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Study on Maturity Indices and Effect of Growing Substrates on Seed Germination of *Litsea glutinosa*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Litsea glutinosa* is a multipurpose, fast-growing tree widely exploited due to its multipurpose utilisation. Species reported low seed viability, hence generally propagated through vegetative methods. We studied the maturity indices of fruits and seeds to determine the optimal collection stage. Studied the effect of different growing substrates and Gibberellic acid (GA3) treatment on the germination percent (GP), mean germination time (MGT), germination value (GV), peak value (PV), and germination index (GI).
Study Design: Study was conducted at Forest Tree Seed Laboratory, ICFRE-FRI. The change in fruit colour from green, dark brown purple, and dark blue stages was compared with the seed parameters, germination percent, mean germination time, germination value, etc. to obtain the most

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suitable stage for fruit and seed maturity. Seed germination experiment was conducted in Complete Randomized Design with four replications of 25 seeds for each treatment. Seeds were sown directly on different germination substrates, such as filter paper, sand, and vermiculite, without treatment and also with 0.05% GA_3 pretreatment. Seeds were kept in the seed germinator at 25±1ºC with a 24-hour photoperiod and high relative humidity (RH > 90%).

Results: The highest germination rate was observed in vermiculite with GA_3 soaking . i.e., 80±3.26%, followed by vermiculite 68±8.64%. The lowest germination percent of 5±4.08% was observed in seeds plated on filter paper with GA_3 soaking. No germination was recorded in the seeds plated on the filter paper.

Conclusion: Most suitable germination medium to maximise the germination of *L. glutinosa seed* is vermiculite. Furthermore, the pretreatment of seeds in GA_3 (0.05%) for 24 hours and sowing in vermiculite improves overall performance with respect to GP, MGT, GV, PV, and GI. In the context of maturity indices, the dark blue stage with a moisture content of around 50% is the right stage for the collection of seeds of the species. This stage is characterised by high GP, GV, and low MGT values as compared to other growth stages.

Keywords: Maturity indices; seed germination; germination substrates; pre treatment.

1. INTRODUCTION

1.1 *Litsea glutinosa*-Conservation Aspect

Litsea glutinosa, also known as the Indian laurel tree or Maida lakdi in Hindi, belongs to the family Lauraceae and is a multipurpose, fast-growing tree. The species is widely exploited due to its multipurpose utilisation. The bark of the tree acts as a binder due to its exceptional viscosity and adhesive properties and is used for the preparation of incense sticks, tablet formulations, and plasters for fractured limbs. The leaves act as an antispasmodic, as well as an emollient used to treat diarrhoea, dysentery, wound healing, etc., and tender parts are used as fodder. Additionally, species are harvested from the wild for the preparation of agriculture tools, ropes, root fibre, seed oil for making candles, soaps, and seed powder for treating skin diseases and burns [1,2] *L. glutinosa* has become threatened and endangered in the wild in Bangladesh and India due to indiscriminate exploitation of its bark [3] and is under consideration for inclusion in a conservation programme [2,4]. Overexploitation of *L. glutinosa* has also occurred in the northern Philippines, where it is reported to be an endangered species [5]. The species is highly recalcitrant in nature and the seeds are very small in size, and the seed germination rate is 17% reported in natural habitats [6,7]. In India, flowering in species reported between March and June, and fructification appears in September and October. Fruits are round and about 8 mm or less in diameter. Species primarily reproduces through vegetative methods such as root-suckering. Conventional propagation is hampered due to low seed viability and no rooting of vegetative cuttings [5].

1.2 Distribution

Litsea glutinosa is native to India, Southern China, Malaysia, Australia, and the Western Pacific islands and has been observed throughout Asia, including several regions of China, the states of Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Odisha, the Western Ghats and outer Himalayas of India, Bhutan, Myanmar, Nepal, the Philippines, Thailand, and Vietnam [8,9]. It is found in mixed primary and secondary forest and thickets throughout India and in the outer Himalayas' [10]. It grows at an altitude of 500–1900 m above sea level, in forest margins, stream sides, sparse forests, or thickets [8]. *Litsea glutinosa* grows where rainfall exceeds 1200 mm per year [11].

