MORPHOLOGICAL CHARACTERIZATION, TISSUE CULTURE AND *IN-VITRO* CONSERVATION STUDIES IN *Alpinia galanga* (L.) WILLD.

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CERTIFICATE

This is to certify that the thesis entitled **'MORPHOLOGICAL CHARACTERIZATION, TISSUE CULTURE AND *IN-VITRO* CONSERVATION STUDIES IN *Alpinia galanga* (L.) WILLD.'** submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (HORTICULTURE) in PLANTATION, SPICES, MEDICINAL AND AROMATIC CROPS** to the College of Horticulture, Mudigere, University of Agricultural and Horticultural Sciences, Shivamogga is a bonafide record of research work carried out by **POOJA, D. A., ID. NO. MH2TAH0205** (poojada25@gmail.com) during the period of study in this university under my guidance and supervision and no part of this thesis has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar titles.

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MORPHOLOGICAL CHARACTERIZATION, TISSUE CULTURE AND *IN-VITRO* CONSERVATION STUDIES IN *Alpinia galanga* (L.) WILLD.

(POOJA, D. A.)

ABSTRACT

An investigation on "Morphological characterization, tissue culture and *in-vitro* conservation studies in *Alpinia galanga* (L.) Willd." was carried out at the Division of Floriculture and Medicinal crops, ICAR-IIHR, Bengaluru during the year 2019-20. Fourteen accessions of ten years old plants were selected for the study, among the fourteen accessions of *Alpinia galanga,* the genotypic and phenotypic coefficient of variations were high for characters *viz.,* petiole length, inflorescence length and leaf area. High heritability coupled with high genetic advance over mean was observed for petiole length, plant height, leaf area, leaf length and inflorescence length. Correlation study revealed that plant height had a highly significant and positive association with yield per tiller at the phenotypic and genotypic level. Among fourteen accessions evaluated, ACC-8 and ACC-10 were found promising. Healthy rhizome buds were cultured on MS medium supplemented with different concentrations and combinations of growth regulators like BAP, Kinetin and NAA. Among various combinations of growth regulators used in tissue culture, the maximum number of shoots per explant (2.07±0.08) and highest shoot length (3.40±0.05) were found in the combination of Kinetin (3.00 mg/l) + NAA (0.5 mg/l), while the maximum number of roots per explant (1.78±0.02) and highest root length (3.63±0.03) were observed in the combination of BAP (3.00 mg/l) + NAA (0.5 mg/l), whereas BAP (2.0 mg/l) + NAA (1 mg/l) combination significantly recorded the maximum number of leaves per explant (4.85±0.02).

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ಅಲ್ಪಿನಿಯಾ ಗಲಂಗ ಸಸ್ಯದ ರೂಪ ವಿಜ್ಞಾನದ ಗುಣಲಕ್ಷಣ, ಅಂಗಾಂಶ ಕೃಷಿ ಮತ್ತು ಇನ್–ವಿಟ್ರೊ ಸಂರಕ್ಷಣೆಯ ಅಧ್ಯಯನ.

(ಪೂಜಾ, ಡಿ. ಎ.)

ಸಾರಾಂಶ

ಅಲ್ಪಿನಿಯಾ ಗಲಂಗ ಸಸ್ಯದ ರೂಪ ವಿಜ್ಞಾನದ ಗುಣಲಕ್ಷಣ, ಅಂಗಾಂಶ ಕೃಷಿ ಮತ್ತು ಇನ್–ವಿಟ್ರೊ ಸಂರಕ್ಷಣೆಯ ಅಧ್ಯಯನವನ್ನು ೨೦೧೯–೨೦ ರ ಅವಧಿಯಲ್ಲಿ, ಭಾರತೀಯ ತೋಟಗಾರಿಕಾ ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಹೆಸರುಘಟ್ಟದ ಹೂವು ಮತ್ತು ಔಷಧಿ ಸಸ್ಯಗಳ ವಿಭಾಗದಲ್ಲಿ ನಡೆಸಲಾಯಿತು. ಹತ್ತು ವರ್ಷ ತುಂಬಿದ ಹದಿನಾಲ್ಕು ಗಿಡಗಳನ್ನು ಅಧ್ಯಯನಕ್ಕಾಗಿ ಬಳಸಲಾಯಿತು. ದೈಹಿಕ ಮತ್ತು ಅನುವಂಶಿಕ ಗುಣಾಂಶಗಳು, ಎಲೆತೊಟ್ಟಿನ ಉದ್ದ, ಹೂಗೊಂಚಲು ಉದ್ದ ಮತ್ತು ಎಲೆಗಳ ವಿಸ್ತೀರ್ಣಗಳಲ್ಲಿ ಹೆಚ್ಚಾಗಿರುವುದು ಕಂಡುಬಂದಿದೆ. ಅತೀ ಹೆಚ್ಚಿನ ಅನುವಂಶಿಕ ವೈವಿಧ್ಯತೆಯು ಎಲೆ ತೊಟ್ಟುಗಳ ಉದ್ದ, ಸಸ್ಯದ ಎತ್ತರ, ಎಲೆಗಳ ವಿಸ್ತೀರ್ಣ, ಎಲೆಗಳ ಉದ್ದ ಮತ್ತು ಮತ್ತು ಹೂಗೊಂಚಲು ಉದ್ದದಲ್ಲಿ ಕಂಡುಬಂದಿರುತ್ತದೆ. ಪ್ರತಿಗಿಡದ ಗೆಡ್ಡೆಗಳ ಇಳುವರಿಗೆ ಗಿಡದ ಎತ್ತರವು ಸಕಾರಾತ್ಮಕವಾಗಿ ಕಾರಣವಾಗಿರುತ್ತದೆ. ಪರೀಕ್ಷಿಸಿದ ಹದಿನಾಲ್ಕು ಗಿಡಗಳಲ್ಲಿ, ಎಸಿಸಿ–೮ ಮತ್ತು ಎಸಿಸಿ–೧೦ ಉತ್ತಮ ಫಲಿತಾಂಶ ಕೊಟ್ಟಿವೆ. ಅಂಗಾಂಶ ಕೃಷಿಯಲ್ಲಿ ವಂಶಾಭಿವೃದ್ಧಿಯನ್ನು ಎಂಎಸ್ ಮಾಧ್ಯಮದಲ್ಲಿ ಆರೋಗ್ಯಕರ ಗೆಡ್ಡೆಗಳ ಮೂಲಕ ಕೈಗೊಳ್ಳಲಾಗಿದ್ದು, ಅದರಲ್ಲಿ ಅನೇಕ ಉಪಚಾರಗಳನ್ನು ಪರಿಗಣನೆ ಮಾಡಿದಾಗ ಕೈನಿಟಿನ್ (೩ ಮಿ.ಗ್ರಾಂ/ಲೀ) + ಎನ್.ಎ.ಎ. (೦.೫ ಮಿ.ಗ್ರಾಂ/ಲೀ) ಉಪಚಾರದಲ್ಲಿ, ಗರಿಷ್ಠ ಕಾಂಡಗಳ ಸಂಖ್ಯೆ (೨.೦೭±೦.೦೮) ಮತ್ತು ಕಾಂಡದ ಉದ್ದ (೩.೪೦±೦.೦೫) ಕಂಡು ಬಂದಿದೆ ಹಾಗೂ ಗರಿಷ್ಠ ಬೇರುಗಳ ಸಂಖ್ಯೆ (೧.೭೮±೦.೦೨) ಮತ್ತು ಬೇರುಗಳ ಉದ್ದ (೩.೬೩±೦.೦೩), ಬಿಎಪಿ (೩ ಮಿ.ಗ್ರಾಂ/ಲೀ) + ಎನ್.ಎ.ಎ. (೦.೫ ಮಿ.ಗ್ರಾಂ/ಲೀ) ಉಪಚಾರದಲ್ಲಿ ಮತ್ತು ಗರಿಷ್ಠ ಎಲೆಗಳ ಸಂಖ್ಯೆಯು (೪.೮೫±೦.೦೨), ಬಿಎಪಿ (೨ ಮಿ.ಗ್ರಾಂ/ಲೀ) + ಎನ್.ಎ.ಎ. (೧ ಮಿ.ಗ್ರಾಂ/ಲೀ) ಉಪಚಾರದಲ್ಲಿ ಕಂಡು ಬಂದಿದೆ.

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ತೋಟಪಟ್ಟಿ, ಸಾಂಬಾರು, ಔಷಧೀಯ ಮತ್ತು
ಸುಗಂಧದ್ರವ್ಯ ಬೆಳೆಗಳ ವಿಭಾಗ
ತೋಟಗಾರಿಕೆ ಮಹಾವಿದ್ಯಾಲಯ, ಮೂಡಿಗೆರೆ
ಕೃಷಿ ಮತ್ತು ತೋಟಗಾರಿಕೆ ವಿಶ್ವವಿದ್ಯಾಲಯ, ಶಿವಮೊಗ್ಗ
ಅಕ್ಟೋಬರ್, ೨೦೨೦

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INTRODUCTION

I INTRODUCTION

India is one of the rich mega biodiversity centres with eight per cent of the global biodiversity in 2.4 per cent land. Floral diversity is much concentrated in the hotspots of India, among which Western Ghats harbour most of the plants with known and unknown medicinal significance (Krishnan *et al.,* 2011). Among various plant species, *Alpinia galanga* is one of the endemic species of Western Ghats grows widely in tropical and subtropical regions, belongs to the family Zingiberaceae. Rhizomes are the economic parts which are extensively used in Indian system of medicine to cure various ailments and it is getting huge global demand all over the world. At the same time conservation measures are to be taken because there is a need for its protection and multiplication in certain ecological areas as it is one among the 195 Red Listed Medicinal Plants species reported by FRLHT (Foundation for Revitalisation of Local Health Tradition, Bengaluru-1997).

Alpinia galanga is a herbal perennial rhizomatous plant (Borthakur *et al.,* 1999), belongs to the medicinally and economically significant family Zingiberaceae. The Latin generic name '*Alpinia*' was given to commemorate Prospero Alpini (1553-1617), an Italian botanist who catalogued and described exotic plants (Ravindran *et al*., 2012). It is commonly known as Greater galangal. There are several vernacular names to this plant in different languages. They are presented in table below (Verma *et al.*, 2011).

Sl. No.	**Language**	**Synonyms**
1	English	Greater galangal
2	Hindi	Kulanjan
3	Kannada	Dhumarasmi
4	Malayalam	Arattha, Kol-inji, PararattaPararatta
5	Tamil	Pera-rattai
6	Telugu	Pedda-dhumpa
7	Sanskrit	Mahabaracach, SugandhaVacha, Rasna

This crop is naturalized in many parts of South East Asia (Ravindran *et al*., 2012). Commonly found in Indonesia, India, China, and Arabic gulf areas, Malaysia, Egypt and Sri Lanka (Jatoi *et al*., 2006). In India it is distributed in the Himalaya and Southern region of Western Ghats. It is often cultivated in Konkan and North Kanara (Chouni and Paul, 2018; Shetty and Monisha, 2015).

The plant grows from slightly aromatic rhizomes in clumps of stiff stalks up to 2-3 meters in height. It has a subterranean, creeping, copiously branched rhizome

from which clumps of leaves are formed at intervals. Rhizomes are 2.5-10.0 cm in thickness, externally reddish-brown in colour whereas light orange-brown internally. Leaves are oblong-lanceolate, acute, green above, paler beneath, with slightly callus white margins with an average length of 30-40 cm and 10-15 cm wide. Flowers are greenish white, in dense flowered, flower bud size varies from 3-4 cm; bracts ovate-lanceolate. Inflorescence is a terminal many-flowered raceme; flowers are fragrant, 3-4 cm long, yellow-white; the fruits are globuse to ellipsoidal capsule, 1-1.5 cm in diameter, orange red to wine red in colour. Rhizome anatomy shows a central stele surrounded by an outer cortical zone. Fibro vascular bundles are distributed throughout the cortex and stele. It produces more than 48 tillers per clump and 13 leaves per tiller (Verma *et al.,* 2011; Ravindran *et al*., 2012).

Flowering season starts from May and it extends upto September, fruiting starts from August. Pollination most usually carried out by Bumble bees. Fruit takes 5 to 6 months for maturation, seeds are small, round and blackish brown in colour, fruit contains 2-5 seeds; The rhizomes are ready for harvesting after one and-a-half years for essential oil extraction.

Pharmacological investigations revealed the presence of diverse flavonoids, tannins and terpenes in extracts of rhizomes which are responsible for therapeutic efficiency (Ghosh and Rangan, 2013). Gas Chromatography analysis revealed that pungent principle of galangal is mainly attributed by the presence of 1'S-1'-acetoxychavicol acetate (galangal acetate), which is also responsible for various biological activities (Chudiwal *et al*., 2010).

Traditionally it is used to treat various ailments including fever, dyspepsia, rheumatic pains, kidney stones, skin diseases, bronchitis, chest pain, disease of heart and lumbago. It also acts as stimulant, aphrodisiac, expectorant and helps in digestion. Recent studies revealed about the anti-cancerous, anti-amoebic, anti-malarial, anti-allergic, anti-diabetic activities and even anti-HIV properties (Verma *et al.,* 2011). Rhizomes also claimed insecticidal activity (Sukhirun *et al.,* 2010). Apart from medicinal uses, rhizomes are also used as flavouring agent, seeds are used as spices and condiment as its smells like cardamom whereas flowers and young shoots are used as vegetable (Shetty and Monisha, 2015).

It grows naturally in open, sunny places, forests and brushwood. It prefers fertile, moist but not swampy; sandy or clay soil with good drainage for better growth. Soil should be well ploughed before planting. It is usually propagated through vegetative means by using rhizome pieces for commercial multiplication. Seed propagation is not feasible due to low germination rate which is a major constraint for large-scale cultivation through seeds.

To know the variation present in the plant, morphological characterization of fourteen accessions of Galanga were undertaken and the correlation studies also conducted to document the amount of influence of various factors on the quantitative characters and also to select the elite plant for further research programme

It is considered as an important spice for local market in the parts of South-East Asia. Most of the South Indian physicians of traditional Ayurveda and Siddha medicine system use *A. galanga* to treat various kinds of diseases starting from fever to cancer (Verma *et al.,* 2011*).* It is gaining lot of importance in pharmaceutical field to be used as a future potent drug for many diseases. There is an increasing demand for *Alpinia* within and across the countries and on the other hand the growing demand is causing great threat to the survival of this plant as it is not getting attention for conservation in the traditional areas of cultivation like Himalaya and Southern region of Western Ghats, hence there is great need to create awareness. The population also decreased exponentially in the last few years in their natural habitat due to harvesting of the entire rhizomes. Hence, there is a need of proper conservation of this species in a systematic way for further utilization is very essential before it lost forever (Parida *et al*., 2011). As practice it is propagated through rhizomes and it requires huge planting material per hectare (5-5.5 tons). Hence there is a need to find out an alternative and faster method to multiply for re-introduction into its natural habitat. *In-vitro* propagation is one such potent tool which fulfill the conservation as well large scale multiplication of plants for efficient and fast propagation (Borthakur *et al.,* 1999*).*

Keeping all the above aspects in view, the present investigation entitled Morphological characterization, tissue culture and *in-vitro* conservation studies in *Alpinia galanga* (L.) Willd. was carried out at Indian Institute of Horticultural Research, Bangalore with the following objectives:

1. Morphological characterization of different accessions of *Alpinia galanga* (L.) Willd.
2. To develop the protocol for multiplication by using tissue culture
3. *In-vitro* conservation of *Alpinia galanga* (L.) Willd.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Alpinia galanga has gained a lot of importance as a medicinal plant, spice and condiment. As it is listed in the category of threatened medicinal plants, more focus is given on the multiplication and conservation. The present investigation was undertaken to study the morphological characterization, *in-vitro* propagation and conservation of this crop. Very limited work has been reported in this species. In this chapter, some literatures specific to *A. galanga* and also literatures of some previous research work done on the various threatened medicinal plants with reference to morphology, tissue culture and conservation is reviewed under the following subtitles.

2.1 Morphological characterization

2.2 *In-vitro* propagation

2.3 *In-vitro* conservation

2.1 Morphological characterization

Morphological characterization of any crop helps to identify the desirable traits, genetic variability, character association and also significant relation between the various traits which there by serves as a prerequisite for selecting elite plants for further research. Research work supporting the morphological characterization in this crop is very meager. Hence, in this chapter literature related to morphological characterization of threatened medicinal plants are reviewed.

2.1.1 Morphological characterization of threatened medicinal plants

Srivastava *et al.* (1993) studied *Terminalia arjuna* to know the genetic variability present in the 15 randomly selected plants consisting of two varieties of the species (var. *angustifolia* and var. *arjuna*). Maximum variation was recorded in leaf yield/plant followed by breadth of leaves. The GCV, PCV and heritability was found to be highest for leaf yield while the highest genetic advance was observed for plant height followed by number of leaves per branch.

Barrett and Fox (1997) conducted a studies on *Santalum album* to know the morphological and nutrient characteristics of pre-parasitic seedlings under the influence of various nutrients. Tallest plants were seen in all nutrient treatment and shortest in treatments without N and K. There was less variation in root length whereas significant differences were in mean shoot length.

