Study on maturity indices and growing medium effect on the seed germination of *Litseaglutinosa.*

Abstract

Aim: *Litseaglutinosa* 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 fruit colour change from the green, dark brown purple, and dark blue stages was compared with seed parameters, germination percent, mean germination time, germination value, etc. to obtain the most preferable stage. 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. 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. i.e., 80±3.26%, followed by vermiculite 68±8.64%. The lowest germination percent of 5±4.08% was observed in filter paper with GA_3. No germination was recorded in the filter paper method.

Conclusion: Most suitable germination medium to maximise the germination of *L. glutinosa*is vermiculite. Furthermore, the treatment of seeds in GA_3 (0.05%) for 24 hours and sowing in vermiculite improves overall performance with respects 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 species. This stage is characterised by high GP, GV, and low MGT values as compared to other growth stages.

Comment [D1]: GA_3

Key words: Maturity indices, seed germination, germination substrates, pre treatment.

INTRODUCTION

Litseaglutinosa-Conservation aspect

Litseaglutinosa, 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 (Haqueet al., 2014; Ramana and Raju, 2017). *L. glutinosa* has become threatened and endangered in the wild in Bangladesh and India due to indiscriminate exploitation of its bark (Kumar, 2011) and is under consideration for inclusion in a conservation programme (Rabena, 2008; Ramana and Raju, 2017). Overexploitation of *L. glutinosa* has also occurred in the northern Philippines, where it is reported to be an endangered species (Rabena, 2010). 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 (Soerianegara, 1995; Ramana and Raju, 2019). 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 are primarily reproduced through vegetative methods such as root-suckering. Conventional propagation is hampered due to low seed viability and no rooting of vegetative cuttings (Rabena, 2010).

Comment [D2]: *et al*

Distribution

Litseaglutinosa 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 (Huang Puhuaet al., 2008; GISD, 2012). It is found in mixed primary and secondary forest and thickets throughout India and in the outer Himalayas' (Kirtikar and Basu, 1981). It grows at an altitude of 500–1900 m above sea level, in forest margins, stream sides, sparse forests, or thickets (Huang Puhua et al., 2008). *Litseaglutinosa* grows where rainfall exceeds 1200 mm per year (Ninot, 2001).

Comment [D3]: *et al*

We studied the maturity indices of the species for developing a 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 (Ekpong and Sukprakarn, 2008). Similarly, harvesting too late may increase the risk of shattering and may decrease the quality of seed due to ageing (Elias and Copeland 2001). The amount of chlorophyll in the seed or seed coat (Ward et al., 1992), moisture content of seeds, seed or seed coat colour, seed weight, seed size (Sripathy and Groot, 2023), 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. (Gairola *et al.,* 2011).

Seeds collected from the same species growing under different conditions may exhibit different germination capacity (Baskin and Baskin, 1998).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 conditionfor the early stages of germination, therefore toobtaining 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. (Simao*et al.,* 2013)

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).

MATERIALS AND METHODS

Seed Collection

Seeds of *Litseaglutinosa* were collected from the Uttarakhand Himalaya, 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 *Syzigiumcumini* and *Mallotusphilippinensis*.

Seed Extraction and Morphological Studies in *Litseaglutinosa*

Seeds of *Litseaglutinosa* were extracted from the pulpy fruits manually and washed with tap water to remove the sticky components from the outer surface of the seeds. Seeds are dried at room temperature for 2 to 3 days. Morphological parameters and the moisture content of the seeds were noted before germination experiments. Seed length, width, colour, weight of 1000 seeds, number of seeds per kg, and fresh moisture content of the seeds were recorded. Various stages of seeds and fruits, including the green stage, intermediate stage, and ripened stage, were noted for studying the maturity indices.

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, sand, sand +GA_3, vermiculite, vermiculite + GA_3 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 except on holidays and weekend.

Germination parameters such as germination percent (ISTA, 2010); Mean Germination Time (Orchard, 1977); Initial Germination Time (IGT) and Final Germination Time (FGT) were calculated. 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. 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. 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.

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.

