OPTIMIZING PROPAGATION TECHNIQUES IN NEELAYAMARI (*Indigofera tinctoria* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"OPTIMIZING PROPAGATION TECHNIQUES IN NEELAYAMARI (*Indigofera tinctoria* L.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Date: 01.10.2019

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CERTIFICATE

Certified that this thesis entitled **"OPTIMIZING PROPAGATION TECHNIQUES IN NEELAYAMARI (*Indigofera tinctoria* L.)"** is a record of research work done independently by **Ms. Mekha Mariam Abraham** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

India is acknowledged as one of the world's richest treasure troves of medicinal plants. Indian sub- continent ranks 10th among the plant genetic resource rich countries in the world. India is also among the top twelve mega diversity centres in the world. It nurtures about 15000- 20000 species of medicinal plants, which account for about 6 per cent of total plant species in the world (Kurian and Sankar, 2007). The flora of India is one of the richest of the world due to wide range of climate, topology and environments in the country.

In India, many indigenous medicinal systems are being practiced since ancient times. Ayurveda, Siddha and Unani are the major ones among them. Although the synthetic drugs and antibiotics have attained greater prominence, they prove to be harmful in the long run. The advantage of plant medicines is that they can be used for treatment of chronic diseases without any deleterious effects. The adverse effects and high cost of production of synthetic drugs also necessitated the preferential use of natural materials for various purposes.

Neelayamari commonly known as 'Indian indigo', is a medicinally as well as commercially useful leguminous plant. It is being cultivated in India, China and other countries of the east as a source of indigo- a commercially valuable dye. The extract of the leaves is reported to have remarkable effect on hair growth and in preventing juvenile greying of hair. In Ayurveda, it has been reported to be one of the major ingredients for the treatment of hydrophobia, epilepsy, nervous disorders and bronchitis. It is also effective in treating sores, old ulcers and haemorrhoids. Due to the presence of many worthy phytochemicals, the crop has been documented in '*Ashtangahridaya*'. This leguminous plant is also important as a green manure crop, catch crop, and transition crop. Owing to its permanent fast colour, indigo has long been used for dyeing and printing cotton, rayon and wool. Many pigments needed for paints, lacquers, rubber and printing ink are prepared from indigo. It is identified as a crop for commercial cultivation in Kerala by National Bank for Agriculture and Rural Development (NABARD) and

is cultivated by several pharmaceutical entrepreneurs, both in public and private sector.

Most of the medicinal plants are propagated through seeds, which is the cheapest method of propagation. Quality seed is a prerequisite for obtaining higher productivity. Seed quality is influenced by pre-harvest and post-harvest factors and also the fruit maturity. Information on fruit and seed development, optimum stage of maturity for seed harvest is essential for obtaining quality seeds. Seeds possess the maximum quality when they are harvested at their physiological maturity. Early or delayed harvesting may result in poor seed vigour. Harvesting at optimum maturity can also aid in preventing seed damage by insect pests, diseases and adverse environmental conditions. Seed being the propagule, its preservation without losing viability is mandatory.

Indigofera tinctoria is commercially propagated through seeds. However, owing to lack of information on optimum stage of harvest, extent of seed viability and germination are reported to be very low. Physiological maturity and dormancy are some of the factors influencing the viability and germination of a seed. Hence, there is a necessity to standardise the physiological maturity index for seed harvest.

Pre-sowing seed treatments are found to improve the rate and uniformity of seed germination, longevity and also seedling vigour. By standardizing the optimum seed treatment technique, seed germination and seed quality can be enhanced and dormancy, if any, can be broken. Despite the immediate improvements in seed performance following pre-sowing treatments, there have been contrasting reports of storage potential of seeds following the treatments. By studying the storability of treated seeds, it would be possible to know whether the initial advantage gained through seed treatment is retained even after storage. A knowledge of proper storage of seeds under ambient conditions at relatively low cost with minimum quality deterioration for next season will be of immense use to farming community and also the seed industry.

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Vegetative propagation has proved to be a cheap alternative to seed propagation in many crops. The possibility of vegetative propagation in Neelayamari is to be ascertained.

Thus, keeping in view of all the facts stated above, the present study 'Optimizing propagation techniques in Neelayamari (*Indigofera tinctoria* L.)' was proposed with the following objectives:

- To standardize the physiological maturity stage in *Indigofera tinctoria* L. for seed harvest
- To standardize the pre-sowing seed treatments to enhance seed quality and longevity
- To standardize the vegetative propagation technique in *Indigofera tinctoria* L. through stem cuttings

Review of literature

2. REVIEW OF LITERATURE

In this chapter, an effort has been made to review the existing literature pertaining to most of the aspects related to the current work.

2.1 GEOGRAPHICAL DISTRIBUTION AND MORPHOLOGY

Indigofera tinctoria L., commonly known as Neelayamari in Malayalam, Indian indigo in English and Neelini/ Neelika/ Renjini in Sanskrit, is a medicinally as well as commercially useful leguminous plant. India is believed to be the birthplace of the crop. The name *Indigofera* is derived from indigo and from the Latin word "ferre" which means to 'bear', thus indicating indigo bearing. The species name *tinctoria* refers to *tinctorius,* meaning 'of dyes' or 'belonging to dyes' (Marafioti, 1970; Simon *et al.,*1984).

Plants in the genus *Indigofera* are found to be prostrate, sub-erect or twining herbs with simple, trifoliate and imparipinnate leaves. Inflorescence is an axillary raceme, panicle or a head. The flower has keels with downward spur on each side with diadelphous (9+1) stamens. Pods are usually oblong, linear, erect or deflexed, straight, curved, torulose, usually dehiscent (Ramamurthy and Pullaiah, 1998). The plant is a shrub with an average height of about 100 to 125 cm. January to February is considered to be the best flowering season. The flowers are produced in spikes and are small, purple to violet in colour. A single spike will bear about 12 to 25 pods of length 1.6 to 2.4 cm and each pod will bear about 4 to 6 seeds.

2.2 ETHNOBOTANICAL USES

It is an excellent source of madras indigo (Gamble, 1915). The leaf extract along with milk is found to be effective in curing hydrophobia, asthma, whooping cough, heart palpitation, certain lung and kidney disorders, bronchitis, haemorrhoids, hepatitis and scorpion sting (Chopra *et al.*, 1956).

A reputed drug called Nilibringadi for lustrous hair growth is produced from the plant (Joy *et al.*, 1998). Root, stem and leaves are medicinally important

parts and are used in various Ayurvedic and Yunani preparations. Root extract is widely used as an antidote against arsenic poisoning. It is also beneficial in curing rheumatism, leprosy and liver complaints. The leaf extract is effective in healing hydrophobia and whooping cough. Oil extracted from seed is used as a cure for epilepsy, sores and bronchitis (Nair and Mohanan, 1998).

Cosmetic, pharmaceutical and dyeing industries make use of the plant greatly. The leaf extract is found to exhibit positive influence on hair growth. It also prevents juvenile greying of hair (Nair *et al.*, 1991). Aerial parts of the plant possess various medicinal properties as anti-hepatotoxic (Sreepriya and Devaki, 2001), anti-dyslipidemic (Narender *et al.*, 2006) and anti-cancerous (Kameswaran and Ramanibhai, 2008). To relieve the pain due to bites and stings of venomous insects and reptiles, and also to cure burns and scalds, leaf decoction is widely used (Stepp, 2004). Methanolic leaf extract was found to possess high antibacterial, antioxidant and cytotoxic activities (Renukadevi and Sulthana, 2011).

2.3 SEED CHARACTERS

The range of viable seeds is about 25 to 50 per cent (Narayanan *et al.*, 1998). Under ambient storage conditions, seed lose their viability in a short span of time (Morris and Hopkins, 2000). *Indigofera tinctoria* seeds will lose viability upto 60 per cent in one year when stored under ambient storage conditions. The seeds have the capacity to germinate at low water potential (Sy *et al.*, 2001). Most of the leguminous plants are characterized by the presence of hard seed coat which is impermeable to water (Hari *et al.*, 2002).

2.4 STUDIES ON PHYSIOLOGICAL MATURITY ON SEEDS

The physiological parameters *viz.*, germinability and vigour play a very important role in judging the quality of a seed. The seed harvested at the physiological maturity will possess maximum germination and vigour. Thereafter, it will decline due to senescence, ageing and eventually no longer be able to germinate (Harrington and Kozlowski, 1972). Hence, the fruits that are harvested

at physiological maturity stage will have maximum dry matter accumulation and seeds attain maximum vigour at this stage and can be stored well for a longer period. Stage of maturity at harvest is one of the most important factors that can influence the quality of seeds. Harvesting too early may result in low yield and quality, because of the partial development of essential structures of seeds, whereas, harvesting too late may increase the risk of shattering and decrease the quality of seeds due to ageing. Adverse environmental conditions such as rainfall or precipitation may also result in sprouting of seeds on mother plants. Therefore, successful seed production depends on detection and prompt harvesting of seeds at appropriate time. This is a pre-requisite for the production of maximum number of high quality seeds (Harrington, 1973). Information available on the seed maturity studies of various crops are reviewed below:

Seed quality is found to be maximum at the physiological maturity stage and also depend on environmental conditions. Prolonged field exposure after the physiological maturity will ultimately result in low germinability, longevity and vigour of seedlings produced (McAlister, 1943).

Shaw and Loomis (1950) described physiological maturity as the point where the seed reaches its maximum dry seed weight.

Seed development and maturation refers to the morphological, physiological and functional changes that occur from the time of fertilization until the mature seeds are ready for harvest (Delouche, 1973).

Seed development studies in two cucumber cultivars, Jed Bai and Puang conducted by Aroonrungsikul *et al.* (2000) revealed that the seeds attained their physiological maturity at 35 days after anthesis in Jed Bai and 30 days after anthesis in Puang.

Sureshbabu *et al.* (2003) reported that in brinjal, highest seed weight (1.18g), germination (85.44%), and vigour index (994) were observed when the fruits were harvested at full yellow colour stage.

Seeds of Eryngo (*Eryngium foetidum* L.) reached their physiological maturity about 40 days after anthesis, with 95.75 per cent germination, when the seed head had just begun to turn brownish black in color. Shattering of pods was observed about 65 days after anthesis (Ekpong and Sukprakarn, 2006).

Physiological maturity of dill (*Anethum graveolens* L.) seeds was reported to be 50 days after anthesis. The per cent of seed germination was found to be highest at this stage (Ekpong and Sukprakarn, 2008).

Seed maturation studies by Ekpong (2009) in *Cleome gynandra* L. indicated that the seeds attain their physiological maturity at 18 days after anthesis. At this stage, the seeds possess maximum dry weight and high germination.

Ghassemi-Golezani *et al.* (2011) investigated the physiological maturity of rapeseed (*Brassica napus* L.) and found that the seed qualities *viz*., viability, germination per cent and seedling dry weight were found to be the highest at 22 days after anthesis.

Maximum seed weight (mass maturity) and moisture content in kenaf (*Hibiscus canabinus*) seeds were achieved at 35 days after anthesis. For high seed quality, kenaf is to be harvested at 35th day of anthesis which is regarded as the point of physiological maturity for seed harvest (Olasoji *et al*., 2012).

A study was conducted to standardise the physiological maturity in mustard (*Brassica juncea*) indicated that at 45 days after anthesis, the germination was the maximum (43 %). Hence, in mustard crop 45 days after anthesis was judged as the physiological maturity stage (Geetha *et al*., 2013).

Jayanthi *et al.* (2013) opined that in davana seeds, physiological maturity was attained on 35th day after anthesis, when the germination (86 %), seedling length (2.3 cm), vigour index (198) and dry matter production (1.23mg) were the highest.

2.5 EFFECT OF PRE-SOWING SEED TREATMENTS ON SEED QUALITY

Pre-sowing seed treatments resulted in improved germinability, better field stand than the corresponding untreated seeds. Reduced seed vigour as a result of ageing can have a considerable effect on harvesting time, quality and yield of crops. Seed treatments were developed to overcome these problems and to improve both the rate and uniformity of germination.

Kissock and Haferkamp (1983) obtained 90 per cent germination in western indigo seeds which were acid scarified, while, the untreated ones showed a lower germination per cent of 17 to 42.

Teketay (1996) investigated the germination requirements of five *Senna* species: *S. bicapsularis, S. didymobotrya, S. multiglandulosa, S. occidentalis* and *S. septemtrionalis* and found that the seeds possess dormancy attributed by their hard seed coats which hamper maximum, uniform and rapid germination. Mechanical scarification of the seeds could help to overcome the dormancy and increase the germination upto 100 per cent.

Kumari and Francis (1998) observed that the treatment of carrot seeds with conc. H_2SO_4 for 4 minutes resulted in increased germination per cent and seedling vigour.

Caseiro *et al.* (2004) reported that pre-sowing soaking in water for 96 h was the most successful method for improving seed germination in onion.

Hossain *et al.* (2005) conducted a study on the effect of different seed treatments on germination and seedling growth attributes of haritaki (*Terminalia chebula*). Improved germination was observed when the depulped seeds were soaked in cold water for 48 hours. This also resulted in the production of quality seedlings.

A study on seed germination and storage characteristics of *Decalepis hamiltonii* was undertaken by Anandalakshmi and Prakash (2010). An improvement in germination (about 98 per cent) was noticed when the seeds were soaked in hot water at 60°C for 24 hours.

Pandita *et al.* (2010) opined that pre-sowing soaking of seeds in water can enhance the germination in crops such as onion, carrot and sorghum.

A study conducted by Tadros and coworkers (2010) suggested that mechanical scarification of seeds of *Acacia farnesiana* was effective in breaking seed dormancy and enhancing seed germination (65 per cent). Soaking of *Leucaena leucocephala* seeds in 70°C water for 20 minutes resulted in maximum germination (97 %).

The effect of presowing treatments on seed germination of *Acacia catechu* and *Elaeocarpus floribundus* seeds was evaluated (Das, 2014). High germination (91.26%) was attained through treatment with hot water (80°C for 10min) treatment in *Acacia catechu*. For *Elaeocarpus floribundus* also, the highest germination (86.35%) was noticed in hot water (100°Cfor12min) treatment.

Mathad *et al.* (2014) studied the effect of dormancy breaking methods in a medicinal legume plant, *Psorolea corylifolia* and reported highest germination (87%) with mechanical puncturing and hot water treatment.

Mechanical scarification was suggested as an effective pre-treatment method in *Acacia polyacantha* seeds in order to enhance the speed and the amount of early seedling growth at the nursery stage (Missanjo *et al.*, 2014).

Nego *et al.* (2015) observed maximum seed germination in onion, when the seeds were soaked in distilled water for 48 h.

Purohit and coworkers in 2015 conducted pre-treatment studies on seeds of *Zanthoxylum armatum* and found out that treatment with diluted (50 per cent) H_2SO_4 for 15 minutes resulted in maximum germination (93.3 per cent).

Treatment of *Celastrus paniculatus* seeds with hot water of 70°C for 3 minutes resulted in increased rate of germination than control (Surya *et al.*, 2015).

Nagar and Meena (2016) claimed that seed scarification in freshly harvested seeds of hairy indigo (*Indigofera astragalina*) can improve the germination up to 94.5 per cent.

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Materechera (2017) conducted a study on *Moringa oleifera* seeds and found that the establishment of the seedlings can be enhanced by pre-treating the seeds. Physical abrasion of the seed coat improved the mean germination time and total germination.

Mechanical scarification and boiling water techniques were recommended as the best pre-sowing seed treatments in *Vachellia rehmanniana* Schinz to overcome seed dormancy (Mathowa *et al.*, 2017).

Miya and Modi (2017) suggested that the mechanically scarified seeds of bambara groundnut (*Vigna subterranea* L.) showed improved germination and seedling vigour.

2.6 INFLUENCE OF SEED STORAGE ON SEED QUALITY

Seed is a living entity and during storage, its quality deteriorates. Relative humidity and temperature are the two most important factors affecting the viability of seeds, in addition to duration of storage (Balesevic- Tubic *et al.*, 2010).

It is widely consented that loss in cell membrane integrity is one of the primary causes for viability loss. During seed ageing, membrane degradation increase electrolyte leakage. Decline in seed germination, field emergence and seedling vigour is associated with high level of electrolyte leakage. Membrane disruption and loss of permeability occur at an early stage during the seed deterioration (Jyoti and Malik, 2013).

The leachate from the seed measured as electrical conductivity was associated with loss of vigour and viability in seeds (Bradknock and Mathews, 1970; Powell and Mathews, 1986).

Abdul-Baki and Anderson (1972) reported that leaching of sugars increased with seed age and ultimately leads to the reduction in metabolic activity and seed quality.

Singh and Setia (1974) opined that the seed germination will decline as the duration of storage increases.

It was observed that the seed longevity is affected by genetics, quality of the seed at the time of storage, moisture content of the seed and the storage environment (Gupta, 1976).

As the storage period of maize seed is increased, the electrical conductivity and the leaching of free amino acid and sugars also increased owing to seed deterioration (Krishnaveni and Ramasamy, 1985).

When the storage period increases, there occurs a notable decrease in germination and seedling vigour (Kurdikeri *et al.*, 1991).

With increase in storage period, membrane permeability rises leading to loss of electrolytes, sugars, aminoacids and phenols (Deswal and Sheoran, 1993).

Meng (1993) reported that soybean seeds when subjected to ageing, cell structure got deteriorated resulting in elevated electrical conductivity and reduced germination.

Dighe *et al.* (1995) observed that seed deterioration in sunflower was judged by increased seed electrical conductivity.

Germination capacity of *Nardostachys jatamansi* seeds was maximum at the time of harvest which decreased during storage. This may be due to the seed deterioration owing to the loss of moisture during storage (Chauhan and Nautiyal, 2007).

A study conducted on soybean seed by Shelar *et al.* (2008) indicated that there is a decline in germination during storage which appeared to be the lowest at the end of storage period.

Zamani *et al.* (2010) opined that in seeds of safflower seeds when stored for longer periods, the cell structures got destroyed leading to elevated membrane permeability and thereby increased loss of sugars and electrolytes from the cells.

Cotton seeds when stored in polythene covers were found to maintain the viability for twelve months (Hemashree *et al.*, 2011).

Maize seeds when exposed to ageing treatments showed reduced vigour and germination. Seedling parameters were also found to be low for those. Thus, the long term storage and ageing leads to deterioration of seed quality in maize (Siadat *et al.*, 2012).

The quality, vigour and germination of cowpea seeds were found to be significantly affected when they were stored for a longer period (Bortey *et al.*, 2016).

Rice seeds when packed in polythene bags retained high seed quality characters such as higher germination, root length, shoot length and lower electrical conductivity of seed leachate after twelve months of storage (Gupta, 2017).

Chilli seeds remain viable for only six months of storage. The viability parameters as germination, field emergence and speed of germination showed a decreasing trend as the storage period increased (Verma *et al.*, 2018).

2.7 EFFECT OF INDOLEBUTYRIC ACID (IBA) ON CUTTINGS

In most cases, the inherent ability of cuttings to root can be altered by chemical treatments. Auxins have been used for many years to promote root initiation. IBA and NAA are the most commonly used rooting hormones. The concentration of these may vary according to the nature of the plant. The role of auxins in stimulating adventitious root formation in stem cuttings was predicted by Went (1938). Dron (1938), based on his studies concluded that the relative amount of auxin present naturally or applied is associated with the formation of root primordia. Root formation in a plant is possible only when there are developing buds or leaves on them. Dormant buds fail to induce rooting. In such instances, rooting is facilitated by the presence of a hormone. It is found that auxins play a role in increasing the rate of formation and final number of root initials.

Sandhu and Singh (1986) studied the effect of IBA treatment on the sweet lime stem cuttings. The treatments 50 and 100 ppm IBA were applied by the prolong dip method and 2000 ppm IBA applied by the quick dip method. 100 percent rooting was observed when the concentration of IBA was the highest. Amount of cuttings having secondary roots also recorded marked improvement with IBA treatment.

Surendran (1990) observed that single node cuttings prepared from mature branches of gamhar (*Gmelia arborea*) achieved 73.3 and 60 per cent rooting when treated with 100 ppm and 1000 ppm IBA respectively.

Vegetative propagation in curry leaf using semi hardwood cuttings was conducted by Mohanalakshmi *et al.* (2000).The cuttings were treated with different concentrations of IBA. The rooting per cent increased with the increase in concentration of IBA and the highest rooting was obtained when the cuttings were treated with 1500 ppm IBA. The semi hardwood cuttings planted during May and July recorded 30 per cent and 20 per cent rooting. The hardwood cuttings also showed higher rate of survival with increased rate of IBA.

The apical shoots of lemon (*Citrus limon* cv. Assam) were treated with IBA at different concentrations (1000, 2000, 3000 and 4000 ppm). Rooting and root characteristics were recorded 70 days after planting. The highest per cent of rooting (98.5%) and survival (100%) were recorded for cuttings treated with 3000 IBA ppm, while the lowest rooting and survival were noted in the control. The treatment also proved to be significantly superior to the rest of the treatments with respect to the number of primary roots, root length, dry weight of roots, number of leaves and shoot length per cutting. IBA at higher concentration (above 3000 ppm) showed an inhibitory effect on rooting and root characters (Nath, 2000).

Patel (2001) investigated the influence of various levels of IBA (1000, 2000, 3000, 4000 and 5000 ppm) on hardwood cutting of *Jasminum sambac* cv. 'Double Mogra'. IBA at4000 ppm was found to be the best treatment for obtaining higher number of rooted cuttings, number of main roots, length and thickness of longest root, sprouting and number of shoots per rooted cuttings, fresh and dry weight of roots and survival.

As per the study conducted by Ranganathappa *et al.* (2002), hardwood stem cuttings of curry leaf registered the highest rooting (37.50 %) and highest per cent survival (91.67 %), when treated with 2000 ppm IBA.

A study conducted in *Rauvolfia serpentine* indicated that the cuttings treated with IBA 2000 ppm showed significantly increased rooting and sprouting compared to the control (Husen, 2003).

Panneerselvam *et al.* (2004) stated that the treatment of softwood, semi-hardwood and hardwood cuttings of stinking tree (*Nothapotydes nimmoniana*) with 2000, 3000 and 4000 ppm IBA resulted in high sprouting, rooting and survival percent when compared to the control. Best results were observed when semi- hardwood cuttings were treated with 3000 ppm IBA in talc form.

Shwetha (2005) opined that IBA at 2000 ppm aided better induction of rooting (66.66%) as against the control (15.33%) in Indian lavender (*Bursera delpechiana*).

Gateble and Pastor (2006) studied the vegetative propagation of *Oxera sulfurea* and reported best rooting rates with IBA at 10g kg^{-1}.

Effectiveness of auxins on rooting of cuttings in Allspice was studied by Rema *et al.* (2008). They obtained 64 per cent rooting of cuttings when treated with 1000 ppm IBA.

Air layers of water apple were treated with various concentrations of IBA. IBA at 1000 ppm was found to be more effective in inducing roots and resulted in production of thicker roots (Paul and Aditi, 2009).

Sharma *et al.* (2009) conducted a study on vegetative propagation of pomegranate through cuttings and observed that semi hardwood and hardwood cuttings showed maximum rooting, root number and root length and field survival when treated with 500 ppm IBA.

The effect of different concentrations of IBA on root formation in stem cuttings of Kagzi-lime was examined by Bhatt and Tomar (2010). The treatment with IBA 500 ppm resulted in increased root formation, length of root, thickening of root and leaf sprouting in shoot.

It was reported that the cuttings of *Tinospora cordifolia* treated with 100 ppm IBA recorded highest sprouting and rooting when compared to control and other auxin treatments (Mishra *et al.*, 2010).

Highest rooting in cuttings of *Premna integrifolia* was observed when they were treated with 1000 ppm IBA (Sharma *et al.*, 2011). Other parameters like number of roots per cutting, fresh and dry weight of roots, field establishment per cent were also found to be highest in the same treatment.

Sharma *et al.* (2009) conducted a study on vegetative propagation of pomegranate through cuttings and observed that semi hardwood and hardwood cuttings showed maximum rooting, root number and root length and field survival when treated with 500 ppm IBA.

Ercisli *et al.* (2012) reported that maximum survival per cent of stem cuttings in two different genotypes of *Rosa spp.* (91% and 89%, respectively) by

using IBA 500 ppm. Whereas, maximum number of roots (50 and 47 roots) and the longest roots (31 and 28 cm) were recorded by using IBA 1000 ppm.

Kuldeep *et al.* (2013) stated that the application of IBA at 2000 ppm was found to be superior in induction of rooting (63.33%), number of roots (30.00), length of roots (12.85cm) and dry weight of roots (0.43g) per cutting after two month of planting in Bougainvillea (var. Thimma) cuttings.

Mehraj *et al.* (2013) investigated the influence of IBA on rooting potential and sprouting of *Bougainvillea spectabilis* stem cuttings. The cuttings were soaked in 500 ppm,1000 ppm and 2000 ppm of IBA. Cuttings treated with 1000 ppm IBA registered maximum rooting and survival.

Das and Jha (2014) investigated the effects of plant growth regulators on root proliferation on *Taxus wallichiana* shoot cuttings and found that better rooting response were exhibited by IBA treated cuttings.

In carnation, high rooting of stem cuttings was recorded when treated with 550 ppm IBA (Kumar *et al.*, 2014).

Saumya *et al.* (2014) reported that the semi- hardwood cuttings of *Salacia fruiticosa* Heyne ex Lawson, when treated with 3000 ppm IBA, resulted in highest rooting (80 %). Maximum root length and number of new leaves were also observed in this treatment.

The rooting (72.3 %), length of roots (36 mm) and number of roots (9.0) were observed to be maximum when treated with IBA 4000 ppm in hard wood cuttings of *Bougainvillea glabra* L (Seyedi *et al.*, 2014).

The influence of IBA on the rooting of mulberry cuttings was investigated by Singh *et al.* (2014) wherein the cuttings were treated with 1000, 1500 and 2000

ppm of IBA by quick dip method. The number of sprouted cuttings, length of roots and longest sprout were higher in 2000 ppm IBA treatment.

Singh *et al.*(2014) obtained the highest number of roots per cutting (43.00), length of roots per cutting (9.28 cm), diameter of roots per cutting (1.67 mm), per cent of rooted cutting (88.00 %), number of sprouts per cuttings (4.34) and the minimum (20.66) days taken to callus formation in stem cuttings of golden duranta when treated with 1400 ppm IBA concentration.