Padmalatha and Prasad (2007) worked on the morphological diversity present in *Pterocarpus santalinus* an endemic and endangered medicinal plants collected from Karnataka, Kerala and Andhra Pradesh and the analysis revealed that there was a significant variation in pod characters, leaf characters, shoot length and number of nodes.

Eighteen accessions of Glory lily were selected to know the mean performance for biochemical characters and also to assess its suitability for seed yield as well as tolerance to leaf blight by Chitra and Rajamani (2010). The accessions showed remarkable variations for all the traits while the dry seed yield per plant showed high correlation with seed length in accession GS 15 (93.90) followed by GS 06 (80.23). They also reported that plant height had positive correlation with pod length, pod girth and seed yield per plant.

Parthasarathy and Nandakishore (2014) did morphological characterization of some important *Garcinia* species, collected from two different ecosystem *viz.,*Western Ghats and Eastern Himalaya and they observed that, morphological characterizations of the species collected from two different ecosystems interestingly exhibited variation within the species of the same ecosystem and similarities in the species of two different ecosystems.

Rawat *et al*. (2014) studied *Aconitum violaceum*, a threatened plant species of the Himalayan Region to investigate the altitudinal and seasonal variation present in the plant. They observed that morphological characters were negatively correlated with an increase in the altitude. Maximum plant height (26.9 ± 3.1 cm) was observed in populations from lower altitude, whereas, the minimum plant height (14.2 ± 2.9 cm) was recorded from the plants of higher altitude.

Dhanasri *et al*. (2015) studied the morphological characteristics of *Salacia reticulata*. To know the variation present in the species, eight accessions were subjected to morphological analysis and they found very little variation among the accessions.

Hrideek *et al*. (2015) studied *Embelia ribes* to analyze the variability present in the juvenile plants grown from the seeds collected from the Southern part of Western Ghats. Significant variation was seen only in the internodal lengh and the other characters showed insignificant variations. The study revealed that the level of genetic variation present in the plant was less.

Munasinghe and Wansapala (2015) conducted an investigation on *Madhuca longifolia* to analyze the morphological variation present in the Seeds. They found significant variation in the length (2.7333-3.4333 cm), width (1.0633-1.2967 cm) and the weight (0.9262-1.4018 g) of the seeds collected from different regions.

Shetty *et al*. (2016) conducted a study on *Decalepis hamiltonii* to know the association for fruit yield and yield traits. The six accessions were subjected to correlation and path analysis studies. According to path analysis studies leaf width, petiole length and fruit length had high positive direct effects on yield per plant where as Plant height, leaf length, pedicel length, fruit diameter, thickness of pericarp and thickness of mesocarp had negative direct effects on yield. They also reported that

petiole length, pedicel length and fruit diameter had direct positive correlation with yield per plant.

Shetty *et al*. (2016 a) studied character association for seed yield and yield traits in *Celastrus paniculatus* Willd and they revealed that yield per plant had high positive correlation with plant height, leaf length, leaf width, petiole length, leaf area, fruit diameter and seed length and negative correlation with Plant height, leaf area and fruit length. They concluded that positively correlating attribute were the prerequisite for selecting elite plants for further research.

Tuppad *et al.* (2017) studied the genotypic variability, heritability and genetic advance for yield and yield contributing traits were studied on 13 *Holostemma ada-kodien* accessions and they recorded the data on the various morphological characters. The investigation reported that leaf width, petiole length, pedicel length, number of fruits per plant has high GCV and PCV indicating variation is more attributed by genetic component and less influence by environmental component.

Saraca asoca (Roxb) was selected to know the character associated with seed yield and yield attributing traits. Correlation study revealed that there is a significant positive correlation between plant height, leaf area, pod length, pod width, leaf length, leaf width, number of pods per plant, number of seeds per pod, seed length and seed width with seed yield per plant and negative correlative between leaf area, number of seeds per pod, pod width, seed length and seed width with the yield (Madhusree *et al*., 2018).

Mastiholi *et al.* (2018) evaluated thirteen accessions of *Salacia chinensis* to know the variability, heritability and genetic advance for yield and other contributing traits. They found highest phenotypic and genotypic coefficient of variation in the fruit yield per plant followed by plant spread and also observed high heritability for fruit weight (99.1 %) followed by fruit yield per plant (98.5 %), whereas low heritability for leaf area (8.29 %) , indicating additive gene effects on this characters.

Shruthi *et al.* (2018) conducted a study on variability, heritability and genetic advance for yield and yield contributing characters in five accessions of *Embelia ribes*. Among the various yield attributing characters studied, the highest magnitude of PCV and GCV were recorded in leaf area, number of inflorescence per plant and fruit yield per plant. High heritability coupled with high genetic advance as per cent over mean was noted in the leaf width (99.00 %), petiole length (91.00 %), plant height (95.20 %), number of inflorescence per plant (95.00 %), fruit weight (91.02) and fruit yield per plant (98.22 %).

Ray *et al.* (2019) conducted a study on morphological diversity in *Hedychium coronarium*. They selected nine quantitative parameters for morphological analysis including plant height, leaf number, leaf length, leaf width, leaf length/leaf width,

petiole length, stem diameter, rhizome diameter and rhizome dry weight. They observed morphological diversity in leaf number, leaf length, leaf width, rhizome diameter and rhizome dry weight.

2.2 *In-vitro* propagation

Increased human intervention has brought most of the medicinal plants under threat status. Large scale cultivation, multiplication and conservation is very essential to bring these plants back to safe and original status. Hence, *in-vitro* propagation is one such effective tool which helps to conserve the rare and endangered plants by making use of limited resources. Since, *Alpinia* requires huge planting material for propagation, *in-vitro* propagation is much needed to minimize the loss of genetic resources and also essential for proper multiplication and conservation. The literature supporting the *in-vitro* propagation is reviewed below:

2.2.1 *In-vitro* propagation in threatened medicinal plants

Vincent *et al*. (1992) studied micro propagation of *Kaempferia galangal* (L.) where the axillary buds are isolated from rhizomes and inoculated on MS medium supplemented with different concentrations and combinations of auxins and cytokinins. The studies revealed that highest number of shoots per explant was obtained from the culture on MS media supplemented with 13.9 µM kinetin and 2.2 µM BAP and maximum number of shoots production was observed after 120 days of inoculation. The axillary bud explants also have the potential to induce multiple shoots as well as roots in the same medium containing BAP alone or BAP + kinetin.

Rout and Das (1993) conducted an investigation on the micro-propagation of *Madhuca longifolia* using apical and axillary meristems derived from ten days old seedling and the explants were cultured on the MS medium supplemented with 1 mg/l BAP alone or in combination with NAA, IAA and IBA. After 18 days of culture, excised shoots were rooted on half strength MS media supplemented IBA at 1 mg/l.

Divatar (1994) studied *in-vitro* propagation of Malabar white pine (*Vateria indica*) to standardize the *in-vitro* technique for multiplication. Nodal segments, intermodal segments and leaf segments were excised and cultured on the MS media supplemented with various growth regulators. Among the various growth regulators used, 2-ip and IBA induced good bud break and shoot production where the primary culture establishment best in half strength MS medium.

Krishnan and Seeni (1994) tried rapid micro-propagation on *Woodfordia fruticosa* using shoot tips on MS media containing various growth regulators. High frequency of axillary shoot buds development was seen within 4-5 weeks in the media supplemented with BAP at 0.2 mg/l.

Micro propagation of *Vitex negundo* was carried out by Sahoo and Chand (1998) by using mature nodal explants. They found highest percentage of nodal segment sprouting (74–75 per cent), the number of secondary shoots per primary shoot (two or three), the shoot length (5–6 cm), the number of new nodal segments generated per active explant (four or five), and the multiplication coefficient (3.5) in the media containing the combination of 4.4 μM BAP and 2.2 μM thidiazuron (TDZ).

Borthakur *et al.* (1999) worked on the micro propagation studies in *Alpinia galanga* by using the emerging buds of rhizomes and inoculated on MS media supplemented with different growth regulators. They observed both shoots and roots production simultaneously in the media supplemented with kinetin 3.0 mg/l within eight weeks of inoculation with an average 8 shoots per explant.

Arya *et al*. (2001) developed the micro propagation protocol for mass multiplication of *Celastrus paniculatus* using nodal shoot explants. They found that lower concentration of BAP (1.5 mg/l) along with NAA (0.1 mg/l) has efficiently produced four to five shoots while the higher concentration of BAP (1.5 mg/l) resulted in shoots with shorter internodes and smaller leaves.

Nair and Seeni (2001) conducted an investigation on the *Celastrus paniculatus*to study the rapid *in-vitro* multiplication and restoration. Nodes, shoot tips, internodes and leaf bases were excised from the vine as an explants and inoculated in Murshige and Skoog (MS) medium containing Sucrose (3%), agar (0.6%) and varied concentration of BAP and Kinetin. Results revealed that all the explants types were regenerative and maximum regeneration (3.60) and frequency (94.00%) of auxiliary shoot formation exhibited by the nodal segments treated with BAP at different concentration (1.0 mg/l).

Raha and Roy (2001) worked on the *in-vitro* plant regeneration in *Holarrhena antidysenterica* by using axillary shoot. The studies revealed that nodal segments cultured on the media containing BAP at15 μM was found to be most effective in inducing bud break, growth and also in initiating multiple shoot (43) per nodal explant.

Nalini and Murali (2002) conducted an investigation on the micro-propagation studies in *Drosera indica* an endangered and threatened medicinal plant. Stem segments were excised and inoculated on the MS media augmented with various growth regulators. Multiple shoots were induced in MS media supplemented with BAP and NAA.

Reddy *et al*. (2002) worked on *Decalepis hamiltoni* to know the effect of triacontanol on the shoot micro propagation. Triacontanol showed highest promotion of axillary shoot proliferation at 20 μg/l while rooting was found to be maximum at 10 μg/l. They concluded that triacontanol can be used as an effective growth regulator

and had a positive effect on shoot growth, chlorophyll content of leaves and also induced root growth.

Martin (2002) studied the rapid propagation of *Holostemma ada-kodien* through axillary bud multiplication and indirect organogenesis and he efficiently developed the protocols of axillary bud multiplication for *Holostemma ada-kodien*. Axillary buds were used as an explants and inoculated in MS medium supplemented with BAP (2 mg/l) and IBA (0.5 mg/l) induced an average of eight shoots per node and also gave the best axillary bud proliferation.

Chand and Singh (2004) developed a protocol for *in vitro* plant regeneration from cotyledonary nodes of *Pterocarpus marsupium* Roxb. Multiple shoots were induced when the cotyledonary nodes were inoculated on Murashige and Skoog medium containing 2.22–13.32 μM BAP or 2.32–13.93 μM kinetin alone or in combination with 0.26 μM NAA. The highest frequency of responding explants (85%) and maximum number of shoots per explant (9.5) were obtained on MS medium supplemented with 4.44 μM BAP and 0.26 μM NAA after 15 week of culture.

Gururaj *et al*. (2004) studied the clonal propagation method of *Decalepis hamiltonii* using shoot tip as explants and developed efficient two stage protocol induction of multiple shoot by using single node under the influence of phloroglucinol and maximum number of shoots per culture was found on the medium containing BAP (1.1 μM), GA3 (5.8 μM) and phloroglucinol (800 μM) and concluded that multiple shoots and adventitious shoots can be enhanced by using phloroglucinol.

In-vitro shoot multiplication studies of *Decalepis hamiltonii* using shoot tip was conducted by Giridhar *et al*. (2005). Shoot tip was cultured on the MS media supplemented with various growth regulators and they found maximum number of multiple shoots (6.5±0.4) on the MS media incorporated with 4.9 μM 2- isopentenyladenine (2-ip).

Malik *et al.* (2005) worked on rapid *in-vitro* multiplication in *Garcinia indica* and developed an efficient protocol for micro propagation. High frequency direct shoot proliferation was noted in seed segments cultured on MS medium supplemented with cytokinins (BAP, kinetin and TDZ) alone and in combination with auxin (NAA).

The basal nodes of 12–16 weeks old greenhouse-grown plants of *Decalepis arayalpathra* were cultured in Murashige and Skoog (MS) medium containing 12.96 μM 6-benzyladenine (BAP), 2.48 μM 2-isopentenyladenine (2-ip) and 2.68 μM α-naphthaleneacetic acid (NAA) and they found 16–17 cm long unbranched robust solitary shoots in 8 weeks. Under same conditions, explants obtained from cotyledonary nodes showed multiple shoot formation and axillary branching, but the shoots were found to be thin, fragile and not suitable for mass propagation. Hence,

use of adult nodal explants is more effective than cotyledonary nodal explants (Sudha *et al.,* 2005).

Hiregoudar *et al.* (2006) conducted a research on rapid clonal propagation of *Vitex trifolia* from mature nodal explants cultured on Murashige and Skoog (MS) medium supplemented with different growth regulators. They observed that multiple shoots were differentiated directly without callus mediation when explants cultured on medium supplemented with cytokinins and noticed maximum number of shoots (9 shoots per explant) production on medium supplemented with 5.0 µM BAP.

Karuppusamy *et al.* (2006) worked on the micro propagation of *Vanasushava pedata* an endangered medicinal plant by using nodal segments of mature plants. Studies revealed that explants cultured on MS medium with the combination of BAP (5.0 mg/l), IAA (0.1 mg/l) and 3 per cent sucrose showed maximum number of shoots (8.6) as well as enhanced shoot lengths. They also noted highest rooting (100%) on half strength MS containing IAA (2.0 mg/l).

Muthan *et al.* (2006) studied the micropropagation of endangered Indian sandalwood. Nodal shoot segments were taken as an explants and cultured on Murashige and Skoog (MS) medium containing 0.53 µM NAA and 11.09 µM BAP to induce multiple shoots. The results showed that combined use of BAP and NAA induced maximum bud break and the highest number of shoots and significantly affected the shoot length.

Pandey *et al.* (2006) studied the shoot initiation and multiplication in *Terminalia arjuna* Roxb. Nodal explants were excised and cultured on Murashige and Skoog (MS) medium fortified with different combination and concentrations of growth regulators. They noticed best response for shoot multiplication on modified MS medium containing 4.44 µM BAP and 0.53 µM NAA.

Rao and Purohit (2006) developed a multiplication protocol for *Celastrus paniculatus* using internodes as an explants. They noticed multiple shoot buds induction when explants were inoculated on Murashige and Skoog's (MS) medium containing different growth regulators and the best results were obtained when the culture inoculated with 4.44 µM 6-benzylaminopurine (BAP).

Bahadur *et al.* (2008) carried an investigation on *in-vitro* micro propagation on *Nepenthes khasiana* - an endangered flowering plant of India. Multi nodes and single nodes were inoculated on the basal woody plant medium + 2 per cent sucrose, +500 mg activated charcoal supplemented with different combinations and concentrations of auxins and cytokinins. Studies revealed that ninety percent of the multi node and fourty percent of the single node responded and survived when treated with different combinations of auxins and cytokinins.

Pati *et al*. (2008) worked on the rapid clonal micro propagation protocol of *Aegle marmelo s*(L.) Corr. *cv*. CISH-B1 by using nodal stem segment of mature bearing tree. They excised three centimeter long shoots having one axillary bud from 10 to 15th nodal region of shoots and cultured on MS medium fortified with BAP (8.84 µM) + IAA (5.7 µM) and results showed the maximum number of proliferated shoots (9.0/explant) on the same media.

Subbu *et al*. (2008) studied the *in-vitro* clonal propagation of *Saraca asoca* by using shoot tip, nodal and intermodal segments. The explants were cultured on MS medium supplemented with different concentration of BAP, Kinetin and 2,4- Dichlorophenoxy acetic acid. Number of adventitious shoots (11.71±0.5) and the frequency of shoot organogenesis (82.00 per cent) was found to be highest in the MS media supplemented with BAP at 0.5mg/l and more callus seen in 2, 4-D.

Balaraju *et al*. (2009) worked on micro propagation of *Swertia chirata* by using shoot tip explants excised from *in-vitro* grown seedlings. They found that BAP (1.0 mg/l) and Kinetin (0.1 mg/l) was found effective in multiple shoots (42.16 ± 1.05) induction per explants.

Mir *et al*. (2009) reported on the *in-vitro* protocol for conservation of *Gardenia gummifera* - an endangered medicinal plant and they excised internodes from the plant and cultured on MS medium supplement with plant growth regulators. Maximum number of shoots were observed in the medium supplemented with BAP 2 mg/l and NAA 0.5 mg/l and roots were found best in IBA 0.5 mg/l.

Rathore and Shekhawat (2009) conducted an experiment on micro propagation of *Pueraria tuberosa* by using tubers. Tubers were surface-sterilized and cultured on Murashige and Skoog (MS) medium supplemented with 8.88 µM benzyladenine, 50 mg/l of ascorbic acid and 25 mg/l of both citric acid and adenine sulphate and they noticed 95 per cent of bud break with 2–4 shoots per node after 10–15 of inoculation on the same medium.