Results

Maturity indices at various stages of fruit growth

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

Maturity indices at various stages of seed growth

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

blue stage, seed length is 8.27±0.2 mm and seed width is 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).

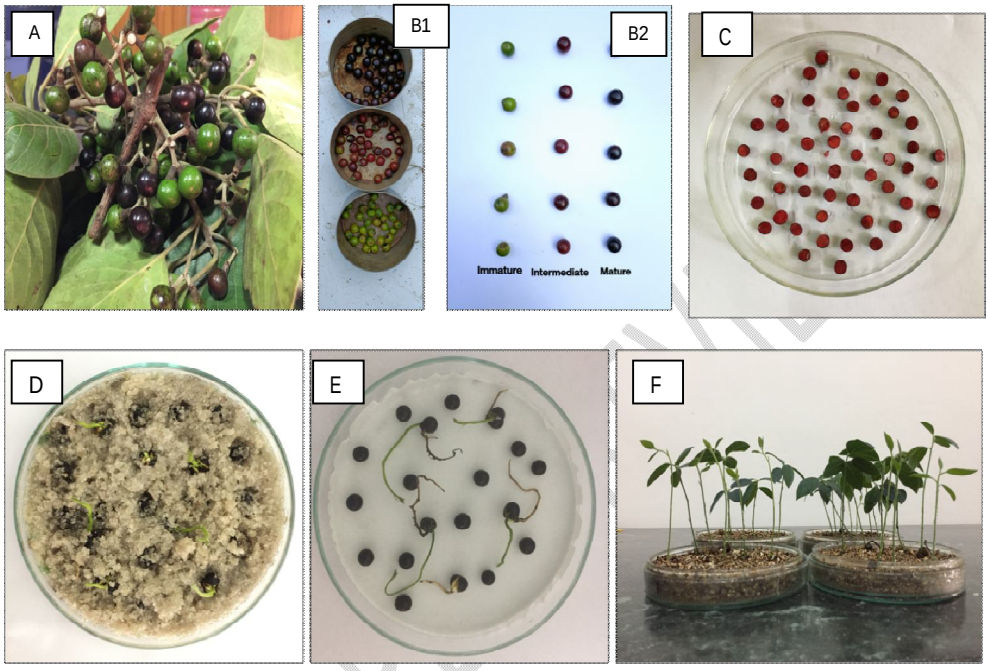


Fig 1 :*Litseaglutinosa*, A:Fruits, B1, B2: Maturity stages, C: TTZ test, D, E,F: Seed germination in sand, filter paper and vermiculite respectively.

Table 1: Fruit maturity indices at three stages of development in *Litseaglutinosa*

