method involves immersing the seed in a 10% solution of bleach (1 tablespoon of bleach plus 9 tablespoons of water) for 30 minutes. Alternatively, hydrogen peroxide serves as an efficient seed sterilant with advantages such as lower toxicity than bleach. It softens the seed coat, facilitating easier entry of water and oxygen, potentially enhancing germination. Seeds can be soaked directly in the antiseptic for up to four

hours(Lamichhane *et al.*, 2017).However, it is essential to exercise caution during sterilization, as both bleach and hydrogen peroxide may require experimentation to determine the most effective solution strength and soaking time. Additionally, it is important to recognize that sterilizing the substrate may eliminate beneficial fungi, bacteria, and insects. Some of these microorganisms are crucial for breaking down organic matter or aiding plants in nutrient absorption. Striking the right balance in sterilization practices is crucial to fostering a healthy germination environment (Ochieno, 2022).

## **Conclusion**

Seed treatments offer significant potential in overcoming seed dormancy, improving germination rates and uniformity, and reducing emergence time. The field of seed technologies is rapidly advancing within global crop protection markets (Sharma *et al.*, 2015).While numerous studies have assessed the efficacy of various methods in breaking dormancy, there is a crucial need for further research. Specifically, investigations should focus on exploring combinations of treatments and leveraging plant growth regulators for enhanced dormancy breaking outcomes. The diverse array of plant species necessitates pre-treatments to address specific dormancy types effectively. The implementation of various pre-sowing treatments not only influences the rate of seed germination but also allows for a substantial reduction in the germination period, transforming it from months to a matter of weeks. The profound understanding of dormancy and its manipulation holds significant practical implications, particularly in the mass production and propagation of high-quality seedling stocks for both indigenous and exotic trees.Ensuring the availability of planting material for afforestation and reforestation, particularly for endangered and valuable forest species, necessitates pre-sowing treatments for dormant seeds. Additionally, to meet the demand for high-quality planting material of commercially valuable species, seed pre-treatment is crucial to breaking dormancy and expediting germination to take up large-scale plantation projects. Furthermore, there is a need for research to standardize treatments aimed at breaking dormancy in responding to abiotic stresses like drought, salt, and temperature variations.

**Table. 2**List of tree species, seed viability and their pre-treatment

| Common Name                                     | Scientific Name                                  | Seed pre-treatment   | Time period      | References   |
|---|--|--|------------------|--|
| Harada  | <i>Terminalia chebula</i> Retz.                  | Cold water   | 48 hrs           | Hossain <i>et al.</i> , 2005   |
| Kindal  | <i>Terminalia paniculata</i> Roth                | Normal Water   | 24 hrs           | Viswanath <i>et al.</i> , 2021   |
| Subabul   | <i>Leucaena leucocephala</i> (Lam.) de Wit       | hot water  | 12 hours         | Hamad and Anwer, 2021  |
| Ritha   | <i>Sapindus mukorossi</i> Gaertn.                | hot water  |                  | Attriet <i>al.</i> , 2015  |
| black wattle, late black wattle or green wattle | <i>Acacia mearnsii</i> De Wild.                  | hot water (80°C)   | 5 min            | São José <i>et al.</i> , 2019  |
| Australian Babul                                | <i>Acacia auriculiformis</i> A. Cunn. ex Benth.  | hot water (80°C); 98% conc. H <sub>2</sub> SO <sub>4</sub>   | 5-10 min         | Azad <i>et al.</i> , 2011; Bisht and Ahlawat, 1999; Olatunjii <i>et al.</i> , 2012 |
| Mangium   | <i>A. mangium</i> Willd.                         | hot water (80°C) for 2 min followed by cold water for 24 hrs | 2 min            | Bisht and Ahlawat, 1999  |
| Bakain  | <i>Melia azedarach</i> L.                        | hot water (80°C)   | 10 min           | Azad <i>et al.</i> , 2010  |
| Pyinkado  | <i>Xylia kerrii</i> Roxb. Taub.                  | hot water (80 °C) immersion                                  | 10 min           | Azad <i>et al.</i> , 2006b   |
| White siris                                     | <i>Albizia procera</i> (Roxb.) Benth.            | Hot water80 °C)  | 10 min           | Ali <i>et al.</i> ,1997  |
| Woman's tongue tree                             | <i>Albizia lebbeck</i> (L.) Benth.               | hot water80 °C)for 10 minute                                 | 10 min           | Azad <i>et al.</i> ,2006a  |
| Gum arabic tree                                 | <i>Acacia nilotica</i> (L.) P.J.H.Hurter & Mabb. | hot water80 °C); sulphuric acid treatment                    | 10 min<br>45 min | Nasr <i>et al.</i> , 2013  |
| Western myall                                   | <i>Acacia papyrocarpa</i> Benth.                 | hot water 100°C  | 5 min            | Pound <i>et al.</i> , 2014   |
| Australian blackwood                            | <i>Acacia melanoxylon</i> R.Br.                  | hot water 100°C  | 60 second        | Burrows <i>et al.</i> , 2009   |
| Teak  | <i>Tectona grandis</i> L.f.                      | Alternate wetting and drying                                 | (12-12hrs)       | Gupta <i>et al.</i> , 1975; Bisht and Ahlawat, 1999; Pamei <i>et al.</i> , 2017    |
| Chironji  | <i>Buchanania</i>                                | Alternate  | (24-12           | Thounaojam   |