Alam *et al*. (2010) conducted a study on micro propagation studies in *Operculina turpethum* by using the nodal segments. They found that explants culture on the Murashige and Skoog medium supplemented with 1.0 mg/l of BAP exhibited rapid shoot bud proliferation (85.33%) along with a maximum of shoots (14) in each bud, while the Indole-3-acetic acid (IAA) at a concentration of 1.0 mg/l was found best for root development.

Annapurna and Rathore (2010) tried direct adventitious shoot induction and plant regeneration in *Embelia ribes* through direct shoot organogenesis from hypocotyl segments cultured on MS medium with various growth regulators. They observed the highest shoot multiplication within 4 weeks on MS medium

supplemented with 2.2 µM BAP and 0.49 µM IBA, while the highest rooting was noted in half strength MS basal medium along with 2.47 µM IBA.

Lal and Singh (2010) developed the mass multiplication protocol for *Celastrus paniculatus* Willd under *in-vitro* conditions. Nodal segments were excised from the mature plant and cultured on MS medium supplemented with various concentrations and combinations of cytokinins and auxins under controlled condition of 16 hours of photoperiod and 8 hours dark period at a temperature of 25 ± 2°C. They obtained hundred per cent bud break with maximum number of shoots (8.9 ± 0.5) in the MS medium supplemented with 1.0 mg/l of BAP, while hundred per cent rooting was observed in MS half strength medium supplemented with 0.5 mg/l NAA.

Balaraju *et al*. (2011) conducted a study on the rapid *in-vitro* propagation of *Pterocarpus santalinus* L. using shoot tip explants. They observed multiple shoots production in the cultures where maximum number of shoot buds (11) per explants with highest frequency for shoot regeneration (83.3 %) was obtained on MS medium supplemented with 1.0 mg/l of BAP along with 0.1 mg/l of thidiazuron after 45 days of culture.

The axillary buds from unsprouted *Alpinia* rhizomes were used as explant and inoculated on MS media containing varying combinations of KIN (3 mg/l), BAP (1-3 mg/l), IAA (0.51.0 mg/l), IBA (1.0 mg/l) NAA (0.5-2.0 mg/l) and adenine sulphate (100 mg/l). Among the different growth regulators used, media containing BAP(3 mg/l) showed more sprouting where the multiplication of shoot buds was seen on the media containing KIN, BAP and NAA combinations (Parida *et al*., 2011).

Rao *et al*. (2011) conducted an experiment on direct and indirect organogenesis of *Alpinia galanga*. Leaf, sheath, rhizome and root were used as an explants and inoculated on MS media augmented with different concentration and combination of growth regulators. Among the various explants used only rhizome responded well to the *in-vitro* multiplication. The shoots were directly raised from the base of the rhizome and it takes hardly 3-4 weeks for multiple shoot initiation from the rhizome. BAP and kinetin found successful in inducing 11.66±1.15 and 10.33±0.63 shoots at the concentration of 3 mg/l respectively where the high frequency of shoot regeneration was observed in Zeatin at 2 mg/l.

Rakkimuthu *et al.* (2011) studied the micro propagation in *Alpinia zerumbet* using rhizome bud explants where rhizome buds were culture on Basal MS medium supplemented with 3% sucrose with different concentrations of BAP in combination with 0.5 mg/l of kinetin. They noticed highest percentage (95%) of explants for shoot induction and multiple shoot (7.9 per explants) in media supplemented with combination of 1.5 mg/l of BAP and 0.5 mg/l of kinetin. Within 6-7 weeks of

inoculation, all the inoculated explants induced multiple shoots and rooting was induced in a medium having half strength MS supplemented with 0.5 mg/l of IBA.

In-vitro propagation strategies for *Amorphophallus paeoniifolius* was developed by Anil *et al*. (2012). They used petiole and corm as an explants and inoculated on Murashige and Skoog (MS) medium containing BAP and NAA. They noticed callus production within four weeks of culture medium with the initiation of adventitious shoot buds, roots and corm like structures (CLS). For corm like structures induction, MS medium supplemented with NAA (2.5 µM) in combination with BAP (5.0 µM) was identified as the best medium.

Warakagoda and Subasinghe (2012) worked on the *in-vitro* propagation of *Pterocarpus santalinus* L. through tissue culture by using stem cuttings as an explants. Explants were cultured on Mc Cowns woody plant medium (WPM) with 0.1 per cent activated charcoal. They observed maximum number of shoots and shoot branches in the Gamberg medium (B5) with 8 µM BAP and 2 µM NAA.

Dhanasri *et al*. (2013) conducted an experiment on the micro propagation studies of *Salacia reticulata* by using nodal segments as explant and explants were cultured on MS supplemented with different growth regulators like BAP, KIN, IBA, IAA and they observed that among the different treatments tried, explants inoculated in the media containing BAP (3.5 mg/l) and IAA (0.5 mg/l) was resulted in efficient shoot multiplication.

Experiment entitled standardization of callus induction in *Saraca indica* was carried out by Mini and Sankaranarayanan (2013) using leaf, stem and flower bud explants. Explants were inoculated on the Murashige and Skoog basal medium containing 3 per cent sucrose and 0.8 per cent agar supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D) and they found that MS medium supplemented with 2, 4-D (2, 3, 4 and 5 mg/l) of both leaf and stem explants produced 100 per cent callusing in all the concentrations.

Parida *et al*. (2013) developed an efficient protocol for *in-vitro* propagation of *Hedychium coronarium*. Axillary bud excised from the plant were cultured on Murashige and Skoog medium fortified with growth regulators. Maximum number of shoots (13.2±0.3) were obtained in the media containing benzylaminopurine (3 mg/l), kinetin (3 mg/l) and thidiazuron (0.2 mg/l).

Sebastinraj *et al*. (2013) conducted a study on micro propagation in *Operculina turpethum* by using cotyledonary nodes as explant. Cotyledonary nodes from 15-20 days old germinated seedlings were excised and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of cytokinins (BAP and KIN) and auxin (IAA). Maximum shoot proliferation was observed on MS

medium supplemented with KIN+IAA (1.5+0.2 mg/l) while highest frequency of root proliferation was seen on the media containing 0.5 mg/l of IBA.

Senapati *et al*. (2013) worked on the multiplication of *Celastrus paniculatus* an endangered medicinal plant using tissue culture. Nodal explants were inoculated on the Murashige and Skoog (MS) basal medium supplemented with various plant growth regulators. Results showed maximum percentage of shoot multiplication (83.4 %) with 8.2 shoots per explants in the media containing 0.5 mg/l BAP and 0.1 mg/l NAA while the maximum rooting of 73.3 per cent with 4.8 roots per shoot was seen on half-strength MS media supplemented with 0.5 mg/l of IAA.

Shah *et al*. (2013) studied the micro propagation of the *Arsitolochia indica* using nodal segments. They established cultures on five different medias such as Murashige and Skoog (MS) medium, Woody Plant Medium (WPM), Gamborg Medium (B5), Nitsch and Nitsch Medium (NN), and Schenk and Hildebrandt Medium (SH) augmented with 10.0 µM each of three cytokinins *viz.*, BAP, thidiazuron, adenosine sulfate and their combinations. The results revealed that SH medium supplemented with 10.0 µM adenosine sulfate has found best for rapid multiplication of *in-vitro* shoots, while the adventitious roots induction was seen best in SH medium supplemented with 10.0 µM NAA.

Shahinozzaman *et al*. (2013) worked on the micro propagation of black turmeric (*Curcuma caesia* Roxb) through *in-vitro* culture. Rhizome buds were cultured on Murashige and Skoog (MS) medium containing 6-Benzyl adenine alone or in combination with α- Naphthalene acetic acid. The results revealed that MS medium containing 3.0 µM BA + 0.5 µM NAA showed the optimum shoot proliferation, while the rooting was observed after 4 weeks of cultivation in half strength MS medium containing IBA and NAA at different concentrations and high frequency of rooting (89.76 %) was obtained in the media containing 3.0 µM IBA (Indole-3-butyric acid).

Gupta *et al*. (2014) conducted an experiment on *in-vitro* propagation of *Terminalia arjuna* by using nodal segment explants obtained from a mature plant. Excised shoot tips were cultured on the MS medium fortified with various concentrations of plant growth regulators. They noticed maximum numbers of shoots (16.50±3.67) on modified Murashige and Skoog medium with 0.5 mg/l of BAP and 0.1 mg/l of NAA.

Mathew *et al.* (2014) investigated on *in-vitro* conservation strategies for the propagation of *Alpinia calcarata Roscoe.* The results indicated that MS medium supplemented with BAP and Kinetin was the best medium for shoot regeneration. The multiple shoots were cultured from rhizome bud explants on MS solid medium

supplemented with BAP 2.5 mg/l and 2.5 mg/l of kinetin. Maximum rooting was obtained in ½ MS medium supplemented with IAA (2 mg/l) and NAA (2 mg/l)

Experiment on *in-vitro* propagation and the acclimatization effect on the synthesis of 2-hydroxy-4-methoxy benzaldehyde in *Decalepis hamiltonii* was carried out using different plant growth regulators (PGRs). Among different combinations of PGRs, Murashige and Skoog's medium (MS) supplemented with 5.0 µM 6-benzyladenine (BAP) + 0.5 µM indole-3-acetic acid (IAA) + 30.0 µM adenine sulphate (ADS) through apical bud sprouting exhibited maximum of 8.20 shoots per explant with mean shoot length of 6.54 cm (Sharma *et al*., 2014).

Singh *et al.* (2014) conducted an experiment to develop efficient protocol for mass propagation of *Alpinia galanga* L. Explants from rhizome buds were cultured on Murashige and Skoog (MS) medium supplemented with 6-Benzylaminopurine (BAP) alone (0 to 5 mg/l) or a combination of BAP (0 to 5 mg/l) and indole 3-acetic acid (IAA) (0 to 2 mg/l). MS medium supplemented with a combination of 5.0 mg/l BAP and 2.0 mg/l IAA and 3.0 mg/l BAP and 0.5 mg/l IAA produced the highest mean number of shoots per explant as compared to other concentrations. The best shoot length was obtained on the medium containing 1.0 mg/l of BAP and 2.0 mg/l IAA. They concluded that combined effects of BAP and IAA improved significantly the shoot growth and proliferation. MS medium supplemented with a combination of 5.0 mg/l BAP and 2 mg/l IAA gave the highest number of roots.

Thangavel *et al*. (2014) carried an investigation on *in-vitro* micro rhizome in *Decalepis hamiltonii* and they found that MS medium supplemented with 2 µM BAP and 6 µM NAA was optimum for rapid callus induction and establishment from leaf disc explant. They also found that micro rhizome formation was found to be increased when yeast extract and poly vinyl pyrrolidone supplemented with plant growth regulators.

Umesh (2014) conducted experiment on *in-vitro* callus induction and antioxidant potential of *Decalepis hamiltonii* and found that callus formation was best on media supplemented with 2 mg/l of 2,4-D where as slow callus formation was noticed on different combinations of NAA and BAP.

Arumugasamy and Shalimol (2015) conducted an experiment on *in-vitro* callus induction of *Smilax wightii* using leaf as an explant. They observed highest number of shoots was in the media containing BAP + Kinetin (2.0+0.04 mg/l). The callus was seen at the concentration of 1.5+0.05 mg/I TDZ with NAA while the rooting was best achieved on half-strength MS medium supplemented with 2.0 mg/l IBA.

Chavan *et al*. (2015) worked on the micro propagation of *Salacia chinensis* L. using different explants cultured on Murashige and Skoog's (MS) medium with

different concentrations and combinations of plant growth regulators (PGRs) namely BAP, kinetin, indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), alpha-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D). MS medium fortified with BAP (2.0 mg/l), NAA (0.8 mg/l) and ascorbic acid (100 mg/l) showed maximum rate of shoot multiplication (75 per cent) and maximum number of shoots (6.7±1.0).

Deepak *et al.* (2015) conducted a study on the clonal propagation of *Salacia oblonga* by using nodal explants cultured on MS media incorporated with various hormonal concentrations of GA_3, TDZ, BAP and they observed maximum shoot response from the axils of nodal explants on MS media, supplemented with BAP (3.5 mg/l) + IBA (1.0 mg/l), followed by BAP (4.0 mg/l) + IAA(1.0 mg/l).

Bhowmik *et al*. (2016) tried direct shoot organogenesis from rhizomes of *Alpinia calcarata* to develop efficient protocol for direct *in-vitro* shoot multiplication and plant regeneration. Results revealed that an average of 6.2 shoots were produced from rhizomatous bud explants inoculated on MS medium supplemented with 5 µM BAP, 10 µM Kinetin and 2.5 µM NAA.

Majid *et al.* (2016) carried out an experiment on rapid mass propagation of *Salacia chinensis* L. by using leaf, node, and shoot tip explants which were inoculated on Murashige and Skoog medium augmented with different concentrations and combinations of plant growth regulators and the studies revealed explants inoculated on the MS media consisting of BAP (1.0 mg/l) and NAA (0.5 mg/l) resulted most efficient shoot regeneration in terms of the proliferation percentage (87.81 ± 3.22 %), number (5.37 ± 0.02) and length of shoots (3.22 ± 0.04 cm).

Shekhawat and Manokari (2016) conducted a micro-propagation studies in *Hemidesmus indicus* using the nodal explants and cultured on Murashige and Skoog (MS) medium supplemented with 2.0 mg/l of BAP and they noticed hundred percent explants response from the nodal meristems with 5.2 ± 0.25 cm long multiple shoots.

Paranthaman *et al*. (2017) conducted a study on *in-vitro* callus induction in *Saraca asoca* using eleven explants such as meristematic shoot tip, nodal segment, internodal segment, leaf bits, axillary bud, cotyledon, embryo, seed, anther, ovary and hypocotyls collected from five different genotypes and cultured on three different media at different concentrations (0.5 to 4.0 mg/l). Among all the genotypes, genotype collected from Periyakulam showed the best response for callus induction in the MS media supplemented with 2,4-D at 2.0 mg/l.

Micro propagation techniques in *Santalum album* L. using shoot tips as explants was carried out by Krishnakumar and Pratibhan (2018) revealed that treatment combination of MS media + Kinetin (5 mg/l) + BAP (2 mg/l) showed the highest multiple shoot induction and root induction was seen best in IBA (3 mg/l).

Mastiholi *et al*. (2018 a) conducted a studies on *Salacia chinensis L*. a high valuable anti-diabetic medicinal plant. Nodal segments were cultured on MS media supplemented with different growth regulators like BAP, NAA, IAA and KIN. Among these treatments, combination of BAP (2 mg/l) and NAA (0.8 mg/l) shows higher shoot length (3.40±0.12 cm), number of shoots (3.27±0.07) and number of leaves per explant (7.00±0.17) were observed.

Bhat *et al*. (2020) worked on the callus induction in *Hydnocarpus pentandra* using shoot tips. Explants were inoculated on the MS media supplemented with different concentration and combination of growth regulators. Among the various treatment used, highest shoot length (2.06 cm) and maximum number of shoots per explants (3.86) were found in the treatment combination BAP (2.4 mg/l) and TDZ (0.5 mg/l).

2.3 *In-vitro* conservation of medicinal plants

In-vitro conservation technique helps to check the survivability and success of *in-vitro* propagated plants, there by maintaining the biological diversity and sustainable ecosystem. Here, some of the reviews to assess the *in-vitro* conservation.

2.3.1 *In-vitro* conservation in threatened medicinal plants

Sharma and Chandel (1992) worked on the low-temperature storage studies on *Rauvolfia serpentina*. Nodal cultures of *Rauvolfia serpentina* plants were exposed to series of controlled temperature of 5 ^{0}C , 10 ^{0}C, 15 ^{0}C and 25 ^{0}C. At 5 ^{0}C and 10 ^{0}C all cultures died within three months, without showing any further growth where the cultures maintained at 15^{0}C showed considerable survivability and excellent health even after nine months. Cultures maintained at 25 ^{0}C and covered with cotton plugs suffered high mortality where those covered with polypropylene caps remained healthy for a longer period. After six months the cultures at 25 ^{0}C with plastic caps started yellowing and very few were alive after nine months, the optimum period of storage was 15 months.

Tyagi *et al*. *(*2004) carried *in-vitro* conservation studies in eight wild species of Curcuma. Rhizome buds were collected from sprouted rhizomes of eight wild species of Curcuma and cultured on MS medium along with 11.1 µM 6- benzyladenine (BAP). Shoots were excised from the two weeks old culture and inoculated on MS media supplemented with various cytokinins. They concluded that Curcuma sp. (unidentified wild species) could be conserved for maximum period of 379 days on MS + 24.6 µM 2-iP followed by *Curcuma aromatica* for 363 days on MS + 22.8 µM zeatin.

A study conducted on *in-vitro* conservation protocol for *Garcinia indica* using shoot cultures as storage material by Malik *et al*. (2005) and they used MS + 0.5 µM BAP as culture condition with an optimum period of storage for 11 months.