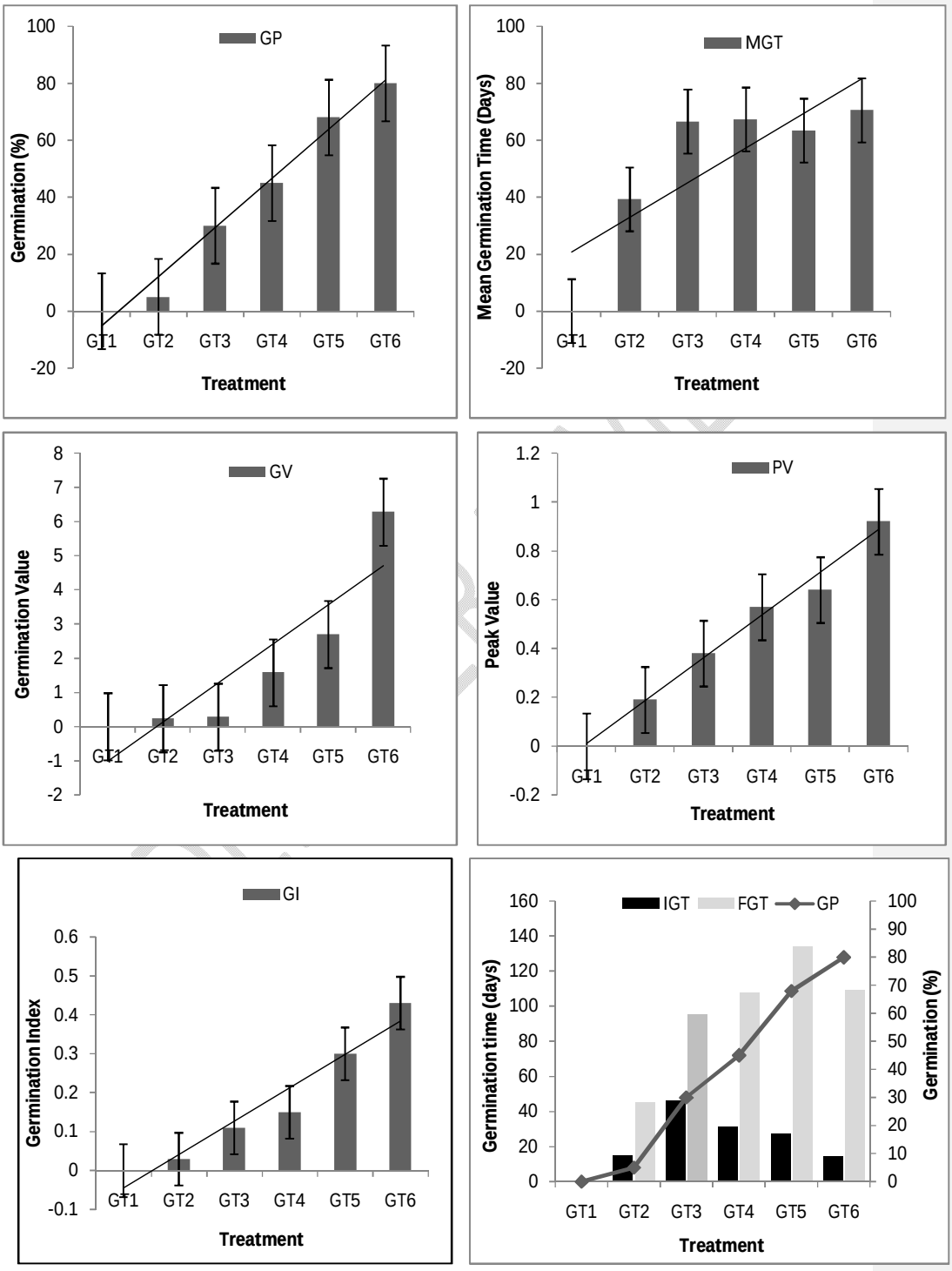
Maturity indices	Green stage	Dark brown purple stage	Dark blue stage
Fruit parameters			
Fruit moisture (%)	54±1	48±1	50±1
Germination rate (%)	21±2	51±2	68±2
1000 fruit weight (gm)	420-425	460-480	565-575
Total number of fruits/kilogram	2400	2200	2000
RHS colour code	N144C	183 C	103 A
RHS colour	Light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

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

Results of seed germination percent under differenttreatments

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□ treatment for 24 hours. In sand media, seeds exhibited30±4.89% germination rate and 45±8.16% germination rate in GA□ treatment. In vermiculite, seeds exhibited 68±8.64% germinationrate and 80±3.26%.germination rate in GA□ treatment.



Graphs 1 : represent the significant effect of seed treatments on germination parameters viz., GP, MGT, GV, PV, GI, IGT and FGTof *Litseaglutinosa seeds.*

Result ofMGT, lGT, and FGT under different treatments

In GT1 method no germination was recorded. In GT2 method, treatment of seeds with GA_3 for 24 hoursfollowed by plating on filter paper shows germination initiation on 15th day of sowing and total 45 days taken for completing the germination experiment with the result of only 5±4.08% germination rate. The mean germination time is 39.25±6.65 days for the experiment.In GT3 method, germination initiates on 46th day and terminate on 95th day with 30±4.89% germination rate. The mean germination time is 66.61±21.49 days for the experiment. In GT4method seed treated with GA□ for 24 hours shows the initiation of seed germination from 31stday and ends on 108th day with 45±8.16% germination rate. The mean germination time is 67.3±5.68 days for the experiment.In GT5 method, germination started at 27th day and ends on 134th day. Mean germination time is 63.42±14.21days with 68±8.64% germination rate.In GT6 method, application of GA_3in seeds for 24 hours followed by sowing invermiculite media shows initiation of germination from 14th day and ends on 109th day.The mean germination time taken for the experiment is 70.52±2.22 days and the germination percent is 80±3.26%.