We studied the maturity indices of the species for identifying suitable time for the collection of species from the wild and investigated the seed germination constraints associated with the species in laboratory conditions by experimenting with various germination media. Additionally, studying the different fruit stages during collection time provides valuable insights into their maturity indices. Seed maturation is one of the main factors in seed quality and a prerequisite for successful germination and emergence. Harvesting seeds too early when there is inadequate development of essential structures and protection mechanisms may result in poor quality [12]. Similarly, harvesting too late may increase the risk of shattering and may decrease the quality of seed due to ageing [13].

The amount of chlorophyll in the seed or seed coat [14]. moisture content of seeds, seed or seed coat colour, seed weight, seed size [15] etc., serve as markers for assessing the level of maturity. The morphological parameters and moisture content of the seeds are crucial in determining their quality and viability for germination experiments. Seed germination depends on several environmental conditions, such as light, temperature, moisture, germination media, etc. [16].

Seeds collected from the same species growing under different conditions may exhibit different germination capacity [17]. An effective option for improving seed germination can be the use of appropriate substrates. The effect of seed germination parameters and seedling growth parameters in different germination substrates has been extensively studied in many agriculture species but limitedly reported in forest species, even though many species require this condition for the early stages of germination, for obtaining healthy seedling growth rates. Filter paper, sterilised sand (quartzite sand), vermiculite, cocopeat, peat moss, etc. are the common substrates used in seed laboratories for sowing seeds. The efficiency of these substrates for seed germination varies from species to species, which may relate to the size of the seed, seed morphology, water availability of the substrates, temperature, etc. [18].

We investigated the effect of different germination substrates and GA_3 combinations on germination percent (GP), mean germination time (MGT), germination value (GV), peak value (PV), and germination index (GI).

2. MATERIALS AND METHODS

2.1 Seed Collection

Seeds of *Litsea glutinosa* were collected from the Uttarakhand, India, at GPS coordinates 29°56´53.93"N latitude and 78°10´50.44"E longitude, at an elevation of 310 m. Seeds were collected from the tropical moist deciduous forest where species is associated with evergreen species such as *Syzigium cumini* and *Mallotus philippinensis*.

2.1.1 Seed extraction and morphological studies in *Litsea glutinosa*

Morphological parameters of freshly collected fruits at various stages of development including the green stage, intermediate stage, and ripened stage were recorded immediately after collection. Moisture content was checked in the fresh fruits of various stages through low constant oven dry method [19].

Seeds were manually extracted from the freshly collected pulpy fruits at green stage, intermediate stage, and ripened stage. Extracted seeds were rinsed with tap water to remove the exterior sticky components. Washed seeds were dried at room temperature for 2 to 3 days to eliminate the excess surface moisture content. Morphological parameters such as seed length, width and colour as well as the weight of 1000 seeds and number of seeds per kg were measured after drying. Further moisture content of the seeds was evaluated using low constant temperature oven dry method at 103ºC for 17 hours [19] in four replications of 5gm seeds in each moisture box. Moisture content was determined on the fresh weight basis (%) using the formula:

$$MC = \frac{Fw - Dw}{Fw} \times 100$$

Where MC is the moisture content, Fw is the initial fresh weight of the replicate and Dw is the dry weight.

2.2 Seed Germination Experiment

Seed germination experiment was conducted in a Complete Randomized Design with four replications of 25 seeds for each treatment. Seed germination experiment was denoted by GT1, GT2, GT3, GT4, GT5, and GT6 which represent filter paper (control), filter paper + GA_3(0.05%), sand, sand + GA_3 (0.05%), vermiculite, vermiculite + GA_3 (0.05%), respectively. Seeds were sown directly on different germination substrates, such as filter paper, sand, and vermiculite, without treatment and also with 0.05% GA_3. Seeds were kept in the seed germinator at 25±1ºC with a 24-hour photoperiod and high relative humidity (RH > 90%). Seed germination was recorded on a daily basis from the day of sowing.