Bhattacharyya *et al.* (2007) carried *in-vitro* conservation studies in *Plumbago indica* – a rare medicinal plant where the *in-vitro* generated plants were kept for short term conservation under reduced culture condition. They noticed that combined application of mannitol (3 per cent) and BAP (2.0 mg/l) resulted in a significant reduction in growth rate and even after 12 months of storage, cultures survived with 100 per cent success by re-growing in regeneration medium.

Rajasekharan *et al.* (2008) carried the *in-vitro* conservation studies for three species of the genus Coleus namely *Coleus forskohlii* Briq, *Coleus parviflorus* Benth., and *Coleus zeylanicus* Benth. Juvenile shoot tips and nodal segments of coleus were cultured on half-strength Murashige and Skoog (MS) medium supplemented with 8.87 µM BAP in combination with 0.54 µM NAA. Rooted cultures were conserved *in-vitro* at 10°C with low light intensity (2.97 $\mu m^{-2}s^{-1}$) for a period of 6 months without subculture and they noticed reduction in shoot length during storage.

Rajasekharan *et al.* (2009) standardized the *in-vitro* conservation in *Tylophora indica. In-vitro* cultured plants were subjected to conservation for six months without subculture with full potential to regenerate, producing viable shoots and nodes and noticed that root production remained unaffected during the conservation and also conserved cultures showed high rooting activity in mannitol and low-temperature treatments.

Rajasekharan *et al.* (2010) studied the *in-vitro* conservation of *Nothapodytes nimmoniana,* an endangered tree species of Western ghats. They cultured various explants of *Nothapodytes nimmoniana* on MS medium consisting of growth regulators. Among the various explants tried, only the isolated seed embryos showed positive response on MS with 0.91 µM thidiazuron (TDZ). After culture establishment, some were shifted to *in vitro* conservation with reduced temperature (10°C) and low light intensity (2.97 $\mu m^{-2}s^{-1}$) for six months. They found that under reduced culture condition, *in-vitro* plants grew well without any sub culturing even after the six months of incubation.

Optimization of protocols for the *in-vitro* multiplication and conservation of *Acorus calamus*, an endangered medicinal plant was carried out by Rajasekharan *et al.* (2010 a). *In-vitro* established cultures of *Acorus calamus* were subjected to conservation under reduced light and temperature conditions (reduced temperature of 10°C and reduced light of intensity of 2.97 $\mu m^{-2}s^{-1}$) and they observed that there was a reduction in multiplication rate when cultures were kept under reduced condition and they also found that medium was best suited for conservation when in there is no supplement of growth regulators.

In-vitro conservation protocol for *Hemidesmus indicus* and *Utlaria salicifolia* using shoot cultures as storage material was standardized by George *et al.* (2010) and

they used 1/2 MS+20 g/l sucrose as culture condition for *Hemidesmus indicus* with an optimum period of storage for 18-22 months and for *Utlaria salicifolia,* they used 1/2 MS+ 40 g/l sucrose as culture condition. The optimum period of storage was 24 months.

Krishnan *et al*. (2011) conducted a study on the conservation of medicinal plants of Western Ghats, India and its sustainable utilization through *in-vitro* technology and concluded that both *in-situ* methods and *ex-situ* methods of conservation through biotechnological tools are required to conserve medicinal plant wealth of Western Ghats.

Parida *et al*. (2011) carried the *in-vitro* conservation studies in *Alpinia galanga* where the cultures of *Alpinia galanga* were maintained for two months with regular subculturing and they also continued *in-vitro* conservation for further two years and noticed the same multiplication rate even after two years of culture production

Verma *et al.* (2012) successfully conducted *in-vitro* conservation of 23 important medicinal plants, which are at the verge of being endangered due to their overexploitation and they collected explants from the different biodiversity zones of India including Western Himalaya, Northeast Himalaya, Gangetic plain, Western Ghats, Semiarid Zone, and Central Highlands and inoculated on MS media consists of different concentration and combination of growth regulators. Out of 23 plants, 18 plants were successfully hardened under glasshouse conditions. *Elaeocarpus spharicus*, *Rheum emodi*, *Saussurea lappa*, *Jurinea mollis* and *Paris polyphylla* were unsuccessfull.

Haque and Ghosh (2013) carried *in-vitro* conservation studies in *Tylophora indica*, where they conducted *in-vitro* conservation of the regenerated plants for up to 21 months without sub-culturing in presence of Mannitol and Sorbitol. They found best results on MS medium supplemented with 3 per cent sucrose and 2 per cent mannitol, where 53.3 ± 3.33 per cent plants survived after 21 months and 86.7 ± 3.33 per cent of these survived plants could turn to normal plants after a re-growth for 2 month

Mohanty *et al.* (2014) carried *in-vitro* conservation studies on nine medicinally and economically important species of Zingiberaceae from Eastern India. *In-vitro* cultured plants were subjected to conservation period of 8–12 months without sub culturing for all species and they found that all the species of Zingiberaceae showed good response on culture media for a minimum period of eight months and they concluded that among all the species of Zingiberaceae, *Curcuma aromatica* and *Kaempferia galangal* were best material for *in-vitro* conservation which could be conserved up to 12 months.

Chattargee and Ghosh (2015) carried *in-vitro* conservation studies in *Plumbago zeylanica* L-an important medicinal plant. Established shoot tips and nodes were dissected from aseptically grown cultures and inoculated on the slow growing media and cultures were maintained at 4°C, 10°C and 18°C into growth chambers under 16 hours photoperiod with fluorescent light. They noticed that the complete plantlets were maintained healthy condition up to 10 months at 10^0C without any subculture by using MS medium in combination with 2 per cent mannitol and 2 per cent sorbitol.

Mastiholi *et al*. (2018) carried *in-vitro* conservation studies in *Salacia chinensis* and they found that, after six month of long storage, *in-vitro* raised plants maintained at 10 ^{0}C showed slower growth in comparison to *in-vitro* plants maintained at standard culture condition and concluded that short term conservation is effective to maintain *in-vitro* cultured plants for prolonged periods without subculture.

Sahoo *et al.* (2020) carried *in-vitro* conservation studies in *Alpinia galangal* where the unsprouted axillary buds of healthy rhizomes were used as explants and rhizomatic buds were cultured on basal MS medium supplemented with different concentration and combination of plant growth regulators. The culture tubes were maintained at 25 ± 1 °C under white fluorescent light with a photoperiod of 16:8 hours light/ dark cycles. They noticed culture establishment after two months and established culture were conserved for six years by frequent sub culturing at an interval of two months.

MATERIAL AND METHODS

III MATERIAL AND METHODS

The present investigation entitled "Morphological characterization, tissue culture and *in-vitro* conservation studies in *Alpinia galanga* (L.) Willd" was carried out at the Division of Floriculture and Medicinal crops, ICAR-IIHR, Bengaluru during the year 2019-20. This chapter consists of the detailed information about the materials used, methodology adopted, observations recorded and statistical procedure adopted during the course of investigation are described under the following subtitles.

3.1 Geographical location and climatic conditions of the experimental site

The experiment on morphological characterization, tissue culture and *in-vitro* conservation studies were carried out at the ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Hesaraghatta Lake Post, Bengaluru. It is located in eastern dry zone of Karnataka at $13^{0}58^{'}$ N latitude and 78^{0} E longitude with an altitude of 890 meters above Mean Sea Level (MSL). It comes under zone-5 of region-3 among the agro-climatic zones of Karnataka and receives South-West and North-East monsoons. The average rainfall is about 768 mm over five to six months (May to October) with a peak in September.

3.2 Experimental details

The details of the experiments conducted are given below:

3.2.1 Morphological characterization of different accessions of Alpinia galanga (L.) Willd.

3.2.1.1 Design and experimental layout

Location: ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru

Design: Randomized Complete Block Design (RCBD)

Genotypes: Fourteen

Replications: Three

Fourteen accessions of 10 years old plants were selected for the study.

3.2.1.2 Observations recorded

Fourteen accessions of 10 years old plants present in the Field Gene Bank for RET medicinal plants were selected to study the morphological characterization with three replication to document the variation present in the accessions with respect to the vegetative and floral characters. With the help of the descriptors of *Alpinia galanga* which is provided by the Division of Floriculture and Medicinal crops, IIHR, Bengaluru. Both qualitative and quantitative characters were recorded and the details of observations recorded are presented below.

3.2.1.2.1 Qualitative characters

3.2.1.2.1.1 Leaf shape and leaf arrangement

The leaf shape of ten leaves on the main stem nearest to the primary branching node was recorded and categorized as oblong and lanceolate. Leaves are arranged in alternate manner with one leaf per node.

3.2.1.2.1.2 Leaf color

For leaf colour assessment, the Royal Horticultural Society (RHS) colour chart was used and categorized as green and yellow green.

3.2.1.2.1.3 Inflorescence type

Observations were recorded at the flowering stage and categorized as panicle type.

3.2.1.2.1.4 Inflorescence position

The inflorescence borne at the terminal positions, arising at the tip of the leaf and the observations were recorded at the flowering stage.

3.2.1.2.1.5 Inflorescence habit

The plant exhibited cluster bearing habit, each flower formed the clusters in the inflorescence and the readings were recorded at the full blooming stage.

3.2.1.2.1.6 Flower bud shape

The flower bud shape of ten randomly selected buds were recorded and categorized as oblong.

3.2.1.2.1.7 Pedicel colour

For the assessment of pedicel colour, the Royal Horticultural Society (RHS) colour chart was used and recorded as green.

3.2.1.2.2 Quantitative characters

3.2.1.2.2.1 Plant height / Tiller height (cm)

The height of the fourteen plants were measured from the base of the plant to the tip of the leaf where three tillers were selected randlomly to measure the height and average was worked out and expressed in centimeters.

3.2.1.2.2.2 Leaf length (cm)

The three leaves were selected randomly from each treatment and the leaf length was measured from the leaf apex to the leaf base using a measuring scale and average was expressed in centimeters.

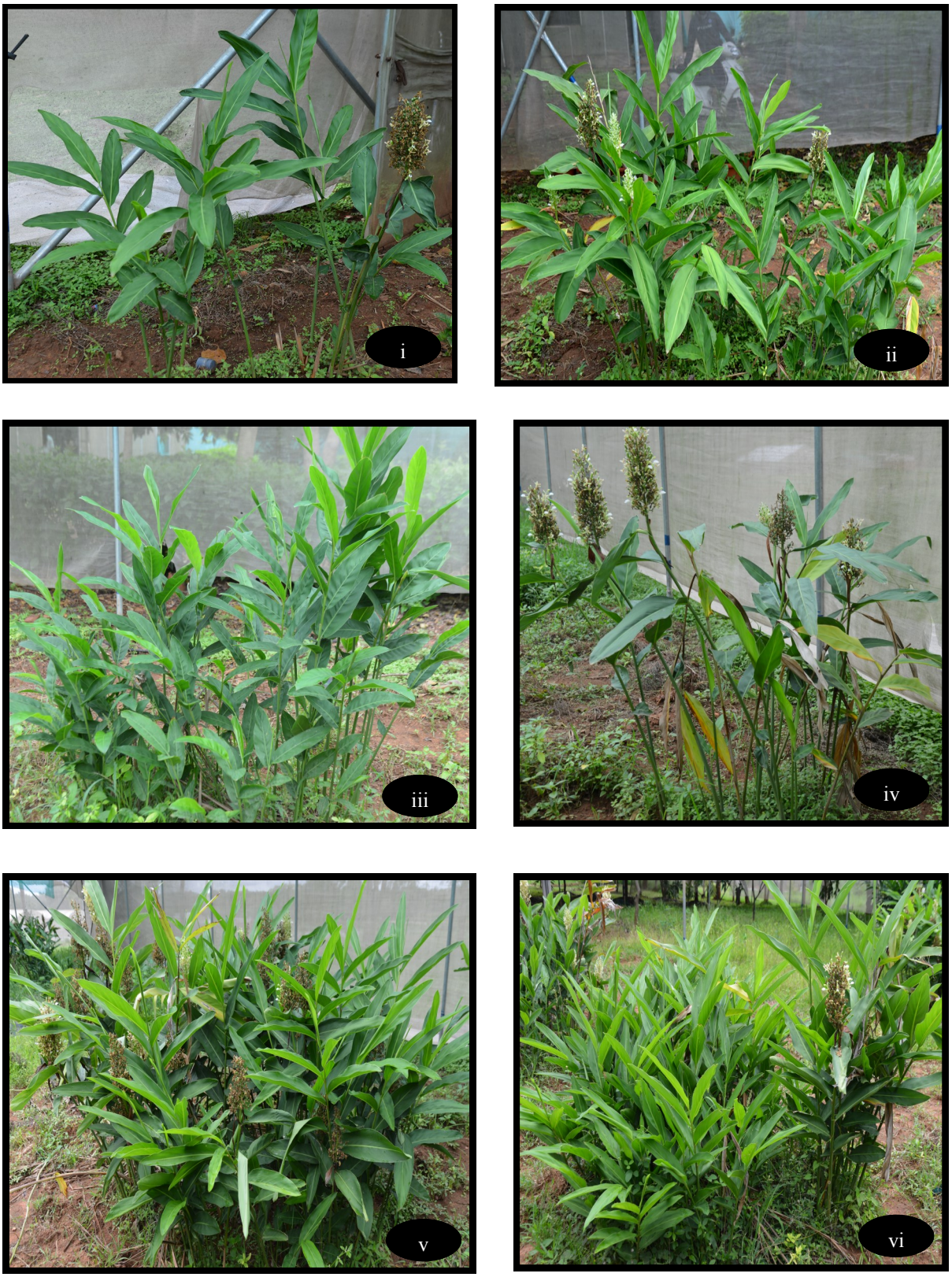

Plate 1a. Different accessions of *Alpinia galanga* (L.) Willd. (i. ACC-1 ii. ACC-2 iii. ACC-3 iv. ACC-4 v. ACC-5 vi .ACC-6)

Plate 1b. Different accessions of *Alpinia galanga* (L.) Willd. (vii. ACC-7 viii. ACC-8 ix. ACC-9 x. ACC-10 xi. ACC-11 xii. ACC-12)

Plate 1c. Different accessions of *Alpinia galanga* (L.) Willd. (xiii. ACC-13 xiv. ACC-14)

Plate 2. Inflorescence bearing flowers and fruits of *Alpinia galanga* (L.) Willd.

Plate 3. Variability in leaf shape

Plate 4. Stages of development of inflorescence in *Alpinia galanga* (L.) Willd. accessions

ACC-1 ACC-2 ACC-3 ACC-4 ACC-5 ACC-6 ACC-7

ACC-8 ACC-9 ACC-10 ACC-11 ACC-12 ACC-13 ACC-14

Plate 5. Variability in the inflorescence of different accessions of *Alpinia galanga* (L.) Willd.

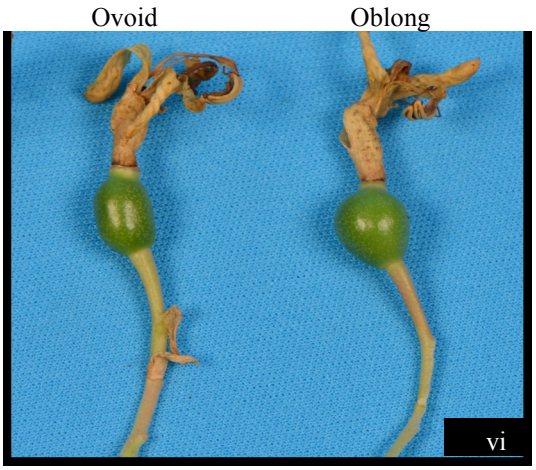

Plate 6. i. Flower of *Alpinia galanga* (L.) Willd.
ii. Stages of development of *Alpinia galanga* (L.) Willd. flowers
iii. Immature fruits of *Alpinia galanga* (L.) Willd
iv. Mature fruits of *Alpinia galanga* (L.) Willd.
v. Seeds of *Alpinia galanga* (L.) Willd.
vi.Variability in fruit shape of *Alpinia galanga* (L.) Willd

3.2.1.2.2.3 Leaf width (cm)

Matured three leaves were randomly selected from each treatment and the width was measured at the broader area using measuring scale and average was expressed in centimeters.

3.2.1.2.2.4 Leaf area (cm^2)

Randomly selected three leaves from each treatment were subjected for calculating leaf area by using leaf area meter (Biovis PSM – L2000) and average was worked out and values were expressed in cm^2.

3.2.1.2.2.5 Petiole length (cm)

The length of stalk attaching the leaf blade to the stem of three randomly selected leaves were measured by using a measuring scale and average was expressed in centimeters.

3.2.1.2.2.6 Inflorescence length (cm)

Randomly selected three inflorescence from each treatment were subjected for measuring inflorescence length and average was expressed in centimeters.

3.2.1.2.2.7 Flower bud size (cm)

Flower bud size three randomly selected flowers were measured and the average values were expressed in centimeters by taking three replication per treatment.

3.2.1.2.2.8 Pedicel length (cm)

The pedicel length of three randomly selected flowers were measured by using a measuring scale and average values were expressed in centimeters.

3.2.1.2.2.9 Number of sepals

The number of sepals per flower was counted manually from randomly selected three flowers and expressed in numbers.

