

Original Research Article

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Development of Seed Cube Technology with Enhanced Seeds of *Albizia lebbek* for Rapid Propagation in Fallow Lands

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ABSTRACT

'Seed balls' are prepared by non-governmental organizations and enthusiastic school children to trees grow trees rapidly for ecosystem restoration. However, there is immense scope for technically improving the seed balls by ensuring the quality of seeds as well as media; further preparing cuboid structures, instead of ball like structure has immense scope in facilitating rooting of the newly emerging seedlings. Against this back drop, a series of experiments were conducted to standardize the seed coating technique, seed priming method and appropriate media for making seed cubes. The results showed that coating of *Albizia lebbek* seeds with TNAU seed coating formulation @ 3g kg⁻¹ can increase the seed germination percentage and vigour index percentage by 24 and 134 percentage over the control. Similarly humid priming of *Albizia lebbek* seeds (24 h soaking in water + 3 days humid incubation) also resulted in significant improvement in seed germination and vigour index to the tune of 32 and 136 percentage over the control. Seeds which were thus enhanced by seed coating and humid priming were enclosed in four different types of cube media. The results revealed that media composed of soil, saw dust, bone meal, vermicompost and VAM recorded the highest seed germination percentage and dry matter production over other media compositions. The control seeds could record only 20 percent of seed germination while coated and primed seeds enclosed in above media composition recorded 62 percent. The new and improved seed cube technology holds immense scope to ensure rapid propagation of *Albizia lebbek* trees in fallow lands or cultivable lands.

Keywords

Seed coating, Seed Priming, *Albizia lebbek*, Seed cubes, Ecosystem

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Introduction

Seed balls are used in farming practice as a source of direct seeding (Fukuoka, 1973). Seed balls can act as short-term soil seed banks and reservoir for protracted germination of seeds. Clay content of seed balls reduces loss of water by increased water potential and helps to prevent foraging of

insects and birds on the seeds. In the recent years, many non-governmental organizations prepare and use seed balls for direct seeding, paving the way for increasing the tree cover. The concept of 'seed ball' is very relevant and has huge potential not only to increase the tree cover but also to improve the awareness among the people about conserving the nature, by promoting tree growing. However,

seed balls that are prepared by various agencies and school children have many lacunas like usage of seeds that have not been tested for seed dormancy or germination percentage, poor seed germination and field establishment. Further, at present, seed balls are made only with a combination of soil and farm yard compost, as the media. There is a need to incorporate latest technological advancements for preparation of seed as well as media to enhance the seedling emergence. Besides, there is also scope to use cuboid structures instead of 'balls', to ensure better rooting and seedling establishment.

Seed coating technology has developed during the past two decades and provides an easy and economical approach to seed enhancement, especially for larger seeded agronomic and horticultural crops. An advantage of seed coating is that the seed enhancement material is placed directly on the seed without obscuring the seed shape. These coatings are extremely thin, which allows multiple layers on the seed with only a 1 to 10% increase in seed weight. The film coating provides a uniform, yet precise placement of chemicals at much lower rates than the traditional seed treatment systems and offers the opportunity to add many enhancement layers as needed to improve performance (Robani, 1993; Watkins, 1994; Tekrony, 2006).

Seed coating materials can improve the germination and increase the seedling emergence at changing soil moisture regime especially in the sub-optimal range (Scott, 1989; Sherin, 2003). The hormone based seed coating formulation developed by Tamil Nadu Agricultural University, Coimbatore, India containing as a combination of hydrophilic polymer, growth hormone, pigment, and stabilizer has been proven to improve seed germination as well as seedling growth characteristics in many agricultural crops and

horticultural crops besides improving the stress tolerance as well as seed storage potential (Karthi, 2017; Leelavathi, 2018, Anil Kumar and Malarkodi, 2019). Anil Kumar and Malarkodi (2019) reported that seeds of *Abelmoschus esculentus* coated with TNAU seed coating formulation @ 3g kg⁻¹ resulted highest physiological potential in terms of germination percentage, total seedling length, dry matter production and vigour index, both in low and medium vigour seeds.

Seed invigouration treatments are physiological treatments that enable an improvement in physiological status of seed, thereby promoting improved germinability, greater storability and better field performance, than the corresponding untreated seeds (Basu, 1994). Seed priming is defined as 'controlled hydration process that involves facilitates the seeds to undergo imbibition and activation phases of germination, to induce pre-germinative physiological and biochemical changes within the seeds, without permitting the radicle protrusion phase'. Chen and Arora, (2013) reported that seed priming process promotes germination-related activities (e.g. respiration, endosperm weakening, and gene transcription and translation, etc.) that facilitate the transition of inactive dry seeds into germinating state and lead to improved germination potential. The seed priming strategy also constitutes 'priming memory' in seeds, which can be recruited upon a subsequent stress-exposure and mediates greater stress-tolerance of germinating primed seeds. The humid priming technique is the process of soaking the seeds in a loosely tied cloth bag for predetermined duration of time, followed by placing the bags in a closed container on an elevated platform that allows to provide humid dark conditions that promotes the invigouration process (Mohamed, 2014; Anil Kumar, 2019).