Germination parameters such as germination percent [19]; Mean Germination Time [20]; Initial Germination Time (IGT) and Final Germination Time (FGT) were calculated. Initial germination time (IGT) is the total of number of days taken for the initiation of seed radicle. Final Germination Time is the total number of days taken for the last initiation of radicle from the viable seed. The following parameters were measured as follows:

Germination percentage (GP) = total number of seeds germinated at end of germination test/total number of seeds plated for germination test.

Mean germination time (MGT) = ΣFx/ΣF; where F is the number of seeds germinated on day x.

Germination value was expressed as ∑DGS/N x (Final cumulative Germination Percent/10.

The peak value is the mean daily germination of the most vigorous component of the seed lot.

Germination index = ΣG/T, where G is the percentage of seed germinated per day, and T is the germination period.

2.3 Statistical Analysis

Statistical analysis of germination data was performed with the SPSS 16.0 software package. The data were subjected to analysis of variance (ANOVA), Post hoc Duncan test was used to check the significance of treatments on GP, MGT, GV, PV and GI.

3. RESULTS

3.1 Maturity Indices at Various Stages of Fruit Growth

At the green stage, fruit length was 7.43±0.5 mm and fruit width was 8.12±0.2mm and moisture content was 54±1%. Number of fruits per kilogram was 2400 and weight of 1000 fruit was 420-425gm. As per the RHS chart, fruit colour code was N144C which was light green colour. At dark brown purple stage, fruit length was 8.85±0.5mm and fruit width was 9.72±0.2mm and 48±1% moisture content. Number of fruits per kilogram was 2200 and weight of 1000 fruit was 460-480gm. As per the RHS chart, fruit colour code was 183C which was dark brown purple colour. At dark blue stage, fruit length was 9.72±0.5mm and fruit width was 9.8±0.2 mm and 50% moisture content. Number of fruits per kilogram was 2000 and weight of 1000 fruit was 565-575gm. As per RHS chart, fruit colour code was 103 A which was dark blue colour (Table 1).