3.2.1.2.2.10 Number of petals

The number of petals present in flower was counted manually from randomly selected three flowers and expressed in numbers.

3.2.1.2.2.11 Number of Panicle

The data pertaining to the number of panicles present in the accession was counted manually and expressed in numbers.

3.2.1.2.2.12 Relative height (cm) between androecium and gynoecium

Relative height between androecium and gynoecium was measured by using a measuring scale and average was worked out and values were expressed in centimeters, where three flowers randomly selected for observation.

3.2.1.2.2.13 Number of tillers

Total number tillers present in each accession was counted manually and expressed in numbers.

3.2.1.2.2.14 Yield per tiller

Rhizomes were harvested from each plant separately by digging up the tillers and rhizomes were separated from tillers and weighed using weighing balance and expressed in grams and total yield per plant was expressed in kilograms.

3.2.1.2.3 Statistical analysis

All the quantitative data obtained during the course of the investigation was tabulated and subjected to statistical analysis by using the method of Analysis of variance (ANOVA).

3.2.1.2.3.1 Analysis of variance

Analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1967) using the mean value of replication from the selected accessions to find out the significance of variation.

Source	**D. F**	**SS**	**MSS**	**Cal. F**
Replication	r-1	RSS	RSS/(r-1)	TrMSS/EMSS
Treatments	t-1	TrSS	TrSS/(t-1)	
Error	(r-1) (t-1)	ESS	ESS/(r-1) (t-1)	
Total	n-1	TSS		

Where,

t = Number of treatments (Accessions)

r = Number of replications

SS = Sum of square

TrSS = Treatment Sum of Square

MSS = Mean Sum of Square

D.F = Degrees of Freedom

TrMSS = Treatment Mean Sum of Square

EMSS = Error Mean Sum of Square

The standard error of mean was calculated as,

$$S.Em = \sqrt{EMSS/r}$$

The significance of treatments mean squares and replication mean squares were tested by comparing with error mean squares referring to 'F' table values at five and one per cent level of probabilities.

3.2.1.2.3.2 Critical difference

In order to compare the means of variance, a critical difference was calculated by using the following formula.

CD = S.Ed ×'t' value at error degrees of freedom

$$S.Ed = \sqrt{\frac{2 \times \text{Error MSS}}{r}}$$

r = Number of replications

t = Table't' value (at 5 per cent or 1 per cent probability level)

3.2.1.2.4 Estimation of genetic parameters

3.2.1.2.4.1 Genotypic and phenotypic variance

The genotypic and phenotypic variances were computed based on the expected mean sum of squares as follows:

$$GV = \frac{M2-M3}{r}$$

$$PV = \frac{M2-M3}{r} + M3$$

Where, M2 – Treatment mean sum of square

M3 – Error mean sum of square

r – Number of replications

3.2.1.2.4.2 Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV)

The Phenotypic and Genotypic Coefficient of Variation was worked out as per the methods suggested by Burton and Devane (1953).

$$\text{Genotypic co} - \text{efficient of variation (GCV): GCV (\%)} = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

$$\text{Phenotypic co} - \text{efficient of variation (PCV):}\quad \text{PCV (\%)} = \frac{\sqrt{\sigma_P^2}}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = General mean

r = Number of replications

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

The PCV and GCV are classified as follows (Subramanian and Menon, 1973).

0 – 10%: Low

10 – 20%: Moderate

Above 20%: High

3.2.1.2.4.3 Heritability

Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage (Falconer, 1981).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

h^2 (%) = Heritability (Broad sense)

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

As suggested by Johnson *et al*. (1955), heritability values are categorized as follows:

Low: Less than 30 %

Moderate: 30 - 60 %

High: More than 60 %

3.2.1.2.4.4 Genetic advance (GA)

Genetic advance (GA) was computed using the formula given by Robinson *et al.* (1949) $GA = i.P.h^2$

Where,

i = Selection differential (2.06) at 5 per cent selection intensity

P = Phenotypic standard deviations

h^2 = Heritability at broad sense

3.2.1.2.4.5 Genetic advance as percentage over mean (GAM)

Genetic advance as percentage over mean was worked out as suggested by Johnson *et al.* (1955)

$$\text{Genetic advance over mean (GAM)} = \frac{GA}{\bar{X}} \times 100$$

Where, GA = Genetic advance

$\bar{X}$ = General mean

The genetic advance as per cent of mean was categorized as suggested by Johnson *et al.* (1955) and the same is given below.

0-10% : Low

10-20% : Moderate

Above 20% : High

3.2.1.2.5 Simple correlation coefficient

The correlation among all the combination of characters was computed using variance and covariance components. Simple correlation was computed as per the formula suggested by Al-Jibouri *et al.,* 1958.

$$\text{Genotypic correlation} = rxy\ (G) = \frac{Cov\ (G)}{\sqrt{Vx(G)\ x\ Vy(G)}}$$

$$\text{Phenotypic correlation} = rxy\ (P) = \frac{Cov\ (P)}{\sqrt{Vx(P)\ x\ Vy(P)}}$$

Where,

Covxy (G) = Genotypic coefficient of variance between x and y

Covxy (P) = Phenotypic coefficient of variance between x and y

Vx (G) = Genotypic variance of character x

Vx (P) = Phenotypic variance of character x

Vy (G) = Genotypic variance of character y

Vy (P) = Phenotypic variance of character y

By comparing t cal value with table‘t’ values at 5 and 1 per cent levels and at n–2 degrees of freedom, the significance were assessed.

3.2.2 Development of multiplication protocols using tissue culture

3.2.2.1 Experimental details

Design: CRD (Completely Randomized Design)

Replications: Three

Treatments: Sixteen

Number of explants per treatment: Fifty

Location: ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru

3.2.2.2 Selection of explant

Rhizome buds were collected from the Field Gene Bank for RET medicinal plants, Division of Floriculture and Medicinal crops, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru. Rhizome buds of 1-2 cm long were used as explants for inoculation.

3.2.2.3 Composition of culture media

Murashige and Skoog's (1962) were used as basal medium for this present investigation as it found to be the best for culture establishment and the composition of Murashige and Skoog's media (1962) is given in Table 1.

3.2.2.4 Preparation of media

The stock solutions were mixed in required proportion along with growth regulators, sucrose and Myo-inositol except agar. The volume was made up by adding distilled water. The pH of the medium was adjusted between 5.8± 0.2 by using either 0.1 N HCl or NaOH with the help of a digital pH meter. The volume was finally fixed and the required quantity of agar was added into the medium while heating. Agar in the medium was melted entirely by gentle heat. Around 30 minutes heating is needed to prepare the media at the proper consistency. Generally, 10-15 ml of medium was poured into 20 mm diameter test tubes and plugged with polypropylene caps.

3.2.2.5 Sterilization of media

The media was autoclaved at 121°C at a pressure of 15 p.s.i for 15-20 minutes, allowed to cool to room temperature and was stored in culture rooms for inoculation.

Table 1. Composition of Murashige and Skoog's (MS) media.

Stock code	Ingredient	Amount (mg/l)	working solution/l
A	$(NH_4)NO_3$	1650	10ml
B	KNO_3	1900	10ml
C	Sulphates		
	$MgSO_4.7H_2O$	370	10ml
	$MnSO_4.4H_2O$	22.3	
	$ZnSO_4.7H_2O$	8.6	
C^1	Halides		
	$CaCl_2.2H_2O$	440	10ml
	KI	8.3	
	$CoCl_2.6H_2O$	0.025	
D	Borates / molybdates		
	H_3BO_3	6.2	10ml
	$Na_2MoO_4.2H_2O$	0.25	
E	Phosphates		
	KH_2PO_4	170	10ml
F	$FeSO_4.4H_2O$	27.8	10ml
	Na_2EDTA	37.3	
Vitamins			
	Glycine	2.0	100µl
	Nicotinic acid	0.5	500µl
	Pyridoxine	0.5	500µl
	Thiamine	0.1	2000µl

Supplements:

Myo- inositol : 100 mg/l

Sucrose: 30.0 g/l

Agar (solidifying agent): 8.0 g/l

Charcoal : 50 mg/l

3.2.2.6 Preparation of explants

Healthy emerging buds of *Alpinia galanga* rhizomes were taken from well established ten years old plants growing in the field gene bank. After harvesting the rhizomes required for inoculation, plant and the rhizomes were separated by cutting the tillers to the base of the rhizomes. Separated rhizomes were then taken to the lab and it is immersed in the water for 30 minutes to remove the adhering soil and again the rhizomes were subjected to repeated washing for 4-5 times. Using a sharp knife, buds were trimmed from the rhizome and placed in beakers and covered with the muslin cloth and washed for 30 minutes under running tap water to remove all the adhering dust particles and microbes from the surface. Later they were washed with liquid detergent (Tween 20) for another 15 minutes and washed adequately in running tap water to remove detergent. Then they were washed with distilled water 4-5 times.

3.2.2.7 Surface sterilization

All the experimental operations were carried under aseptic conditions in a horizontal laminar airflow fitted with a bactericidal ultraviolet tube (15W, peak emission 2537 A^0). The floor of the chamber was thoroughly wiped with cotton dipped in alcohol. The surface of all the vessels and other accessories such as spatula, forceps, needles, scalpel and spirit lamp was also cleaned with 70 per cent alcohol. The fresh materials to be inoculated were kept in sterile *Petri* dish covered with a piece of black paper to protect it from the harmful effects of ultraviolet rays. The chamber was then sterilized with ultraviolet tubes kept continuously on for 20 min. Under the sterile conditions, the explants were treated with $HgCl_2$ (0.2 %) solution for 5-7 minutes. The explants were thoroughly washed (4-5 washings) with sterilized distilled water to remove traces of $HgCl_2$. Thereafter the explants were dipped in 70 per cent (v/v) ethanol for 1-2 minutes, then the alcohol dipped explants were exposed to flame for 1-2 seconds in order to facilitate easy peeling of the leaves. Outer leaves were removed aseptically and explants of 1-2 cm size were used for inoculation.

3.2.2.8 Treatment details

MS media consists of following growth regulators at different concentrations are given below.

Table 2. Hormones used either singly or in combination for shoot induction

Sl. No.	Growth regulator (mg/l)
1	Kinetin (3.00)
2	Kinetin (2.00)
3	Kinetin (1.00)
4	BAP (3.00)
5	BAP (2.00)
6	NAA (1.00)
7	NAA (0.5)
8	Kinetin (3.00) + NAA (0.5)
9	Kinetin (3.00) + NAA (1.00)
10	Kinetin (2.00) + NAA (0.5)
11	Kinetin (2.00) + NAA (1.00)
12	Kinetin (4.00) + NAA (0.5)
13	BAP (3.00) + NAA (0.5)
14	BAP (3.00) + NAA (1.00)
15	BAP (2.00) + NAA (0.5)
16	BAP (2.00) + NAA (1.00)

Solvent used for preparation of growth hormones as given below:

Table 3. Preparation of growth hormones

Sl. No.	Hormones	Solvents
1	6-Benzyl amino purine (6-BAP)	1N NaoH
2	6-furfuryl amino purine (KIN)	1N NaoH
3	Naphthalene acetic acid (NAA)	1N NaoH

3.2.2.9 Inoculation

Sterilized explants were aseptically inoculated on the previously autoclaved MS medium supplemented with 3 per cent sucrose, 0.8 per cent agar and supplemented with different concentrations and combinations of growth regulators. The inoculated tubes were capped and sealed with parafilm and placed in culture stands.

3.2.2.10 Culture conditions

All the cultures were maintained in a Standard Culture Condition (SCC) at a temperature of 26 ± 2^0C. The source of illumination consisted of 4 feet long fluorescent tubes (40W) and incandescent bulb (25W) with a photoperiod of 16-hour white light and 8-hour dark per day. The intensity of illumination was 3500 lux at the level of cultures.

3.2.2.11 Observations recorded

3.2.2.11.1 Callus induction

Callus induction was not seen, as galanga buds directly induces shoot proliferation when cultured on MS medium supplemented with various concentrations and combinations of plant growth regulators.

3*.2.2.11.2 Number of days for shoot initiation*

Number of days for shoot initiation was recorded by counting the time between the day of inoculation and the day of shoot initiation.

3.2.2.11.3 Number of shoots

The number of shoots regenerated was recorded by taking random five cultures for observation in each treatment.

3.2.2.11.4 Shoot length (cm)

The length of shoots was measured by using a measuring scale and the values were expressed in centimeter.

3.2.2.11.5 Number of leaves

The number of opened leaves was recorded by taking random five cultures for observation in each treatment.

3.2.2.12 Statistical analysis

The experiments were laid out in Completely Randomized Design (CRD) by following the procedure outlined by Panse and Sukhatme (1967). The analyzed data were subjected to ANOVA with critical difference values tabulated at one per cent level of significance of the corresponding degree of freedom.

Source of variation	D. f	MSS	Expected MSS
Treatments	t-1	Treatment sum of squares / (t-1)	Treatment sum of squares / Error sum of squares
Error	n-t	Error sum of squares / (n-t)	
Total	n-1		

Where, t = treatments, n = number of observations

3.2.3 In-vitro conservation

Short term conservation of *in-vitro* raised plants of *Alpinia galanga* was attempted for three months to slow down its growth by providing limited light intensity for growth. In order to accomplish this, an equal number of eight-week old tissue cultured plants from each treatment (MS medium + Hormonal combinations) were taken and kept under low light intensity (2.97 μm^{-2} s^{-1}) in a chamber having ambient temperature maintained at 10^0 C. Equal numbers of replicates from each treatment were kept under Standard culture conditions (SCC) to compare it with those which are kept under Reduced culture condition (RCC). Observations on growth parameters were recorded at regular intervals.

3.2.3.1 Treatment details

Eight weeks old tissue cultured plants from each treatment were subjected to *in-vitro* conservation for three months and the following observations were recorded.

3.2.3.2. Observations recorded

3.2.3.2.1 Survival per cent

The number of the tissue cultured plant survived in both Standard culture conditions (SCC) and Reduced culture conditions (RCC) was recorded at an interval of one, two and three month and survival per cent was calculated by comparing the difference exhibited by the tissue culture plants in both the condition.

3.2.3.2.2 Shoot length (cm)

The shoot length of plants was recorded in both Standard culture conditions (SCC) and Reduced culture condition (RCC) at an interval of one, two and three months and the values were expressed in centimeter.

3.2.3.2.3 Number of shoots

The number of shoots was recorded in both Standard culture conditions (SCC) and Reduced culture condition (RCC) at an interval of one, two and three months by taking random five cultures for observation in each treatment.

3.2.3.2.4 Number of leaves

The number of opened leaves was recorded in both Standard culture conditions (SCC) and Reduced culture condition (RCC) at an interval of one, two and three months by taking random five cultures for observation in each treatment.

3.2.3.3 Statistical analysis

The experiments were laid out in Completely Randomized Design (CRD) by following the procedure outlined by Panse and Sukhatme (1967). The analyzed data were subjected to ANOVA with critical difference values tabulated at one per cent level of significance of the corresponding degree of freedom.

Source of variation	**D. f**	**MSS**	**Expected MSS**
Treatments	t-1	Treatment sum of squares / (t-1)	Treatment sum of squares/ Error sum of squares
Error	n-t	Error sum of squares / (n-t)	
Total	n-1		

Where, t = treatments, n = number of observations

IV EXPERIMENTAL RESULTS

The present investigation on "Morphological characterization, tissue culture and *in-vitro* conservation studies in *Alpinia galanga* (L.) Willd." was conducted during the year 2019-20 at Division of Floriculture and Medicinal crops, ICAR – IIHR, Bengaluru. The results obtained during the course of investigation have been presented under the following headings.

4.1 Morphological characterization of *Alpinia galanga* (L.)Willd.

The present investigation on morphological characterization was carried out by taking fourteen accessions and three replications. It covers the qualitative and quantitative characterization of *Alpinia galanga* genotypes and provides details of the genetic variability present between the accessions. The mean performance of the accessions was analyzed by calculating the critical difference following the analysis of variance for the different traits. To understand the nature of different traits, genetic parameters *viz*., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance as per cent of mean (GAM) were worked out.

4.1.1 Performance of qualitative characters

Among the different qualitative traits *viz*., leaf shape, leaf arrangement, inflorescence type, inflorescence position, inflorescence habit and flower bud shape, only leaf shape was found to have variation among the genotypes. The morphological variation exhibited by these characters were listed in Table 4. For the assessment of leaf colour, petiole colour, pedicel colour and the harvested rhizome colour, the Royal Horticultural Society (RHS) colour chart was used and all the observations were presented in detail below.

4.1.1.1 Leaf shape

Leaf shape showed variability and based on the leaf shape; the accessions were grouped into lanceolate leaf type and oblong leaf type. The ACC-4, ACC-7and ACC-13 was grouped under oblong leaf type and the remaining ACC-1, ACC-2, ACC-3, ACC-5, ACC-6, ACC-8, ACC-9, ACC-10 ACC-11, ACC-12 ACC-14 were grouped under lanceolate leaf type (Table 4).