The stem cuttings of Rose cv. Bajazzo produced the maximum number of roots (30.25) when treated with IBA 2000 ppm, whereas the control produced the least number of roots (Abbas *et al.*, 2015).

Al Zebari and Al Brifkany (2015) investigated the effect of IBA on rooting capacity of citron stem cuttings. It was found that increase in IBA concentration resulted in increased rooting, shoot diameter, length of roots, number of leaves and leaf chlorophyll content. Semi-hardwood cuttings treated with 500 ppm and 1000 ppm IBA performed better in terms of root per cent, length of shoot, number of leaves and length of roots.

Husen *et al.* (2015) reported that highest per cent rooting and sprouting in nodal shoot cuttings of *Grewia optiva* Drummond was observed when treated with IBA 3000 ppm.

The propagation studies of difficult to root *Lemon verbena* cuttings conducted by Ibrahim *et al.* (2015) suggested that the highest value of rooting of cuttings and root length were recorded with 250 ppm of IBA in semi-hardwood cuttings.

Jana *et al.* (2015) treated woody stem cuttings of Asian pear (*Pyrus puyrifolia*) with different concentrations of IBA and reported that IBA treatment

at 1500 ppm accounted for minimum time for rooting with maximum success percent.

The effect of maturity stage of cuttings on the survival per cent of *Salacia reticulate* propagation was examined by Nayana *et al.* (2015). The semi hardwood cutting produced the highest number of sprouts and showed maximum survival per cent compared to softwood and hardwood cuttings.

Softwood cuttings of *Cordyline terminalis* exhibited enhanced rooting of 88 per cent and higher number of roots when treated with IBA 1000 ppm (Rahdari *et al.*, 2015).

Singh and Tomar (2015) conducted an experiment to examine the effect of different levels of IBA on rooting of phalsa cuttings. Treatment of hardwood cuttings with 4000 ppm IBA resulted in maximum survival per cent (67.2), rooting per cent (73.2) number of roots (10.87) and shoots (6.23) and longest root (10.63) followed by IBA 2000 ppm.

Singh *et al.* (2015) stated that treatment with 8000 ppm IBA recorded maximum spouting (81.90) and survival per cent (88.70) in cuttings of lemon cv. Pant lemon -1 as compared to control.

Singh *et al.* (2015) examined the effect of hormonal treatments on rooting ability and survival of *Citrus limon* cuttings. Maximum number of sprouts (2.42) and highest survival per cent (81.68 %) were recorded when the cuttings were treated with IBA 5000pm concentration. Spring season was found superior in terms of number of sprouts (2.29) and number of primary roots (7.3%) per cutting. The survival per cent (77.37 %) and number of secondary roots (15.30 %) were more in spring season.

Compared to control, rooting was high (36.63 %) in hardwood and semi-hardwood cuttings of guava when treated with 3000 ppm IBA (Soni *et al.*, 2015).

Yeshiwas *et al.* (2015) reported that the number of roots (54.2), root length (11.29 cm), shoot length (14.4 cm), fresh weight of shoot (2.05 g), dry weight of shoot (0.61 g) and dry weight of root (0.21 g) were maximum in stem cuttings of Rose on treatment with IBA 1000 ppm. However, fresh weight of root (0.90 g) wasthe highest at IBA 1500 ppm.

Sundharaiya *et al.* (2016) conducted an experiment to standardise vegetative propagation of Vellerukku (*Calotropis procera*) using three types of cuttings and three types of growth regulators both alone and in combination. The results indicated that softwood cuttings treated with 500 ppm IBA recorded the highest rooting per cent, number of roots, highest survival per cent and longest root and shoot.

Chater *et al.* (2017) experimented on the vegetative propagation of pomegranate and reported highest per cent of rooting and survival when the cuttings were treated with 2000 ppm IBA. Higher callus formation, number of primary and secondary roots and length of roots were also recorded under the treatment. Maximum success was recorded in hardwood cuttings compared to semi hard wood cuttings.

Semi-hardwood cuttings of pomegranate cv. Bhagwa were treated with IBA for 8 hours at different concentrations. The cuttings treated with 1500 ppm IBA recorded the highest sprouting per cent, highest rooting per cent, maximum number of sprouts, greatest diameter of sprout, longest roots and widest diameter of roots (Seiar, 2017).

Ahmad *et al.* (2018) investigated the effect of IBA on rooting and shooting parameters of Rangpur lime cuttings. Among the various treatments used in the

experiment, IBA 1000 ppm resulted in the best response with respect to all the parameters studied *viz*., rooting/cutting (45.37 %), survival per cent of rooted cuttings (60.00 %), maximum number of roots/cutting (3.93), girth of the thickest root (1.38 mm), length of the longest root (4.47 cm), number of leaves/cutting (2.27), number of secondary branches/cutting (1.87) and number of leaves/secondary branch (4.60).

Dahale *et al.* (2018) studied the effect of various concentrations of IBA on rooting and survival of fig hardwood cuttings. Treatment with combination of 1000 ppm IBA registered maximum survival (82.50 %), rooted cuttings (58.66%), root and shoot growth.

Successful rooting in *Taxus baccata* juvenile stem cuttings was aided by treatment with IBA at lower concentration (1000 ppm). But a higher concentration of 2000 ppm was necessary for successful rooting of mature cuttings (Das and Jha, 2018).

Izhaki *et al.* (2018) reported that the softwood cuttings of persimmon (*Diospyros virginiana*) treated with 6000 ppm IBA rooted better than the untreated ones.

The rooting performance of stem cuttings of *Aegle marmelos* (Bael) under three different doses of IBA was examined by Kabir *et al.* (2018). The highest rooting (60 %) was recorded with 2g/l and 4g/l IBA followed by 8g/l IBA (40%). Longest root (3 cm) was observed on treatment with 4g/l IBA followed by 2g/l IBA (1.2 cm). The maximum root number (2.25) and root diameter (2 mm) were obtained from cuttings treated with 8g/l IBA followed by 4g/l IBA (2 and 1.9 mm respectively). Survival of the cuttings (the rooted cuttings) increased significantly on exogenous IBA application. The highest survival per cent (73.5%) was observed for the cuttings treated with 4g/l IBA followed by 8g/l IBA (68.5%).

Mehta *et al.* (2018) observed that in pomegranate stem cuttings, the number of sprouted cuttings, number of leaves on new shoot, length of longest sprout and per cent of rooted cuttings were high on treatment with 500 ppm IBA treatment.

Tanuja and Rana (2018) stated that the stem cuttings of Karonda (*Carissa carandas*) cv. Pant Manohar recorded maximum percent of sprouting and rooting when treated with 8000 ppm IBA.

Materials and methods

3. MATERIALS AND METHODS

The study on 'Optimizing propagation techniques in Neelayamari (*Indigofera tinctoria* L.)' was conducted in Kerala Agricultural University (KAU) during 2017-2019. The study was conducted with the objectives of standardizing the physiological maturity stage for seed harvest, pre-sowing seed treatments for improving seed quality and longevity and also vegetative propagation technique through stem cuttings in Neelayamari. The details regarding the materials and techniques that have been used for the research work are described below:

3.1 LOCATION AND CLIMATE

The experiment was conducted at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara P. O., Thrissur 680656. The location experiences a humid tropical climate, located 40 m above MSL at $10^0$54' North latitude and $76^0$28' East longitude.

3.2 EXPERIMENT DETAILS

3.2.1 Experiment 1: Standardization of physiological maturity stage for seed harvest in Neelayamari

3.2.1.1 Experimental material

The crop was raised during 2018-2019 in the field of Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University (KAU). Management practices were followed as per the Package of Practices Recommendations of Kerala Agricultural University (2016).

Flowers were tagged on the day of anthesis. Pods were harvested at an interval of 5 days starting from the 10^{th} day of anthesis up to the pod splitting stage. The pods were shade dried for 24 hours and then the seeds were extracted. The seeds were sown in sterilised sand medium on extraction.

3.2.1.2 Experimental details

Five plants were randomly selected in each replication of a treatment and tagged. Observations were recorded in these tagged plants at appropriate growth stages.

Design : Completely Randomised Design (CRD)

No. of treatments : 8

No. of replications : 3

Treatments are :

T_1	10 DAA
T_2	15 DAA
T_3	20 DAA
T_4	25 DAA
T_5	30 DAA
T_6	35 DAA
T_7	40 DAA
T_8	45 DAA
T_9	50 DAA
T_{10}	55 DAA
T_{11}	60 DAA
T_{12}	Control (Pod splitting stage)

3.2.1.3 Observations

Following observations were taken:

Plant height at flowering (cm)

Height of the plant when the crop reached fifty percent flowering was recorded and expressed in centimetres.

Number of days for flowering

Total number of days taken from sowing to fifty per cent of flowering was recorded.

Days to pod set

Total number of days required for pod setting in fifty percent of the plants in the plot, from the day of planting was recorded.

Per cent of pod setting

Total number of pods produced in an inflorescence in five randomly selected plants in each replication was recorded. Per cent of pod set was calculated as detailed below and averaged.

$$\text{Per cent of pod set per plant} = \frac{\text{Total number of pods produced per inflorescence}}{\text{Total number of florets in an inflorescence}}$$

Pod length (cm)

Length of 75 pods was measured from the stalk end excluding the stalk to the distal end and the average pod length expressed in centimetres.

Pod thickness (mm)

Thickness of 75 pods was measured and the average was expressed in millimetres.

Fresh pod weight (mg)

Freshly harvested pods (75 nos) were weighed and the average weight expressed in milligrams.

Dry pod weight (mg)

The harvested pods were dried in a hot air oven, their weight recorded and the average was expressed in milligrams.

Number of seeds per pod

Seeds from pods (75 nos) were extracted and the total number of seeds per pods recorded and average arrived at.

Fresh seed weight per pod (mg)

Seeds separated from individual pods (75 nos) were weighed and the average expressed in milligrams.

Seed length (mm)

Length of seeds (100 seeds) were measured and the average expressed in millimetres.

Seed breadth (mm)

Breadth of seeds (100 seeds) were measured and the average was expressed in millimetres.

100 seed weight (g)

One hundred seeds selected randomly from five plants in each replication were weighed to determine the 100 seed weight and the average expressed in grams.

Seed moisture content (%)

Moisture content of the seeds was determined using low constant temperature hot air oven method as per the procedure advocated by ISTA (1999). Fresh weight of the sample was determined using an electronic balance. Seed samples were then dried in hot air oven, maintained at 103^0C for 24 hours and then the dry weight of the sample was noted. The moisture content was determined on wet basis by using the following formula and expressed as per cent (ISTA, 1999).

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Germination (%)

Three replications of seeds (100 seeds/ replication) were sown in sterilized sand medium under ambient conditions. The initial and final germination counts were recorded on 7^{th} and 14^{th} days respectively by recording the total number of normal seedlings produced. The mean number of normal seedlings produced to the number of seeds sown was averaged and expressed in per cent.

Seedling root length(mm)

At the end of germination test period *i.e.,* on the 14^{th} day, ten normal seedlings were selected at random and the length between the collar region and root tip was measured as the root length and the average expressed in centimetres.

Seedling shoot length (cm)

The length between the collar region and the leaf tip in the seedlings in which root length was recorded, was measured in centimetres and the mean value arrived at.

Seedling dry weight(mg)

Ten normal seedlings were randomly selected and their fresh weight were recorded. They were air dried and put in a butter paper cover and then dried in hot air oven maintained at 60^0C for 24 hours as per ISTA (2007). Then the dried seedlings were cooled for 45 minutes and the dry weight assessed using an electronic balance. The dry weight of a single seedling was then calculated and expressed in milligrams.

3.2.2 Experiment 2: Effect of pre-sowing treatments on seed quality and longevity in Neelayamari

3.2.2.1 Effect of pre-storage treatments on seed quality

3.2.2.1.1 Experimental details

Design : Completely Randomised Design (CRD)

Number of treatments : 8

Treatments :

T_1	Treatment with Con.H_2SO_4 for 5 min
T_2	Treatment with Con. H_2SO_4 for 10 min
T_3	Treatment with Con. H_2SO_4 for 15 min
T_4	Mechanical scarification with sand
T_5	Hot water treatment at 80°C for 20 min
T_6	Hot water treatment at 60°C for 30 min
T_7	Hydration for 24 h
T_8	Absolute control

Seeds extracted immediately after harvest were primed as per the standard procedure. Unprimed seeds served as the control. The seeds were sown at 5 days interval starting from the day of treatment upto 30 days and observations recorded.

3.2.2.1.2 Observations

Observations on germination (%), seedling root length (cm), seedling shoot length (cm) and seedling dry weight (mg) were recorded as enumerated under *3.2.1.3.*

3.2.2.2 Effect of pre-storage treatments on seed longevity

The primed seeds were dried to ≤8 per cent moisture, packed in 700 gauge polyethylene bags and were stored at ambient condition. Seed quality parameters were recorded at the start of storage and subsequently at monthly intervals up to 6 months.

Design : Completely Randomised Design (CRD)

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Number of treatments : 8 priming treatments (same as the above experiment)

Storage period : 6 months

Observations on germination (%), seedling root length (cm), seedling shoot length (cm) and seedling dry weight (mg) were recorded as enumerated under *3.2.1.3.*

Seed moisture content

Moisture content of the seeds was determined using low constant temperature hot air oven method as per the procedure advocated by ISTA (1999). Fresh weight of the sample was determined using an electronic balance. Seed samples were then dried in hot air oven, maintained at 103^0C for 24 hours and then dry weight of the material was noted. The moisture content was determined on wet basis by using the following formula and expressed as per cent (ISTA, 1999).

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Electrical conductivity (dSm^{-1})

Fifty randomly selected seeds in each replication were soaked in 50 ml of water for 8 hours and then the leachate was used for measuring the electrical conductivity using an EC meter (ISTA, 1999).

3.2.3 Experiment 3: Standardization of vegetative propagation techniques in Neelayamari through cuttings

3.2.3.1 Experimental material

The cuttings required for the experiment were collected from plants grown in field under the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University (KAU).

3.2.3.2 Experimental details

Design : Completely Randomised Design (CRD)

No. of cuttings per treatment : 30

No. of Treatments : 27

a. Types of cuttings : 3 (softwood, semi-hardwood and hardwood)
b. Dosage of IBA : 9 Nos. (enumerated below)

Treatments are:

C_1	Hardwood cuttings
C_2	Semi- hardwood cuttings
C_3	Softwood cuttings
T_1	250 ppm IBA for 15 min
T_2	500 ppm IBA for 15 min
T_3	750 ppm IBA for 15 min
T_4	1000 ppm IBA for 1 min
T_5	1500 ppm IBA for 1 min
T_6	2000 ppm IBA for 1 min
T_7	2500 ppm IBA for 1 min
T_8	Charcoal slurry dip for 1 min
T_9	Control (Untreated)

The prepared cuttings were exposed to different treatments for different time periods. Exposure period for treatments T_1 to T_3 is 15 minutes and for T_4 to T_8 is 1 minute. The cuttings were planted immediately after treatment period in the growing medium and were maintained in mist chamber.

3.2.3.3 Observations

The observations recorded were

Days to sprout

The number of days taken from the day of planting to the appearance of first sprout was recorded.

Number of emerged leaves

Number of leaves after sprouting in each cuttings were counted after 30 days of planting.

Length of emerged leaves(cm)

Length of the emerged leaves was measured after 30 days of planting and expressed in centimetres.

Width of emerged leaves

Width of the emerged leaves was measured after 30 days of planting and expressed in centimetres.

Number of roots

Number of primary roots of rooted cuttings after 60 days of planting was counted.

Length of roots (cm)

Length of the longest primary root was measured and expressed in centimetres.

Cuttings establishment (%)

Number of sprouted cuttings that had survived till 60 days after planting were counted.

3.3 STATISTICAL ANALYSIS

The data obtained from experiment 1 and 2 was analysed using SPSS software package. Duncan's Multiple Range Test was employed to test the significance of difference between means of treatments. Data of vegetative propagation studies was analysed using OPSTAT software package (two factor analysis).

Results

4. RESULTS

The results of the current investigation on 'Optimizing propagation techniques in Neelayamari (*Indigofera tinctorial* L.)' are presented below.

4.1 STANDARDIZATION OF PHYSIOLOGICAL MATURITY STAGE FOR SEED HARVEST IN NEELAYAMARI

At physiological maturity, the seeds reaches its maximum germination potential. Thereafter the seed quality declines, owing to senescence and ageing.

For optimizing the physiological maturity stage for seed harvest, the pod, seed and seedling characters were studied. The flowers were tagged on the day of anthesis and the pods were harvested in 5 days interval starting from 10 days after anthesis upto 63 days after anthesis (pod splitting stage). Pods were shade dried for 24 hours and then the seeds were extracted. The seeds were then sown in sterilised sand medium.

4.1.1 Flowering characters

Morphological characters of plants such as plant height, days to flowering, days to pod set and pod setting per cent were calculated (Table 1). Plant height at flowering was found to be 127.35 cm. Plants started to flower on 134.56 days and the pod setting was observed at 140.35 days. Pod setting per cent was about 30.26 per cent.

Table 1. Plant height, days to flowering and pod set in Neelayamari

Characters	Mean values
Plant height at flowering (cm)	127.35
Days to flowering	134.56 days
Days to pod set	140.35 days
Pod setting (%)	30.26

4.1.2 Visual characters of pods and seeds

Visual changes in pods during different developmental stages are described in Table 2. During the initial stages, the pods were slender and curvy with calyx adhering to them (Plate 1). They were dark green coloured with purple to brown shading at inner surface. As the pods developed, their curviness disappeared and they became straight. Their colour also changed at different developmental stages. There was a transition from dark green to brown. The initial dark green colour faded to green and then to light green. As the pods mature, they became yellowish coloured. During later stages, they became brown coloured indicating the drying.

Table 2. Visual characters of pods at different stages of maturity

Days after anthesis (DAA)	Morphological characters of pods
T1 (10 DAA)	Curved, slender, adhering calyx, dark green with purple to brown shading at inner side
T2 (15 DAA)	Slightly curved, slender, dark green with purple to brown shading at inner side, seed setting
T3 (20 DAA)	Curved at tip, purple to brown shading at inner side
T4 (25 DAA)	Curved at tip, fading of purple shading
T5 (30 DAA)	Straight, green colour
T6 (35 DAA)	Straight, bold and round, green colour
T7 (40 DAA)	Straight, bold, green to light green colour
T8 (45 DAA)	Straight, plumpy, yellowish colour
T9 (50 DAA)	Straight, plumpy, yellowish to light brown
T10 (55 DAA)	Straight, shrivelling of pods, light brown
T11 (60 DAA)	Straight, shrivelling of pods, light to dark brown
T12 (63 DAA)	Pods split open, dark brown

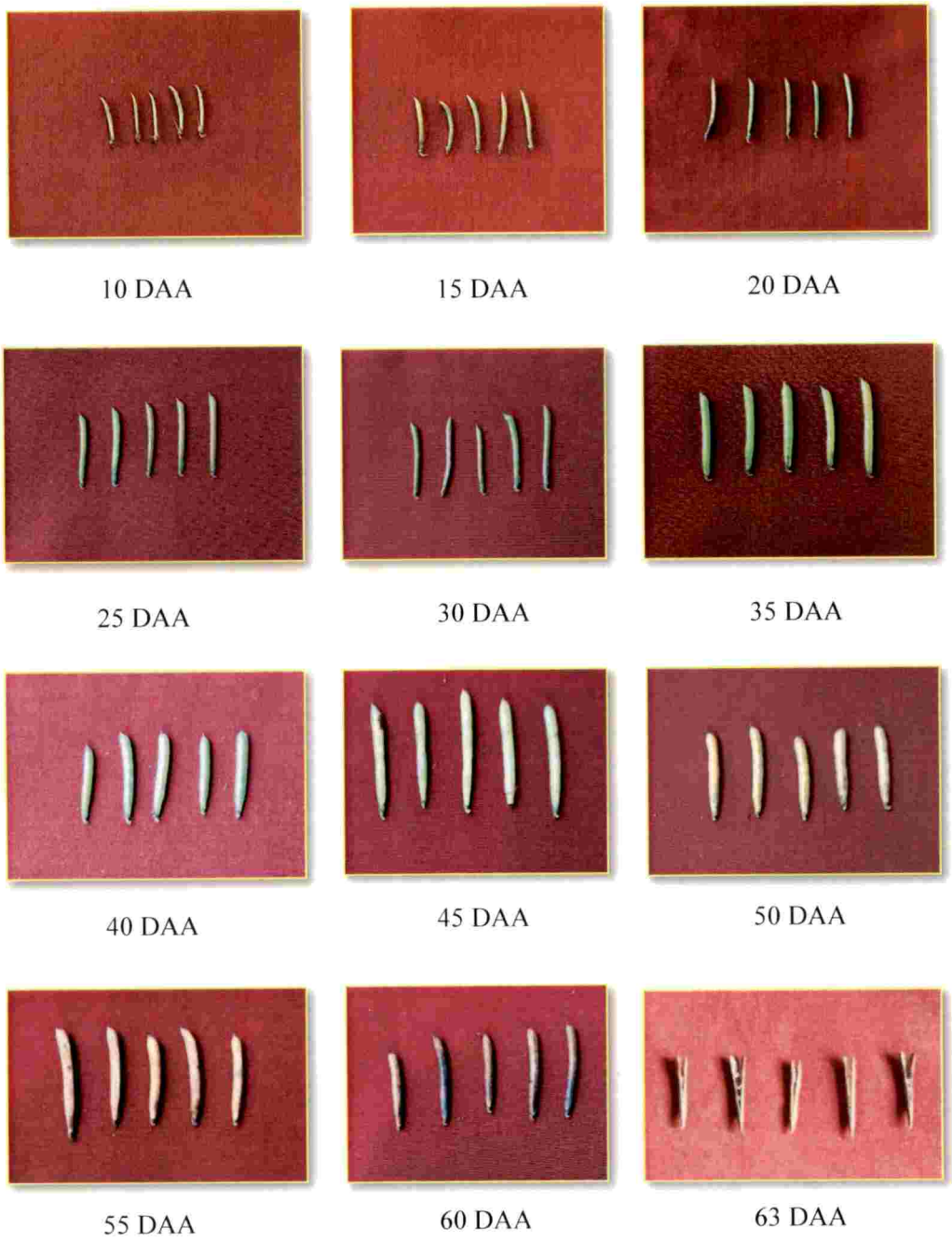

Plate 1: Pods at different developmental stages

The seed morphology at various developmental stages are recorded in Table 3. During the initial developmental stages, the seeds were very minute that they were not clearly visible with naked eyes (Plate 2). They were dark green coloured and could not be separated from the pod. At later stages, they became much larger and bold. Colour transition was observed through developmental stages. At the end of development, they became dark brown with prominently visible black hilum.

Table 3. Visual characters of seeds at different stages of maturity

Days after anthesis (DAA)	Morphological characters of fresh seeds
T1 (10 DAA)	Very minute seeds, inseparable from pod and difficult to observe with naked eyes
T2 (15 DAA)	Very minute, dark green, inseparable from pod
T3 (20 DAA)	Minute, dark green, translucent
T4 (25 DAA)	Minute, dark green, translucent
T5 (30 DAA)	Small, dark green
T6 (35 DAA)	Small, dark green
T7 (40 DAA)	Large, bolder, dark green
T8 (45 DAA)	Large, bolder, yellowish green
T9 (50 DAA)	Large, bolder, brownish green
T10 (55 DAA)	Light brown, black prominent hilum
T11 (60 DAA)	Dark brown, black prominent hilum
T12 (63 DAA)	Dark brown, black prominent hilum

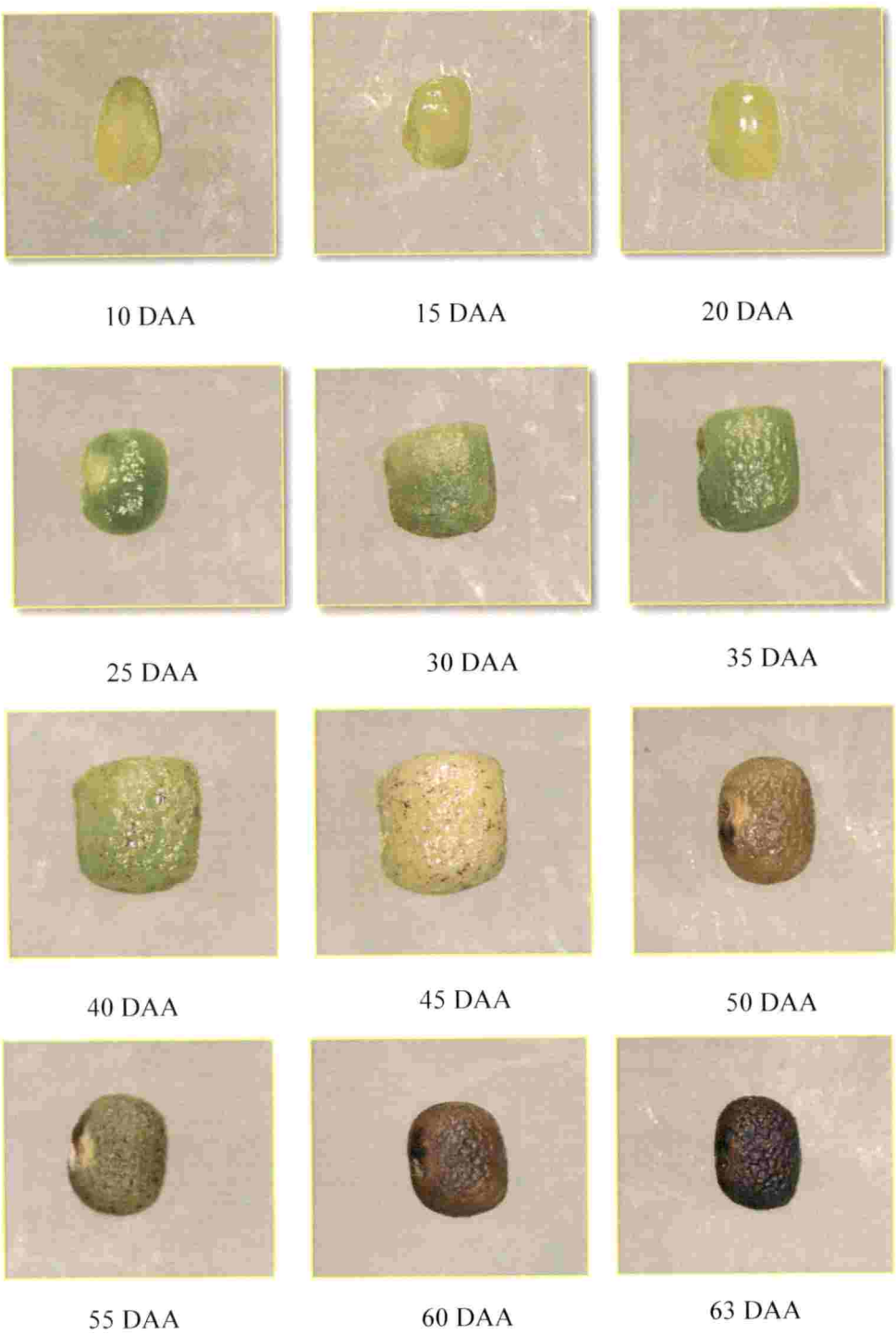

Plate 2: Seeds at different developmental stages (10x)

Pod characters (Quantitative)

Observations on pod characters *viz*., length, thickness, fresh and dry weight were taken and the details are given in Table 4.