Albizia lebbbeck Benth. is a tall, fast growing deciduous tree which belongs to the family Mimosaceae, distributed throughout India from the plains up to 900 m in the Himalayas (Babu *et al.*, 2009). Trees attain an average maximum height of 18 to 25 m and 50 to 80 cm d.b.h. (Parrotta, 1988). Owing to quick germination and fast growing nature, there is immense scope to improve tree cover by direct seeding of *Albizia lebbbeck*.

Against this background, a study was conducted to overcome the limitations of seed ball technology and by incorporation advanced technologies, seed coating, seed priming and seed germination testing. The study will be highly useful to the non-governmental organizations and eco-conservationists for preparation of technically sound 'seed cubes' of *Albizia lebbbeck* which will eventually provide a simplified technology for rapid propagation of these trees.

Materials and Methods

A sequence of experiments were conducted using the seeds of *Albizia lebbbeck* for making seed cubes containing the seeds that are enhanced through coating and priming techniques and enclosing of seeds with optimal composition of media. The seeds of *Albizia lebbbeck* were collected from Forest College and Research Institute, Mettupalayam and experiments were conducted in Department of Forest Biology and Tree Improvement, Forest College & Research Institute, Mettupalayam.

Experiment I: Standardization of seed coating technique for *Albizia lebbbeck* seeds

Seeds of *Albizia lebbbeck* were subjected to seed coating with 'TNAU seed coating polymer formulation' @ 2g, 3g and 4g kg⁻¹ of seeds. The weighed quantity of polymer

formulation was mixed with optimum quantity of water, mixed well and uniformly coated over the seeds by stirring with glass rod. The coated seeds were dried at room temperature to attain original moisture content and subjected to germination test in raised bed conditions maintained inside a shade net. Three replications of 100 seeds were sown. Daily observations for germination of seeds were recorded and speed of emergence was calculated using the formula proposed by Magurie (1962). After 14 days, the number of normal seedlings was counted and the mean was expressed as germination percentage. Root length and shoot length were measured and expressed in cm. After measuring the total seedling length (root length + shoot length), seedlings were kept in a paper cover, shade dried for 24 h and then dried in a hot air oven at 80±2⁰ C for 24 h to record the dry matter production (mg). Vigour index values were calculated using the formula suggested by Abdul-Baki and Anderson and the mean values were expressed as whole numbers.

Experiment II: Standardization of humid priming technique for *Albizia lebbbeck* seeds

The humid priming technique consists of imbibing the seeds in water, incubating it in dark, humid conditions and drying back to original moisture content. In this experiment, the *Albizia lebbbeck* seeds were soaked in water for two different durations *viz.*, 12 and 24 hours in a separate containers. Later, the water was drained and the imbibed seeds were tied in a wet cloth bags and placed in a container, over an elevated platform, so as to allow excess water to drain. The containers were closed tightly and placed in dark condition to allow incubation of seeds. Seeds were drawn after 1, 2 and 3 days of incubation and then shade dried for 12 h to attain original moisture content. These seeds were subjected to germination test as

described above and observations were made on speed of emergence, seed germination per cent, root length, shoot length, total seedling length, dry matter production and vigour index.

Experiment III: Developing and evaluating different seed cube media for *Albizia lebbek* seeds

Four seed cubing media were prepared. The composition of the four media treatments are as follows:

The media components of the respective treatments were mixed well with water to make a semi-solid paste. A specially designed wooden dye with cavities of cuboid dimension of 1.5 cm (h) x 3 cm (l) x 3 cm (b) was used for making seed cubes. Seed cubes were prepared by applying the semi-solid paste in cavities of the wooden frame. Seeds subjected to best seed coating + humid priming treatment were embedded and enclosed in the cube media to make seed cubes @ two seeds cube⁻¹. The wet seed cubes were extracted from the cavities of wooden frame by gentle tapping, and were dried well for 24 h in a hot air oven maintained at 40°C.

The dried seed cubes were submitted to germination test as described in the above experiments along with 'control raw seeds' that were not subjected to either seed coating or humid priming. The seed cubes were placed on the surface of the bed and watered with rose can, daily. Observations were made on speed of emergence, seed germination percentage, root length, shoot length, total seedling length, dry matter production and vigour index.