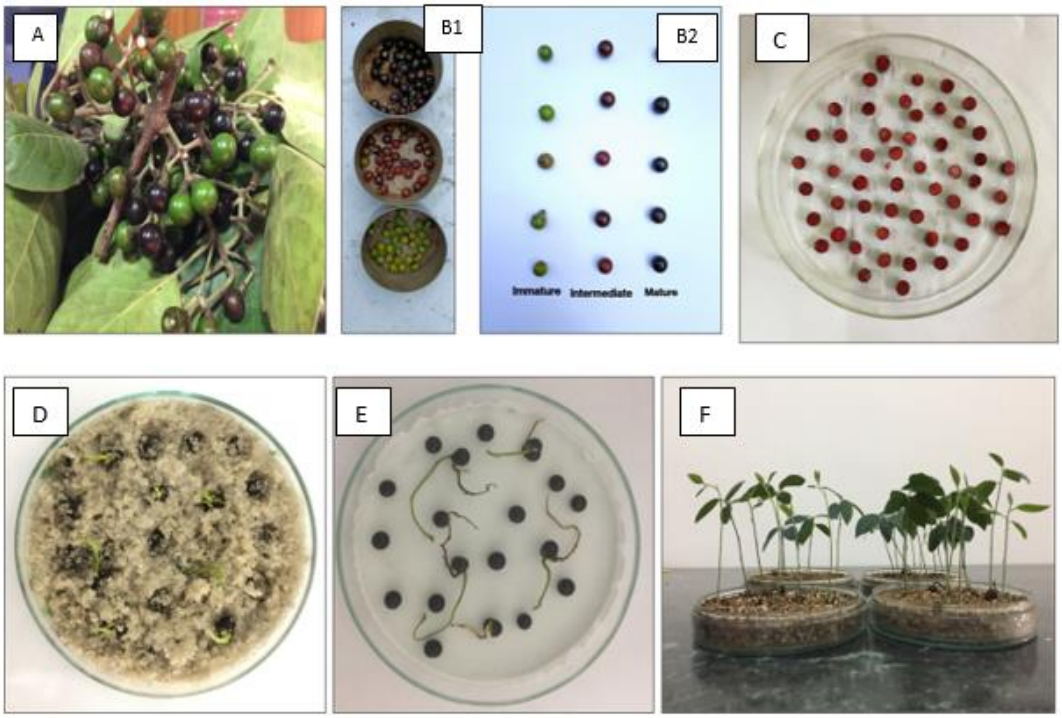

Fig. 1. *Litsea glutinosa*, A:Fruits, B1, B2: Maturity stages, C: TTZ test, D, E,F: Seed germination in sand, filter paper and vermiculite respectively

3.2 Maturity Indices at Various Stages of Seed Growth

The green stage of the seed was characterized by a moisture content of 35±1% and a germination percentage of 21±2% (Table 2). There were approximately 5200 seeds per kilogram and the weight of 1000 seeds was 180-190 grams. As per the RHS chart, the seed colour at this stage was classified as 200 B, indicating dark brown. The seed in the dark brown purple stage had a moisture content of 33±1% and a germination percentage of 51±2%. There were approximately 4900 seeds per kilogram, and the weight of 1000 seeds was 215-225grams. According to the RHS chart, the seed colour at this stage was classified as 200 B, indicating dark brown colour. In the dark blue stage, seed has 31±1% moisture content and 68±2% germination percent. Number of seeds per kilogram was 4500 and weight of 1000 seed was 245-255gm. As per the RHS chart, seed