4.1.3 Pod length (cm)

Pod length differed significantly with increase in days after anthesis. It varied from 0.90 cm at 10 days after anthesis to 2.33 cm at 45 days after anthesis and showed a gradual decrease thereafter. Pod length at 45 days after anthesis was on par with that of 50 days after anthesis (2.32 cm) and 55 days after anthesis (2.26 cm). At 63 days (pod splitting stage) after anthesis, the length of pod was 1.92 cm.

4.1.4 Pod thickness (mm)

Significant variation in pod thickness was noted among the treatments (Table 4). Pod thickness was found to be increasing with increase in days after anthesis with 0.74 mm at 10 days after anthesis and reached the maximum at 45 days after anthesis (2.79 mm) and then it showed a gradual decline till the pods reach splitting stage at 63 days after anthesis (1.88 mm).

4.1.5 Fresh pod weight (mg)

Fresh pod weight differed significantly along different developmental stages (Table 4). At 10 days after anthesis, the fresh weight of pods was recorded as 28.43 mg. Fresh pod weight was recorded as 97.40 mg at 45 days after anthesis. At the pod splitting stage (63 days after anthesis), the fresh weight of pod was considerably low (32.13 mg).

4.1.6 Dry pod weight (mg)

As the pods developed, their dry weight also increased and reached the maximum (44.23 mg) at 45 days after anthesis. In later stages of development, there was a gradual decline in dry weight of pods (Table 4).The dry weight of pods was observed to be 9.83 mg at 10 days after anthesis and it gradually increased and reached the maximum at 45 days after anthesis (44.23 mg). At 60

days after anthesis, the dry pod weight reduced and reached 29.07 mg, which became 23.27 mg at pod splitting stage (63 days after anthesis).

4.1.7 Number of seeds per pod

There was no significant variation in number of seeds per pod along the developmental stages and it ranged from 4.8 to 5.7 (Table 4).

Table 4. Pod characters at different maturity stages

Days after anthesis (DAA)	Pod length (cm)	Pod thickness (mm)	Fresh pod weight (mg)	Dry pod weight (mg)	Number of seeds per pod
10 DAA	0.90^{h}	0.74^{k}	28.43^{k}	9.83^{k}	5.1^{a}
15 DAA	1.26^{g}	0.95^{j}	31.20^{j}	14.53^{j}	4.9^{a}
20 DAA	1.47^{f}	1.34^{h}	36.43^{h}	21.67^{i}	5.3^{a}
25 DAA	1.77^{e}	1.77^{g}	41.47^{g}	26.37^{g}	5.1^{a}
30 DAA	1.91^{d}	2.12^{e}	56.90^{e}	32.93^{e}	5.7^{a}
35 DAA	1.98^{c}	2.26^{d}	72.50^{d}	35.87^{d}	5.1^{a}
40 DAA	2.14^{b}	2.57^{c}	85.70^{c}	37.60^{c}	4.8^{a}
45 DAA	2.33^{a}	2.79^{a}	97.40^{a}	44.23^{a}	5.3^{a}
50 DAA	2.32^{a}	2.62^{b}	88.73^{b}	40.23^{b}	5.6^{a}
55 DAA	2.26^{a}	2.24^{f}	54.43^{e}	32.63^{e}	5.1^{a}
60 DAA	2.15^{b}	2.17^{f}	43.80^{f}	29.07^{f}	5.4^{a}
63 DAA	1.92^{cd}	1.88^{i}	32.13^{i}	23.27^{h}	4.9^{a}

Table 5. Seed characters at different maturity stages

Days after anthesis (DAA)	Fresh seed weight per pod (mg)	Seed length (mm)	Seed breadth(mm)	100 seed weight (g)	Seed moisture (%)
30 DAA	39.83[f]	2.41[g]	1.86[d]	0.69[f]	42.12[e]
35 DAA	46.60[d]	2.79[e]	1.94[c]	0.78[d]	45.73[d]
40 DAA	58.03[c]	2.92[c]	2.18[b]	1.03[c]	59.83[b]
45 DAA	69.88[a]	3.14[a]	2.38[a]	1.22[a]	64.73[a]
50 DAA	61.56[b]	3.02[b]	2.20[b]	1.12[b]	53.48[c]
55 DAA	42.59[e]	2.91[c]	1.91[c]	0.73[e]	37.31[f]
60 DAA	37.31[g]	2.87[d]	1.49[e]	0.59[g]	28.78[g]
63 DAA	23.20[h]	2.73[f]	1.25[f]	0.39[h]	17.66[h]

Seed characters

Details as fresh seed weight, seed length and breadth, 100 seed weight and seed moisture are given under Table 5. During the initial stages of pod development till 25 days after anthesis, the seeds were very minute and was difficult to extract them from the pods. Thus, the observations on seed characters are lacking during early developmental stages.

4.1.8Fresh seed weight per pod (mg)

Fresh seed weight per pod (Table 5) showed an increasing trend during the developmental stages from 30 days after anthesis (39.83 mg) till 45 days after anthesis (69.88 mg). At 50 days after anthesis, the value of fresh seed weight was 61.56 mg, which declined to 42.59 mg by 55 days after anthesis. A decreased value of 37.31 mg was observed at 60 days after anthesis. At 63 days after anthesis, the fresh seed weight per pod was further reduced to 23.20 mg.

4.1.9 Seed length (mm)

The length of seeds differed significantly along the developmental stages. This increased during early development stages and reached a maximum value at 45 days after anthesis, which gradually declined in later stages (Table 5). The seed length increased from 2.41 mm (30 days after anthesis) to 3.14 mm (45 days after anthesis). Later on, a gradual decline was noticed and it reached 3.02 mm by 50 days after anthesis and further declined to 2.91 mm at 55 days after anthesis. At 60 days after anthesis, seed length was measured to be 2.87 mm, which again declined and reached 2.73 mm at 63 days after anthesis.

4.1.10 Seed breadth (mm)

There was significant variation in seed breadth along different developmental stages. It was observed that the seed breadth gradually increased during the initial stages, which after reaching the maximum value started to decline (Table 5). The seed breadth was measured as 1.86 mm during 30 days after anthesis, which increased to1.94 mm at 35 days after anthesis. The maximum seed breadth was recorded at 45 days after anthesis (2.38 mm), which declined thereafter and reached 2.20 mm by 50 days after anthesis. At 63 days after anthesis (pod splitting stage), a further reduced value of 1.25 mg was recorded.

4.1.11 Seed weight

100 seed weight was found to increase during initial stages which declined in later stages of development, after attaining a maximum value (Table 5). At 30 days after anthesis, 100 seed weight was recorded as 0.69 g which increased to 0.78 g and 1.03 g by 35 and 40 days after anthesis, respectively. 100 seed weight was found maximum (1.22 g) at 45 days after anthesis. Seed weight then started to decline and reached 1.12 mg (50 days after anthesis) and to 0.73 g (55 days after anthesis). The seed weight again declined and reached 0.59 mg (60 days after anthesis) and 0.39 g (63 days after anthesis, pod splitting stage).

4.1.12 Seed moisture (%)

A gradual increase in moisture content was noted during the earlier stages of seed development (Table 5). After reaching the maximum seed moisture content, the value then reduced. At 30 days after anthesis, moisture content in seeds was measured to be 42.12 per cent. At 45 days after anthesis, moisture content in seeds became maximum and the value was noted as 64.73 per cent. Later, moisture content reduced to 53.48 per cent (50 days after anthesis) and to 37.31 per cent (55 days after anthesis). Moisture content again declined considerably and reached to 28.78 per cent by 60 days after anthesis, and to 17.66 per cent by 63 days after anthesis (pod splitting stage).

4.1.13 Germination per cent

Observations on germination of seeds at different maturity stages were taken and are given in Table 6. During initial stages of development, there was no seed germination till 25 days after anthesis. A very low germination of 3.53 per cent was noted at 30 days after anthesis which increased to 13.97 per cent by 35 days after anthesis. There was a drastic increase in germination at 40 days after anthesis (60.10 %), which again increased and reached at its peak at 45 days after anthesis (73.80 %). After this stage, a decline in germination was noted and was observed to be 69.80 % at 50 days after anthesis. Germination per cent reduced considerably at 55 days after anthesis (58.53 %) and at 60 days after anthesis, it reached 51.13 per cent. When the pods attained splitting stage, a highly declined germination rate of 31.33 per cent was noted.

Table 6. Germination and seedling characters at different maturity stages

Days after anthesis (DAA)	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (mm)
30 DAA	3.53^{h}	1.83^{f}	3.04^{f}	4.87^{h}	1.62^{g}
35 DAA	13.97^{g}	2.10^{e}	3.27^{d}	5.37^{f}	2.32^{e}
40 DAA	60.10^{c}	2.28^{c}	3.48^{c}	5.76^{c}	2.96^{c}

45 DAA	73.80[a]	2.59[a]	3.98[a]	6.57[a]	3.99[a]
50 DAA	69.80[b]	2.41[b]	3.76[b]	6.18[b]	3.39[b]
55 DAA	58.53[d]	2.21[d]	3.24[d]	5.48[d]	2.79[d]
60 DAA	51.13[e]	2.18[d]	3.12[e]	5.31[e]	2.43[e]
63 DAA	31.33[f]	1.82[f]	2.38[g]	4.21[g]	2.08[f]

Seedling characters

Seedling characters *viz.*, root length, shoot length, seedling length and dry weight were measured and are given in Table 6.

4.1.14 Root length (cm)

During initial developmental stages till 25 days after anthesis, no germination was noticed and hence root length cannot be observed in those stages (Table 6). During remaining stages, the root length showed a gradual increase till it reached its highest and then declined (Table 6). At 30 days after anthesis, the root length was 1.83 cm, which reached to 2.10 cm and 2.28 cm by 35 days and 40 days after anthesis, respectively. The maximum root length was obtained at 45days after anthesis (2.59 cm), and it declined later on. Root length was only 1.82 cm at 63 days after anthesis.

4.1.15 Shoot length (cm)

During initial developmental stages till 25 days after anthesis, no germination was noticed and hence shoot length cannot be observed in those stages (Table 6). At 30 days after anthesis, shoot length was measured as 3.04 cm, which later increased to 3.27 cm and 3.48 cm by 35 and 40 days after anthesis, respectively. Shoot length reached its peak at 45 days after anthesis (3.98 cm)and later decreased and reached to 3.76 cm at 50 days after anthesis. It again declined and reached 3.24 cm at 55 days after anthesis and 3.12 cm at 60 days

after anthesis. By 63 days after anthesis (pod splitting stage), the shoot length was reduced to 2.38 cm.

4.1.16 Seedling length (cm)

Seedling length also showed a trend similar to root length and shoot length, along the developmental stages (Table 6). It was recorded as 4.87 cm at 30 days after anthesis, which increased to 5.37 cm at 35 days after anthesis and to 5.76 cm at 40 days after anthesis. Maximum seedling length was noticed at 45 days after anthesis (6.57 cm), succeeded by a reduced seedling length of 6.18 cm at 50 days after anthesis. The seedling length declined further and reached 5.48 cm at 55 days after anthesis and again declined to 5.31 cm by 60 days after anthesis. At 63 days after anthesis, it further reduced and reached 4.21 cm.

4.1.17 Seedling dry weight (mg)

Seedling dry weight also showed a similar trend wherein it gradually increased during initial stages, reached the maximum at 45 days after anthesis (3.99 mg) and in later stages of development, there was a gradual decline (Table 6). The dry weight of seedling was observed to be 1.62 mg at 30 days after anthesis and it gradually increased to 2.32 mg and 2.96 mg by 35 days and 40 days after anthesis. Seedling dry weight reached the maximum of 3.99 mg by 45 days after anthesis, which became 2.08 mg at pod splitting stage (63 days after anthesis).

Table 7. Vigour Indices of seedlings at different maturity stages

Days after anthesis (DAA)	Vigour Index 1	Vigour Index 2
30 DAA	17^{h}	6^{h}
35 DAA	75^{g}	32^{g}
40 DAA	346^{c}	178^{c}
45 DAA	485^{a}	294^{a}

50 DAA	431[b]	236[b]
55 DAA	321[d]	163[d]
60 DAA	271[e]	124[e]
63 DAA	132[f]	65[f]

Calculated values of Vigour Indices (I & II) are given in Table 7.

4.1.18 Vigour Index I

Significant difference in Vigour Index I was observed between the treatments (Table 7). Vigour Index I showed an increasing trend during initial stages, reached its maximum and then had undergone a gradual decline during the later stages of development. Vigour Index I at 30 days after anthesis was calculated as 17, which increased to 75 by 35 days after anthesis. A drastically higher Vigour Index I was obtained at 40 days after anthesis (346) which further rose to 485, reaching its maximum. Then the value of Vigour Index I decreased and became 431 at 50 days after anthesis, succeeded by a smaller value of 321 at 55 days after anthesis. Vigour Index I values again declined and reached to 271 (60 days after anthesis) and then to 132 (63 days after anthesis).

4.1.19 Vigour Index II

Vigour Index II also showed an increasing trend during initial stages, reached its maximum and then had undergone a gradual decline during the later stages of development (Table 7). At 30 days after anthesis, value of Vigour Index II was found to be very low (6), which later increased to 32 by 35 days after anthesis. It again increased and reached to 178 by 40 days after anthesis. Highest Vigour Index II value was obtained at 45 days after anthesis (294). Later it decreased to 236(50 days after anthesis), it became 236 and to 163 (55 days after anthesis). It again declined and reached 124 at 60 days after anthesis and 65 at 63 days after anthesis.

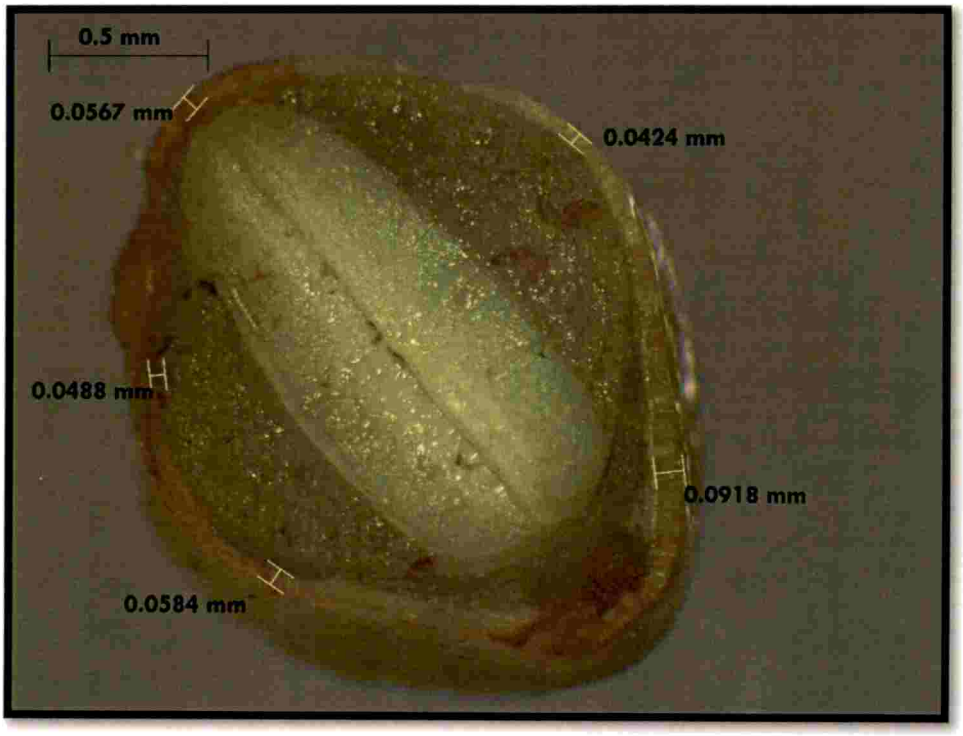

Plate 3: Cross- section of seed at 45 days of anthesis (15x)

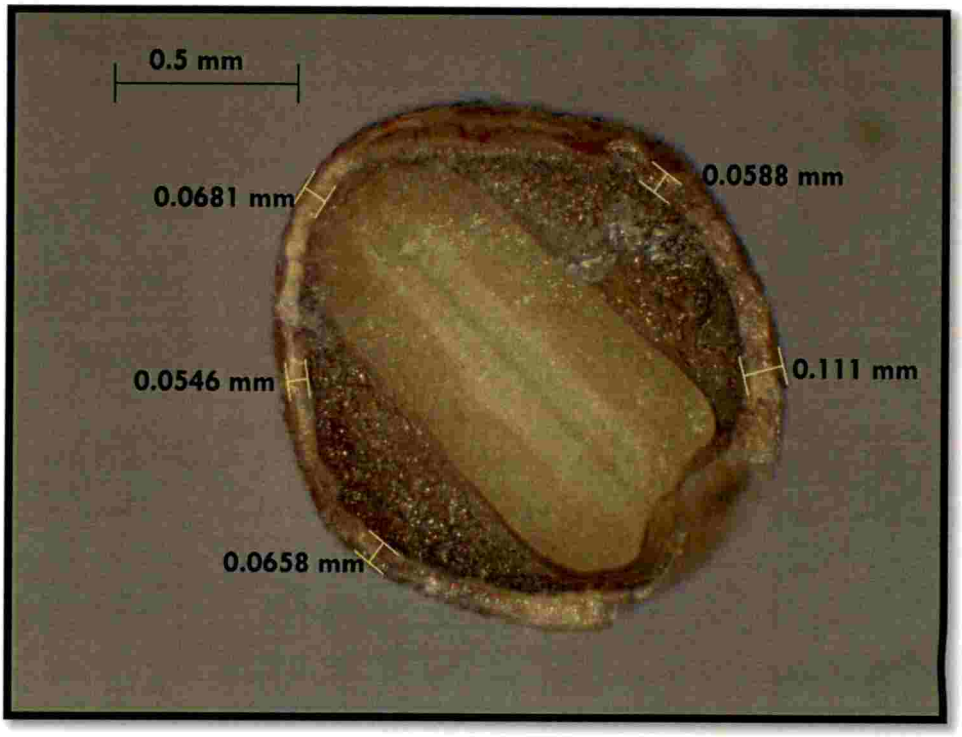

Plate 4: Cross-section of seed at 45 days of anthesis (15x)

4.1.20 Anatomical studies of seeds

The cross section of seeds were observed under stereomicroscope (15x) so as to understand the thickness of seed coat during the developmental stages. It was found that the seed coat was thin in seeds at 45 days of anthesis with an average value of 0.059 mm (Plate 3) while that of seeds at 63 days of anthesis was 0.072 mm (Plate 4).

4.2 EFFECT OF PRE-SOWING TREATMENTS ON SEED QUALITY AND LONGEVITY IN NEELAYAMARI

4.2.1 Effect of pre-sowing treatments on seed quality in Neelayamari

The experiment was conducted using treated Neelayamari seeds. Fresh seeds after extraction were primed as per the standard procedure. Unprimed seeds were considered as the control. The treated seeds and the control were sown at 5 days interval from the day of treatment till 30 days of treatment (Plates 5 & 6). Germination and related observations were taken as per the standard procedure.

4.2.1.1 Germination (%)

Germination per cent of different pre-sowing seed treatments till 30 days of treatment are given in Table 8. The effect of seed treatment on germination was observed to be highly significant. There was a slight increase in the germination per cent in the treatments which decreased marginally later. On the day of treatment, maximum germination was observed in mechanically scarified seeds (95. 83%) followed by T7, hydration of seeds for 24 hours (93.27%). T5, where the seeds were treated with hot water at 80^0C for 20 minutes (76.70 %) also showed an elevated germination rate than control (74.33 %). T6 (Treatment with hot water at 60^0C for 30 minutes) showed a germination per cent of 67.5 per cent and it is inferior to control. Treatments involving sulphuric acid resulted in lowest germinations. In T1 where the seeds were treated with conc. H_2SO_4 for 5 minutes, germination rate was 54.70 per cent. In T2 and T3 where the seeds were treated with conc. H_2SO_4 for 10 and 15 minutes, drastically reduced germination rates of 34.34 per cent and 22.33 per cent respectively were recorded.

Plate 5: Germinated seedlings in sterilized sand medium

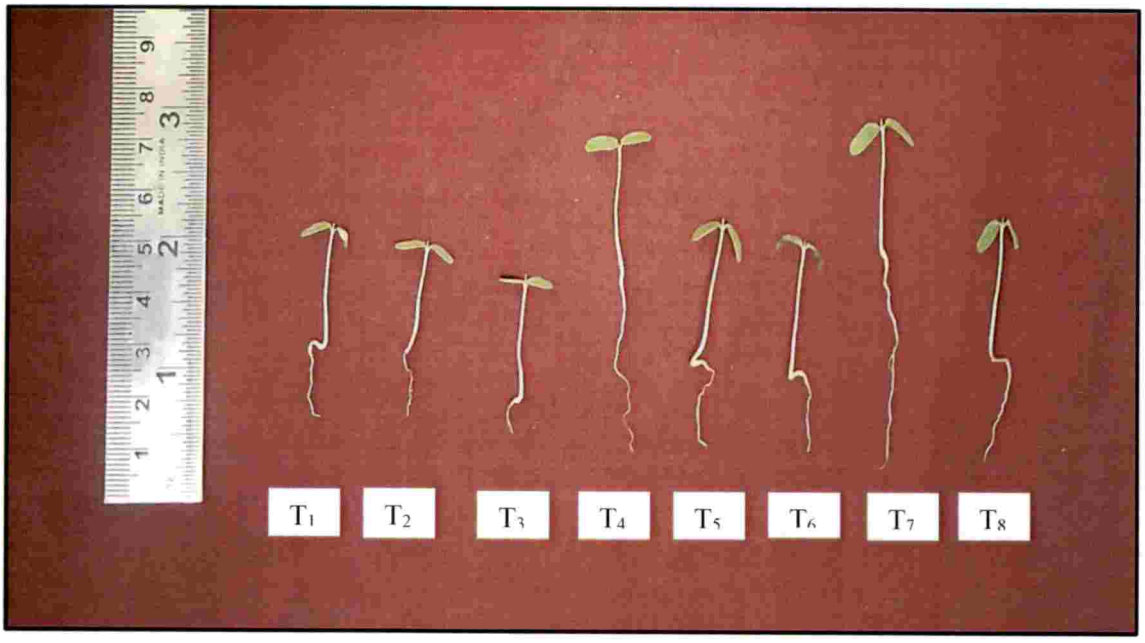

Plate 6: Seedlings of different treatments

4.2.1.2 Seedling root length (cm)

Root length of seedlings from the day of treatment to the 30th day of treatment are given in Table 9. Seed treatments imposed a significant effect on root length. Highest root lengths were noticed in treatments T7 (2.95 cm) and T4 (2.89 cm) when sown on the day of treatment. T5 (2.62 cm) also exhibited a higher root length than the control (2.57 cm). T6 (2.53 cm), T2 (2.46 cm), T1 (2.41 cm) and T3 (2.39 cm) appeared inferior to the control.

T7 (2.98 cm) showed a slight increase in root length on 5th day after treatment and then gradually decreased to reach 2.89 cm by 30th day after treatment. T1 and T2 also showed enhanced root lengths of 2.50 cm and 2.48 cm respectively at 5th day which gradually declined to 2.35 cm and 2.32 cm by 30th day after treatment. At 30 days after treatment, root length of T3 gradually decreased to 2.27 cm. T4, T5 and T6 also showed gradual decrease in root length so as to reach 2.81 cm, 2.58 cm and 2.44 cm respectively by 30th day after treatment. Untreated seeds also showed a decline in root length as the days increased and reached 2.53 cm by 30th day after treatment.

Table 8. Effect of seed treatments on germination per cent

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	54.70[d] (33.22)	57.75[d] (35.14)	57.15[d] (34.89)	56.63[d] (34.52)	55.83[e] (33.99)	53.34[d] (32.27)	51.92[d] (31.36)
T_2	34.34[e] (20.09)	33.28[e] (19.46)	34.62[e] (20.27)	34.12[e] (19.97)	33.59[f] (19.97)	33.26[e] (19.44)	30.67[e] (17.86)
T_3	22.33[f] (12.91)	21.69[f] (12.54)	21.65[f] (12.52)	21.23[f] (12.27)	20.56[f] (11.87)	20.21[f] (11.66)	18.34[f] (10.58)
T_4	95.83[a] (74.07)	95.93[a] (74.06)	95.66[a] (73.22)	95.32[a] (73.46)	95.13[a] (72.15)	94.86[a] (71.84)	94.52[a] (71.01)
T_5	76.70[b] (50.14)	76.89[b] (50.37)	75.17[b] (48.79)	75.58[b] (49.17)	75.56[c] (49.13)	75.23[b] (48.87)	74.56[b] (48.27)
T_6	67.5[c] (42.49)	67.72[c] (42.66)	67.12[c] (42.22)	66.35[c] (41.62)	66.11[d] (41.41)	64.53[c] (40.24)	63.90[c] (39.78)
T_7	93.27[a] (69.41)	93.31[a] (69.72)	93.11[a] (68.89)	92.89[a] (68.72)	92.63[b] (67.99)	92.32[a] (67.67)	92.33[a] (67.74)
T_8	74.33[bc] (48.10)	74.51[bc] (48.22)	74.26[b] (48.02)	74.03[b] (47.84)	73.63[c] (47.48)	72.48[b] (46.53)	73.67[b] (47.59)

***values in parenthesis are the transformed ones**

4.2.1.3 Seedling shoot length (cm)

Observations on shoot length of different treatments are showed in Table 10. Different treatments have significant effect on shoot length along the storage. Highest shoot length was noticed in treatments T7 (4.53 cm) followed by T4 (4.45 cm) when sown on the day of treatment. Shoot length in these were found to be superior to the control (3.95 cm). T5 also showed a higher shoot length of 4.02 cm. Shoot lengths of T6 (3.89 cm), T1 (3.56 cm), T2 (3.46 cm) and T3 (3.37 cm) were found to be inferior to the control, the lowest being that of T3.