Data were analyzed using an analysis of variance (ANOVA) as a factorial combination of treatments. Means were separated on the basis of least significant difference (LSD)

only if F test of ANOVA for treatments was significant at the 0.05 or 0.01 probability level. Values in percent data were arcsine transformed before analysis.

Results and Discussion

Development of seed cube technology for *Albizia lebbek*, consisted of three experiments that included standardization of techniques such as coating and priming techniques as well as cube media.

The first experiment was conducted to study the effect of seed coating with 'TNAU seed coating formulation' on *Albizia lebbek*, by subjecting the seeds to three dosages (2, 3 and 4 g kg⁻¹) (Table 1). The hormone based seed coating formulation developed by Tamil Nadu Agricultural University, Coimbatore, India consists of hydrophylic polymer, growth hormone, pigment, and stabilizer. Among the dosages, 3g kg⁻¹ demonstrated the highest potential to enhance the seed germination and seedling vigour of *Albizia lebbek* by registering the increased germination percentage (54%), speed of germination (4.32), root length (7.18 cm), shoot length (7.06 cm), total seedling length (14.24 cm), dry matter production (900.00 mg seedling⁻¹⁰) and vigour index (48600) which was 80, 80.7, 15.8, 10.8, 13.28, 30.4 and 134.7 per cent higher than the control seeds, respectively (Table 1).

This results in concordance with the findings of Karthi (2017) who reported that the TNAU seed coating formulation containing hydrophylic polymer, and gibberellic acid enhanced the seed germination and seedling vigour improvement in a wide range of agricultural crops (rice, maize, bajra, sorghum, ground nut, cotton *etc.*) and horticultural crops (tomato, brinjal, chilli, onion, bhendi, ridge gourd, bitter gourd, *etc.*, cotton seeds).

The gibberellic acid contained in the seed coating formulation could have been instrumental in improve the performance of the *Albizia lebbeck* seed. Gibberellic acid improves the membrane permeability and nutrient level in leaves which ultimately leads to better seedling growth and total biomass (Tuna *et al.*, 2007). Gibberellins promote the mobilization of stored reserves and weaken the mechanical resistance of endosperm around the radical tip thus promoting radical protrusion during seed germination (Groot and Karssen, 1987).

When the seeds of *Albizia lebbeck* were subjected to humid priming treatments involving, two different durations (12 and 24 hours) and then humid incubation for three different durations (1,2 and 3 days). These results revealed that 24 hours of soaking followed by 3 days of humid incubation was superior to rest of the treatments by producing

best germination of 68 percent whereas control recorded the lowest value of 36 per cent germination. The speed of emergence, seedling growth and vigour index were also found to be superior compared to other humid priming treatments. At the same time the control seeds were found to record the lowest seed germination, seedling growth and vigour index. Beneficial effects of seed priming such as rapid, uniform and increased germination, improved seedling vigour and growth under a broad range of environments resulting in better stand establishment and alleviation of phytochrome-induced dormancy have been reported in many crops (Khan, 1992; Basu, 1994; McDonald, 2000; Copeland *et al.*, 2001). Seed priming has been found to improve RNA and DNA synthesis (Salehzade *et al.*, 2009). Physiological studies have indicated increased rate of metabolic activity of primed seeds during germination (Malnassy, 1971) (Table 2 and 3).

Treatment

Treatments	Material composition
Media I :	Soil
Media II :	Soil (50 % by volume) + Saw dust (50 % by volume)
Media III :	Soil (40% by volume) + Saw dust (40% by volume) + Bone meal (20% by volume)
Media IV :	Soil (40% by volume) + Saw dust (40% by volume) + Bone meal (10% by volume) + Vermicompost (5% by volume) + VAM (5% by volume)

Table.1 Effect of seed coating on seed germination and seedling vigour of *Albizia lebbek* seeds

Treatments		Germination (%)	Speed of emergence	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Dry matter production (mg/ seedlings ⁻¹⁰)	Vigour index
Seed coating formulation	2g/kg	42 (40.40)	4.18	6.60	6.55	13.15	745.24	31300
	3g/kg	54 (47.29)	4.32	7.18	7.06	14.24	900.00	48600
	4g/kg	44 (41.55)	3.01	6.56	6.59	13.15	814.55	35840
Control		30 (33.21)	2.39	6.20	6.37	12.57	690.00	20700
Mean		42.50 (40.61)	3.47	6.64	6.64	13.28	787.45	34110.00
SEd		0.9382	0.0729	0.1398	0.1386	0.2781	16.9138	779.6777
CD(P=0.05)		2.1634	0.1681	0.3224	0.3197	0.6414	39.0038	1797.9603