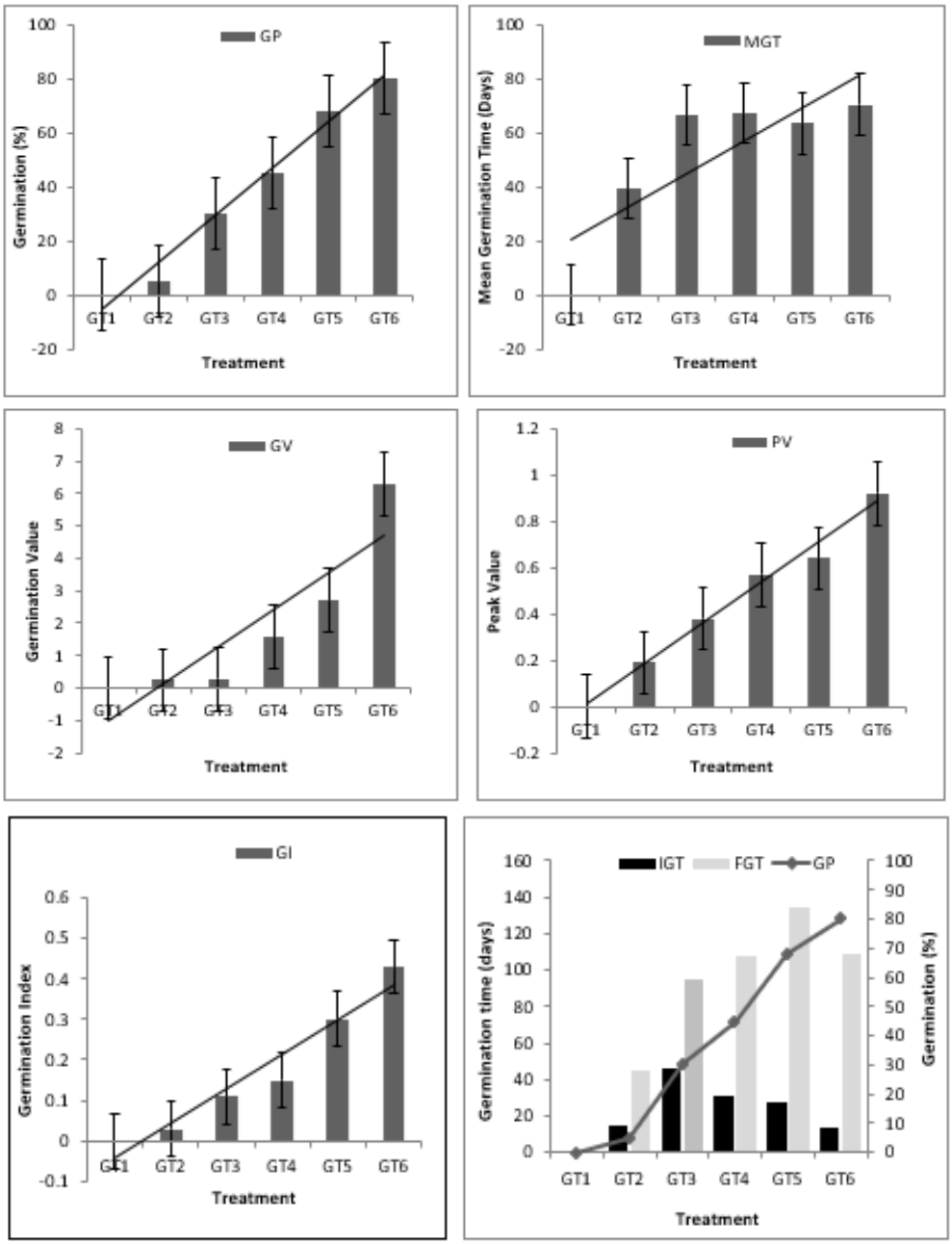

Fig. 2. Effect of seed treatments on germination parameters viz., GP, MGT, GV, PV, GI, IGT and FGT of *Litsea glutinosa seeds*

Table 1. Fruit maturity indices at three stages of development in *Litsea glutinosa*

Maturity indices	Green stage	Dark brown purple stage	Dark blue stage
Fruit parameters			
Fruit moisture (%)	54±1	48±1	50±1
Germination rate (%)	21±2	51±2	68±2
1000 fruit weight (gm)	420-425	460-480	565-575
Total number of fruits/kilogram	2400	2200	2000
RHS colour code	N144C	183 C	103 A
RHS colour	Light green	Dark brown purple	Dark blue colour
Fruit length	7.43±0.5	8.85±0.5	9.27±0.5
Fruit width	8.12±0.2	9.72±0.2	9.8±0.2

Table 2. Seed maturity indices at three stages of development in *Litsea glutinosa*

Maturity indices	Green stage	Dark brown purple stage	Dark blue stage
Seed parameters			
Seed moisture (%)	35±1	33±1	31±1
Germination rate (%)	21±2	51±2	68±2
Mean Germination Time (days)	90±6.8	85±9.6	63.42±14.21
Germination Value	0.13±26	1.24±0.31	2.7±0.97
1000 seed weight (gm)	180-190	215-225	245-255
Total number of seeds/kilogram	5200	4900	4500
RHS colour code	200B	200B	200B
RHS colour	Dark brown	Dark brown	Dark brown
Seed length (mm)	7.23±0.2	7.72±0.2	8.27±0.2
Seed width (mm)	7.32±0.1	7.62±0.1	8.02±0.1

Table 3. ANOVA and post hoc test. Effect of seed treatment on germination parameters of *Litsea glutinosa*