Result ofGV, 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 ranges 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).

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

Treatment	Code	GP±SD	MGT±SD	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□	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□	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□	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 is 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).

Discussion

Standardization of maturity indices of Litseaglutinosa

Litseaglutinosa exhibited different maturity stage 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 seed at the green stage has a higher moisture content and lower germination percentage compared to the seed 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 progresses from the green stage to the brown purple stage, there is 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 improves significantly, increasing by 47% from brown purple stage to dark blue stage.When the fruit turns dark blue colour from dark brown purple it is the right stage for the collection of species. This stage is 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 (1995) 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 *Kydiacalycina*, 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 change from green to dark red during maturity (Shah at al., 2010), Fruits of *Mallotusphilippenesis* changed colour from reddish-green to dark red and seeds from whitish-yellow to black at maturity (Tewari *et al.,* 2016). The change in pod colour from green to dark red and the seed colour from green to whitish brown is a useful indicator of maturity in *Bauhinia retusa* (Upadhyay *et al.,* 2006). The change in fruit colour from dark green to pale red or red was a useful indicator of seed maturity in *Prunuscerasoides*(Tewari*et al.,* 2011).

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 shows the highest GP in GT6 and GT5, which are significantly different ($P<0.05$) from other treatments. Application of gibberellic acid (GA□) significantly affects the germination rate of seeds in all three germination substrates. In vermiculite, the

germination rate is increased by 12% (i.e., 80±3.26% GP) with GA□ treatment compared to vermiculite alone. In sand, the germination rate is increased by 15% (i.e., 45±8.16% GP) with GA□ treatment. In the GT1 method, zero percent germination is reported in the control condition, but the application of GA_3 has provided 5% germination. *Litseaglutinosa,* 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 is 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* recorded maximum GP in vermiculite (85.00±9.01%) over sand (82.50±6.61%) and filter paper (55.00±6.61%) (Gairola et al., 2011); In addition, several studies have reported the efficiency of vermiculite over other substrates in *Styraxcamporum*(Simao et al., 2013) and in *Cordia trichotoma* (Grzybowski, 2022). Germination substrates like perlite and cocopeat are also reported as suitable material for many species. Cocopeat is used for most horticultural species. Coco peat has been reported as suitable germination substrates for *Pterocarpusmacrocarpus* (Kijkar, 1991), *Eucalyptus tereticornis*(Kumar and Marimuthu 1997),*Swietenia macrophylla* (Woods et al. 1998), *Gonystylusbancanus* (Utami et al 2006), *Oroxylumindicum* (Trivedi and Joshi 2012), *Stereospermumsuaveolens*(Trivedi and Joshi, 2014). Similarly sowing on perlite medium reported germination rate as two times than control in four species Goniolimon.(Manolova*et al.,* 2015).

Several experimental studies have also reported the effectiveness of filter paper (92.05%) over sand (by 13.60%) in sunflower (Ovuka*et al.,* 2010). 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. In laboratory condition, medium-sized seeds with a round shape get a continuous moisture regime for the initiation of germination using substrates such as sand, and vermiculite yielded a higher germination percent, e.g., *Santalum album, Cinnamomumcamphora*, *Putranjivaroxburghii*, etc. Medium sized, flat or oval-shaped seeds show uniform and good germination potential in filter paper as well as sand and vermiculite, e.g., *Bauhinia* spp., *Nyctanthesarbotristis, Acacia* spp. (*A. Catechu, A. nilotica, A. holosericea, A. mangium,* etc.). (Results observed from laboratory experiments, unpublished data).

Conclusion

The present study concluded that the most suitable germination medium to maximise the germination of *L. glutinosa*is vermiculite. Furthermore, the treatment of seeds in GA_3(0.05%) for 24 hoursand sowing in vermiculiteimproves 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 species. This stage is 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.

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