On the 5th day after treatment, T1 and T6 showed a slightly increased shoot lengths of 3.61 cm and 3.91 cm respectively. These gradually decreased along the storage so as to reach 3.44 cm and 3.86 cm by 30th day after treatment. T2, T3, T5 and T7 exhibited their highest shoot lengths of 3.49 cm, 3.39 cm, 4.06 cm and 4.56 cm respectively, on 10th day after treatment. After this, all showed a slight decreasing trend along storage and reached 3.25 cm, 3.26 cm, 3.94 cm and 4.45 cm respectively on 30th day after treatment. T4 showed gradual decline in shoot length along storage and was noted to be 4.32 cm at 30th day after treatment.

4.2.1.4 Seedling length (cm)

Seedling lengths of different treatments from the day of treatment till 30th day of treatment are showed in Table 11. Seed treatments imposed a significant effect on seedling length. Highest seedling length was noticed in treatments T7 (7.48 cm), followed by T4 (7.34 cm) when sown on the day of treatment. Both these were superior when compared to control (6.52 cm). T5 (6.65 cm) also exhibited a higher seedling length than the control. Seedling lengths of T6 (6.41 cm), T1 (5.97 cm), T2 (5.92 cm) and T3 (5.76 cm) appeared to be inferior to that control, the lowest being that of T3.

T1 showed a slight increase in seedling length from initial to reach 6.03 cm at 10th day after treatment which gradually declined to 5.79 cm. In T2, T3, T5 and T8 seedling length showed slight decrease on 5th day after treatment (5.89 cm, 5.71 cm, 6.61 cm and 6.45 cm), which slightly increased on 10th day after

treatment (5.92 cm, 5.77 cm, 6.72 cm and 6.51 cm). This then gradually decreased to 5.57 cm, 5.53 cm, 6.52 cm and 6.37 cm respectively on 30th day after treatment. T7 and T4 gradually declined along storage and reached 7.34 cm and 7.13 cm by 30th day after treatment.

4.2.1.5 Seedling dry weight (mg)

The observations on seedling dry weight till 30 days after treatment are given in Table 12. Seedling dry weight was found to be significantly influenced by various treatments. Highest seedling dry weight was noticed in treatments T7 (4.59 mg), followed by T4 (4.49 mg) when sown on the day of treatment. These were superior to that of control (3.99 mg). All remaining treatments produced seedlings with lower dry weight than that of control. Seedling dry weights of T1, T2, T5 and T6 were 3.52 mg, 3.45 mg, 3.92 mg and 3.73 mg respectively. The lowest value was notice din T3 (3.31 mg). In T6, highest seedling dry weight was obtained on 5th day after treatment (3.75 mg). This later became 3.62 mg on 30th day after treatment. Highest seedling dry weights in T1, T3, T4, T5, T7 and T8 were attained on 10th day after treatment and were 3.54 mg, 3.33 mg, 4.50 mg, 3.93 mg, 4.60 mg and 4.00 mg respectively. These showed declining trend and reached 3.41 mg, 3.18 mg, 4.34 mg, 3.78 mg, 4.42 mg and 3.82 mg respectively. T2 didn't show any increase from initial seedling dry weight and gradually declined to reach 3.37 cm by 30th day after treatment.

4.2.1.6 Vigour Index I

Observations on vigour index I of different treatments till one month of storage are noted in Table 13. Seed treatments were found to have significant influence on vigour index I. Highest vigour index I was exhibited by T4 (703) followed by T7 (698) when sown on the day of treatment. T5 also showed a

Table 9. Effect of seed treatments on seedling root length (cm)

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	2.41^{e}	2.50^{c}	2.45^{cd}	2.43^{cd}	2.38^{cd}	2.37^{cd}	2.35^{d}
T_2	2.46^{de}	2.48^{c}	2.43^{cd}	2.38^{d}	2.39^{cd}	2.36^{cd}	2.32^{d}
T_3	2.39^{e}	2.36^{d}	2.38^{d}	2.31^{d}	2.34^{d}	2.30^{d}	2.27^{d}
T_4	2.89^{a}	2.89^{ab}	2.87^{a}	2.81^{ab}	2.83^{a}	2.85^{a}	2.81^{a}
T_5	2.62^{b}	2.61^{bc}	2.66^{b}	2.62^{bc}	2.61^{b}	2.59^{b}	2.58^{b}
T_6	2.53^{cd}	2.51^{c}	2.55^{bcd}	2.53^{cd}	2.50^{bc}	2.47^{bc}	2.44^{c}
T_7	2.95^{a}	2.98^{a}	2.92^{a}	2.94^{a}	2.89^{a}	2.89^{a}	2.89^{a}
T_8	2.57^{bc}	2.52^{c}	2.56^{bc}	2.51^{cd}	2.53^{b}	2.51^{b}	2.53^{b}

Table 10. Effect of seed treatments on seedling shoot length (cm)

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	3.56^{d}	3.61^{c}	3.58^{d}	3.52^{c}	3.44^{c}	3.43^{c}	3.44^{d}
T_2	3.46^{de}	3.41^{cd}	3.49^{de}	3.39^{cd}	3.35^{c}	3.28^{cd}	3.25^{e}
T_3	3.37^{e}	3.35^{d}	3.39^{e}	3.28^{d}	3.25^{c}	3.21^{d}	3.26^{e}
T_4	4.45^{a}	4.41^{a}	4.43^{a}	4.39^{a}	4.39^{a}	4.35^{a}	4.32^{b}
T_5	4.02^{b}	4.00^{b}	4.06^{b}	3.98^{b}	3.94^{b}	3.93^{b}	3.94^{c}
T_6	3.89^{c}	3.91^{b}	3.82^{c}	3.9^{b}	3.79^{b}	3.78^{b}	3.86^{c}
T_7	4.53^{a}	4.50^{a}	4.56^{a}	4.51^{a}	4.49^{a}	4.45^{a}	4.45^{a}
T_8	3.95^{bc}	3.93^{b}	3.95^{b}	3.91^{b}	3.90^{b}	3.88^{b}	3.84^{c}

Table 11. Effect of seed treatments on seedling length (cm)

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	5.97^{d}	5.99^{cd}	6.03^{d}	5.95^{c}	5.94^{c}	5.80^{c}	5.79^{e}
T_2	5.92^{de}	5.89^{d}	5.92^{d}	5.77^{c}	5.74^{cd}	5.64^{c}	5.57^{f}
T_3	5.76^{e}	5.71^{d}	5.77^{d}	5.59^{c}	5.59^{d}	5.51^{c}	5.53^{f}
T_4	7.34^{a}	7.30^{a}	7.30^{a}	7.2^{a}	7.22^{a}	7.20^{a}	7.13^{b}
T_5	6.65^{b}	6.61^{b}	6.72^{b}	6.60^{b}	6.55^{b}	6.52^{b}	6.52^{c}
T_6	6.41^{c}	6.42^{bc}	6.37^{c}	6.43^{b}	6.29^{b}	6.25^{b}	6.30^{d}
T_7	7.48^{a}	7.48^{a}	7.48^{a}	7.45^{a}	7.38^{a}	7.34^{a}	7.34^{a}
T_8	6.52^{bc}	6.45^{bc}	6.51^{bc}	6.42^{b}	6.43^{b}	6.39^{b}	6.37^{cd}

Table 12. Effect of seed treatments on seedling dry weight (mg)

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	3.52^{f}	3.51^{d}	3.54^{d}	3.49^{d}	3.47^{d}	3.43^{d}	3.41^{d}
T_2	3.45^{g}	3.41^{de}	3.45^{de}	3.42^{d}	3.39^{d}	3.37^{d}	3.37^{d}
T_3	3.31^{h}	3.28^{e}	3.33^{e}	3.29^{e}	3.24^{e}	3.21^{e}	3.18^{e}
T_4	4.49^{a}	4.47^{a}	4.50^{a}	4.48^{a}	4.42^{a}	4.37^{a}	4.34^{a}
T_5	3.92^{d}	3.90^{bc}	3.93^{b}	3.87^{b}	3.83^{b}	3.80^{b}	3.78^{b}
T_6	3.73^{e}	3.75^{c}	3.70^{c}	3.69^{c}	3.65^{c}	3.65^{c}	3.62^{c}
T_7	4.59^{b}	4.56^{a}	4.60^{a}	4.53^{a}	4.48^{a}	4.42^{a}	4.42^{a}
T_8	3.99^{c}	3.98^{b}	4.00^{b}	3.94^{b}	3.87^{b}	3.85^{b}	3.82^{b}

Higher vigour index I of 510. All these were superior to that of control (485). Vigour indices of T6 (433), T1 (327) and T2 (203) were lower than that of control. Lowest vigour index I was obtained in T3 (129).

In T1 and T6, highest vigour index I was noticed on 5th day after treatment (347 and 435 respectively), which declined along storage to reach 301 and 403 on 30th day after treatment. In T2, highest vigour index I was noticed on 10th day after treatment (205) and later declined to reach 171 at 30 DAS. Vigour index I of T3, T4, T5, T7 and T8 gradually declined and reached 101, 674, 486, 678 and 469 respectively on 30th day after treatment. At 30th day also, vigour index was observed to be higher in T7and T4.

4.2.1.7 Vigour Index II

Vigour Index II of different treatments starting from the day of treatment till 30th day after treatment are shown in Table 14. Different seed treatments have significant influence on vigour index II of the seeds. On the day after treatment, highest vigour index II was noticed in T4 (430) followed by T7 (428). T5 also possessed a higher vigour index II of 301. All these were superior to that of control (297). Lower vigour index II was observed in T6 (252), T1 (193) and T2 (119), the lowest being that of T3 (74). In T1, highest vigour index II was noted on 5th day after treatment (203), which later declined gradually to reach 177 by 30th day after treatment. In T2, a slight dip was noticed on 5th day after treatment (113), which showed a slight increase on 10th day after treatment (119). This later declined to 103 on 30th day after treatment. Vigour index II of T3 gradually declined along storage and reached 58 on 30th day after treatment. Highest vigour index II in T4 was noted on 10th day after treatment (431) which gradually declined to 409 by 30th day after treatment. In T5, T7 and T8, vigour indices gradually declined and reached 282, 402 and 282 respectively on 30th day after treatment. In T6, highest vigour index was noted on 5th day after treatment (254) which became 232 by 30th day after treatment.

Table 13. Effect of seed treatments on vigour index I

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	327^{d}	347^{d}	345^{d}	337^{d}	332^{d}	310^{d}	301^{d}
T_2	203^{e}	196^{e}	205^{e}	197^{e}	193^{e}	188^{e}	171^{e}
T_3	129^{f}	124^{f}	125^{f}	119^{f}	115^{f}	111^{f}	101^{f}
T_4	703^{a}	701^{a}	699^{a}	687^{a}	687^{a}	683^{a}	674^{a}
T_5	510^{b}	509^{b}	506^{b}	499^{b}	495^{b}	491^{b}	486^{b}
T_6	433^{c}	435^{c}	428^{c}	427^{c}	415^{c}	404^{c}	403^{c}
T_7	698^{a}	698^{a}	696^{a}	692^{a}	684^{a}	678^{a}	678^{a}
T_8	485^{b}	481^{bc}	484^{b}	476^{bc}	474^{b}	464^{b}	469^{b}

Table 14. Effect of seed treatments on vigour index II

Treatments	Days after treatment						
	0	5	10	15	20	25	30
T_1	193[d]	203[d]	202[d]	198[d]	194[d]	183[d]	177[d]
T_2	119[e]	113[e]	119[e]	116[e]	114[e]	112[e]	103[e]
T_3	74[f]	71[f]	72[f]	70[f]	67[f]	65[f]	58[f]
T_4	430[a]	429[a]	431[a]	427[a]	421[a]	414[a]	409[a]
T_5	301[b]	300[b]	295[b]	292[b]	289[b]	286[b]	282[b]
T_6	252[c]	254[c]	248[c]	244[c]	241[c]	236[c]	232[c]
T_7	428[a]	426[a]	428[a]	421[a]	415[a]	408[a]	408[a]
T_8	297[b]	296[b]	297[b]	292[b]	285[b]	279[b]	282[b]

4.2.2. Effect of pre-storage treatments on seed longevity

Fresh seeds after extraction were primed as per the standard procedure. The primed seeds were then dried to ≤8 per cent moisture, packed in 700 gauge polyethylene bags and stored at ambient condition. Seed quality parameters were recorded at the start of storage and subsequently at monthly intervals upto 6 months.

4.2.2.1 Germination (%)

The effect of seed treatments on germination was found to be high and significant throughout the storage period (Table15). Germination in all the treatments was found to be declining over storage. Before storage (immediately after treatment- 0 MAS),germination ranged from 22.33 per cent in T3 to 95.83 per cent in T4. Highest germination (95.83 %) was exhibited by the mechanically scarified seeds which was on par with that noted in T7, hydrated seeds (93.27 %). These treatments differed significantly from the control (74.33 %). Germination of seeds treated with hot water at 80^0C for 20 minutes (76.70 %) was significantly higher than that of control.

After one month of storage, germination ranged from 18.34 (T3- conc. H_2SO_4 for 15 minutes) to 94.52 (T4- Mechanical scarification). Hydrated seeds (92.33 %) was on par with that of mechanically scarified seeds, but differed significantly from all other treatments. Untreated seeds showed 73.67 per cent germination.

Table 15. Effect of seed treatment on germination per cent during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	54.70^{d} (33.22)	51.92^{d} (31.36)	48.32^{c} (28.94)	37.20^{d} (21.85)	25.54^{f} (14.81)	20.72^{e} (11.96)	9.84^{d} (5.64)
T_2	34.34^{e} (20.09)	30.67^{e} (17.86)	26.67^{d} (15.47)	25.64^{e} (14.88)	18.33^{g} (10.58)	10.33^{f} (5.94)	4.67^{e} (2.67)
T_3	22.33^{f} (12.91)	18.34^{f} (10.58)	15.69^{e} (9.01)	9.67^{f} (5.55)	5.33^{h} (3.06)	2.67^{g} (1.53)	0^{f} (0)
T_4	95.83^{a} (74.07)	94.52^{a} (71.01)	90.67^{a} (65.47)	87.67^{a} (61.84)	84.67^{a} (57.95)	77.34^{a} (50.76)	65.67^{a} (41.08)
T_5	76.70^{b} (50.14)	74.56^{b} (48.27)	65.29^{b} (40.78)	64.35^{b} (40.08)	53.80^{d} (32.58)	41.80^{d} (24.72)	35.14^{c} (20.58)
T_6	67.50^{c} (42.49)	63.90^{c} (39.78)	55.00^{c} (33.42)	53.80^{c} (32.57)	48.38^{e} (28.95)	39.50^{d} (23.28)	31.13^{c} (18.16)
T_7	93.27^{a} (69.41)	92.33^{a} (67.74)	89.67^{a} (63.92)	84.67^{a} (58.09)	78.33^{b} (51.61)	69.67^{b} (44.24)	63.67^{a} (39.59)
T_8	74.33^{bc} (48.10)	73.67^{b} (47.59)	68.34^{b} (43.19)	65.34^{b} (40.93)	61.33^{c} (37.86)	54.34^{c} (32.94)	44.33^{b} (26.35)

***Values in parenthesis are the transformed ones * MAS- months after storage**

Seeds treated with hot water at 80^0C for 20 minutes exhibited 74.56 per cent germination. All the remaining treatments exhibited poor germination compared to control.

After two months storage, highest germination was noted in mechanically scarified seeds (90.67 %), which was on par with that of hydrated seeds (89.67 %). Untreated seeds exhibited 68.34 per cent germination. All the remaining treatments showed lower germination rate than that of control. Seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes exhibited 65.29 per cent and 55.00 per cent germination respectively. Treatment of seeds with conc. sulphuric acid for 5, 10 and 15 minutes resulted in 48.32 per cent, 26.67 per cent and 15.69 per cent germinations respectively.

After three months storage period, the germination per cent was highest in mechanically scarified seeds (87.67 %) and hydrated seeds (84.67 %). This is followed by 65.34 per cent in the untreated seeds. All the remaining treatments were found to be lower than that of untreated seeds. Hot water treatment of seeds at 80^0C for 20 minutes and 60^0C for 30 minutes resulted in 64.35 per cent and 53.80 per cent germinations respectively. Seed treatment with conc. sulphuric acid for 5, 10 and 15 minutes resulted in 37.20 per cent, 25.64 per cent and 9.67 per cent germinations respectively.

After a storage period for four months, mechanically scarified seeds (84.67 %) showed highest germination followed by that of hydrated seeds (78.33 %). Untreated seeds recorded a germination of 61.33 per cent, which was higher than the remaining treatments. Seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes exhibited germination of 53.80 per cent and 48.38 per cent respectively. Treatment of seeds with conc. sulphuric acid for 5, 10 and 15 minutes resulted in 25.54 per cent, 18.33 per cent and 5.33 per cent germination respectively.

After five months storage, highest germination was exhibited by mechanically scarified seeds (77.34%) followed by that of hydrated seeds (69.67%). A germination of 54.34 per cent was recorded in untreated seeds, while the remaining treatments failed to exhibit a higher germination than this. Hot water treatment of seeds at 80^0C for 20 minutes and 60^0C for 30 minutes resulted in 41.80 per cent and 39.50 per cent germination respectively. Seed treatment with conc. sulphuric acid for 5, 10 and 15 minutes resulted in 20.72 per cent, 10.33 per cent and 2.67 per cent germination respectively.

At the end of storage period, germination exhibited by mechanically scarified seeds (65.74%) and hydrated seeds (63.67%) were on par followed by the untreated seeds (44.33%). Remaining treatments recorded poor germination. Seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes exhibited germination of 35.14 per cent and 31.13 per cent respectively. Treatment of seeds with conc. sulphuric acid for 5 and 10 minutes resulted in 9.84 per cent and 4.67 per cent germination respectively. Conc. Sulphuric acid treatment of seeds for 15 minutes failed to germinate at this stage.

When the seeds were treated with conc. sulphuric acid for 5 minutes (T1), the germination was found to be 54.70 per cent immediately after treatment which later decreased and reached 51.92 per cent in one month storage period. At the end of 2 months of storage, germination further declined to 65.29 per cent and then to 64.35 per cent by the end of 3 months of storage. After 4 months, germination was 53.80 per cent which declined to 41.80 per cent by the end of 5 months. Seeds after storing for 6 months showed a germination rate of 35.14 per cent only.

Initial germination of seeds that were treated with conc. H_2SO_4 for 10 minutes (T2) were noted as 34.34 per cent, lower than the control. This germination declined during storage to reach 30.67, 26.67 and 25.64 per cent by the end of 1, 2 and 3 months storage respectively. Again germination declined and reached 18.33 per cent (4 months) and 10.33 per cent (5 months) and by the end of 6 months of storage, germination reduced to 4.67 per cent.

The seeds treated with conc. H_2SO_4 for 15 minutes (T3) drastically reduced the germination and was noted as 22.33 per cent before storage, which later decreased to 18.34 per cent and 15.69 per cent at the end of 1 and 2 months of storage respectively. After 3 months germination was found to be 9.67 per cent which again declined to 5.33 per cent by storing one month more. Germination after storing 5 months was recorded as 2.67 per cent. After 6 months of storage, the seeds failed to germinate.

Mechanically scarified seeds (T4) showed an initial germination of 95.83 per cent, which was the highest among all the treatments which became 94.52 per cent after storing for one month. After two months of storage, germination reduced to 90.67 per cent and later to 87.67 per cent at 3 months storage period. Germination further reduced to 84.67 per cent and 77.34 per cent by the end of 4 and 5 months of storage. This later declined to 65.67 per cent after storage for 6 months.

Hot water treatment of seeds at 80°C for 20 minutes (T5) drastically reduced the germination and was initially recorded as 76.70 per cent, which became 74.56 per cent after storing for a month. This again reduced to 65.29 per cent, 2 months after storage. Germination of 64.35 per cent was noted after storing for 3 months. Germination rate of 53.80 per cent and 41.80 per cent were recorded after storing for 4 months and 5 months respectively. The seeds showed a germination rate of 35.14 percent after six months storage period.

Seeds treated with hot water at 60°C for 30 minutes (T6) also showed an initial germination of 67.50 per cent. After storing for a month, it became 63.90 per cent which further declined to 55.00 per cent after 2 months of storage. Storing for one more month reduced the germination to 53.80 per cent and then to 48.38 per cent by the end of 4 months of storage. It further reduced to 39.50 per cent and 31.13 per cent by storing for 5 months and 6 months respectively.

Hydration of seeds for 24 hours (T7) resulted in an initial germination rate of 93.27 per cent, which became 92.33 after storing for a month. Storing seeds for

2 months resulted in a germination rate of 89.67 per cent, which declined to 84.67 per cent (3 months storage) and 78.33 per cent (4 months storage). Germination reduced to 77.34 per cent and then to 63.67 per cent by the end of 5 and 6 months of storage.

Untreated seeds (T8) showed an initial germination rate of 74.33 per cent which became 73.67 per cent after one month of storage. It reduced to 68.34 per cent and 65.34 per cent after storing for 2 months and 3 months respectively. After storing for 4 months, germination became 61.33 per cent, which further decreased to 54.34 per cent and to 44.33 per cent after 5 and 6 months of storage.

4.2.2.2 Seedling root length (cm)

Root length of the seedlings were found to be influenced by seed treatments. The root length also showed a decreasing trend along the storage (Table16). The highest initial root length was recorded in T7 (2.95 cm) which was on par with T4 (2.89 cm). T5 showed a root length of 2.62 cm. all the remaining treatments produced shorter roots than that of control (2.57 cm). The lowest was recorded in seeds that were treated with conc. sulphuric acid for 15 minutes (2.39 cm). In T1 (conc. H_2SO_4 for 5 minutes), the initial root length was recorded as 2.41 cm which showed a gradual decline during storage and reached 1.91 cm at the end of storage. The seeds treated with conc. H_2SO_4 for 10 minutes (T2) noted an initial root length of 2.46 cm which reached 1.76 cm by 6 months of storage. Root length of seeds treated with conc. H_2SO_4 for 15 minutes (T3) produced the shortest roots among all (2.39 cm) initially, which was recorded as 1.45 cm at the end of storage period of five months. Root length at 6 months after storage cannot be observed as there was no germination noticed. Mechanically scarified seeds produced roots with 2.89 cm immediately after the treatment which further declined and reached to a lower value of 2.36 cm at 6 months of storage. The seeds those were treated with hot water at 80°C for 20 minutes showed a root length of 2.62 cm which decreased along the storage period and reached

Table 16. Effect of seed treatment on seedling root length (cm) during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	2.41^{e}	2.35^{d}	2.26^{d}	2.19^{e}	2.11^{d}	2.06^{d}	1.91^{e}
T_2	2.46^{de}	2.32^{d}	2.26^{d}	2.18^{e}	2.02^{e}	1.86^{e}	1.76^{f}
T_3	2.39^{e}	2.27^{d}	2.17^{e}	1.82^{f}	1.54^{f}	1.45^{f}	-
T_4	2.89^{a}	2.81^{a}	2.73^{a}	2.61^{b}	2.56^{a}	2.42^{a}	2.36^{a}
T_5	2.62^{b}	2.58^{b}	2.51^{b}	2.48^{c}	2.41^{b}	2.31^{b}	2.25^{b}
T_6	2.53^{cd}	2.44^{c}	2.40^{c}	2.33^{d}	2.25^{c}	2.11^{d}	2.03^{d}
T_7	2.95^{a}	2.89^{a}	2.80^{a}	2.73^{a}	2.59^{a}	2.48^{a}	2.32^{a}
T_8	2.57^{bc}	2.53^{b}	2.48^{b}	2.43^{c}	2.39^{b}	2.22^{c}	2.17^{c}

2.25 cm at 6 months of storage. Hot water treatment at 60°C for 30 minutes produced seedlings of root length, 2.53 cm which declined to 2.03 cm at the end of six months of storage. Hydrated seeds produced seedlings with longest root length of 2.95 cm which reached 2.32 cm by 6 months of storage. Untreated seeds initially produced roots of length 2.57 cm, which later declined to 2.17 cm after storage for 6 months.