Treatment	Code	GP±SD (%)	MGT±SD (days)	GV±SD	PV±SD	GI±SD
Filter paper	GT1	$0±00^{a}$	$0±00^{a}$	$0±00^{a}$	$0±00^{a}$	$0±00^{a}$
Filter paper + GA_3	GT2	$5±4.08^{a}$	$39.25±6.65^{b}$	$0.24±0.39^{ab}$	$0.19±0.19^{b}$	$0.03±0.02^{a}$
Sand	GT3	$30±4.89^{b}$	$66.61±21.49^{c}$	$0.28±0.13^{ab}$	$0.38±0.00^{c}$	$0.11±0.00^{b}$
Sand + GA_3	GT4	$45±8.16^{c}$	$67.3±5.68^{c}$	$1.58±0.53^{bc}$	$0.57±0.5^{d}$	$0.15±0.02^{b}$
Vermiculite	GT5	$68±8.64^{d}$	$63.42±14.21^{c}$	$2.7±0.97^{c}$	$0.64±0.07^{d}$	$0.3±0.05^{c}$
Vermiculite + GA_3	GT6	$80±3.26^{e}$	$70.52±2.22^{c}$	$6.28±1.88^{d}$	$0.92±0.03^{e}$	$0.43±0.07^{d}$
F		132.29	24.36	27.94	53.34	58.4
P		<0.05	<0.05	<0.05	<0.05	<0.05

Duncan test was used for testing the significance of GP, MGT, GV, PV and GI between treatments. P<0.05 statistically significant difference; P>0.05 statistically no significant difference. The values with different superscript letters in a column are significantly different (p<0.05)

colour was 200 B (dark brown). At the dark blue stage, seed length was 8.27±0.2 mm and seed width was 8.02±0.1mm. Highest germination value was recorded in the dark blue stage and the lowest GV was found in green stage. Similarly, lowest MGT was observed in the dark blue stage and highest MGT was found in green stage (Table 2).

3.3 Influence of Seed Treatment on Germination Percent

The seed experiment was conducted in three germination media such as filter paper, sand, and vermiculite without any pretreatment in one set and with gibberellic acid treatment (0.05%) for 24 hours in second set. Seeds plated on the filter paper shows 0% germination rate at control condition and 5±4.08% germination rate under GA_3 treatment for 24 hours. In sand media, seeds exhibited30±4.89% germination rate and 45±8.16% germination rate in GA_3 treatment. In vermiculite, seeds exhibited 68±8.64% germinationrate and 80±3.26%. Germination rate in GA_3 treatment.

3.4 MGT, IGT, and FGT under Different Treatments

In GT1 method no germination was recorded. In GT2 method, treatment of seeds with GA_3 for 24 hours followed by plating on filter paper shows

germination initiation on 15th day of sowing and it took 45 days for completion of the germination experiment with the result of only 5±4.08% germination rate. The mean germination time was 39.25±6.65 days for the experiment. In GT3 method, germination initiated on 46th day and completed on 95th day with 30±4.89% germination rate. The mean germination time was 66.61±21.49 days for the experiment. In GT4 method seed treated with GA_3 for 24 hours shows the initiation of seed germination occurred on 31stday and ends on 108th day with 45±8.16% germination rate. The mean germination time was 67.3±5.68 days for the experiment. In GT5 method, germination started at 27th day and ended on 134th day. Mean germination time was 63.42±14.21days with 68±8.64% germination rate. In GT6 method, application of GA_3 in seeds for 24 hours followed by sowing in vermiculite media showed initiation of germination on 14th day and concluded on 109th day. The mean germination time taken for the experiment was 70.52±2.22 days and the germination percent was 80±3.26%.