4.1.1.2 Leaf color

Variability in leaf colour was observed and it ranged from green to yellow-green colour and the data pertaining to observed leaf colour are presented in Table 5. Green 137 A colour was observed in ACC-1, ACC-2, ACC-11 and ACC-12 and Green 137 C was noted in ACC-9, Green 143 B was observed in ACC-3 AND ACC-7, Yellow-green 147A was noted in ACCC-4, ACC-5, ACC-6, ACC-8, ACC-10, ACC-13 and ACC-14.

Table 4. Various qualitative traits of *Alpinia galanga* (L.) Willd. accessions.

Accessions	Leaf shape	Leaf arrangement	Inflorescence			Flower bud shape
			Type	Position	Habit	
ACC- 1	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 2	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 3	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 4	Oblong	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 5	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 6	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 7	Oblong	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 8	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 9	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 10	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 11	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 12	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 13	Oblong	Alternate	Panicle	Terminal	Cluster	Oblong
ACC- 14	Lanceolate	Alternate	Panicle	Terminal	Cluster	Oblong

Table 5. Colour estimation of various qualitative traits of *Alpinia galanga* (L.) Willd. accessions.

Accessions	Leaf colour	Petiole colour	Flower colour	Pedicel colour	Rhizome colour (outside)	Rhizome colour(inside)
ACC- 1	Green NN137 A; Fan 3	Yellow green 144B; Fan 3	White 155 C; Fan 4	Yellow green 144B; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 C; Fan 4
ACC- 2	Green NN137 A; Fan 3	Yellow green 144B; Fan 3	White 155 C; Fan 4	Yellow green 144B; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 C; Fan 4
ACC- 3	Green 143 B; Fan 3	Green 143 C; Fan 3	White 155 B; Fan 4	Yellow green 144C; Fan 3	Greyed yellow 161 C; Fan 4	Yellow white 158 B; Fan 4
ACC- 4	Yellow Green 147 A; Fan 3	Yellow green 144B; Fan 3	White 155 C; Fan 4	Yellow green 144 C; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 B; Fan 4
ACC- 5	Yellow Green 147 A; Fan 3	Yellow green 144B; Fan 3	White 155 C; Fan 4	Yellow green 144 C; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 C; Fan 4
ACC- 6	Yellow Green 147 A; Fan 3	Green 143 C; Fan 3	White 155 C; Fan 4	Yellow green 144B; Fan 3	Greyed yellow 162 A; Fan 4	Yellow white 158 B; Fan 4
ACC- 7	Green 143 B; Fan 3	Yellow green 144C; Fan 3	White 156 D; Fan 4	Yellow green 146 D; Fan 3	Greyed yellow 161 C; Fan 4	Yellow white 158 A; Fan 4

Table 5 (Cont…). Colour estimation of various qualitative traits of *Alpinia galanga* (L.) Willd. accessions.

Accessions	Leaf colour	Petiole colour	Flower colour	Pedicel colour	Rhizome colour (outside)	Rhizome colour(inside)
ACC- 8	Yellow Green 147 A; Fan 3	Yellow green 144B; Fan 3	White 155 B; Fan 4	Yellow green 144B; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 B; Fan 4
ACC- 9	Green NN137 C; Fan 33	Yellow green 144B; Fan 3	White 155 C; Fan 4	Yellow green 146C; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 C; Fan 4
ACC- 10	Yellow Green 147 A; Fan 3	Yellow green 148C; Fan 3	White 155 B; Fan 4	Yellow green 144D; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 B; Fan 4
ACC- 11	Green NN137 A; Fan 3	Yellow green 147C; Fan 3	White 155 C; Fan 4	Yellow green 146C; Fan 3	Greyed yellow 162 A; Fan 4	Yellow white 158 B; Fan 4
ACC- 12	Green NN137 A; Fan 3	Yellow green 147 B; Fan 3	White 155 C; Fan 4	Yellow green 144B; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 C; Fan 4
ACC- 13	Yellow Green 147 A; Fan 3	Yellow green 144C; Fan 3	White 156 D; Fan 4	Yellow green 144B; Fan 3	Greyed yellow 161 C; Fan 4	Yellow white 158 A; Fan 4
ACC- 14	Yellow Green 147 A; Fan 3	Yellow green 144C; Fan 3	White 155 C; Fan 4	Yellow green 144C; Fan 3	Greyed yellow 162 A; Fan 4	Greyed yellow 160 B; Fan 4

4.1.1.3 Petiole colour

Variation in the petiole colour was assessed using the RHS colour chart and it is presented in Table 5. The green colour was noted in ACC-3 and ACC-6, whereas all the other accessions showed yellow-green colour.

4.1.1.4 Flower colour

Variation in flower colour was observed at the peak flowering stage and are presented in Table 5. There is no much variation between the accession and white colour was observed.

4.1.1.5 Pedicel colour

Variation in the pedicel colour was assessed using the RHS colour chart and recorded as yellow-green in all the accessions and it is presented in Table 5.

4.1.1.6 Rhizome colour

Variation in rhizome colour both outside and inside was assessed using the RHS colour chart and it is presented in Table 5. Externally there is no much variation in the rhizome colour and showed greyed yellow colour whereas internally rhizome colour ranged from greyed yellow to yellow-white.

4.1.1.7 Other qualitative characters

There no variation between the accession with respect to leaf arrangement, inflorescence type, inflorescence position, inflorescence habit, and flower bud shape and it is presented in Table 4.

4.1.2 Performance of quantitative characters

The mean performances of fourteen accessions were computed after the least significant difference by analysis of variance.

4.1.2.1 Plant height / Tiller height (cm)

Plant height for fourteen accessions varied significantly and it ranged from 157.01 to 211.67 cm with a grand mean of 187.36 cm (Table 6). ACC-8 (211.67 cm) was found to be tallest followed by ACC-10 and ACC-13 (207.67 cm) whereas, ACC-2 (157.01 cm) was the shortest.

4.1.2.2 Leaf length (cm)

Leaf length varied significantly among the accessions and ranged from 29.85 to 44.41 cm with a mean of 39.97 cm (Table 6). The longest leaves were found in ACC-14 (44.41 cm) followed by ACC-12 (44.30 cm) and the smallest leaves were found in ACC-7 (29.85 cm).

4.1.2.3 Leaf width (cm)

The leaf width of fourteen accessions ranged from 8.91 to 11.93 cm with a mean of 10.46 cm (Table 6). ACC-8 registered largest leaf width (11.93 cm) followed by ACC-6 (11.73 cm) and smallest leaf width was found in ACC-4 (8.91 cm).

4.1.2.4 Leaf area (cm^2)

Leaf area varied significantly among the accessions and ranged from 230.14 to 416.79 cm^2 with a mean of 331.50 cm^2. The highest leaf area was found in ACC-11 (416.79 cm^2) followed by ACC-14 (406.72 cm^2) whereas, lowest leaf area was found in ACC-1 (230.14 cm^2) (Table 6).

4.1.2.5 Petiole length (cm)

Petiole length varied significantly among the fourteen accessions and ranged from 0.37 to 0.98 cm with a mean of 0.63 cm (Table 6). The petiole length was found to be highest in ACC-10 (0.98 cm) followed by ACC-8 (0.93 cm) and the least was found in ACC-5 (0.37cm).

4.1.2.6 Inflorescence length (cm)

Inflorescence length ranged from 13.33 to 33.81 cm with a grand mean of 25.01cm (Table 7). ACC-10 recorded maximum inflorescence length (33.81 cm) followed by ACC-8 (31.47 cm)and minimum inflorescence length was recorded in ACC-7 (13.33 cm).

4.1.2.*7 Flower bud size (cm)*

Flower bud size varied significantly among the accessions and ranged from 2.73 to 3.51 cm with a grand mean of 3.08 cm (Table 7). ACC-14 recorded maximum flower bud size (3.51 cm) followed by ACC-6 (3.33 cm) and minimum inflorescence length was recorded in ACC-2 (2.73 cm).

4.1.2.*8 Pedicel length (cm)*

A significant difference was observed with respect to pedicel length among the different accessions ranged from 0.48 to 0.78 cm with a grand mean of 0.62 cm (Table 7). The highest pedicel length was observed in ACC-9 (0.78 cm) and the least was exhibited by ACC-6 (0.48cm).

4.1.2.*9 Relative height (cm) between androecium and gynoecium*

Among fourteen different accessions, ACC- 14 (3.96 cm) recorded maximum relative height between androecium and gynoecium followed by ACC- 9 (3.86 cm) whereas least was recorded by ACC-7 (2.26 cm) with grand mean of 3.50 cm (Table 7).

Table 6: Mean values for different vegetative parameters in *Alpinia galanga* (L.) Willd. accessions.

Accessions	Mean values of different characters				
	Plant height (cm)	**Leaf length (cm)**	**Leaf width (cm)**	**Leaf area (cm^2)**	**Petiole length (cm)**
ACC- 1	166.67	38.06	9.83	230.14	0.65
ACC- 2	157.01	34.17	10.06	236.90	0.50
ACC- 3	193.67	35.30	8.97	352.82	0.55
ACC- 4	166.67	40.91	8.91	282.48	0.50
ACC- 5	183.23	43.23	10.13	316.47	0.37
ACC- 6	178.98	41.08	11.73	347.05	0.40
ACC- 7	200.24	29.85	10.37	245.67	0.70
ACC- 8	211.67	41.86	11.93	370.29	0.93
ACC- 9	199.67	42.23	11.01	340.01	0.75
ACC- 10	207.67	42.26	11.23	406.82	0.98
ACC- 11	188.67	44.16	10.23	416.79	0.77
ACC- 12	186.12	44. 30	11.36	339.92	0.65
ACC- 13	207.67	38.13	10.36	348.78	0.55
ACC- 14	178.33	44.41	10.43	406.72	0.53
Mean	**187.36**	**39.97**	**10.46**	**331.50**	**0.63**
S. Em ±	**5.45**	**1.58**	**0.46**	**23.39**	**0.05**
CD @ 5%	**15.85**	**4.58**	**1.35**	**68.00**	**0.13**

Table 7: Mean values for different reproductive parameters in *Alpinia galanga* (L.) Willd. accessions.

Accessions	Mean values of different characters				
	Inflorescence length (cm)	**Flower bud size (cm)**	**Pedicel length (cm)**	**Relative height (cm) b/w androecium and gynoecium**	**No. of panicles/ plant**
ACC- 1	23.53	3.06	0.71	3.56	4.50
ACC- 2	26.96	2.73	0.61	3.37	6.50
ACC- 3	19.33	3.32	0.51	3.16	2.50
ACC- 4	24.16	3.06	0.53	3.63	7.00
ACC- 5	27.06	3.07	0.65	3.56	31.50
ACC- 6	25.93	3.33	0.48	3.83	25.50
ACC- 7	13.33	2.90	0.55	2.26	7.00
ACC- 8	31.47	3.12	0.55	3.73	41.00
ACC- 9	25.33	3.06	0.78	3.86	11.00
ACC- 10	33.81	3.23	0.61	3.41	28.00
ACC- 11	25.43	2.91	0.76	3.70	15.50
ACC- 12	27.85	2.93	0.61	3.42	16.50
ACC- 13	20.33	3.14	0.71	3.73	4.50
ACC- 14	26.92	3.51	0.56	3.96	10.00
Mean	**25.01**	**3.08**	**0.62**	**3.50**	**13.96**
S. Em ±	**1.82**	**0.11**	**0.04**	**0.20**	**5.38**
CD @ 5%	**5.29**	**0.32**	**0.11**	**0.57**	**16.60**

4.1.2.10 Number of panicles

Wide variability was observed between different accessions with respect to the number of panicles per plant. ACC-8 (41) recorded maximum number of panicles per plant followed by from ACC-5 (31.50) and the minimum was recorded by ACC-3 (2.50) with a grand mean of 13.96 (Table 7).

4.1.2.11 Number of tillers per plant

A significant difference was observed with respect to the number of tillers per plant. ACC-10 (83.00) recorded maximum number of tillers per plant followed by ACC-8 (81.50) and the minimum was recorded by ACC-1 (14.00) with a grand mean of 51.67 (Table 8).

4.1.2.12 Yield per tillers

Yield per tillers varied significantly among the accessions and ranged from 279.74 to 457.74 gramwith a grand mean of 374.43 grams (Table 8). ACC-8 recorded maximum yield (457.74 g) followed by ACC- 10 (446.29 g) and minimum yield was recorded in ACC-1 (279.74 g).

4.1.2.13 Number of sepals and petals

The number of sepals and petals was found to be three in number in all the flowers and observation was recorded at the full blooming stage.

4.1.3 Analysis of variance

The Analysis of variance showed significantly higher amount of variability among the accessions for all the characters studied *viz.*, plant height, leaf length, leaf width, leaf area, petiole length, inflorescence length, flower bud size, pedicel length, relative height between androecium and gynoecium and yield per tiller (Table 9).

4.1.4 Genetic parameters

Genetic parameters *viz.*, phenotypic and genotypic coefficient of variations, heritability in a broad sense and genetic advance as per cent of mean of various quantitative attributes were recorded and presented in Table 10.

4.1.4.1 Phenotypic and genotypic coefficient of variation

The Genotypic Coefficient of Variation (GCV) was found to be less than the Phenotypic Coefficient of Variation (PCV) in all the characters studied. The phenotypic coefficient of variation (PCV) as well as the genotypic coefficient of variation (GCV) was higher for the characters *viz*., petiole length, inflorescence length and leaf area. Among all these growth and yield characters, highest PCV and GCV were recorded for petiole length 30.71 and 28.03 per cent respectively, followed by inflorescence length (22.31 % and 18.40 %) and leaf area (21.28 % and 17.42 %) respectively. Moderate PCV and GCV of 10 to 20 per cent were recorded for the traits

viz., yield per tiller (18.42 % and 13.05 %), pedicel length (17.56 % and 14.15 %), relative height between androecium and gynoecium (14.37 % and 10.55 %) and leaf length (12.26 % and 10.18 %). Remaining plant height, leaf width and flower bud size found to have low PCV and GCV per cent.

4.1.4.2 Heritability

The heritability per cent ranged from 44.24 per cent in flower bud size to 83.35 per cent in petiole length. Broad sense heritability values revealed high heritability for characters like petiole length 83.35 percent, plant height (74.47 %), leaf length (68.95 %) which was reported to be on par with leaf area and inflorescence length (68.03 %) and pedicel length (64.87 %). Moderate heritability was recorded for the traits like relative height between androecium and gynoecium (53.97 %), yield per tiller (52.14 %). All these traits had heritability estimates of more than 40 per cent.

4.1.4.3. Genetic advance as per cent of mean

The computed genetic advance as per cent of mean in different traits ranged from 7.61 per cent (flower bud size) to 52.72 per cent (petiole length). Higher estimates of genetic advance were recorded for traits like petiole length (52.72 %), inflorescence length (31.26 %), leaf area (29. 38 %) and pedicel length (23.47 %). Moderate estimates of genetic advance as per cent of mean were recorded in yield per tiller (19.25 %), leaf length (17.41 %), relative height between androecium and gynoecium (15.97 %) and plant height (15.84 %) and leaf width (10.99 %) whereas, lowest genetic advance as per cent of mean was observed in flower bud size (7. 61 %).

4.1.5 Correlations

The genotypic and phenotypic correlations among yield and its attributes were worked out to know the nature of association existing among the characters. The results are presented in tables 11 and 12.

At genotypic level plant height exhibited positive and highly significant association with yield per tiller (1.03), petiole length (0.63) and leaf area (0.51) whereas, positive and significant association with leaf width (0.38). At phenotypic level also plant height exhibited positive and highly significant association with yield per tiller (0.64), petiole length (0.49) and leaf area (0.47) whereas, positive significant association with leaf width (0.36).

Leaf length exhibited positive and highly significant association with relative height between androecium and gynoecium (0.91), inflorescence length (0.85) and leaf area (0.71). At the same time, it also showed significant positive correlation with leaf width and flower bud size (0.36) at genotypic level. Leaf length also showed positive and highly significant correlation with relative height between androecium and gynoecium (0.63), leaf area (0.62), inflorescence length (0.54), and leaf width (0.40) at phenotypic level.

Table 8. Mean values for yield parameters in *Alpinia galanga* (L.) Willd.

Accessions	No. of tillers/ plants	Yield/tiller (g)	Total yield/plant (Kg)
ACC- 1	14.00	279.74	3.91
ACC- 2	35.50	295.58	10.49
ACC- 3	44.50	394.66	17.56
ACC- 4	32.00	333.10	10.65
ACC- 5	78.50	436.82	34.29
ACC- 6	69.50	355.03	24.67
ACC- 7	63.50	394.13	25.02
ACC- 8	81.50	457.74	37.30
ACC- 9	29.50	394.93	11.65
ACC- 10	83.00	446.29	37.09
ACC- 11	79.00	393.61	31.09
ACC- 12	43.00	369.99	15.90
ACC- 13	39.50	391.86	15.47
ACC-14	30.50	298.52	9.10
Mean	51.67	374.43	17.518
S. Em ±	**2.93**	**27.54**	_
CD @ 5%	**8.73**	**80.05**	_

Table 9. Analysis of variance (Mean sum of square) for morphological and yield parameter in *Alpinia galanga* (L.) Willd.