|   |  |  |           |   |
|---|--|--|-----------|---|
|   | <i>lanzan</i> Spreng.                          | wetting and drying   | hrs)      | and Dhaduk 2020   |
| Tamarind tree   | <i>Tamarindus indica</i> L.                    | Scarified with sand paper                                    |           | Azad <i>et al.</i> , 2013                                 |
| Kusum tree  | <i>Schleichera oleosa</i> Lour.                | Scarified with sand paper                                    |           | Tanjina <i>et al.</i> , 2014                              |
| Cow tamarind, monkey pod  | <i>Albizia saman</i> (Jacq.) Merr.             | Scarified with Nail clipping (one side of the seeds)         |           | Alamgir andHossain 2005                                   |
| Vilaiti keekar,Mesquite   | <i>Prosopis juliflora</i> (Sw.) DC.            | manual cutting   |           | Asif <i>et al.</i> , 2020                                 |
| Shisham; North Indian rosewood                                  | <i>Dalbergia sissoo</i> Roxb.                  | manual cutting   |           | Asif <i>et al.</i> , 2020                                 |
| Bombay blackwood; Indian rosewood                               | <i>Dalbergia latifolia</i> Roxb.               | tap water  | 12 hours  | Kumar and Chavan, 2018                                    |
| Khair   | <i>Acacia catechu</i> (L. f.) Willd.           | hot water (80°C)   | 10 min    | Das, 2014   |
| Golden Shower Tree; Amaltas                                     | <i>Cassia fistula</i> L.                       | Conc. H <sub>2</sub> SO <sub>4</sub>                         | 2 Min     | Humtsoet <i>al.</i> , 2018                                |
| Marking nut   | <i>Semecarpus anacardium</i> Linn.             | 98% conc. H <sub>2</sub> SO <sub>4</sub>                     | 1 min     | Panda and Hazra, 2009                                     |
| Anola; Indian gooseberry  | <i>Phyllanthus emblica</i> L.                  | GA <sub>3</sub> 200ppm                                       |           | Chiranjeevi <i>et al.</i> , 2017                          |
| Physic Nut, Barbados Nut  | <i>Jatropha curcas</i> L.                      | GA <sub>3</sub> 50 ppm or CaCl <sub>2</sub> , Shell cracking | 12 hours  | Sunil <i>et al.</i> , 2016; Marcello <i>et al.</i> , 2015 |
| West Indian lime, bartender's lime, Omani lime, or Mexican lime | <i>Citrus aurantifolia</i> (Christm.) Swingle  | GA <sub>3</sub> 80 ppm                                       | 12 hours  | Dilip <i>et al.</i> , 2017                                |
| Gamhar; khamer  | <i>Gmelina arborea</i> Roxb.                   | GA <sub>3</sub>  |           | Maharana <i>et al.</i> ,2018                              |
| Sandalwood; Indian Chandan                                      | <i>Santalum album</i> L.                       | GA <sub>3</sub> @ 300ppm                                     | 12 hrs    | Hemalatha and Chaudhari, 2021                             |
| Chaulmoogra;Hydnocarpus Oil Tree                                | <i>Hydnocarpus pentandra</i> (Buch.-Ham.) Oken | GA <sub>3</sub> @350 ppm                                     | overnight | Bhat <i>et al.</i> , 2020                                 |
| Tamanu, oil-nut, mastwood, beach calophyllum                    | <i>Calophyllum inophyllum</i> L.               | Seeds without pericarp; mechanical scarification             | -         | Gunagaet <i>al.</i> , 2011                                |
| Ber, Indian Jujube  | <i>Zizyphus mauritiana</i> Lam.                | GA <sub>3</sub> @ 400 ppm                                    | 24 hr     | Kumar <i>et al.</i> , 2020                                |
| <b>Herb</b>   |  |  |           |   |

|                                    |  |   |              |                              |
|------------------------------------|--|---|--------------|------------------------------|
| Chickpea milkvetch                 | <i>Astragalus cicer</i> L.             | physical scarification  | -            | Statwick, 2016               |
| Chili pepper; Cayenne Pepper Plant | <i>Capsicum annuum</i> L.              | Sulphuric acid  | 10 min       | Evans and Blazich, 1999      |
| Brazil macca                       | <i>Mimosa bimucronata</i> (DC.) Kuntze | hot water at 80 °Cwith post-treatment immersion in water at room temperature for 24 hours | 1 min        | Giasson <i>et al.</i> , 2019 |
| Aswagandha                         | <i>Withania somnifera</i> (L.) Dunal   | GA <sub>3</sub> 100ppm  | 48 hrs       | Madhumita and Ghosh, 2004    |
| Rose root; Arctic root             | <i>Rhodiola rosea</i> L.               | GA <sub>3</sub> 100ppm  | 12 or 18 hrs | Aiello and Fusani,2008       |
| Eastern gamagrass                  | <i>Tripsacum dactyloides</i> (L.) L.   | GA <sub>3</sub> 1000 ppm  | 24 hrs       | Rogis <i>et al.</i> , 2004   |
| Indian Noni, vomit fruit, awl tree | <i>Morinda citrifolia</i> L.           | GA <sub>3</sub> at 800 ppm  | 24 hours     | Chandra and Sagar, 2013      |

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