3.5 GV, PV and GI under Different Treatments

Germination value of the experiment ranged from 0±00to 6.28±1.88. Highest GV was observed in GT6 (6.28±1.88), followed GT5 (2.7±0.97) and lowest GV value was observed in GT1, GT2 and GT3. Peak value derived from the experiments ranged from 0±00 to 0.92±0.03. Highest value recorded in GT6 (0.92±0.03), followed by GT5 (0.64±0.07) and the lowest value was observed in GT1 (0±00) followed by GT2 (0.19±0.19). Germination Index of the experiment ranged from 0±00 to 0.43±0.07. Highest GI was observed in GT6 (0.43±0.07) followed by GT5 0.3±0.05and the lowest value was observed in GT1 (0±00) followed by GT2 (00.03±0.02).

4. DISCUSSION

4.1 Standardization of Maturity Indices of *Litsea glutinosa*

Litsea glutinosa exhibited different maturity stages during fruit collection were categorized as green stage, brown purple stage and dark blue stage based on the colour code of RHS colour chart. The study attempted to investigate the maturity indices, viability status of seeds at each stage and also studied the moisture content and morphological parameters of seeds. The seeds at the green stage had a higher moisture content and lower germination percentage compared to the seeds at the brown purple stage and dark blue stage. These stages are crucial in the seed's development and undergo significant changes in size, moisture content and germination percent.

As the seed progressed from the green stage to the brown purple stage, there was a decrease in moisture content and an increase in germination percent. The moisture content of the seed increases slightly from the brown purple stage to the dark blue stage. The germination percentage improved significantly, increasing to 47% from brown purple stage to dark blue stage. When the fruit turns dark blue colour from dark brown purple it was the right stage for the collection of species. This stage was characterized by high GP, GV and low MGT value as compared to earlier stages. Physiologically mature fruits provide good quality seeds in terms of germination and vigour compared to early and intermediate stage of maturity and enhances the storage potential of seeds.

Maturity indices based on physical characteristics like colour change can impact seed germination as reported by Negi and Todaria [21] in some forestry species. In *Acer oblongum*, fruit colour change from greenish grey to light grey recorded seed germination of 6.67(±0.58)% and 83.33(±0.58)% respectively. In *Kydia calycina*, fruit colour changes from green to dark brown exhibited seed germination of 0.0(±0)% and 36.67(±1.15)% respectively. Fruit colour change from yellowish green to pinkish red reported seed germination of 0.0(±0)% and 36.67(±2.08)% in *Terminalia tomentosa*. In *Terminalia chebula,* the light green to yellow with black spots stage of fruit reported a germination rate of 3.3(±0.58)% and 16.67(±1.15)% respectively. In *Terminalia bellerica,* the green colour stage to the bright brown stage of fruit reported germination of 6.67(±0.58)% and 96.67 (±3.21)%, respectively. In *Myrica esculenta,* fruit colour changed from green to dark red during maturity [22], Fruits of *Mallotus philippenesis* changed colour from reddish-green to dark red and seeds from whitish-yellow to black at maturity [23]. The change in pod colour from green to dark red and the seed colour from green to whitish brown was a useful indicator of maturity in *Bauhinia retusa* [24] The change in fruit colour from dark green to pale red or red was a useful indicator of seed maturity in *Prunus*

cerasoides [25]. Ratheish and Negi [26] standardized the seed maturity indices of *Semecarpus anacardium* and reported that ground collected and fully ripened seeds showed significant results with respect to germination percentage, germination capacity, sedling vigour index,collar diameter and height.

4.2 Seed Germination Experiment

The present study on the seed germination experiment revealed a significant difference ($P<0.05$) in the germination percent, mean germination time, germination value, peak value, and germination index under different treatments. Post hoc Duncan test was used to check the significance between different treatments.