Sl. No.	Source of variation	Replication	Treatments	Error	S. Em ±	CD @ 5%
	Degrees of freedom	**2**	**13**	**26**		
1	Plant height (cm)	66.50	870.08**	89.22	5.45	15.85
2	Leaf length (cm)	0.48	57.15**	7.46	1.58	4.58
3	Leaf width (cm)	0.21	2.54**	0.65	0.46	1.35
4	Leaf area (cm^2)	1170.03	11646.33**	1641.61	23.39	68.00
5	Petiole length (cm)	0.01	0.10**	0.01	0.05	0.13
6	Inflorescence length (cm)	7.93	73.43**	9.95	1.82	5.29
7	Flower bud size (cm)	0.10	0.13**	0.04	0.11	0.32
8	Pedicel length (cm)	0.00	0.03**	0.00	0.04	0.11
9	Relative height (cm) b/w androecium and gynoecium	0.04	0.53**	0.12	0.20	0.57
10	Yield/tiller (g)	1614.83	9446.73**	2274.91	27.54	80.05

*** Significant @ 5 %** **** Significant @ 1**

Table 10. Estimation of mean, range, components of variance, heritability and genetic advances for growth and yield parameters of *Alpinia galanga* (L.) Willd.

Sl. No.	Characters	Mean	GV	PV	GCV (%)	PCV (%)	ECV (%)	h^2 (%)	GAM (%)
1	Plant height (cm)	187.36	260.28	349.50	8.61	9.98	5.04	74.47	15.84
2	Leaf length (cm)	39.97	16.56	24.02	10.18	12.26	6.83	68.95	17.41
3	Leaf width (cm)	10.46	0.63	1.28	7.60	10.82	7.70	49.31	10.99
4	Leaf area (cm^2)	331.50	3355.65	4976.71	17.42	21.28	12.62	68.03	29.38
5	Petiole length (cm)	0.63	0.03	0.04	28.03	30.71	12.53	83.35	52.72
6	Inflorescence length (cm)	25.00	21.16	31.11	18.40	22.31	12.61	68.03	31.26
7	Flower bud size (cm)	3.08	0.03	0.07	5.56	8.35	6.24	44.24	7.61
8	Pedicel length (cm)	0.62	0.01	0.01	14.15	17.56	10.56	64.87	23.47
9	Relative height(cm) b/w androecium and gynoecium(cm)	3.505	0.13	0.25	10.55	14.37	9.75	53.97	15.97
10	Yield/tiller (g)	374.43	2390.65	4665.50	13.05	18.24	12.73	52.14	19.25

Where, GV: Genotypic variance, PCV: Phenotypic coefficient of variance, PV: Phenotypic variance, h^2: Broad sense heritability, GCV: Genotypic coefficient of variance, GAM: Genetic advances as per cent of mean

Table 11. Genotypic correlation coefficient for growth and yield parameters in *Alpinia galanga* (L.) Willd.

	1	2	3	4	5	6	7	8	9	10
1	**1.000**									
2	0.040	**1.000**								
3	0.388*	0.361*	**1.000**							
4	0.518**	0.718**	0.297	**1.000**						
5	0.635**	0.111	0.487**	0.385*	**1.000**					
6	-0.097	0.856**	0.606**	0.499**	0.323*	**1.000**				
7	0.176	0.361*	0.043	0.655**	-0.110	0.163	**1.000**			
8	0.290	0.230	-0.067	-0.022	0.225	0.056	-0.371*	**1.000**		
9	-0.176	0.918**	0.311*	0.602**	-0.130	0.709**	0.455**	0.377*	**1.000**	
10	1.037**	-0.068	0.411**	0.355*	0.501**	-0.228	-0.145	0.141	-0.387*	**1.000**

Correlation r value 5 % = 0.304 1 %= 0.393 *Significant @ 5 % **Significant @ 1 %

1. Plant height (cm), 2. Leaf length (cm), 3. Leaf width (cm), 4. Leaf area (cm^2), 5. Petiole length (cm), 6. Inflorescence length (cm)

7. Flower bud size (cm), 8. Pedicel length (cm), 9. Relative height (cm) between androecium and gynoecium, 10. Yield per tiller (g)

Table 12. Phenotypic correlation coefficient for growth and yield parameters in *Alpinia galanga* (L.) Willd.

	1	2	3	4	5	6	7	8	9	10
1	**1.000**									
2	0.098	**1.000**								
3	0.360**	0.402**	**1.000**							
4	0.477**	0.626**	0.485**	**1.000**						
5	0.497**	0.121	0.344*	0.301	**1.000**					
6	-0.036	0.543**	0.440**	0.392*	0.317*	**1.000**				
7	0.166	0.293	0.136	0.408**	-0.049	0.080	**1.000**			
8	0.174	0.251	0.062	0.129	0.208	-0.020	-0.413**	**1.000**		
9	-0.039	0.634**	0.165	0.367*	-0.087	0.491**	0.389*	0.122	**1.000**	
10	0.642**	-0.010	0.252	0.238	0.410**	-0.069	-0.062	0.098	-0.169	**1.000**

Correlation r value 5 % = 0.304 1 %= 0.393 *Significant @ 5 % **Significant @ 1 %

1. Plant height (cm), 2. Leaf length (cm), 3. Leaf width (cm), 4. Leaf area (cm^2), 5. Petiole length (cm), 6. Inflorescence length (cm)

7. Flower bud size (cm), 8. Pedicel length (cm), 9. Relative height (cm) between androecium and gynoecium, 10. Yield per tiller (g)

At genotypic level leaf width exhibited positive and highly significant correlation with inflorescence length (0.60), petiole length (0.48) and yield per tiller (0.41) while it showed positive and significant correlation with plant height (0.38), leaf length (0.36) and relative height between androecium and gynoecium (0.31). Whereas, leaf width had a positive and highly significant association with leaf area (0.48), inflorescence length (0.44), and leaf length (0.40) it showed positive and significant correlation with plant height (0.36), and petiole length (0.344) at phenotypic level.

Leaf area exhibited positive and highly significant association with leaf length (0.71), flower bud size (0.65), relative height between androecium and gynoecium (0.60), plant height (0.51), inflorescence length (0.49), and yield per tiller (0.35) while it showed positive and significant correlation with petiole length (0.38) at genotypic level. While it also exhibited positive and highly significant correlation with leaf length (0.62), leaf width (0.48), yield per tiller (0.47) and flower bud size (0.40) while it showed positive and significant correlation with inflorescence length (0.39), and relative height between androecium and gynoecium (0.36) at phenotypic level.

At genotypic level, petiole length exhibited positive and highly significant correlation with plant height (0.63), yield per tiller (0.50) and leaf width (0.48) while it showed positive and significant correlation with leaf area (0.38) and inflorescence length (0.32). At phenotypic level petiole length exhibited positive and highly significant correlation with plant height (0.49) and yield per tiller (0.41) while it showed positive and significant correlation with leaf width (0.34) and inflorescence length (0.31).

Inflorescence length exhibited positive and highly significant association with leaf length (0.85), leaf width (0.60), leaf area (0.49) and relative height between androecium and gynoecium (0.70) while it showed positive and significant correlation with petiole length (0.32) at genotypic level. While it also exhibited positive and highly significant correlation with leaf length (0.54), relative height between androecium and gynoecium (0.70), yield per tiller (0.49) and leaf width (0.44) while it showed positive and significant correlation with leaf area (0.39) and petiole length (0.31) at phenotypic level.

At genotypic level flower bud size exhibited positive and highly significant correlation with leaf area (0.65) and relative height between androecium and gynoecium (0.45) while it showed positive and significant correlation with leaf length (0.36) however, it showed negative and significant correlation with and pedicel length (-0.37). At phenotypic level flower bud size exhibited positive and highly significant correlation with leaf area (0.40) while it showed positive and significant correlation with relative height between androecium and gynoecium (0.38) however, it showed negative and highly significant correlation with and pedicel length (-0.41).

Pedicel length exhibited positive and significant association with relative height between androecium and gynoecium (0.37). It showed negative and significant correlation with flower bud size (-0.37) at genotypic level While it exhibited negative and highly significant correlation with flower bud size (-0.41) at phenotypic level.

At genotypic level relative height between androecium and gynoecium exhibited positive and highly significant correlation with leaf length (0.91), inflorescence length (0.70), leaf area (0.60) and flower bud size (0.45) while it showed positive and significant correlation with pedicel length (0.37) and leaf width (0.31) however, it showed negative and significant correlation with yield per tiller (-0.38). At phenotypic level flower bud size exhibited positive and highly significant correlation with leaf length (0.63), inflorescence length (0.49) while it showed positive and significant correlation with flower bud size (0.38) and leaf area (0.36).

Yield per tiller exhibited positive and highly significant association with plant height (1.03), petiole length (0.50) and leaf width (0.41) while it showed positive and significant correlation with leaf area (0.35) however, it showed negative and significant correlation with relative height between androecium and gynoecium (-0.38) at genotypic level. While yield exhibited positive and highly significant association with plant height (0.64) and petiole length (0.41) at phenotypic level.

4.2 Development of multiplication protocol in *Alpinia galanga* using tissue culture

Healthy rhizome buds were collected from field gene bank (ACC-8) and cultured on MS medium supplemented with various concentrations and combinations of growth regulators and new shoots began to proliferate within eight weeks of inoculation. Direct organogenesis was found, *i.e.*, shoots were directly induced from explant without producing callus. Shoot initiation and proliferation was seen in all the treatments except T_6 and T_7 and estimation of growth parameters carried after 12 weeks of inoculation. Days taken for shoot initiation, number of shoots per explants, shoot length, number roots per explants, root length and number of leaves per explants were recorded in Standard culture conditions (SCC). Data were statistically analyzed by analysis of variance (ANOVA) and significance was calculated and the details of observation recorded are presented in table 13 and table 14.

4.2.1 Number of days taken for shoot initiation

Shoot initiation was observed after an average of eight weeks of inoculation and the results are presented in table 13. MS medium supplemented with Kinetin (3.00 mg/l) + NAA (0.5 mg/l) has taken less days for shoot initiation (53.12±0.88) whereas BAP (3.00 mg/l) + NAA (0.5 mg/l) has taken more days for shoot initiation (61.23±1.45).

Table 13. Effect of growth regulators on number of days taken for shoot initiation in *Alpinia galanga* (L.) Willd.

Treatment	MS media + Growth regulator (mg/l)	Number days taken for shoot initiation (Mean±SE) *
T_1	Kinetin (3.00)	56.16±0.88
T_2	Kinetin (2.00)	55.83±1.20
T_3	Kinetin (1.00)	59.11±1.15
T_4	BAP (3.00)	58.64±1.73
T_5	BAP (2.00)	58.97±1.45
T_6	NAA (1.00)	–
T_7	NAA (0.5)	–
T_8	Kinetin (3.00) + NAA (0.5)	53.12±0.88
T_9	Kinetin (3.00) + NAA (1.00)	57.78±.0.57
T_{10}	Kinetin (2.00) + NAA (0.5)	55.13±1.20
T_{11}	Kinetin (2.00) + NAA (1.00)	59.01±1.20
T_{12}	Kinetin (4.00) + NAA (0.5)	57.14±0.88
T_{13}	BAP (3.00) + NAA (0.5)	61.23±1.45
T_{14}	BAP (3.00) + NAA (1.00)	58.57±0.52
T_{15}	BAP (2.00) + NAA (0.5)	60.75±1.76
T_{16}	BAP (2.00) + NAA (1.00)	58.63 ±.1.45

***Mean value of growth parameters of 5 plantlets per treatment.**

Table 14. Effect of growth regulators on *in-vitro* shoot multiplication in *Alpinia galanga* (L.) Willd. after 12 weeks of inoculation.

Treatment	MS media + Growth regulator (mg/l)	No. of shoots/ explants (Mean±SE) *	Shoot length (cm) (Mean±SE) *	No. of roots/ explants (Mean±SE) *	Root length (cm) (Mean±SE) *	No. of leaves/ explants (Mean±SE) *
T_1	Kinetin (3.00)	1.28±0.04	2.93±0.88	1.17±0.06	3.18±0.04	4.18±0.04
T_2	Kinetin (2.00)	1.13±0.06	2.60±0.05	1.02±0.04	2.83±0.03	3.87±0.03
T_3	Kinetin (1.00)	1.15±0.05	2.71±0.05	1.12±0.01	2.67±0.03	3.65±0.02
T_4	BAP (3.00)	1.23±0.66	3.07±0.08	1.15±0.05	2.55±0.02	4.20±0.05
T_5	BAP (2.00)	1.31±0.07	2.73±0.08	1.28±0.01	2.73±0.03	4.33±0.08
T_6	NAA (1.00)	–	–	–	–	–
T_7	NAA (0.5)	–	–	–	–	–
T_8	Kinetin (3.00) + NAA (0.5)	2.07±0.08	3.40±0.05	1.70±0.02	3.55±0.02	4.78±0.01
T_9	Kinetin (3.00) + NAA (1.00)	1.38±0.04	2.73±0.08	1.52±0.06	2.83±0.03	4.48±0.06
T_{10}	Kinetin (2.00) + NAA (0.5)	1.42±0.07	2.93±0.08	1.62±0.06	3.27±0.12	4.38±0.06
T_{11}	Kinetin (2.00) + NAA (1.00)	1.27±0.33	3.07±0.08	1.37±0.03	3.43±0.08	4.50±0.05
T_{12}	Kinetin (4.00) + NAA (0.5)	1.80±0.05	3.30±0.05	1.55±0.02	2.81±0.05	4.22±0.06
T_{13}	BAP (3.00) + NAA (0.5)	1.32±0.06	3.20±0.05	1.78±0.02	3.63±0.03	4.68±0.06
T_{14}	BAP (3.00) + NAA (1.00)	1.58±0.06	3.07±0.08	1.48±0.04	3.20±0.05	4.42±0.06
T_{15}	BAP (2.00) + NAA (0.5)	1.87±0.12	3.20±0.05	1.62±0.01	2.97±0.06	4.62±0.04
T_{16}	BAP (2.00) + NAA (1.00)	2.03±0.06	3.32±0.07	1.75±0.02	3.45±0.02	4.85±0.02

***Mean value of growth parameters of 5 plantlets per treatment.**

4.2.2 Number of shoots per explant

As far as number of shoots are concerned, MS medium supplemented with Kinetin (3.00 mg/l) + NAA(0.5 mg/l) responded significantly high in terms of number of shoots (2.07±0.08) followed by treatment combination of BAP (2.00 mg/l) + NAA (1.00 mg/l) which gave good number of shoots (2.03±0.06). Whereas MS medium supplemented with Kinetin (2.0 mg/l) alone showed less number of shoots (1.13±0.06) which was reported to be on par with Kinetin (1.0 mg/l) which also showed less number of shoots (1.15±0.05).

4.2.3 Length of shoots (cm)

MS medium supplemented with Kinetin (3.00 mg/l) + NAA(0.5 mg/l) shown significantly highest shoot length (3.40.±0.05) which was reported to be on par with of BAP (2.00 mg/l) + NAA (1.00 mg/l) which showed high shoot length of 3.32±0.07. MS medium supplemented with Kinetin (2.0 mg/l) alone showed significantly minimum shoot length (2.60 ±0.05).

4.2.4 Number of roots per explant

As far as number of roots are concerned, MS medium supplemented BAP (3.00 mg/l) + NAA (0.5 mg/l) responded significantly high in terms of number of roots per explant (1.78±0.02) followed by treatment combination of BAP (2.0 mg/l) and NAA (1 mg/l) which gave good number of roots (1.75±0.02), whereas less number of roots (1.02±0.04) were produced in Kinetin (2.0 mg/l).

4.2.5 Length of roots

Root length was found maximum in MS medium supplemented with BAP (3.00 mg/l) + NAA (0.5 mg/l) and recorded root length of 3.63±0.03 followed by treatment combination of Kinetin (3.00 mg/l) + NAA (0.5 mg/l) and recorded root length of 3.55±0.02. MS medium supplemented with BAP (3.0 mg/l) alone showed significantly less roots length (2.55±0.02).

4.3.6 Number of leaves per explant

MS medium supplemented with BAP (2.0 mg/l) +NAA (1 mg/l) showed significantly high number of leaves per explant (4.85±0.02) followed by MS media supplemented with Kinetin (3.00 mg/l) + NAA (0.5 mg/l) also showed more number of leaves per explants (4.78±0.01). MS medium supplemented with Kinetin (1.0 mg/l) alone showed significantly less number of leaves per explants (3.65±0.02).

4.3 *In-vitro* conservation

In-vitro established plants were further kept for short term conservation without any sub-culture during the entire conservation period. Observations were recorded at an interval of one, two and three month of incubation for survival per cent, shoot length, number of shoots, number of leaves, root length, number of roots and number of leaves and kept under low light intensity (2.97 $\mu m^{-2}s^{-1}$) in a chamber having ambient temperature maintained at 10 ^{0}C and observations recorded were presented in tables below (Table 15, 16 and 17). Comparisons were also made based on growth parameters between *in-vitro* conserved plants in Reduced Culture Condition (RCC) and other *in-vitro* raised plants which were maintained in Standard Culture Condition (SCC) and observations during this period were presented in Table 18. Following are the observations made for *in-vitro* conserved plantlets after three months of conservation. Data was statistically analyzed by analysis of variance (ANOVA) and significance was calculated.