4.2.2.3 Seedling shoot length (cm)

Different seed treatments were found to have influence on shoot length of the germinated seedlings. There was a decrease in shoot length as the storage time goes on increasing (Table 17). Initially, the highest shoot length was observed in T7 (4.53 cm) where the seeds were soaked in water for 24 hours, which was on par with mechanically scarified seeds (4.45 cm) while the lowest was in T3 (3.37 cm) where the seeds were treated with conc. sulphuric acid for 15 minutes. Hot water treatment at 80°C for 20 minutes (T5) recorded a shoot length of 4.03 cm. Untreated seeds showed a shoot length of 3.95 cm. All the remaining treatments recorded shorter shoot lengths. In T1(Treatment with conc. H_2SO_4 for 5 minutes), the initial shoot length observed before storage was 3.56 cm which reduced to 2.81cm after 6 months of storage under ambient conditions. When the seeds were treated with conc. H_2SO_4 for 10 minutes (T2), the initial shoot length was observed to be 3.46 cm which later reduced to 2.47 cm at the end of storage period. The seeds treated with conc. H_2SO_4 for 15 minutes (T3) initially produced seedlings with the shortest shoot length of 3.37 cm which gradually declined along the storage period and reached 2.49cm after 5 months of storage. Observations at sixth month of storage couldn't be taken as no germination was noted. Initially, shoot length of 4.45 cm was observed in seedlings of mechanically scarified seeds (T4), which declined to 4.19 cm after 2 months of storage. At 4 months of storage, it was noted as 3.95 cm, which further declined to 3.56 cm at the end of 6 months of storage. Seeds treated with hot water at 80°C for 20 minutes (T5) produced seedlings with shoot length of 4.03 cm immediately

Table 17. Effect of seed treatment on seedling shoot length (cm) during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	3.56^{d}	3.44^{d}	3.27^{d}	3.14^{e}	3.01^{c}	2.93^{c}	2.81^{d}
T_2	3.46^{de}	3.25^{e}	3.19^{d}	3.02^{f}	2.86^{d}	2.59^{d}	2.47^{e}
T_3	3.37^{e}	3.26^{e}	3.08^{e}	3.01^{f}	2.88^{d}	2.49^{d}	-
T_4	4.45^{a}	4.32^{b}	4.19^{b}	3.95^{b}	3.95^{a}	3.71^{a}	3.56^{a}
T_5	4.03^{b}	3.94^{c}	3.82^{c}	3.74^{c}	3.61^{b}	3.49^{b}	3.38^{abc}
T_6	3.89^{c}	3.86^{c}	3.74^{c}	3.62^{d}	3.59^{b}	3.39^{b}	3.13^{e}
T_7	4.53^{a}	4.45^{a}	4.31^{a}	4.18^{a}	4.01^{a}	3.77^{a}	3.54^{ab}
T_8	3.95^{bc}	3.84^{c}	3.79^{c}	3.64^{d}	3.54^{b}	3.38^{b}	3.25^{bc}

after the treatment, which declined during storage and became 3.38 cm after 6 months of storage. In T6 (Hot water treatment at 60°C for 30 minutes), shoot length was 3.89 cm when the seeds were sown before storage. This decreased along storage and reached 3.13 cm at the end of six months of storage. Highest shoot length of 4.53 cm was recorded in T7 (Hydration for 24 hours) when sown immediately after treatment, which declined during storage and was 4.31 cm after storing for two months. This further declined to 4.01 cm after four months and then became 3.54 cm at the end of six months of storage. Untreated seeds recorded a shoot length of 3.95 cm before storage, which gradually declined to reach 3.25 cm after 6 months.

4.2.2.4 Seedling length (cm)

Length of seedlings were significantly influenced by different seed treatments and also showed a declining trend along the storage (Table 18). The highest initial seedling length was recorded in T7 (7.48cm) which was on par with T4 (7.34cm). T5 showed a seedling length of 6.65cm. All the remaining treatments produced shorter seedlings than that of control (6.52 cm). The lowest was recorded in seeds that were treated with conc. sulphuric acid for 15 minutes (5.76 cm). In T1 (conc. H_2SO_4 for 5 minutes), the initial seedling length was recorded as 5.97 cm which showed a gradual decline during storage and reached 4.72 cm at the end of storage. The seeds treated with conc. H_2SO_4 for 10 minutes (T2) noted an initial seedling length of 5.92 cm which reached 4.23 cm by 6 months of storage. Seeds treated with conc. H_2SO_4 for 15 minutes (T3) produced the shortest seedlings among all (5.76 cm) initially, which was recorded as 3.95 cm at the end of storage period of five months. Seedling length after 6 months of storage could not be observed as there was no germination noticed. The seeds those were treated with hot water at 80°C for 20 minutes produced seedlings with length of 6.65 cm which decreased along the storage period and reached 5.61cm after 6 months of storage. Hot water treatment at 60°C for 30 minutes produced seedlings of length, 6.41 cm which declined to 5.17 cm at the

Table 18. Effect of seed treatment on seedling length (cm) during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	5.97^{d}	5.79^{e}	5.53^{e}	5.33^{f}	5.12^{d}	4.99^{d}	4.72^{d}
T_2	5.92^{d}	5.57^{f}	5.45^{e}	5.20^{f}	4.89^{e}	4.45^{e}	4.23^{e}
T_3	5.76^{e}	5.53^{f}	5.25^{f}	4.83^{g}	4.42^{f}	3.95^{f}	-
T_4	7.34^{a}	7.13^{b}	6.92^{a}	6.56^{b}	6.51^{a}	6.13^{a}	5.92^{a}
T_5	6.65^{b}	6.52^{c}	6.33^{b}	6.22^{c}	6.04^{b}	5.80^{b}	5.61^{b}
T_6	6.41^{c}	6.30^{d}	6.14^{d}	5.95^{e}	5.84^{c}	5.51^{c}	5.17^{c}
T_7	7.48^{a}	7.34^{a}	7.11^{a}	6.91^{a}	6.60^{a}	6.25^{a}	5.93^{a}
T_8	6.52^{bc}	6.37^{cd}	6.28^{bc}	6.07^{d}	5.93^{bc}	5.60^{c}	5.42^{bc}

end of six months of storage. Hydrated seeds produced seedlings with longest length of 7.48 cm which reached 5.93 cm by 6 months of storage. Untreated seeds initially produced seedlings of length 6.52 cm, which later declined to 5.42 cm after storage for 6 months.

4.2.2.5 Seedling dry weight (mg)

Different seed treatments were found to have significant influence on dry weight of seedlings and regardless of type of treatment, it declined during storage (Table 19). Highest seedling dry weight was noticed in hydrated seeds (4.59 mg) which was on par with that of the mechanically scarified seeds (4.49 mg) and were superior to that of the untreated seeds. All the remaining treatments resulted in seedlings with lower dry weight than the control. Untreated seeds (T8) recorded a seedling dry weight of 3.99 mg when sown immediately after treatment. This declined during storage and reached 3.06 mg after storing for 6 months. In T1 (Treatment with conc. H_2SO_4 for 5 minutes), seedling dry weight was 3.52 mg before storage which reduced during storage and reached 2.52 mg after six months of storage. Seeds treated with conc. H_2SO_4 for 10 minutes (T2) recorded an initial seedling dry weight of 3.45 mg which became 2.49 mg at the end of six months of storage. Seedling dry weight was 3.31 mg in T3 (Treatment with conc. H_2SO_4 for 15 minutes) when sown immediately. This declined gradually on storage and reached 2.37 mg after storing for five months. There was no germination noted after sixth month. Mechanically scarified seeds (T4) recorded a seedling dry weight of 4.49 mg when sown before storage. After six months of storage, it was reduced to 3.51 mg. Seedling dry weight in T5 (Hot water treatment at 80°C for 20 minutes) was 3.92 mg when sown immediately after treatment. This declined during storage and reached 3.04 mg after 6 months of storage. Hot water treatment of seeds at 60°C for 30 minutes resulted in an initial seedling dry weight of 3.73 mg, which declined to 2.97 mg by six months of storage. Maximum seedling dry weight of 4.59 mg was noted in T7 (Hydration for 24 hours) when sown before storage. This decreased along the storage period and reached 3.35 mg at the end of six months of storage.

Table 19. Effect of seed treatment on seedling dry weight (mg) during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	3.52^{e}	3.41^{d}	3.32^{e}	3.16^{cd}	3.07^{cd}	2.78^{e}	2.52^{d}
T_2	3.45^{f}	3.37^{d}	3.2^{f}	3.12^{d}	3.03^{d}	2.67^{f}	2.49^{d}
T_3	3.31^{g}	3.18^{e}	3.04^{g}	2.8^{e}	2.43^{e}	2.37^{g}	-
T_4	4.49^{a}	4.34^{a}	4.19^{b}	4.01^{a}	3.81^{a}	3.63^{a}	3.51^{a}
T_5	3.92^{c}	3.78^{b}	3.43^{d}	3.26^{c}	3.18^{bc}	3.13^{c}	3.04^{c}
T_6	3.73^{d}	3.62^{c}	3.38^{de}	3.21^{cd}	3.13^{cd}	3.03^{d}	2.97^{c}
T_7	4.59^{a}	4.42^{a}	4.29^{a}	4.05^{a}	3.78^{a}	3.51^{b}	3.35^{b}
T_8	3.99^{b}	3.82^{b}	3.66^{c}	3.49^{b}	3.31^{b}	3.19^{c}	3.06^{c}

4.2.2.6 Vigour index I

The effect of seed invigoration on vigour index I of seeds was found to be highly significant. Irrespective of the treatment applied, vigour index I showed a declining trend along the storage period (Table 20). Before storage (immediately after treatment- 0 MAS) when vigour index I was calculated, there was a significant variation noted among the treatments. Vigour index I ranged from 129 in T3 to 703 in T4. Highest vigour index I (703) was exhibited by the mechanically scarified seeds which was on par with that noted in T7, hydrated seeds (698). Both these treatments were found to be far more superior to the control with a vigour index I of 485. Seeds treated with hot water at 80^0C for 20 minutes (76.70 %) showed a higher vigour index I (510) than that of control. All the remaining treatments failed to enhance vigour index I and were found to result in lower vigour index I than the control. Seeds treated with hot water at 60^0C for 30 minutes exhibited a vigour index I of 433 while those treated with conc. H_2SO_4 for 5, 10 and 15 minutes possessed vigour indices of 327, 203 and 129 respectively.

After one month of storage, vigour index I ranged from 101 (T3- conc. H_2SO_4 for 15 minutes) to 678 (T7- Hydration for 24 hours). Mechanically scarified seeds showed a vigour index I of 674 which was on par with that of hydrated seeds. Untreated seeds showed vigour index I of 469. Seeds treated with hot water at 80^0C for 20 minutes exhibited vigour index I 486. After two months storage, highest vigour index I was noted in hydrated seeds (645), which was on par with that of mechanically scarified seeds (621). Untreated seeds exhibited a vigour index I of 429. All the remaining treatments showed lower vigour index I than that of control. Seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes exhibited vigour index I of 414 and 338 respectively. Treatment of seeds with conc. sulphuric acid for 5, 10 and 15 minutes resulted in vigour index I of 267, 145 and 82 respectively.

Table 20. Effect of seed treatment on vigour index I during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	327[d]	301[d]	267[d]	198[d]	131[e]	104[d]	47[d]
T_2	203[e]	171[e]	145[e]	134[e]	90[f]	46[e]	23[de]
T_3	129[f]	101[f]	82[f]	47[f]	24[g]	11[f]	-
T_4	703[a]	674[a]	621[a]	575[a]	551[a]	474[a]	389[a]
T_5	510[b]	486[b]	414[b]	401[b]	325[c]	242[c]	198[c]
T_6	433[c]	403[c]	338[c]	320[c]	283[d]	218[c]	162[c]
T_7	698[a]	678[a]	645[a]	602[a]	541[a]	460[a]	378[a]
T_8	485[b]	469[b]	429[b]	397[b]	364[b]	304[b]	241[b]

After three months storage period, the vigour index I was highest in hydrated seeds (602) and mechanically scarified seeds (575). This was followed by 397 in the untreated seeds. All the remaining treatments were found to be lower than that of untreated seeds. Vigour index I of seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes were 401 and 320 respectively. Seeds treated with conc. sulphuric acid for 5, 10 and 15 minutes showed vigour index I of 198, 134 and 47 respectively.

After a storing for four months, mechanically scarified seeds (551) showed highest germination which was on par with that of hydrated seeds (541). Untreated seeds recorded a vigour index I of 364, which was higher than the remaining treatments. Seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes exhibited vigour index I of 325 and 283 respectively. Treatment of seeds with conc. sulphuric acid for 5, 10 and 15 minutes resulted in vigour index I of 131, 90 and 24 respectively.

At the end of storage period, vigour index I exhibited by mechanically scarified seeds (389) and hydrated seeds (378) were on par followed by the untreated seeds (241). Remaining treatments recorded poor vigour index I. Seeds treated with hot water at 80^0C for 20 minutes and 60^0C for 30 minutes exhibited vigour index I of 198 and 162 respectively. Treatment of seeds with conc. sulphuric acid for 5 and 10 minutes resulted in vigour index I of 47 and 23 respectively. Conc. Sulphuric acid treatment of seeds for 15 minutes failed to germinate at this stage.

Throughout the period of observation, the vigour index I in treatments T1,T2, T3 and T6 remained inferior to control. The highest vigour index I was observed in treatment T4 (703), where the seeds were mechanically scarified using sand, which was followed by T7 (698) where the seeds were soaked in water for 24 hours. These were on par with each other and were also superior to the control.

Untreated seeds (T8) showed an initial vigour index of 485 which diminished during storage to reach 469 after one month. Vigour again declined and reached 429 and 397 by the end of 2 months and 3 months of storage. After storing for 4 months, it declined to 364 and then to 304 after 5 months storage. Vigour index I became 241 at the end of 6 months storage.

Before storage, vigour index I of T1 (Treatment with conc. H_2SO_4 for 5 minutes) was found to be 327 which declined during storage and reached 301 after one month. This further declined to 267 and 198 after 2 and 3 months of storage. When stored for a month more, vigour index I further reduced to 131. After 5 months of storage, it became 104 and again declined to 47 at the end of storage period of 6 months.

T2 (Conc. Sulphuric acid for 10 minutes) showed an initial vigour index I of 203 which decreased to 171 by storing for a month. This became 145 after storing for one more month. After 3 months of storage, vigour index I was calculated as 134 which declined to 90 and 46 by the end of 4 months and 5 months storage respectively. Vigour index I after 6 months storage was calculated as 23.

Initial vigour index I of T3 (conc. Sulphuric acid for 15 minutes) seeds before storage was calculated as 129 which declined along storage. It reached 101 after storing for a month and again decreased to 82 by the end of 2 month storage. Vigour index I further declined to 47 by storing for a month more. It again decreased to 24 and 11 after storing for 4 months and 5 months respectively. Observations after six months could not be taken as the seeds failed to germinate.

Highest initial vigour index I was recorded in T4 (703), where the seeds were scarified with sand. This vigour showed a declining trend as the storage period increased and reached 674 after storing for a month. After 2 months, this declined to 621 and then to 575 after storing for a month more. It further decreased to 551 and 474 when recorded after 4 and 5 months storage. At the end of six months of storage, it reached a reduced value of 389.

In T5, initial vigour index was recorded as 510, which further declined during storage to 486 after storing for a month. It further declined to 414 and 401 after being stored for 2 and 3 months respectively. After 4 months, vigour index I became 325 which declined to 242 after storing for a month more (5 MAS). Vigour index during sixth month was noticed as 198.

Initial vigour index I of T6 was 433, which became 403 after 1 month of storage. It became 338 by the end of 2 months storage. Vigour index I of 320 was recorded after 3 months, which decreased to 283 in a month. After storing for 5 months, it became 218 which later reduced to 162 after 6 months storage.

Hydrated seeds showed an initial vigour index I of 698, higher than that of control following T4. This decreased to 678 by storing for a month and then to 645 after storing for a month more. After 3 months, the value reached to 602 and then to 541 after storing for 4 months. By the end of 5 months storage, vigour index I was calculated as 460, which later declined to 378 after 6 months storage.

4.2.2.7 Vigour index II

Vigour index II of the seeds was greatly influenced by different seed treatments. Regardless of the treatments applied, vigour declined during storage (Table 21) vigour index II of treatments T4 and T7 was found to be superior to the control along the storage period. Remaining treatments T1, T2, T3, T6 and T5 recorded lower values than the control *viz.,* 204, 173, 116, 69 and 32 respectively. The highest vigour index II was noted in T4 (430) and was on par with that of T7 (428).

Untreated seeds showed an initial vigour index II of 297 which reduced to 282 after a month. It became 250 after 2 months storage which again decreased to 228 by the end of third month. This further declined to 203 and 173 after 4 and 5 months of storage. Vigour index II became 136 by six months of storage.

In T1, vigour index II was 193 before initiating the storage and it reached 177 after storing for a month. By the end of second month of storage, it reached 160 and then 119 by storing for a month more. Vigour index further reduced to 79

when stored for a month more. This later reduced to 58 and 125 by the end of 5 and 6 months of storage.

Initial vigour index II of T2 was recorded as 119 which reduced to 103 after one month. This decreased to 85 and then to 80 after storing for 2 and 3 months. Vigour index II decreased to 56 and 28 after storing for 4 and 5 months. It declined to 13 at the end of six months storage.

Vigour index II of T3 was calculated as 74 initially at the beginning of storage and is the least among all the treatments. This gradually declined to reach 58 after storing for a month. 2 months storage reduced the vigour index II to 48 and then to 27 after storing for a month more.

Mechanically scarified (T4) seeds showed highest initial vigour index II of 430 which decreased to 409 after one month. This became 376 and 352 after storing for 2 and 3 months respectively. By the end of 4 months, vigour index II reduced to 323, which further declined to 281 by storing for a month more. By the end of 6 months, vigour index II became 231.

Initial vigour index II of T5 was 301 which was lowest among all and reduced to 282 after a month. Storage for one more month further reduced the vigour index II to 224. At the end of third month, it became 210. This again declined to 172, 131 and 107 by the end of 4, 5 and 6 months of storage respectively.

In T6, initial vigour index II was calculated as 252 which became 232 by the end of a month. It became 186 after 2 months storage which again decreased to 173 by the end of third month. This further declined to 154 and 120 after 4 and 5 months of storage. Vigour index II became 93 by six months of storage.

Table 21. Effect of seed treatment on vigour index II during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	193[d]	177[d]	160[e]	119[d]	79[e]	58[e]	25[e]
T_2	119[e]	103[e]	85[f]	80[e]	56[f]	28[f]	13[e]
T_3	74[f]	58[f]	48[g]	27[f]	13[g]	6[g]	-
T_4	430[a]	409[a]	376[a]	352[a]	323[a]	281[b]	231[a]
T_5	301[b]	282[b]	224[c]	210[b]	172[d]	131[d]	107[d]
T_6	252[c]	232[c]	186[d]	173[c]	154[d]	120[d]	93[c]
T_7	428[a]	408[a]	389[a]	343[a]	296[b]	244[a]	213[b]
T_8	297[b]	282[b]	250[b]	228[b]	203[c]	173[c]	136[d]

T7 seeds showed an initial vigour index II of 428, higher than that of control following T4. This decreased to 408 by storing for a month and then to 389 after storing for a month more. After 3 months, the value reached to 343 and then to 296 after storing for 4 months. By the end of 5 months storage, vigour index I was calculated as 244, which later declined to 213 after 6 months storage.

4.2.2.8 Seed moisture (%)

The seeds after different treatments were dried to 8 per cent moisture level and stored in 700 gauge polythene packs. There was no significant variation in seed moisture content during storage for all the treatments (Table 22).

4.2.2.9 Electrical conductivity (dSm^{-1})

Electrical conductivity (EC) of the seeds was influenced by different treatments and showed an increasing trend along the storage (Table 23). Initially, among the different treatments, T3 (conc. H_2SO_4 for 15 minutes) exhibited the highest EC of 0.0063 $dSm^{-1}$and the lowest was for T7 (0.0011 dSm^{-1}) which was on par with T4 (0.0012 dSm^{-1}). All the other treatments had higher EC values than the control (0.0022 dSm^{-1}). This trend continued along till the end of storage period. In T1, initial EC was found to be 0.0042 dSm^{-1}which later increased to 0.0043 dSm^{-1} in one month storage and to 0.0044 dSm^{-1}in 3 months storage. This then increased along storage so as to reach 0.0052 dSm^{-1} in 6 months of storage. Seeds treated with conc. H_2SO_4 for 10 minutes (T2) noted an initial EC of 0.005 dSm^{-1}which reached 0.0053 $dSm^{-1}$by storing for a month. This further increased to 0.0055 dSm^{-1}in 3 months and then to 0.0064 $dSm^{-1}$by 6 months of storage. EC of seeds treated with conc. H_2SO_4 for 15 minutes (T3) showed the highest initial EC among all (0.0063 dSm^{-1}), which was recorded as 0.0073 dSm^{-1} after storing for 3 months. At the end of storage period of six months, the value was 0.0073 dSm^{-1}. Mechanically scarified seeds (T4) exhibited an EC of 0.0012 dSm^{-1}immediately after the treatment. This increased in storage and reached to a higher value of 0.0020 dSm^{-1} at 6 months of storage. The seeds treated with hot water at 80°C (T5) for 20 minutes exhibited an initial EC of 0.0033 dSm^{-1} which

Table 22. Effect of seed treatment on seed moisture (%) during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	8.00	8.01	8.01	8.03	8.04	8.05	8.07
T_2	8.00	8.02	8.03	8.04	8.05	8.07	8.10
T_3	8.00	8.01	8.01	8.03	8.07	8.08	8.10
T_4	8.00	8.01	8.02	8.03	8.04	8.04	8.05
T_5	8.00	8.01	8.01	8.03	8.04	8.05	8.07
T_6	8.00	8.01	8.02	8.04	8.04	8.08	8.09
T_7	8.00	8.01	8.02	8.03	8.04	8.04	8.05
T_8	8.00	8.02	8.02	8.04	8.04	8.08	8.09

Table 23. Effect of seed treatment on electrical conductivity (dSm^{-1}) during storage

Treatments	0 MAS	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1	0.0042^{c}	0.0043^{c}	0.0044^{c}	0.0044^{c}	0.0046^{c}	0.0049^{c}	0.0052^{c}
T_2	0.0051^{b}	0.0053^{b}	0.0054^{b}	0.0055^{b}	0.0056^{b}	0.0060^{b}	0.0064^{b}
T_3	0.0063^{a}	0.0064^{a}	0.0065^{a}	0.0066^{a}	0.0067^{a}	0.0071^{a}	0.0073^{a}
T_4	0.0012^{g}	0.0012^{g}	0.0013^{g}	0.0015^{g}	0.0017^{g}	0.0019^{g}	0.0020^{g}
T_5	0.0033^{e}	0.0034^{e}	0.0035^{e}	0.0036^{e}	0.0037^{e}	0.0040^{e}	0.0043^{e}
T_6	0.0035^{d}	0.0036^{d}	0.0037^{d}	0.0039^{d}	0.0042^{d}	0.0045^{d}	0.0048^{d}
T_7	0.0011^{g}	0.0012^{g}	0.0013^{g}	0.0014^{g}	0.0015^{h}	0.0016^{h}	0.0018^{h}
T_8	0.0022^{f}	0.0023^{f}	0.0024^{f}	0.0024^{f}	0.0026^{f}	0.0029^{f}	0.0032^{f}

increased along the storage period and reached 0.0043 dSm^{-1}at 6 months of storage. In T6, EC value initially noted was 0.0035 dSm^{-1} which further increased to 0.0048 dSm^{-1} after 6 months of storage. Hydrated seeds (T7) possessed the lowest EC of 0.0011 dSm^{-1} initially, which later increased and reached 0.0018 $dSm^{-1}$by 6 months of storage. Untreated seeds initially showed an EC of 0.0022 dSm^{-1} which later increased to 0.0032 dSm^{-1} after storage for 6 months.

4.3 STANDARDIZATION OF VEGETATIVE PROPAGATION TECHNIQUE THROUGH CUTTINGS

In the third experiment, vegetative propagation through stem cuttings in *Indigofera tinctoria* was undertaken. Effect of different concentrations of IBA (250 ppm, 500 ppm, 750 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm) on rooting of softwood, semi-hardwood and hardwood stem cuttings was evaluated in two seasons viz., summer and rainy seasons (Plate 7, 8 and 9). The simple effect of IBA, cutting types (softwood, semi-hardwood and hardwood cuttings) and interaction effect of IBA with cutting types are described in the Table 24 to Table 36.

4.3.1 Impact of varying doses of IBA and type of cutting on vegetative propagation in Neelayamari during summer season

4.3.1.1 Days to sprouting

There was no significant variation in days to sprouting in different treatments during the summer season (Table 24). Among them, T_6 (2000 ppm IBA) took minimum days to sprout (12.08) and control took maximum days to sprout (13.67 days). Among the cuttings, softwood cuttings took least days (9.68 days) to sprout, while semi-hardwood and hardwood took 12.25 and 12.37 days respectively.

There was no significant variation in the interaction effect of IBA and cuttings during summer season. In this, C_3T_6 (softwood cuttings × 2000 ppm IBA) took least days (9.31 days) to sprout while C_1T_9 (hardwood cuttings × 0 ppm IBA) took 16.52 days to sprout.

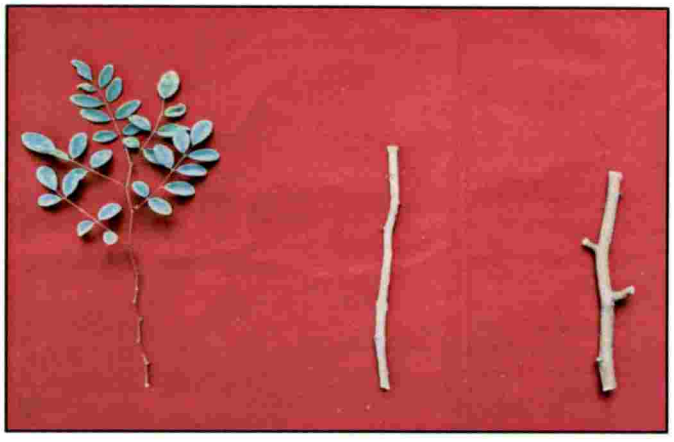

Plate 7: Types of cuttings (softwood, semihardwood and hardwood cuttings)

Plate 8: Cuttings planted and maintained in mist chamber

Plate 9: Sprouted cuttings at 30 days after planting

4.3.1.2 Sprouting (%)

The extent of sprouting of different types of cuttings under different treatments during summer season are shown in Table25. Highest sprouting was noticed when IBA was used at 2500 ppm (12.03%) and 2000 ppm (11.99%), followed by 1500 ppm (10.01%). Least amount of sprouting was noticed in untreated cuttings. While considering the type of cuttings, softwood cuttings exhibited the highest sprouting of 9.31 per cent followed by semi-hardwood (8.46 %) and hardwood cuttings (6.05%).

In interaction, C_3T_6 (softwood cuttings × 2000 ppm IBA) exhibited the highest amount of sprouting (16.57%) followed by C_3T_5, 14.45 per cent (softwood cuttings × 1500 ppm IBA) and C_2T_7 ,14.45 per cent (semi-hardwood cuttings × 2500 ppm IBA). Lowest sprouting rate was noted in C_3T_9 (hardwood cuttings × 0 ppm IBA).