Figures in the parenthesis indicates arc sine value

Table.2 Effect of humid priming on seed germination and seedling vigour of *Albizia lebbek* seeds

Humid priming		Germination %	Speed of emergence	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Dry matter production (mg seedlings ⁻¹⁰)	Vigour index
Soaking	Humid priming							
12 h	1 d	30 (33.21)	1.79	5.63	5.94	11.57	783.33	23500
	2 d	50 (45)	3.77	6.33	6.82	13.15	880.00	44000
	3 d	42 (40.39)	3.06	6.18	6.80	12.98	910.48	38240
24 h	1 d	32 (34.45)	2.60	6.30	6.94	13.24	761.88	24380
	2 d	52 (46.15)	4.20	6.32	7.05	13.37	862.31	44840
	3 d	68 (55.55)	5.01	6.42	7.14	13.56	1024.41	69660
Control		36 (36.86)	2.28	5.56	6.25	11.81	821.11	29560
Mean		44.29 (41.66)	3.24	6.11	6.71	12.81	863.36	39168.57
SEd		1.16	0.09	0.16	0.17	0.33	21.89	1033.19
CD(P=0.05)		2.54	0.20	0.35	0.38	0.72	47.70	2251.15

Figures in the parenthesis indicates arc sine value

Table.3 Effect of cube media composition on seed germination and seedling growth of coated and humid primed seeds of *Albizia lebbek*

Treatments		Germination %	Speed of emergence	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Dry matter production (mg /seedlings ⁻¹⁰)	Vigour index
Media	I	20 (26.57)	1.43	5.57	8.48	14.05	505	10100
	II	28 (31.95)	2.44	5.65	8.37	14.02	647.14	18120
	III	42 (40.40)	3.77	5.81	7.55	13.36	506.67	21280
	IV	62 (51.94)	5.79	6.25	8.50	14.75	676.77	41960
Control (raw seeds)		20 (26.57)	1.10	5.92	7.74	13.66	492	9840
Mean		34.40 (35.48)	2.90	5.84	8.13	13.97	565.52	20260
SEd		0.99	0.09	0.14	0.20	0.34	14.83	645.25
CD (P=0.05)		2.22	0.20	0.32	0.45	0.76	33.05	1437.72

Figures in the parenthesis indicates arc sine value

The third experiment conducted to standardize the media for preparing the seed cubes after subjecting the seeds to seed enhancement treatments *viz.*, seed coating (3 g kg⁻¹) + humid priming (24 h soaking + 3 days humid incubation). Among the four media compositions, cube media containing soil, saw dust, bone meal, vermicompost and VAM were found to record highest germination percent then the rest of the media composition. This media (IV) was found to produce 62 per cent germination whereas a lower germination percent were recorded in rest of the treatment compositions *viz.*, media I (20%), media II (28%) and media III (42%). The speed of emergence was found to record 426 % higher than the untreated control seeds. The root length, shoot length, dry matter production and vigour index of enhanced seeds embedded in seed cubes made up of media IV recorded the highest percentage of increase *viz.*, 5, 9, 37.5% and 326% respectively over the control. The results obtained from the present experiment were found to be in concordance with that of Anilkumar (2019) who reported that combining of humid priming (12 h soaking + 4 h humid incubation) and seed coating @ rate of 3g kg⁻¹ using TNAU seed coating formulation were effective in bringing about increased anatomical potential as well as seed germination and seedling growth of *Abelmoschus esculentus* probably due to initiation of membrane reorganization, synthesis of RNA, and repair and synthesis of mitochondria and DNA.

The saw dust added to the media could have act as a pore-forming agent (Eliche-Quesada *et al.*, 2012) facilitating better germination and better root growth (Agbo and Omaliko, 2005). Bone meal contains large amount of nitrogen, phosphorus and calcium that could act as a effective fertilizer (Salomonsson *et al.*, 1994) besides functioning as organic input for facilitating better growth of rhizosphere

microorganisms (Kahiluoto and Vestberg, 1998). The use of vermicompost (Edwards and Burrows, 1988) and VAM (Ravikumar *et al.*, 1997) could have enhanced the seedling growth and vigour, by adding up to the organic content of the soil. Thus, the media containing the mixture of soil, saw dust, bone meal, vermicompost and VAM had more potential for registering better germination percent and seedling growth compared to other media compositions.

From the above experiments, it is established that enclosing of enhanced seeds in an improved media can successfully enhance the germination percent and seedling vigour than the seed balls containing untreated raw seeds and unimproved media. Therefore, it is recommended that, non-governmental organizations and other agencies interested in ecosystem restoration can encourage preparation and direct seeding of improved seed cubes instead of conventional seed balls.

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