Post hoc Duncan test showed that the highest GP in GT6 and GT5, which were significantly different ($P<0.05$) from other treatments. Application of gibberellic acid (GA_3) significantly affects the germination rate of seeds in all three germination substrates. In vermiculite, the germination rate was increased by 12% (i.e., 80±3.26% GP) with GA_3 treatment compared to vermiculite alone. In sand, the germination rate was increased by 15% (i.e., 45±8.16% GP) with GA_3 treatment. In the GT1 method, no germination was reported in the control condition, but the application of GA_3 yielded 5% germination. *Litsea glutinosa,* having high moisture content at the initial harvesting stage, required a continuous moisture regime during its germination stage. Growth promoters alone cannot significantly affect the germination percent, as has been proven through the experiments in filter paper methods that have been used for the majority of the seeds in laboratory experiments. Germination substrates have a great role in the germination percent, MGT, and other germination parameters like GV, PV, and GI. The germination percent of seeds alone in vermiculite media provides 68% GP, which was comparatively higher than GT1, GT2, GT3, and GT4. Vermiculite has been reported to have a high cation exchange capacity, air space, and water holding capacity, and to maintain a pH range suitable for species. It contains magnesium and potassium, which, in their earlier stages, promote the growth of seedlings and hold nutrients in reserve for later release.

Similar studies in *Jatropha curcus* reported maximum GP in vermiculite (85.00±9.01%) over sand (82.50±6.61%) and filter paper (55.00±6.61%) [16], In addition, several studies have reported the efficiency of vermiculite over other substrates in *Styrax camporum* Simao et al., [18] and in *Cordia trichotoma* [27]. Germination substrates like perlite and cocopeat were also reported as suitable material for many species. Cocopeat was used for most horticultural species. Coco peat has been reported as suitable germination substrates for *Pterocarpus macrocarpus* [28], *Eucalyptus tereticornis* [29], *Swietenia macrophylla* [30], *Gonystylus bancanus* [31], *Oroxylum indicum* (Trivedi and Joshi [32]. *Stereospermum suaveolens* [33] Similarly sowing on perlite medium reported germination rate as two times than control in four species Goniolimon [34].

Experimental studies in sunflower reported the effectiveness of filter paper (92.05%) over sand (by 13.60%) [35]. The study highlights that, in addition to an ideal temperature range, light, and relative humidity, many seeds require a continuous moisture regime for maximum germination potential. So the substrate has a paramount role in maintaining the moisture level, aeration, and nutrient holding capacity. Vermiculite substrate was preferable over sand although filter paper has not been recommended. Both sand and vermiculite provide continuous water supply and aeration; however, the vermiculite maintains the pH, has high cation exchange capacity and contains magnesium and potassium which improve seed germination potential. Despite the long mean germination time, germination rate of species after GA_3 treatment and nutrient rich vermiculite substratum indicate certain endogenous dormancy in species associated with immature/underdeveloped embryos or due to some chemical inhibitors. The time to completion of germination of a species depended on the dormancy status of species Baskin and Baskin [36,37].

In laboratory condition, medium-sized seeds with a round shape require a continuous moisture regime for the initiation of germination and usage of substrates such as sand, and vermiculite yielded a higher germination percent, e.g., *Santalum album, Cinnamomum camphora*, *Putranjiva roxburghii*, etc. Medium sized, flat or oval-shaped seeds show uniform and good germination potential on filter paper as well as sand and vermiculite, e.g., *Bauhinia* spp., *Nyctanthes arbotristis, Acacia* spp. (*A. catechu,*

A. nilotica, A. holosericea, A. mangium, etc.). (Results observed from laboratory experiments, unpublished data).

5. CONCLUSION

The present study concluded that the most suitable germination medium to maximise the germination of *L. glutinosa* was vermiculite. Furthermore, the treatment of seeds with GA_3 (0.05%) for 24 hours and sowing in vermiculite improves overall performance with respect to GP, MGT, GV, PV, and GI. In the context of maturity indices, the dark blue stage with a moisture content of around 50% was the right stage for the collection of seeds of species. This stage was characterised by high GP, GV, and low MGT values as compared to other growth stages. The methodology devised for seed collection and germination experiments can be applied for faster and higher germination of the species to produce vigorous seedlings for plantation and conservation programs of *L. glutinosa*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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