4.3.1.3 Seedling establishment (%)

Though sprouting was observed in all treatment combinations, only the softwood cuttings treated with T_6 (2000 ppm IBA) and T_5 (1500 ppm IBA) survived after 60 days of planting, with an establishment rate of 8.89 per cent and 5.53 per cent (Table 26). All the other sprouts dried off.

4.3.1.4 Number of roots

Number of roots in C_3T_6 (softwood cuttings × 2000 ppm IBA) was noted as 5.15 and that of C_3T_5 (softwood cuttings × 1500 ppm IBA) was observed to be 4.28 (Table 27).

4.3.1.5 Length of roots (cm)

Root length (Table 28) was found to be 4.12 cm in C_3T_6 (softwood cuttings × 2000 ppm IBA) and 3.27 cm in C_3T_5 (softwood cuttings × 1500 ppm IBA).

Table 24. Effect of IBA and types of cuttings on days to sprout during summer season

Treatments	C_1	C_2	C_3	Mean
T_1	16.19	12.58	10.82	13.19
T_2	16.07	12.36	10.54	12.99
T_3	15.76	12.19	10.16	12.70
T_4	15.52	11.86	9.75	12.38
T_5	15.47	11.72	9.42	12.20
T_6	15.35	11.58	9.31	12.08
T_7	15.34	11.46	9.65	12.15
T_8	15.44	12.05	9.87	12.45
T_9	16.52	13.78	10.72	13.67
Mean	12.37	12.25	9.68	

Factors	C.D (0.05)	SE (m)
Cuttings	0.52	0.18
IBA	NS	0.32
Interaction	NS	0.55

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1=250 ppm IBA

T_2 =500 ppm IBA

T_3 =750 ppm IBA

T_4=1000 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

Table 25. Effect of IBA and types of cuttings on sprouting (%) during summer season

Treatments	C_1	C_2	C_3	Mean
T_1	3.33	5.56	5.56	4.81
T_2	3.33	7.78	5.56	5.56
T_3	5.56	7.78	7.78	7.04
T_4	6.67	6.67	10.00	7.78
T_5	7.78	7.78	14.45	10.01
T_6	7.78	11.64	16.57	11.99
T_7	10.00	14.45	11.64	12.03
T_8	7.78	10.00	8.89	8.89
T_9	2.22	4.44	3.33	3.33
Mean	6.05	8.46	9.31	

Factors	C.D (0.05)	SE (m)
Cuttings	0.62	0.14
IBA	1.08	0.36
Interaction	1.87	0.53

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_5= 1500 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

Table 26. Effect of IBA and types of cuttings on seedling establishment (%) during summer season

Treatments	C_1	C_2	C_3
T_1	0.00	0.00	0.00
T_2	0.00	0.00	0.00
T_3	0.00	0.00	0.00
T_4	0.00	0.00	0.00
T_5	0.00	0.00	5.53
T_6	0.00	0.00	8.89
T_7	0.00	0.00	0.00
T_8	0.00	0.00	0.00
T_9	0.00	0.00	0.00

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_5 = 1500 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

Table 27. Effect of IBA and type of cuttings on number of roots during summer season

Treatments	C_1	C_2	C_3
T_1	0.00	0.00	0.00
T_2	0.00	0.00	0.00
T_3	0.00	0.00	0.00
T_4	0.00	0.00	0.00
T_5	0.00	0.00	4.28
T_6	0.00	0.00	5.15
T_7	0.00	0.00	0.00
T_8	0.00	0.00	0.00
T_9	0.00	0.00	0.00

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

Table 28. Effect of IBA and type of cuttings on length of roots during summer season

Treatments	C_1	C_2	C_3
T_1	0.00	0.00	0.00
T_2	0.00	0.00	0.00
T_3	0.00	0.00	0.00
T_4	0.00	0.00	0.00
T_5	0.00	0.00	3.27
T_6	0.00	0.00	4.12
T_7	0.00	0.00	0.00
T_8	0.00	0.00	0.00
T_9	0.00	0.00	0.00

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_5 = 1500 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

Table 29. Effect of IBA and type of cuttings on days to sprout during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	13.16	10.24	8.32	10.57
T_2	12.04	10.16	8.14	10.11
T_3	12.84	10.04	8.00	10.29
T_4	12.56	9.65	7.78	9.99
T_5	12.95	9.65	7.36	9.98
T_6	12.42	9.51	7.18	9.70
T_7	12.20	9.31	7.52	9.67
T_8	13.16	9.81	7.84	10.31
T_9	13.40	10.50	8.41	10.77
Mean	12.76	9.88	7.83	

Factors	C.D (0.05)	SE (m)
Cuttings	0.33	0.16
IBA	0.18	0.09
Interaction	0.56	0.19

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_5 = 1500 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

4.3.2 Impact of varying doses of IBA and type of cutting on vegetative propagation in Neelayamari during rainy season

4.3.2.1 Days to sprouting

Number of days taken for the appearance of first green coloured sprout on the stem cuttings in each season was recorded and values are presented in Table.29. The least number of days for sprouting (9.67 days) was observed when the cuttings were treated with 2500 ppm IBA (T7) followed by 9.70 days in T6 (2000 ppm IBA). Both were found to be significantly on par with each other. The maximum number of days for sprouting (10.77 days) was recorded in cuttings where no treatment was applied (control). While considering the effect of cutting type on days to sprouting, the softwood cuttings recorded lowest number of 7.83 days for sprout initiation and maximum days for sprouting was recorded by hardwood cuttings (12.76 days). Semi-hard wood cuttings took 9.88 days for sprouting.

It was evident from the given data (Table 29) that the interaction effect of IBA × cutting type recorded a wide variation on number of days for sprouting. The least number of days for sprout initiation (7.18 days) was recorded in C_3T_6 (softwood cuttings × 2000 ppm IBA) followed by 7.36 days in C_3T_5 (softwood cuttings × 1500 ppm IBA) and 7.52 days in C_3T_7 (softwood cuttings × 2500 ppm IBA) respectively. All these were found to be on par with each other. The maximum days for sprouting (13.40 days)was recorded in C_1T_9 (hardwood cuttings × 0 ppm IBA) followed by13.26 days in C_1T_1 (hardwood cuttings × 250 ppm IBA). Both were on par with each other.

4.3.2.2.Sprouting (%)

The per cent sprouting was found to be significantly influenced by different concentrations of IBA (Table 30). The maximum sprouting of 46.93 per cent was recorded in cuttings which were treated with 2000 ppm IBA (T_6)

followed by 2500 ppm IBA (T_7) where the sprouting was noticed to be 45.95 per cent. They were found to be on par with each other followed by T_5 (42.68 %).

Lowest sprouting per cent was noted in control (11.63 %). Sprouting per cent was also found to be influenced by the type of cutting and had shown significant variation among different type of cuttings. In rainy season, maximum sprouting (47.98 %) was exhibited by soft wood cuttings (C_3) while the lowest sprouting (23.37 %) was observed with hardwood cuttings (C_1).

In terms of interaction effect, C_3T_6 (softwood cuttings × 2000 ppm IBA) resulted in maximum sprouting of 76.58 per cent. This was followed by C_1T_5 (softwood cuttings × 1500 ppm IBA) where the sprouting was noted to be 68.37 per cent (Table 30). The lowest sprouting of 6.67 per cent was recorded in C_1T_9 (hardwood cuttings × 0 ppm IBA).

Table 30. Effect of IBA and type of cuttings on sprouting (%)during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	17.74	23.14	31.25	24.04
T_2	20.19	25.65	35.41	27.08
T_3	23.67	26.24	38.67	29.53
T_4	25.17	29.57	54.75	36.49
T_5	28.45	31.24	68.37	42.68
T_6	30.67	33.54	76.58	46.93
T_7	36.54	37.52	63.80	45.95
T_8	21.25	24.97	46.49	30.90
T_9	6.67	11.61	16.61	11.63
Mean	23.37	27.05	47.98	

Factors	C.D (0.05)	SE (m)
Cuttings	0.66	0.24
IBA	1.53	0.53
Interaction	4.59	1.61

4.3.2.3. Number of emerged leaves

The number of emerged leaves per cutting recorded considerable variation among various treatments (Table 31). T_6 (2000 ppm IBA) recorded maximum number of leaves (5.10) which was on par with T_7 (2500 ppm IBA) where the number of leaves were 4.81. This was followed by T_5 (1500 ppm IBA) where the number of leaves per cutting was 4.27. The lowest number of leaves (3.36) was recorded by T_9 (control). In case of effect of cutting types, softwood cuttings (4.38) and semi-hardwood cuttings (4.31) recorded maximum number of leaves followed by hardwood cuttings (3.88).

It was concluded from the data that the interaction effect C_3T_6 (softwood cuttings × 2000 ppm IBA) recorded maximum number of leaves (5.90) which was on par with C_2T_7 (semi-hardwood cuttings × 2500 ppm IBA), where number of leaves were recorded as 5.53. The minimum number of leaves (3.03) was observed in C_2T_9 (semi-hardwood cuttings × 0 ppm IBA) and C_1T_9 (hardwood cuttings × 0 ppm IBA).

Table 31. Effect of IBA and type of cuttings on number of emerged leaves during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	3.7	4.20	3.17	3.69
T_2	4.16	4.80	3.77	4.24
T_3	4.03	4.53	4.20	4.26
T_4	3.20	3.70	5.00	3.97
T_5	4.23	4.17	4.40	4.27
T_6	4.37	5.03	5.90	5.10
T_7	4.10	5.53	4.80	4.81
T_8	4.10	4.37	4.20	4.22
T_9	3.03	3.03	4.00	3.36
Mean	3.88	4.31	4.38	

Factors	C.D (0.05)	SE (m)
Cuttings	0.26	0.09
IBA	0.45	0.16
Interaction	0.77	0.28

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_5 = 1500 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

4.3.2.4. Length of emerged leaves (cm)

From the Table 32, it was evident that the cuttings that were treated with T_7 (2500 ppm IBA) recorded highest length (8.11 cm) which was on par with T_6 (8.10 cm) followed by 7.85 cm recorded in T_5 (1500 ppm IBA) while the lowest leaf length (5.98 cm) was observed with T_1 (250 ppm IBA). Among the type of cuttings, softwood cuttings (C_1) recorded the highest leaf length of 7.75 cm followed by semi-hardwood cuttings (7.45 cm) and hardwood cuttings (6.34 cm).

The interaction effect C_3T_6 (softwood cuttings × 2000 ppm IBA) recorded highest leaf length (8.66 cm) which was on par with C_3T_5 (softwood cuttings × 1500 ppm IBA) where leaf length was noted to be 8.29 cm. The lowest value was recorded in C_1T_9 (hardwood cuttings × 0 ppm IBA) where leaf length of 5.04 cm was recorded.

Table 32. Effect of IBA and type of cuttings on length of emerged leaves (cm) during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	5.23	6.24	6.43	5.98
T_2	5.54	6.45	7.15	6.38
T_3	6.46	7.36	7.51	7.11
T_4	6.84	7.50	8.15	7.49
T_5	7.15	8.12	8.29	7.85
T_6	7.48	8.17	8.66	8.10
T_7	7.88	8.21	8.25	8.11
T_8	5.42	7.66	8.03	7.03
T_9	5.04	7.15	7.42	6.54
Mean	6.34	7.45	7.75	

Factors	C.D (0.05)	SE (m)
Cuttings	0.09	0.03
IBA	0.17	0.05
Interaction	0.39	0.17

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8= Charcoal slurry dip

T_9 = Control

Table 33. Effect of IBA and type of cuttings on width of emerged leaves(cm) during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	3.84	3.95	4.56	4.12
T_2	3.97	4.05	4.92	4.31
T_3	3.87	4.19	4.78	4.28
T_4	4.12	4.52	5.35	4.67
T_5	4.34	4.63	5.28	4.75
T_6	4.52	4.86	5.72	5.03
T_7	4.87	5.28	5.41	5.22
T_8	4.54	4.81	5.52	4.92
T_9	3.79	4.45	4.32	4.19
Mean	4.21	4.53	5.09	

Factors	C.D (0.05)	SE (m)
Cuttings	0.05	0.02
IBA	0.10	0.03
Interaction	0.16	0.07

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

T_9 = Control

4.3.2.5. Width of emerged leaves (cm)

Leaf width (Table 33) was observed to be highest (5.22 cm) in the cutting that were treated with T_7 (2500 ppm IBA) followed by T_6 (2000 ppm IBA) which recorded a leaf width of 5.03 cm. The lowest leaf width (4.12) was noticed in T_1 (250 ppm IBA). The highest value for leaf width (5.09 cm) was observed with softwood cuttings (C_3) followed by semi-hardwood cuttings (4.53 cm) and softwood cuttings (4.21 cm)

The interaction effect C_3T_6 (softwood cuttings × 2000 ppm IBA) recorded highest leaf width (5.72 cm) followed by C_3T_8 softwood cuttings × charcoal dip) where leaves attained a width of 5.52 cm. The lowest leaf width was noticed in C_3T_9 (hardwood cuttings × 0 ppm IBA) where leaf width was recorded as 3.79 cm.

4.3.2.6. Seedling establishment (%)

Seedling establishment per cent (Table 34) was found to be the highest in T_6 (2000 ppm IBA) which was recorded as 40.26 per cent followed by T_7- 38.60 per cent (2500 ppm IBA). The lowest establishment was noted in control (8.07). In cuttings, highest establishment of seedlings occurred in softwood cuttings (42.74 %) followed by semi-hardwood cuttings (21.77 %) and the lowest in hardwood cuttings (17.90 %).

While considering the interaction effect, the treatment C_3T_6 (softwood cuttings × 2000 ppm IBA) resulted in the highest establishment per cent of 70.67 per cent, which was followed by C_3T_5 (softwood cuttings × 1500 ppm IBA) with an establishment rate of 64.44 per cent. The lowest establishment (3.34 %) was recorded in C_1T_9 (hardwood cuttings × 0 ppm IBA).

Table 34. Effect of IBA and type of cuttings on seedling establishment (%)during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	13.34	19.00	26.56	19.63
T_2	16.67	20.24	29.42	22.11
T_3	18.54	21.74	34.46	24.91
T_4	20.14	23.56	48.51	30.74
T_5	21.87	25.21	64.44	37.13
T_6	23.65	26.48	70.67	40.26
T_7	27.23	30.41	58.17	38.60
T_8	16.33	20.71	40.18	25.74
T_9	3.34	8.56	12.21	8.07
Mean	17.90	21.77	42.74	

Factors	C.D (0.05)	SE (m)
Cuttings	1.27	0.56
IBA	1.47	0.87
Interaction	5.36	1.95

4.3.2.7. Number of roots (cm)

Number of roots (Table 35) was highest (8.74) in T_6 (2000 ppm IBA) which was followed by 7.74 in T_7 (2500 ppm IBA) and T_5 (1500 ppm IBA) where the number of roots was 7.41. The lowest number of roots were noticed in control (2.95). Among the cuttings, highest number of roots was noted in softwood cuttings (6.10), followed by semi-hardwood (5.93) and hardwood cuttings (5.46).

When the interaction effect was considered, highest number of roots (9.56) were recorded in C_3T_6 (softwood cuttings × 2000 ppm IBA) followed by 8.14 in C_3T_5 (softwood cuttings × 1500 ppm IBA). The lowest amount of roots were noticed in C_1T_9 (hardwood cuttings × 0 ppm IBA)-2.73.

Table 35. Effect of IBA and type of cuttings on number of roots of cuttings during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	4.78	5.19	4.36	4.78
T_2	5.02	5.47	5.12	5.20
T_3	5.29	6.18	5.04	5.50
T_4	6.41	6.52	7.02	6.65
T_5	6.89	7.21	8.14	7.41
T_6	7.32	7.34	9.56	8.74
T_7	7.51	7.79	7.94	7.74
T_8	3.22	4.74	4.50	4.15
T_9	2.73	2.93	3.21	2.95
Mean	5.46	5.93	6.10	

Factors	C.D (0.05)	SE (m)
Cuttings	0.09	0.03
IBA	0.17	0.05
Interaction	0.32	0.11

Table 36. Effect of IBA and type of cuttings on length of roots (cm) of cuttings during rainy season

Treatments	C_1	C_2	C_3	Mean
T_1	1.09	1.61	1.69	1.46
T_2	1.44	1.72	1.64	1.60
T_3	2.01	1.89	1.72	1.87
T_4	2.61	2.47	2.75	2.61
T_5	2.52	2.94	3.12	2.86
T_6	2.94	3.14	4.52	3.53
T_7	3.68	4.02	3.84	3.85
T_8	1.02	1.41	1.69	1.37
T_9	0.86	1.01	1.38	1.08
Mean	3.88	4.31	4.37	

Factors	C.D (0.05)	SE (m)
Cuttings	0.11	0.08
IBA	0.19	0.14
Interaction	0.34	0.14

C_1 = Hardwood cuttings

C_2 = Semi-hardwood cuttings

C_3 = Softwood cuttings

T_1 = 250 ppm IBA

T_2 =500 ppm IBA

T_3 = 750 ppm IBA

T_4 = 1000 ppm IBA

T_5 = 1500 ppm IBA

T_6= 2000 ppm IBA

T_7= 2500 ppm IBA

T_8 = Charcoal slurry dip

4.3.2.8. Length of roots (cm)

Length of roots (Table 36) was found to be highest (3.85 cm) in T_7 (2500 ppm IBA) which was followed by 3.53 cm in T_6 (2000 ppm IBA). This was followed by T_5 (1500 ppm IBA), where the root length was 2.86 cm. The lowest root length was noticed in control (1.08 cm). Among the cuttings, highest length of roots was noted in softwood cuttings (4.37 cm), which was on par with semi-hardwood (4.31 cm) and he lowest in hardwood cuttings (3.88).

While considering the interaction effect of the treatments on length of roots, highest root length (4.52 cm) was noted in the treatmentC_3T_6 (softwood cuttings × 2000 ppm IBA) followed by 4.02 cm in C_2T_7 (semi-hardwood cuttings × 2500 ppm IBA). Lowest root length was recorded in C_1T_9 (hardwood cuttings × 0 ppm IBA).

Discussion

5. DISCUSSION

5.1 STANDARDIZATION OF PHYSIOLOGICAL MATURITY STAGE FOR SEED HARVEST IN NEELAYAMARI

Quality seed is an essential requisite for any crop production. Harvesting stage has a significant impact on the quality of a seed. The seeds when harvested either at an early stage or during later stage of physiological maturity, their quality will be diminished along with reduced yield. The changes that occurred in the seeds beyond physiological maturity is mainly dehydration, without accumulation of any reserves.

The development pattern of pods and seeds of *Indigofera tinctoria* was studied at an interval of 5 days starting from 10 days after anthesis till the pod splitting stage (63 days after anthesis), so as to standardise the optimum stage of seed harvest or the physiological maturity stage of the crop.

The study revealed that characters as pod length, pod thickness, pod fresh weight, pod dry weight, seed length, seed breadth, germination per cent, seedling length and vigour index increased from the day of anthesis and reached a maximum value at 45 days after anthesis and decreased thereafter.

Length of the pod was found to increase from the day of anthesis. Length of the pod attained its maximum value at 45 days after anthesis (2.33 cm) and gradually decreased thereafter and reached 1.92 cm at pod splitting stage (63days after anthesis). Thickness of the pod also increased from the day of anthesis and reached the maximum at 45 days after anthesis (2.79 mm) and declined during later developmental stages to reach 1.88 mm at pod splitting stage (63days after anthesis). Similar trend was observed in sesame, wherein the length, width and thickness of capsules increased during initial stages and reached a maximum value of 2.10 cm, 0.73 cm and 0.87 cm respectively at 35 days after anthesis, which was concluded as the physiological maturity stage of the crop (Monalisa *et al.*, 2018).

Fresh weight and dry weight of the developing pod increased during initial stages and reached their maximum at 45 days after anthesis (97.40 mg and 44.23 mg respectively) (Figure 1), which showed a declining trend during later stages and reached 32.13 mg and 23.27 mg respectively by pod splitting stage (63 days after anthesis). The growing fruit is an active sink that diverts water and solutes from other parts of plant. The central theme of fruit growth is the mobilisation of substances to the developing fruit. During early stages of fruit development, new cell formation occurs followed by enlargement of the cells which contribute to the increasing weight (Harrington and Kozlowski, 1972). Along this period, development of embryo takes place. Pods after attaining the maximum weight at a particular stage, their weight decreased towards later stages. Such a trend during fruit development was reported in snakegourd variety Baby, where the maximum fruit weight was attained on 36 days after anthesis, which is identified as the physiological maturity stage of the crop and decreased later on (Dhobi, 2015). The reduction in weight observed during later stages could be related to the loss of moisture in the maturing pod (Ganar *et al.*, 2004).

Number of seeds did not vary with the developmental stages and it ranged from 4-6.

Fresh seed weight per pod gradually increased, reaching a maximum value at 45 days after anthesis (69.88 mg) and then declined during later stages reaching23.20 mg at pod splitting stage (63 days after anthesis). Similar result was noticed in soybean, where the highest seed weight was recorded at physiological maturity (12.23 g), while lower seed weight (10.52 g) was observed at field maturity stage(Nichal *et al.*, 2018).

The seed length and breadth also tended to vary with the developmental stages. During initial stages till 25 days after anthesis, the seeds were difficult to separate from the pod. Those seeds were translucent and tiny, which later became thick and bold. The seeds were very minute initially which made them difficult to observe with naked eyes. Seeds then increased gradually in size and attained the

maximum length and breadth at 45 days after anthesis (3.14 mm and 2.38 mm respectively), and thereafter, a gradual decline was noticed till the pod splitting stage. Lima *et al.* (2012) observed that, during the physiological maturation, the seeds of *Poincianella pyramidalis* gradually increased in length and width along the maturation process, reaching the maximum estimated values of 106.08 mm and 12.32 mm, respectively at the 129th day after anthesis, which later declined towards later stages, owing to loss of moisture.

100 seed weight showed an increasing trend during initial stages, reaching its maximum value at 45 days after anthesis (1.22 g) and then started to decline leading to a smaller value at pod splitting stage. The increase in seed weight during initial developmental stages is associated with greater accumulation of photosynthates and moisture in to the seeds up to physiological maturity stage after which a reduction in weight noticed due to decrease in photosynthesis and accumulation of photosynthates and also moisture loss as reported by Olasoji *et al.* (2012) in *Hibiscus canabinus* which attained the physiological maturity at 35 days after anthesis with a higher seed weight of 14.2 mg. Venudevan (2008) in glory lily also observed similar growth pattern in orthodox group of medicinal plants, wherein the fresh weight of seed attained their maximum at 18 days after anthesis and decreased thereafter due to disintegration occurred between source to sink and depletion of moisture content from seed. But, Delouche (1973) has already opined that reduction in weight is also due to the depletion of volatile substances in semi fluid state that might have escaped along with water. Similar reductions in weight due to desiccation drying that is specific to orthodox species was also reported in *Phyllanthus niruri* (Revathi, 2001),a medicinal plant of tropical region.

Seed moisture showed an increasing trend during initial developmental stages before reaching its maximum of 64.73 per cent (45 DAA) which later declined during later stages to reach 17.66 per cent at pod splitting stage. Similar trend was noticed in mustard where the seed moisture at physiological maturity stage was the highest (28.02 %) as reported by Geetha *et al.* (2013).

Germination capacity is an indicator of seed maturation. In this crop, germination rate of seeds exhibited an increasing trend during initial developmental stages, which reached maximum at 45 days after anthesis and then the rate declined gradually (Figure 2). During initial development stages till 30 days after anthesis, there was no germination noticed. At 30 days after anthesis, germination rate was 3.53 per cent, which later reached the maximum at 45 days after anthesis (73.80 %). Germination gradually decreased thereafter and became 31.33 per cent at the pod splitting stage. Similar trend in germination was observed in davana seeds (*Artemisia pallens*), wherein the seeds collected between 5 and 10 days after anthesis failed to germinate due to immaturity of the embryo, while the seeds collected at 15 days after anthesis attained germinability to a minimum of 15per cent, which steadily increased to the maximum of 86 per cent at 35 DAA, but decreased to 69 per cent with advances in maturation (40 DAA) (Jayanthi *et al.*, 2013). The maximization of germination at 45 days after anthesis might be due to attainment of potentiality for reproduction by the presence of a mature embryo with essential structures and activation of the enzymes and nutrients required for regeneration of miniature plant. The increasing trend in germination per cent till attaining the maximum on 45 days after anthesis might be attributed to the maximum dry matter content in seeds. Low seed quality at the early stages of seed development was due to immaturity while the decline in quality parameter at later stages was caused by seed ageing (Ghassemi-Golezani and Mazloomi-Oskooyi, 2008).

The root length, shoot length, total length and dry weight of the seedlings increased with the advancement in maturation of seeds and were the maximum at 45 days after anthesis (2.59 cm, 3.98 cm, 6.57 cm and 3.99 mg respectively). Similar results were obtained in soybean, where the root length (14.34 cm), shoot length (13.77 cm), total length (28.11 cm) and dry weight (82 mg) of the seedlings were the highest at the physiological maturity stage (Nichal *et al.*, 2018).

Seedling vigour is an inherent ability to survive well under wide range of conditions (Heydecker, 1979) and is considered as one of the important parameter

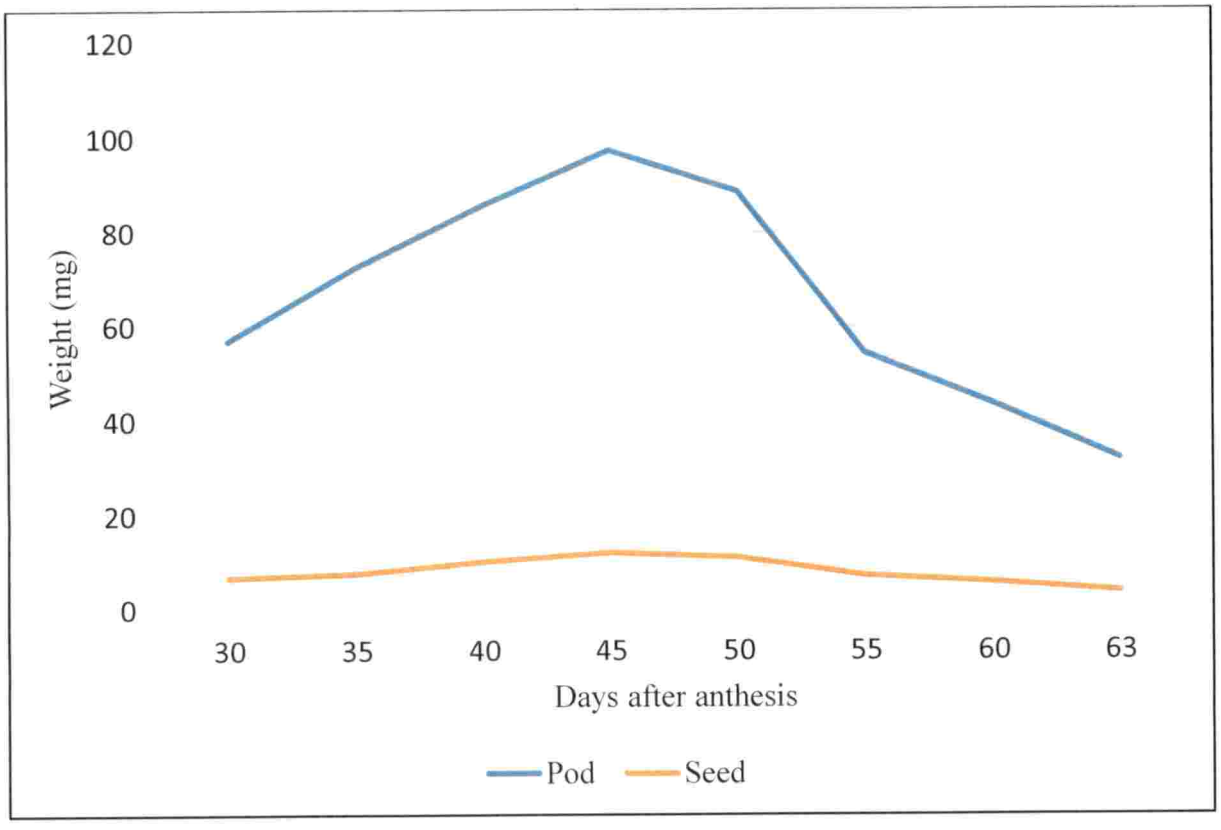

Figure 1: Fresh pod and seed weight at developmental stages

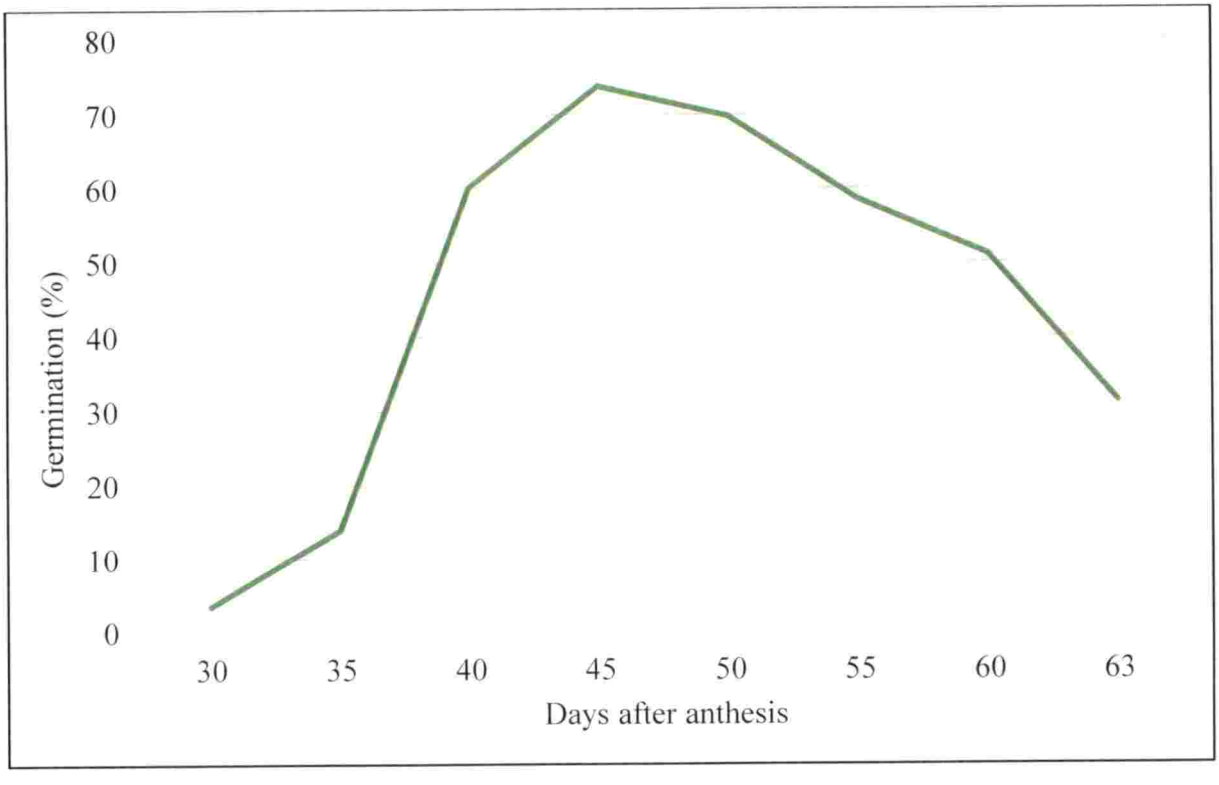

Figure 2: Germination of seeds at different developmental stages

indicating the seed maturity. The seedling vigour (vigour index I and II) exhibited an increasing trend during initial developmental stages and attained the maximum vigour on 45 days after anthesis (485 and 294 respectively) (Figure 3). The computed vigour index value was maximum at 45 days after anthesis coinciding with the higher germination, seedling length and dry weight. This result was in conformity with the findings of Jayanthi *et al.*(2013) in davana seeds, which exhibited the highest vigour index value of 198 at its physiological maturity stage (35 days after anthesis) and decreased during later stages.

The seeds attain their maximum quality at the physiological maturity stage which is indicated by maximum dry weight, germination and vigour of the seeds (Harrington and Kozlowski, 1972). Hence, from the present study, it can be inferred that the *Indigofera tinctoria* seeds attain their physiologically mature stage for harvesting at 45 days after anthesis, as all the seed characteristics and the seedling characters were maximum at 45 days after anthesis and thereafter declined. This decline may be mainly due to acquired dormancy owing to hardening of seed coat, making it impermeable to water or oxygen during seed drying. The quality parameters of seeds harvested at 45 days after anthesis would thus be superior to that obtained through the traditional practice, where the seeds are harvested when the pod dries.

5.2 EFFECT OF PRESOWING TREATMENTS ON SEED QUALITY AND LONGEVITY IN NEELAYAMARI

Seed occupies a cardinal role in all the agriculture systems. Obviously, all the defects in seed such as seed dormancy, uneven germination, poor germination and reduced seed vigour can lead to drastic economic loss to the farmers. Various pre sowing seed treatments are found to have positive impact on seed germination and vigour in many crops. Since, *Indigofera tinctoria* seeds are found to have low germination and vigour, the study was conducted to identify the most suitable seed treatment to enhance germination and vigour and also to identify the storability of treated seeds.

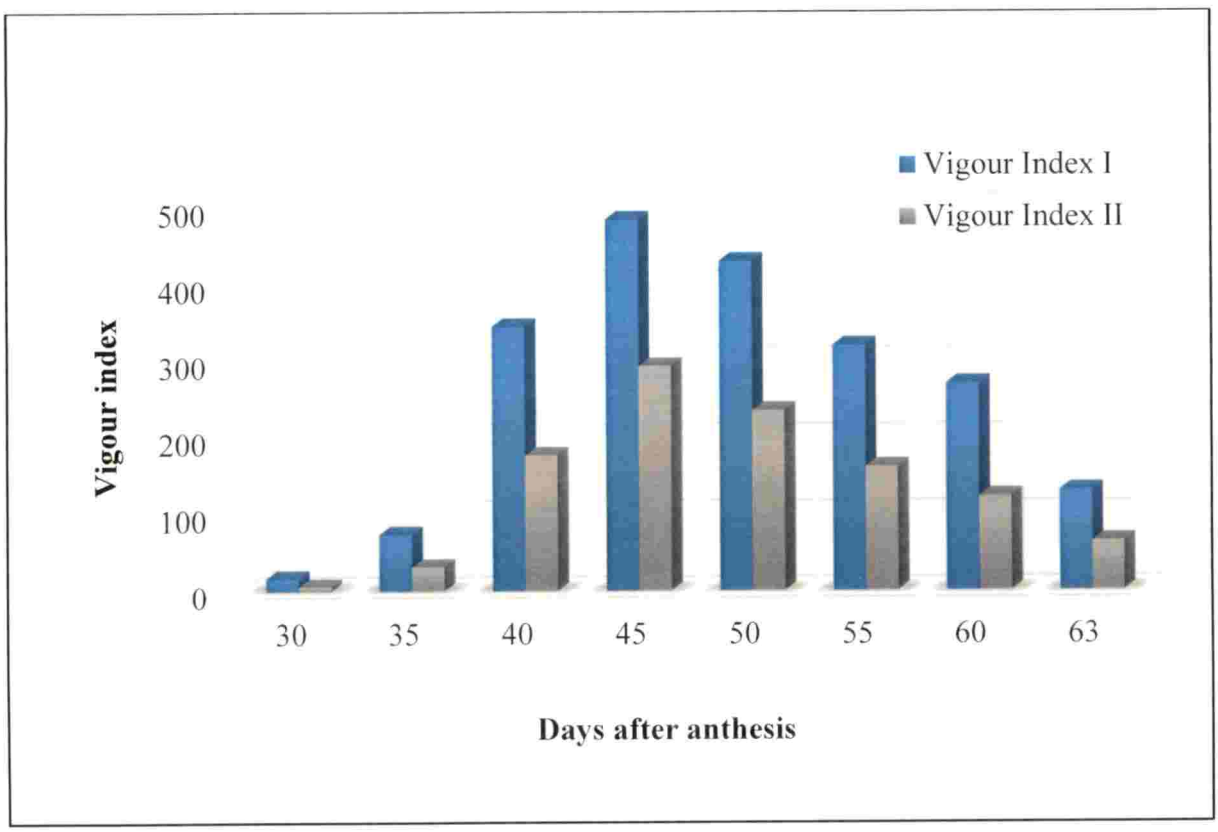

Figure 3: Vigour Indices of seedlings at different developmental stages

Highest germination immediately after treatment was noted in seeds that were mechanically scarified (95.83 %). It was about 28.9 per cent higher than that of the untreated seeds (Figure 4). Mechanical scarification was reported to be quite effective in increasing the germination rate of forage legume seeds (Rusdy, 2017).Chisha-Kasumu *et al.* (2007) reported that the highest rate of germination in *Pterocarpus angolensis* seeds was obtained when they were mechanically scarified. These results demonstrated the effectiveness of mechanical scarification in overcoming physical dormancy, which is imposed by a hard seed coat. Lignified palisade cell layer in the seeds could be damaged after scarification and germination occurs. Mechanical scarification cracks part of the hard seed coat which acts as a barrier to uptake water and undergo gaseous exchange and allow germination to proceed (Yildiztugay and Kucukoduk, 2012). By facilitating water uptake and gas exchanges through vertical cracks on seed coat, germination was improved. This indicates that the hard seed coat caused a major physical restraint to seed germination.

Hydration for 24 hours was also found to have higher germination (93.27%) and was on par with that of mechanically scarified seeds. This is in line with the result obtained in *Ocimum basilicum*, which showed a germination per cent of 90.66 per cent when soaked in water for 24 hours (Hosseein and Kasra, 2011). Another study conducted in *Salvia officinalis* also showed about 25.5 per cent higher germination than the control (Fahimi *et al.*, 2013). Water is an essential requirement in many biochemical reactions and also serve as a medium for life processes. In case of seeds, water is an essential factor in the external environment for the stimulation of germination. Seeds when soaked in water at room temperature, leads to softening of seed coats, removal of germination inhibitors and also increases germination. It also helps in reduction of time required for germination. Water imbibition will also result in the enlargement of embryo (Hartmann and Kester, 1979).

Hot water treatment of the seeds at 80^0C for 20 minutes resulted in a germination rate of 76.7 per cent, which was only about 3.19 per cent higher than

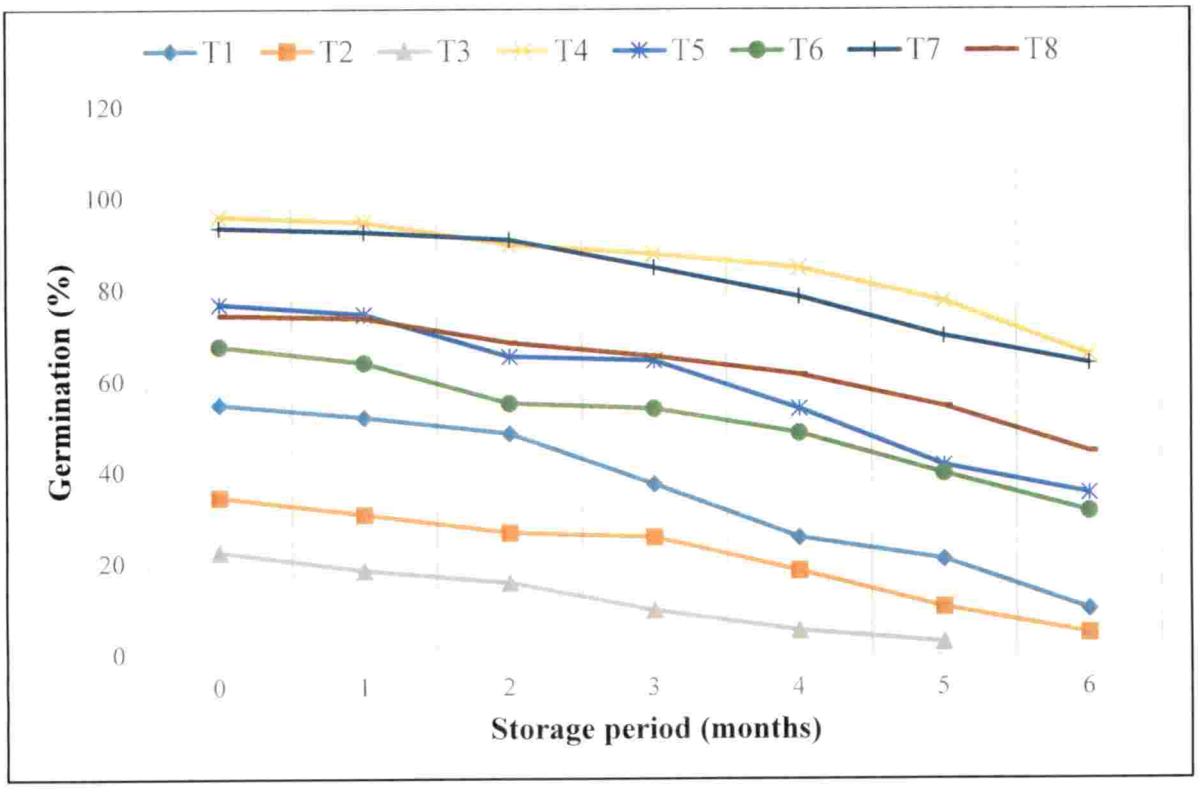

Figure 4: Effect of seed treatment on germination (%) during storage

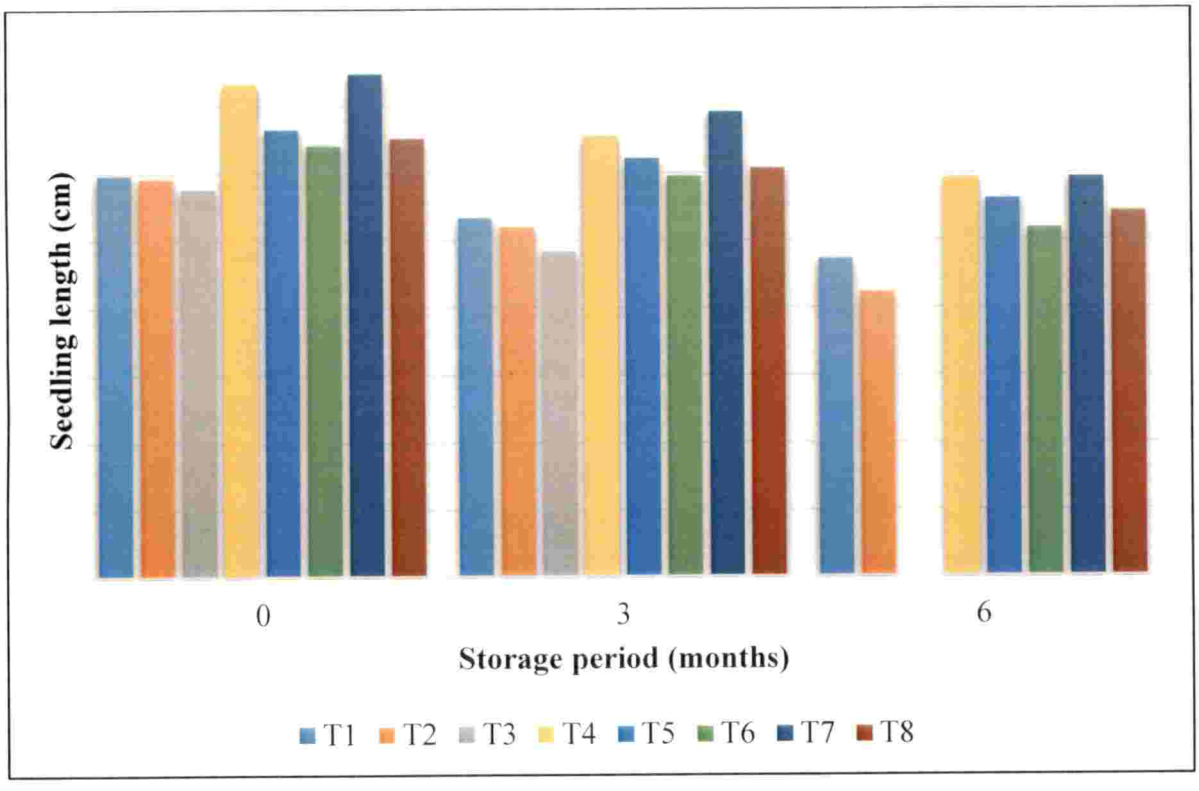

Figure 5: Effect of seed treatment on seedling length (cm) during storage

that of control. The seeds that were treated with water at 60^0C for 30 minutes showed a germination of 67.50 per cent, which was lower than the untreated ones. The lower germination per cent in the hot water treatment may be due to death of seeds and embryo due to prolonged exposure of the seeds to higher temperature. Similar trend was noticed in *Centrosema pubescens* where reduced seed germination and more dead seeds were noted in hot water treatment (Pe *et al.*, 2012).

Seed treatment with conc. sulphuric acid for 5, 10 and 15 minutes resulted in reduced germination of 54.7 per cent, 34.34 per cent and 22.33 per cent respectively. These were found to be far inferior than the control. This indicated that acid scarification for this period was detrimental to seed quality. This was in agreement with the result obtained in *Corchorus tridens* where the seeds exposed to conc. sulphuric acid for more than 5 minutes significantly decreased the germination capacity. The acid being corrosive in nature, it might have damaged the embryos of some of the seeds resulting in reduced germination (Emonger *et al.*, 2004).

Germination showed a declining trend along the storage period both in control and the treated seeds. In mechanically scarified seeds, the germination per cent experienced a slight decline of 1.31 per cent to reach 94.52 per cent at the end of one month of storage. This again reduced along different months of storage to reach 65.67 per cent by 6 months of storage. In hydrated seeds, initial germination rate declined by 0.94 per cent to reach 92.33 per cent after storing for a month. This further declined in consecutive storage months to reach 63.67 per cent by the end of storage period. Untreated seeds reached a lower germination of 44.33 per cent after 6 months of storage. Seeds treated with hot water at 80^0C for 20 minutes showed a declined germination rate of 35.14 per cent and those treated at 60^0C for 30 minutes showed a yet lower value of 31.13 per cent after the storage period. Treatment with conc. sulphuric acid resulted in already lower initial germination rates which further reduced during storage. Similar declining trend in germination over storage period was reported by Shelar *et al.* (2008) in

soybean seeds. The reduction in final germination rate could be due to the expansion of external osmotic pressure that influences the water assimilation by the seeds. Accumulation of ions such as sodium (Na^+) and chloride (Cl^-) in the embryo will influence the metabolic processes related to germination, leading to cell death in embryo. This can also contribute to the reduced germination along storage (Maher *et al.*, 2013).

It was also noticed that the seed treatment helped in extending the viability of the seeds. Mechanically scarified and hydrated seeds retained their germination above 60 per cent for six months during storage (65.67% and 63.67% respectively), while, in untreated seeds, germination reached 61.33 per cent at four months of storage and declined to 44.34 per cent by six months of storage.

Seedling length (Figure 5) was found to be highest in the mechanically scarified seeds (7.48 cm), this was followed by that of hydrated seeds (7.34 cm), similar to that reported in onion seeds by Caseiro *et al.* (2004). Seeds treated with hot water at 80^0C for 20 minutes also showed a higher seedling length than the control. All the remaining treatments produced seedlings with reduced lengths. Despite the type of treatment applied, seedling length declined along the storage period. Mechanically scarified seeds exhibited a reduced seedling length of 5.93 cm after storing for 6 months, while that of hydrated seeds was noted to be 5.92 cm. Similar result was noticed by Das (2012) in cowpea.

Highest initial seedling dry weight was observed in mechanically scarified seeds (4.59 mg). This is in corroboration with the result reported in *Leucaena leucocephala*, a leguminous forage plant by Koobonye *et al.* (2018). Seedling dry weight exhibited by hydrated seeds was higher (4.49 mg) and statistically on par with that of mechanically scarified seeds. Similar result was obtained in *Tagetes erecta* when soaked in water for 20-24 hours (Pramila *et al.*, 2013).Seedling dry weights observed in remaining treatments were inferior to that of control and may be due to the deterioration the treatments have caused on the seeds and the embryo.

All the seedlings despite the type of treatment, showed reduced dry weight as the storage period increased. Mechanically scarified seeds produced seedlings with a reduced dry weight of 3.51 mg after storing for 6 months. Hydrated seeds produced seedlings with a smaller dry weight of 3.35 mg at the end of storage period. This is found to be in agreement with the result reported by Leelavathi (2017) in green gram where a reduction in dry weight along the storage period was noted irrespective of the treatment applied.

Vigour index I was found to be significantly influenced by seed treatments. Initial vigour index I was observed to be highest in mechanically scarified seeds (703). Similar result was reported by Miya and Modi (2017) in *Vigna subterranean* L. Hydrated seeds also exhibited higher vigour index I (698). This was in agreement with a study conducted by Hosseein and Kasra (2011) in *Ocimum basilicum.* All the remaining treatments resulted in lower vigour index I than the control (492), except for hot water treatment at 80^0C for 20 minutes, where the seeds showed a value of 510. All these observations showed a declining trend along the storage period such that the lowest value of vigour index I of respective treatments were recorded after storing for six months. Vigour index I of mechanically scarified seeds became 389 after six months of storage, while that of hydrated seeds reduced to 378. Similar result was recorded in green gram when stored after different seed treatments (Leelavathi, 2017).

Vigour index II of the seeds was also influenced by different seed treatments. Mechanically scarified seeds exhibited the highest vigour index II (430). Study conducted in *Vigna subterranean* L by Miya and Modi (2017) also came up with similar result. This is followed by hydrated seeds (428), similar to the result reported by Hosseein and Kasra (2011) in *Ocimum basilicum*. Seeds exposed to hot water treatment at 80^0C for 20 minutes also showed a vigour index II (301) higher than that of control (297). All the remaining treatments resulted in inferior vigour of the seedlings, which might be due to damage done to seeds and embryo. As the seeds are stored, ageing of the seeds resulted in depletion of quality and vigour of the seeds. This is quite evident by decreasing trend of vigour

index II along the storage period. The mechanically scarified seeds showed a much reduced vigour index II of 224 and that of hydrated seeds became 220 after storing for six months. The lowest vigour index II after six months storage was noted in seeds treated with conc. H_2SO_4 for 15 minutes (13) which showed an initial vigour index II of 119. Similar decreasing trend in vigour index II was recorded in green gram when stored after different seed treatments (Leelavathi, 2017).

The differential EC values recorded among the different seed treatments implied that the nature and extent of membrane protection that was being offered might not be the same for all the treatments (Kurdikeri *et al.*, 1991). Electrical conductivity (Figure 6) was highest in seeds that were treated with conc. H_2SO_4 for 15 minutes (0.0063 dSm^{-1}) at 0 months of storage which continued to increase along the storage. The lowest electrical conductivity was noted in hydrated seeds (0.0011 dSm^{-1}). The lower electrical conductivity of hydrated seeds might be because the hydration facilitates early DNA replication, RNA and protein synthesis and embryo growth. This might also help in repairing of deteriorated seed parts and reduces the leakage of metabolites (Adebisi *et al.*, 2011). Occurrence of membrane repair during hydration process can also be pointed out as a cause for reduced electrical conductivity as stated by Rudrapal and Nakamura (1988) in radish and Basra *et al.* (2003) in rice. During storage, the seeds undergo ageing which has considerable effect on seed viability and vigour. Loss of cellular membrane integrity is considered as one of the main causes of loss of viability during storage. In aged seeds, the electrical conductivity will be higher due to increased membrane permeability, which is attributed by the destruction of phospholipid layer of cell membrane by lipid peroxygenase (Harrington, 1959). In the present study, electrical conductivity of the seeds increased along storage irrespective of the type of treatment. It became 0.0018 dSm^{-1} and 0.0020 dSm^{-1} for hydrated seeds and mechanically scarified seeds respectively. Highest electrical conductivity was noticed in seeds treated with conc. H_2SO_4 for 15 minutes (0.0073 dSm^{-1}) after the storage period.

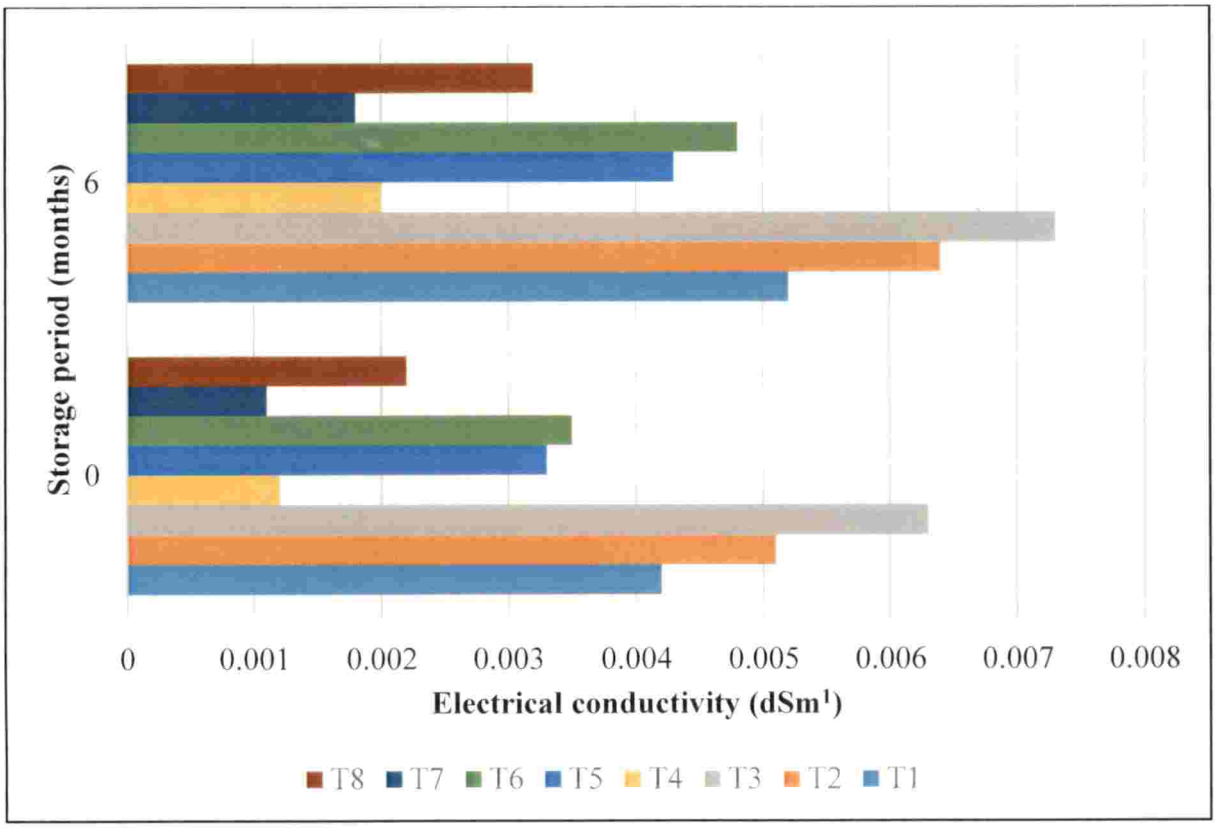

Figure 6: Effect of seed treatment on electrical conductivity (dSm^{1}) during storage

The results thus indicated that mechanical scarification of seeds or hydropriming for 24 h can improve the seed germination. They also can prolong the longevity of the seeds.

5.3 STANDARDIZATION OF VEGETATIVE PROPAGATION TECHNIQUE THROUGH CUTTINGS

Adventitious root formation in stem cuttings is a critical physiological process for clonal propagation of many plant species. In spite of a thorough control of environmental factors during propagation, there is still insufficient rooting observed in many plants. The success of rooting in cuttings depends upon the species and cultivar, condition of cutting wood, type of cuttings (hardwood, semi-hardwood cuttings and softwood), season, amount of internal auxin and many other factors (Hartmann *et al.*, 2007). Auxin is an essential requirement for rooting commencement. Cuttings fail to initiate roots when the internal auxin amount is insufficient to accelerate the rooting. Hence, external auxin application is widely practiced in stem cuttings for accelerating the formation of adventitious roots (Blythe *et al.*, 2007).Application of auxin to cuttings causes metabolic changes during the adventitious root formation which consists of three successive phases- induction, initiation and expression. The induction phase depicts the molecular and biochemical events without visible changes, the initiation phase is characterized by cell divisions and root primordia organization, while the expression phase denotes the intra-stem growth of root primordia and the emergence of roots. Rooting is a high-energy-demanding process, and hence, rooting ability of cuttings has been frequently discussed in relation to soluble and storage carbohydrate contents. Auxins also helps in mobilization of carbohydrates in leaves and upper stem, and accelerates their transport to the rooting zone (Husen *et al.*, 2015).

In this study, three types of cuttings *viz.,* hardwood, semi-hardwood and softwood cuttings of Neelayamari were treated with different concentrations of IBA (250 ppm, 500 ppm, 750 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm)

and charcoal slurry and were planted and maintained in a mist chamber. The experiment was conducted during summer season and rainy season.

5.3.1. Days to sprout initiation

Number of days taken for the appearance of first green coloured sprout on the stem cutting was recorded. Irrespective of season of propagation and auxin treatment, there was significant difference in time taken for sprouting in softwood, semi-hardwood and hardwood cuttings. Softwood cuttings took the least time to sprout both during summer and rainy (9.68 and 7.18 days) than semi-hardwood and hardwood cuttings. This earliness in sprouting may be due to the delicate and leafy nature of the softwood cuttings.

Among different concentrations of IBA used, lowest sprouting days was noticed in 2000 ppm IBA (12.08 days) in summer and 2500 ppm IBA (9.67 days) in rainy season. In case of interaction effect, softwood cuttings that were treated with 2000 ppm IBA took least time for sprouting both in summer and in rainy season. This is in line with the results obtained by Husen (2003) in *Rauvolfia serpentina*, where the softwood stem cuttings treated with 2000 ppm IBA took the shortest time to sprout.

During the rainy season, the cutting took lower time to germinate than in summer. It was reported that time taken for sprouting in summer increases due to the rise in temperature and dropping of humidity (Hartmann *et al.*, 2007).

5.3.2 Sprouting (%)

Per cent of sprouting significantly varied in all seasons and treatment combinations. Softwood cuttings recorded maximum per cent of sprouting while hardwood cuttings with no treatment recorded lowest sprouting in both the seasons. During summer season 2000 ppm IBA noted highest sprouting while during summer it was 2000 and 2500 ppm IBA. The better number of sprouts per cutting with IBA treatment may be due to the better absorption and translocation

of nutrients. Maximum sprouting was observed in rainy season and minimum in summer.

In rainy season, the maximum sprouting of 46.93 per cent was recorded when cuttings were treated with 2000 ppm IBA while the control recorded the minimum (11.63 %). With respect to the effect of cutting type was concerned, maximum sprouting (47.98 %) was recorded by softwood cuttings (C_3) while the lowest (23.37 %) with hardwood cuttings (C_1).In terms of interaction effect, maximum sprouting was recorded in C_3T_6 (softwood cuttings × 2000 ppm IBA) followed by C_3T_5 (softwood cuttings × 1500 ppm IBA). The lowest sprouting of 6.67 was recorded in C_1T_9(hardwood cuttings × 0 ppm IBA).

The maximum sprouting in summer was recorded in cuttings those were treated with 2500 ppm IBA (12.03%) and 2000 ppm IBA (11.99%). Among the different types of cuttings, maximum sprouting (9.31%) was recorded by softwood cuttings (C_3) while the lowest sprouting of 6.05 % in hardwood cuttings (C_1). Better sprouting of the softwood cuttings may be due to the accumulation of endogenous growth promoters in their tissues. Exogenous IBA application synergistically influence those endogenous hormones and favour better sprouting (Ullah *et al.*, 2005). The interaction effect, C_3T_6 (softwood cuttings × 2000 ppm IBA) recorded maximum sprouting of 16.57 per cent. The lowest sprouting of 2.22 per cent was recorded in hardwood cuttings × 0 ppm IBA. It was found that higher concentration of IBA (2500 ppm) produced lesser number of sprouts compared to 2000 ppm. This may be due to inhibition of bud and root development by higher IBA concentrations. Under such instances, the hormone will be translocated to upper parts of the cuttings, where it will inhibit the bud growth and favours increased ethylene synthesis (Hartmann *et al.*, 2007).

The lowest sprouting success was recorded in summer due to unfavourable weather parameters especially due to high temperature. Singh *et al.* (2015) reported maximum number of sprouts (17.77) and sprouted cuttings (6.29) when treated with 2000 ppm of IBA in lemon. Gautam *et al.* (2010) reported that poor

growth of cuttings during summer may be due to poor activity of cambium and its proliferation under unfavourable environmental conditions.

5.3.3 Number of emerged leaves

Maximum number of leaves per cutting was observed in rainy season than in summer season. Among different IBA concentrations, 2000 ppm IBA resulted in highest number of leaves. The softwood cuttings treated with 2000 ppm IBA produced maximum number of leaves among different combinations.

This is in corroboration with the results reported in lemon (*Citrus limon* cv. Assam) when the stem cuttings were treated with different concentrations of IBA, where the softwood cuttings treated with 2000 ppm IBA resulted in highest sprouting along with highest number of leaves among all the treatment combinations (Nath, 2000).

5.3.4 Length and width of emerged leaves

Longest and widest leaves were emerged during rainy season. Length of leaves were highest in cuttings treated with 2500 ppm and 2000 ppm IBA. Softwood cuttings treated with 2000 ppm IBA produced longest and widest leaves, among different types of cuttings. This is similar to results obtained in lemon stem cuttings when treated with different concentrations of IBA (Nath, 2000).

5.3.5 Root characters

The observations on rooting were taken after 60 days after planting. Maximum survival rate and rooting were observed to be higher in rainy season than in summer. This is in line with the results obtained in phalsa cuttings which recorded maximum rooting (68.33 %) in rainy season when compared to other seasons (Singh and Tomar, 2015). Maximum seedling establishment (70.67 %) was recorded in softwood cuttings treated with 2000 ppm IBA (Figure 7). Similar results were noted in golden duranta where the survival of rooted cuttings

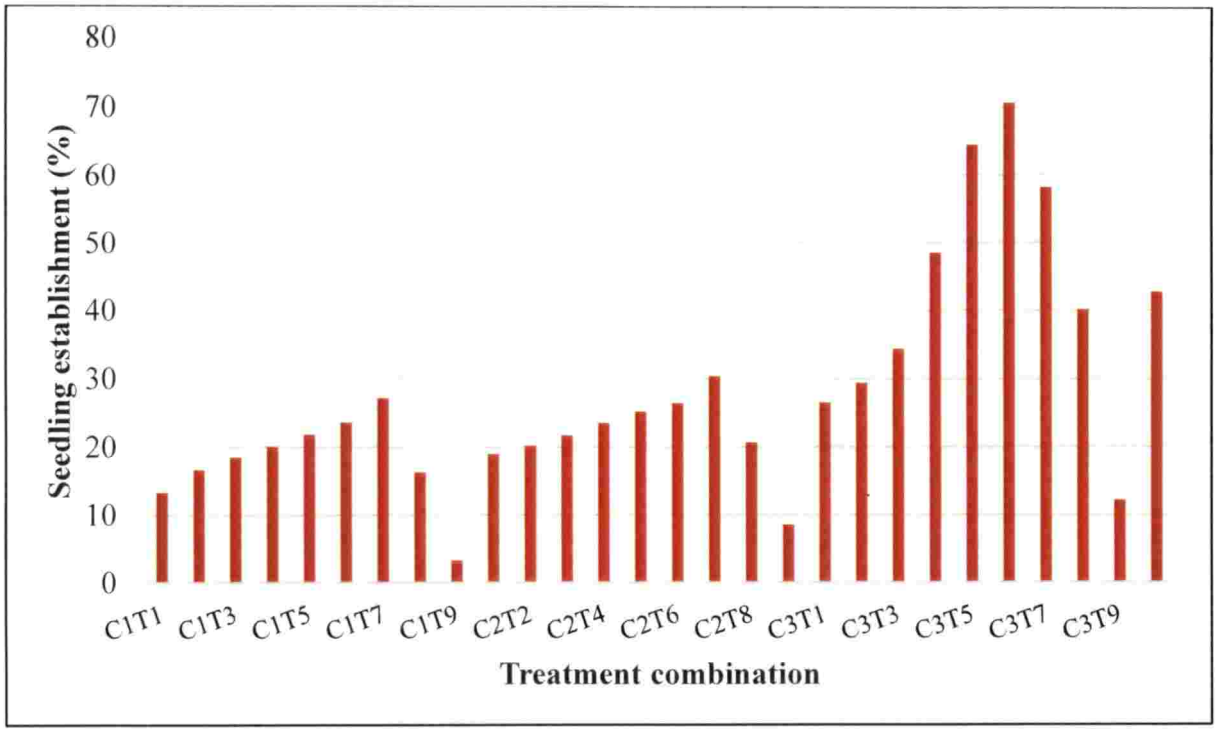

Figure 7: Effect of IBA and cuttings on seedling establishment (%) in rainy season

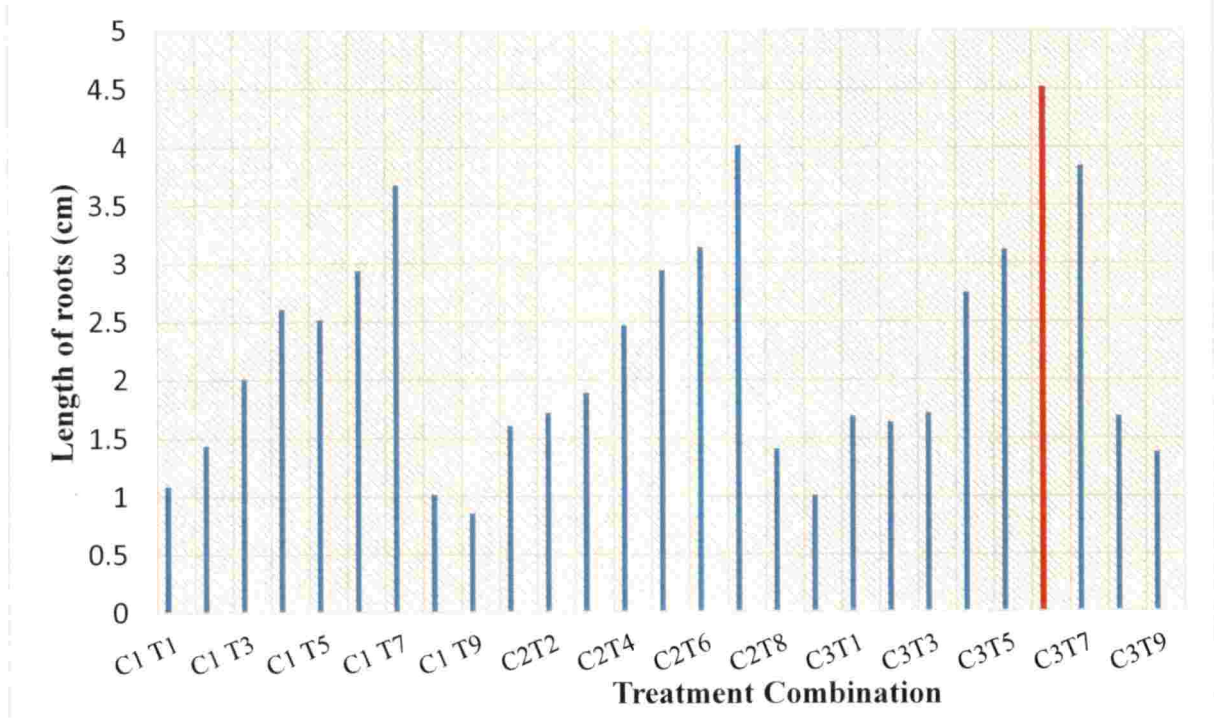

Figure 8: Effect of IBA and cuttings on length of roots (cm) in rainy season

extended up to 80 per cent (Singh *et al.*, 2014). The increased establishment per cent in softwood cuttings may be due to more number of roots coupled with longer roots. This is in line with the results obtained in guava softwood cuttings treated with IBA (Ullah *et al.*, 2005).

Maximum number of roots along with longest roots (Figure 8) were noticed in softwood cuttings treated with 2000 ppm IBA, both during summer and rainy season. Similar results were recorded in *Juglans cinerrea* where the softwood cuttings with IBA treatment provided highest number of roots along with longer roots (Pijut and Moore, 2002). The induction of more number of roots in treated cuttings may be due to the stimulation of cambial activity linked with root initiation, by the growth regulators in many species as reported by Digby and Wannerman (1985).

It can be concluded that IBA treatment is effective in vegetative propagation of Neelayamari. There was a noted reduction in number of days to sprouting in IBA treated cuttings when compared to control. Sprouting (%), number, length and width of emerged leaves were also positively influenced. This might be due to the activation of shoot growth and cell division by IBA. Better mobilization and utilization of carbohydrates, nitrogen and other nutrients might be the reason for increase in shoot length (Chandramouli *et al.*, 2003). Semi-hardwood and hardwood cuttings failed to give good results even under IBA application. This might be due to presence of high amount of resins, tannins and phenols. This could adversely affect the rooting capacity of the cuttings as observed in *Embelia ribes* (Lal and Mishra, 2016).

Thus, softwood cuttings treated with 2000 ppm IBA is the best among different treatments and can be considered as a feasible alternate propagation technique in Neelayamari especially during rainy season.

Summary

6. SUMMARY

Medicinal plants are so important for health care of human beings in respect to ancient medicine system. The indigenous systems of medicines, developed in India for centuries, make use of many medicinal herbs and one among them is Neelayamari (*Indigofera tinctoria* L.). It is commonly known as 'Indian indigo' and is a medicinally important leguminous plant. The extract of the leaves is reported to have remarkable effect on hair growth and in preventing juvenile greying of hair. Poor germination and vigour of seeds is a major problem in Neelayamari propagation, along with heavy loss in seed yield due to the splitting of pods at maturity. Keeping in view of the facts, the present study 'Optimizing propagation techniques in Neelayamari (*Indigofera tinctoria* L.)' was proposed with the objectives of standardizing the physiological maturity stage in *Indigofera tinctoria* L. for seed harvest, pre-sowing seed treatments to enhance seed quality and longevity and vegetative propagation technique in *Indigofera tinctoria* L. through stem cuttings.

The salient findings of the study are summarized below:

- The plant flowered in 134.56 days, at a height of 127.35 cm
- Pod setting took place in 140.35 days from the day of planting with a pod setting rate of 30.26 per cent
- Pod, seed and seedling characters increased up to 45 days after anthesis and thereafter declined
- Pod length and thickness were found to be low during initial stages (0.90 cm and 0.74 mm respectively at 10 days after anthesis) which increased along the growing phase and reached the highest value at 45 days after anthesis (2.33 cm and 2.79 mm respectively). Further, there was a decline in the values reaching 1.92 cm and 1.88 mm respectively at the pod splitting stage (63 days after anthesis)
- Fresh and dry pod weights of the pods also showed a similar trend where in the highest values were obtained at 45 days of anthesis (97.40 mg and 44.23 mg respectively)

- No significant variation in number of seeds noticed among different developmental stages and it ranged from 4 to 6
- Fresh seed weight per pod was highest (69.88 mg) on the 45th day after which it declined to reach 23.20 mg at the pod splitting stage (63 days after anthesis)
- Seed length and seed breadth were also the highest at 45th day of anthesis (3.14 mm and 2.38 mm) and declined to 2.73 mm and 1.25 mm respectively at 63 days after anthesis
- Seed moisture increased along developmental stages and reached maximum on 45th day (64.73%)
- Highest germination (73.80%) and seedling vigour indices (485 and 294 respectively) were also at the 45th day of anthesis
- Among different treatments applied, highest germination was recorded in mechanically scarified (95.83 %) and hydrated seeds (93.27%). Untreated seeds showed a germination of 74.33 per cent
- Vigour index I and II were also highest in mechanically scarified (703 and 430 respectively) and hydrated seeds (698 and 428 respectively), while those in untreated seeds were 485 and 295 respectively
- All the remaining treatments recorded poor germination compared to control
- No increase from initial germination and vigour was noticed in both treated and untreated seeds
- Irrespective of the treatments applied, the germination rate showed a declining trend along the storage
- After 6 months of storage, mechanically scarified seeds showed a lowered germination of 65.67 per cent and that of hydrated seeds became 63.67 per cent. In untreated seeds, germination reached 44.33 per cent by six months of storage
- Seed moisture did not show any significant variation among the treatments along the storage

- Electrical conductivity of the seeds was the lowest in hydrated seeds (0.0011 dSm^{-1}) and mechanically scarified seeds (0.0012 dSm^{-1}).
- Untreated seeds exhibited an electrical conductivity of 0.0022 dSm^{-1}.
- Highest electrical conductivity was recorded in seeds treated with conc. sulphuric acid for 15 minutes (0.0063 dSm^{-1})
- In hydrated seeds, electrical conductivity was 0.0018 dSm^{-1} after storage and for mechanically scarified seeds, it was 0.0020 dSm^{-1}
- For sulphuric acid treated seeds, electrical conductivity became 0.0073 dSm^{-1}after storage
- During summer, softwood cuttings treated with 2000 ppm IBA took lowest days to sprout (9.31 days) with the highest sprouting of 16.57 per cent
- After 60 days of planting, only the softwood cuttings treated with 2000 (8.89%) and 1500 ppm IBA (5.53%) survived, while others withered and dried off
- During rainy season, softwood cuttings and 2000 ppm IBA (7.18 days) took the minimum days to sprout
- Among the treatment combinations, highest sprouting was noted in softwood cuttings treated with 2000 ppm IBA (76.58%) with a survival rate of 70.67 per cent after sixty days of planting

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OPTIMIZING PROPAGATION TECHNIQUES IN NEELAYAMARI (*Indigofera tinctoria* L.)

by

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Abstract

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ABSTRACT

India is acknowledged as one of the world's richest treasure trove of medicinal plants. Neelayamari (*Indigofera tinctoria* L.) commonly known as 'Indian indigo', is a commercially grown leguminous plant of medicinal importance. The extract of the leaves is reported to have remarkable effect on hair growth and in preventing juvenile greying of hair. Due to the presence of many worthy phytochemicals, the crop is being documented in '*Ashtangahridaya*' and is being cultivated by several pharmaceutical entrepreneurs, both in public and private sector. Like most of the medicinal plants, the cheapest method of propagation of this crop is through seeds. However, poor germination and vigour of seeds is a major problem in Neelayamari. In addition, heavy loss in seed yield occurs due to the splitting of pods at maturity.

The study 'Optimizing propagation techniques in Neelayamari (*Indigofera tinctoria* L.)' was conducted in the Department of Plantation crops and Spices, with the objectives of standardizing the physiological maturity stage in *Indigofera tinctoria* L. for seed harvest, presowing seed treatments to enhance seed quality and longevity and vegetative propagation technique in *Indigofera tinctoria* L. through stem cuttings. In order to assess the stage of attainment of physiological maturity for seed harvest in Neelayamari, flowers were tagged on the day of anthesis and the pods were harvested at five days interval from 30 days of anthesis up to the pod splitting stage (63 days after anthesis). It was observed that the values of pod, seed and seedling characters increased up to 45 days after anthesis to reach their highest and thereafter declined till the pod splitting stage (63 days after anthesis). Germination and vigour indices at 45 days after anthesis were 73.80 per cent, 485 and 294 respectively and those at the pod splitting stage were found to be 31.33 per cent, 132 and 65 respectively. Hence, it was inferred that the seeds of Neelayamari reached physiological maturity stage by 45 days after anthesis.

The seeds harvested at physiological maturity were dried to 8 per cent moisture content and subjected to various seed treatments before being packed in 700 gauge polyethylene bags. Untreated seeds served as the control. The seeds were

stored under ambient conditions upto six months. The scarification treatments included treatment with Conc. H_2SO_4 for 5, 10 and 15 minutes, mechanical scarification with sand, hot water treatment at 80°C for 20 minutes and 60°C for 30 minutes and hydration for 24 hours. The quality parameters of the stored seeds were recorded at monthly intervals during the storage period and were put for germination test in sterilized sand media. Results pointed out that most of the seed treatments were effective in enhancing germination. High initial germination was recorded in mechanically scarified (95.83 %) and hydrated seeds (93.27%), while the untreated seeds showed a germination of 74.33 per cent. Vigour index I (703 and 698 respectively) and II (430 and 428 respectively) were also the highest in these treatments. Seed treatment with sulphuric acid, however, proved to be detrimental.

Seed treatment also helped in extending the viability of the seeds. Mechanically scarified and hydrated seeds retained their germination above 60 per cent for six months during storage (65.67% and 63.67% respectively), while, in untreated seeds, germination reached 61.33 per cent at four months of storage and declined to 44.34 per cent by six months of storage. Seeds treated with sulphuric acid never attained the germination of sixty per cent throughout the storage period and those treated with sulphuric acid for 15 minutes failed to germinate at sixth month of storage. EC was found to be the lowest in hydrated seeds (0.0011 dSm^{-1}) and mechanically scarified seeds (0.0012 dSm^{-1}). The results thus indicated that mechanical scarification of seeds or hydropriming for 24 h can not only improve seed germination and seed quality but also prolong the longevity of the seeds. It was also noticed that all the seed quality parameters declined along the storage period.

Attempt to assess the possibility of vegetative propagation in Neelayamari was carried out using hardwood, semi-hardwood and softwood cuttings, exposed to varying doses of IBA (250 ppm, 500 ppm, 750 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm) and charcoal slurry dip. The cuttings were planted in polythene bags and were maintained in a mist chamber. Initially, the experiment was

conducted during summer season and only the softwood cuttings treated with 2000 (8.89%) and 1500 ppm IBA (5.53%) survived after 60 days of planting, while all others withered and dried off. The experiment was then repeated during rainy season and the best results were obtained in the season. Softwood cuttings treated with 2000 ppm IBA during rainy season exhibited early sprouting (7.18 days) and a field establishment of 70.67 per cent. In comparison, the semi-hardwood and hardwood cuttings exhibited very low establishment rates of 21.77 per cent and 17.90 per cent respectively. Hence, it was evident that vegetative propagation using softwood cuttings treated with 2000 ppm IBA during rainy season can be relied upon as an alternative propagation method in Neelayamari.