4.3.1 Survival per cent

In-vitro conserved plants of galanga showed 100 percent survivability even after three months of conservation period in most of the treatments and only T_3 and T_{13} showed lowest survival per cent (80 %).

4.3.2 Number of shoots

MS medium supplemented with Kinetin (3.0 mg/l) +NAA (0.5 mg/l) has showed maximum number of shoots per explant (3.27±0.08) followed by MS medium supplemented with BAP (2.0 mg/l) +NAA (1 mg/l) shown high number of shoots (3.03±0.10). Whereas, MS medium supplemented with Kinetin (2.0 mg/l) showed less shoots number per explant (1.39±0.08) followed by MS medium supplemented with BAP (3.0 mg/l) which also shown less number of shoots (1.63±0.03).

4.3.3 Shoot length (cm)

MS medium supplemented with Kinetin (3.0 mg/l) +NAA (0.5 mg/l) has showed significantly higher shoot length (4.07±0.08) followed by MS medium supplemented Kinetin (4.0 mg/l) +NAA (0.5 mg/l) showed shoot length of 3.83±0.08. Whereas, MS medium supplemented with Kinetin (2.0 mg/l) showed significantly less shoot length (2.50±0.05).

4.3.4 Number of roots

As far as number of roots per explant is concerned, MS medium supplemented with BAP (3.0 mg/l) +NAA (0.5 mg/l) showed significantly high number of roots per explants (3.12±0.08) followed by Kinetin (2.0 mg/l) +NAA (0.5 mg/l) which also shown high number of roots per explant (2.83±0.08). MS medium supplemented with Kinetin (1.0 mg/l) showed less roots number per explant (1.93±0.08).

Table 15. Estimation of growth after one month of *in-vitro* conservation in *Alpinia galanga* (L.) Willd.

Treatment	MS media + Growth regulator (mg/l)	Survival per cent	No. of shoots/ explants (Mean±SE) *	Shoot length (cm) (Mean±SE) *	No. of roots/ explants (Mean±SE) *	Root length (cm) (Mean±SE) *	No. of leaves/ explants (Mean±SE) *
T_1	Kinetin (3.00)	100	1.18±0.04	1.77±0.03	1.06±0.04	0.39±0.03	2.48±0.04
T_2	Kinetin (2.00)	100	1.09±0.03	1.45±0.02	1.13±0.02	0.37±0.08	2.40±0.05
T_3	Kinetin (1.00)	100	1.13±0.03	1.65±0.02	1.11±0.03	0.31±0.05	2.17±0.06
T_4	BAP (3.00)	100	1.08±0.04	1.65±0.02	1.03±0.01	0.34±0.01	2.17±0.03
T_5	BAP (2.00)	100	1.12±0.03	2.07±0.06	1.07±0.03	0.39±0.08	2.40±0.05
T_6	NAA (1.00)	–	–	–	–	–	–
T_7	NAA (0.5)	–	–	–	–	–	–
T_8	Kinetin (3.00) + NAA (0.5)	100	1.49±0.04	2.28±0.04	1.53±0.03	0.49±0.01	2.65±0.02
T_9	Kinetin(3.00) + NAA	100	1.10±0.01	1.45±0.02	1.17±0.03	0.42±0.01	2.55±0.02
T_{10}	Kinetin (2.00) + NAA (0.5)	100	1.18±0.03	1.65±0.02	1.43±0.03	0.40±0.03	2.43±0.08
T_{11}	Kinetin (2.00) + NAA	100	1.07±0.02	1.63±0.06	1.23±0.03	0.43±0.05	2.63±0.03
T_{12}	Kinetin (4.00) + NAA (0.5)	100	1.43±0.05	2.18±0.04	1.13±0.03	0.41±0.06	2.65±0.02
T_{13}	BAP (3.00) + NAA (0.5)	100	1.08±0.02	1.85±0.02	1.63±0.03	0.52±0.03	2.33±0.03
T_{14}	BAP (3.00) + NAA (1.00)	100	1.28±0.02	1.97±0.06	1.2±0.02	0.44±0.05	2.62±0.01
T_{15}	BAP (2.00) + NAA (0.5)	100	1.39±0.08	2.07±0.08	1.23±0.03	0.42±0.08	2.40±0.05
T_{16}	BAP (2.00) + NAA (1.00)	100	1.46±0.02	2.18±0.04	1.43±0.03	0.50±0.02	2.70±0.05

***Mean value of growth parameters of 5 plantlets per treatment.**

Table 16. Estimation of growth after two month of *in-vitro* conservation in *Alpinia galanga* (L.) Willd.

Treatment	MS media + Growth regulator (mg/l)	Survival per cent	No. Of shoots/ explants (Mean±SE) *	Shoot length(cm) (Mean±SE) *	No. of roots/ explants (Mean±SE) *	Root length(cm) (Mean±SE) *	No. of leaves/ explants (Mean±SE) *
T_1	Kinetin (3.00)	100	1.55±0.02	2.20±0.05	1.53±0.08	0.72±0.01	3.55±0.07
T_2	Kinetin (2.00)	100	1.23±0.33	2.07±0.08	1.65±0.33	0.52±0.01	3.33±0.03
T_3	Kinetin (1.00)	100	1.38±0.04	1.93±0.88	1.73±0.03	0.41±0.01	3.21±0.05
T_4	BAP (3.00)	100	1.30±0.05	2.07±0.08	1.67±0.06	0.46±0.01	3.45±0.02
T_5	BAP (2.00)	100	1.57±0.01	2.45±0.02	1.58±0.05	0.79±0.01	3.62±0.04
T_6	NAA (1.00)	_	_	_	_	_	_
T_7	NAA (0.5)	_	_	_	_	_	_
T_8	Kinetin (3.00) + NAA (0.5)	100	2.35±0.05	2.70±0.05	2.20±0.05	0.87±0.01	3.65±0.02
T_9	Kinetin(3.00) + NAA (1.00)	100	1.63±0.06	2.17±0.03	2.13±0.03	0.59±0.06	3.13±0.03
T_{10}	Kinetin (2.00) + NAA (0.5)	100	1.53±0.03	2.48±0.04	2.27±0.03	0.71±0.01	3.38±0.04
T_{11}	Kinetin (2.00) + NAA (1.00)	100	1.80±0.05	2.32±0.07	2.20±0.57	0.56±0.05	3.42±0.06
T_{12}	Kinetin (4.00) + NAA (0.5)	100	1.83±0.03	2.48±0.04	2.35±0.02	0.79±0.08	3.53±0.04
T_{13}	BAP (3.00) + NAA (0.5)	100	1.67±0.03	2.20±0.05	2.43±0.03	0.90±0.01	3.61±0.05
T_{14}	BAP (3.00) + NAA (1.00)	100	2.17±0.03	2.17±0.03	2.13±0.03	0.69±0.08	3.55±0.06
T_{15}	BAP (2.00) + NAA (0.5)	100	1.60±0.05	2.37±0.03	2.23±0.06	0.69±0.01	3.55±0.02
T_{16}	BAP (2.00) + NAA (1.00)	100	2.13±0.03	2.58±0.04	2.33±0.03	0.82±0.08	3.75±0.02

***Mean value of growth parameters of 5 plantlets per treatment.**

Table 17. Estimation of growth after three month of *in-vitro* conservation in *Alpinia galanga* (L.) Willd.

Treatment	MS media + Growth regulator (mg/l)	Survival per cent	No. of shoots/ explants (Mean±SE) *	Shoot length(cm) (Mean±SE) *	No. of roots/ explants (Mean±SE) *	Root length(cm) (Mean±SE) *	No. of leaves/ explants (Mean±SE) *
T_1	Kinetin (3.00)	100	2.60±0.03	2.63±0.03	2.33±0.03	0.98±0.01	5.87±0.12
T_2	Kinetin (2.00)	100	1.39±0.03	2.50±0.05	2.20±0.05	0.73±0.01	5.47±0.12
T_3	Kinetin (1.00)	80	1.93±0.08	2.80±0.10	1.93±0.08	0.67±0.03	5.40±0.10
T_4	BAP (3.00)	100	1.63±0.03	3.03±0.12	2.23±0.06	0.69±0.03	5.43±0.08
T_5	BAP (2.00)	80	2.21±0.06	3.40±0.05	2.21±0.05	0.94±0.01	5.77±0.03
T_6	NAA (1.00)	_	_	_	_	_	_
T_7	NAA (0.5)	_	_	_	_	_	_
T_8	Kinetin (3.00) + NAA (0.5)	100	3.27±0.08	4.07±0.08	2.81±0.07	1.00±0.01	6.07±0.88
T_9	Kinetin(3.00) + NAA (1.00)	100	2.55±0.02	3.50±0.05	2.43±0.03	0.80±0.01	5.52±0.06
T_{10}	Kinetin (2.00) + NAA (0.5)	100	2.20±0.05	3.52±0.04	2.83±0.08	0.90±0.02	5.78±0.05
T_{11}	Kinetin (2.00) + NAA (1.00)	80	2.38±0.08	3.17±0.03	2.40±0.05	0.79±0.01	5.63±0.08
T_{12}	Kinetin (4.00) + NAA (0.5)	100	2.69±0.01	3.83±0.08	2.80±0.05	0.95±0.01	6.23±0.08
T_{13}	BAP (3.00) + NAA (0.5)	100	2.87±0.08	3.37±0.03	3.12±0.08	1.27±0.04	5.67±0.08
T_{14}	BAP (3.00) + NAA (1.00)	100	2.55±0.07	3.07±0.08	2.30±0.05	0.89±0.01	6.21±0.08
T_{15}	BAP (2.00) + NAA (0.5)	100	2.57±0.06	3.40±0.05	2.37±0.66	1.07±0.03	5.83±0.03
T_{16}	BAP (2.00) + NAA (1.00)	100	3.03±0.06	3.79±0.06	2.73±0.03	1.17±0.01	6.31±0.08

***Mean value of growth parameters of 5 plantlets per treatment.**

Table 18. Comparisons of growth for tissue cultured plantlets kept under Standard Culture Condition (SCC) and Reduced Culture Condition (RCC) after three months in *Alpinia galanga* (L.) Willd.

Treatment	MS media + Growth regulator (mg/l)	Survival percent		No. of shoots/ explants (Mean±SE) *		Shoot length(cm) (Mean±SE) *	
		SCC	RCC	SCC	RCC	SCC	RCC
T_1	Kinetin (3.00)	100	100	2.83±0.03	2.60±0.03	5.90±0.11	2.63±0.03
T_2	Kinetin (2.00)	80	100	1.90±0.05	1.39±0.03	5.10±0.15	2.50±0.05
T_3	Kinetin (1.00)	80	80	2.26±0.08	1.93±0.08	4.60±0.05	2.80±0.10
T_4	BAP (3.00)	100	100	2.38±0.10	1.63±0.03	4.70±0.11	3.03±0.12
T_5	BAP (2.00)	80	100	2.78±0.06	2.21±0.06	5.77±0.08	3.40±0.05
T_8	Kinetin (3.00) + NAA (0.5)	100	100	3.86±0.12	3.27±0.08	8.13±0.13	4.07±0.08
T_9	Kinetin (3.00) + NAA (1.00)	100	100	3.00±0.10	2.55±0.02	5.63±0.12	3.50±0.05
T_{10}	Kinetin (2.00) + NAA (0.5)	100	100	2.80±0.05	2.20±0.05	6.45±0.08	3.52±0.04
T_{11}	Kinetin (2.00) + NAA (1.00)	80	100	3.36±0.08	2.38±0.08	5.48±0.04	3.17±0.03
T_{12}	Kinetin (4.00) + NAA (0.5)	100	100	3.76±0.08	2.69±0.01	7.20±0.05	3.83±0.08
T_{13}	BAP (3.00) + NAA (0.5)	100	80	3.66±0.06	2.87±0.08	5.27±0.08	3.37±0.03
T_{14}	BAP (3.00) + NAA (1.00)	100	100	3.26±0.08	2.55±0.07	6.53±0.08	3.07±0.08
T_{15}	BAP (2.00) + NAA (0.5)	100	100	3.55±0.02	2.57±0.06	7.40±0.11	3.40±0.05
T_{16}	BAP (2.00) + NAA (1.00)	100	100	3.63±0.03	3.03±0.06	7.67±0.08	3.79±0.06

***Mean value of growth parameters of 5 plantlets per treatment.**

Table 18 (Cont…). Comparisons of growth for tissue cultured plantlets kept under Standard Culture Condition (SCC) and Reduced Culture Condition (RCC) after three months in *Alpinia galanga* (L.) Willd.

Treatment	MS media + Growth regulator (mg/l)	No. of roots/ explants (Mean±SE) *		Root length(cm) (Mean±SE) *		No. of leaves/ explants (Mean±SE) *	
		SCC	RCC	SCC	RCC	SCC	RCC
T_1	Kinetin (3.00)	2.85±0.08	2.33±0.03	4.60±0.05	0.98±0.01	8.63±0.08	5.87±0.12
T_2	Kinetin (2.00)	2.65±0.05	2.20±0.05	4.68±0.06	0.73±0.01	7.53±0.12	5.47±0.02
T_3	Kinetin (1.00)	2.23±0.06	1.93±0.08	4.17±0.06	0.67±0.03	8.42±0.06	5.40±0.05
T_4	BAP (3.00)	2.43±0.33	2.23±0.06	4.43±0.08	0.69±0.03	8.10±0.11	5.43±0.08
T_5	BAP (2.00)	2.70±0.05	2.21±0.05	5.10±0.11	0.94±0.01	9.07±0.08	5.77±0.03
T_8	Kinetin (3.00) + NAA (0.5)	4.02±0.01	2.81±0.07	5.67±0.08	1.00±0.01	9.30±0.05	6.07±0.88
T_9	Kinetin (3.00) + NAA (1.00)	3.48±0.04	2.43±0.03	4.93±0.08	0.80±0.01	8.77±0.06	5.52±0.06
T_{10}	Kinetin (2.00) + NAA (0.5)	3.70±0.05	2.83±0.08	5.18±0.04	0.90±0.02	8.30±0.11	5.78±0.05
T_{11}	Kinetin (2.00) + NAA (1.00)	3.38±0.02	2.40±0.05	5.03±0.06	0.79±0.01	8.63±0.08	5.63±0.08
T_{12}	Kinetin (4.00) + NAA (0.5)	3.92±0.05	2.80±0.05	5.82±0.06	0.95±0.01	7.73±0.58	6.23±0.08
T_{13}	BAP (3.00) + NAA (0.5)	4.07±0.08	3.12±0.08	6.13±0.12	1.27±0.04	8.73±0.08	5.67±0.08
T_{14}	BAP (3.00) + NAA (1.00)	3.30±0.05	2.30±0.05	5.25±0.02	0.89±0.01	9.13±0.08	6.21±0.08
T_{15}	BAP (2.00) + NAA (0.5)	3.37±0.06	2.37±0.66	5.43±0.08	1.07±0.07	8.80±0.05	5.83±0.03
T_{16}	BAP (2.00) + NAA (1.00)	3.53±0.03	2.73±0.03	6.05±0.07	1.17±0.01	9.52±0.07	6.31±0.08

***Mean value of growth parameters of 5 plantlets per treatment.**

4.3.5 Root length (cm)

MS medium supplemented with BAP (3.0 mg/l) +NAA (0.5 mg/l) has showed significantly high root length (1.27±0.04) followed by MS medium supplemented with BAP (2.0 mg/l) +NAA (1 mg/l) showed root length of 1.17±0.01. Whereas, MS medium supplemented with Kinetin (1.0 mg/l) showed significantly less root length (0.67±0.03).

4.3.6 Number of leaves

As far as number of leaves per explant is concerned, MS medium supplemented with BAP (2.0 mg/l) +NAA (1 mg/l) showed significantly high number of leaves per explants (6.31±0.08) followed by MS medium supplemented with Kinetin (4.0 mg/l) +NAA (0.5 mg/l) showed high number of leaves per explant (6.23±0.18). MS medium supplemented with Kinetin (1.0 mg/l) shown significantly less number of leaves per explant (5.40±0.10) which was on par with BAP (3.0 mg/l) and Kinetin (2.0 mg/l) which shown less number of leaves per explants of 5.43±0.08 and 5.47±0.03 respectively.

4.3.7 Comparisons between in-vitro conserved plantlets and tissue cultured plantlets regenerated in standard culture conditions for some growth related traits

A comparison was made between *in-vitro* conserved plants in Reduced Culture Condition (RCC) and other *in-vitro* raised plants maintained in Standard Culture Condition (SCC) for survival per cent, shoot length, number of shoots, root length, number of roots and number of leaves. Observation recorded during the three months duration were recorded below with the following headings.

4.3.7.1 Survival per cent

Without sub-culturing in both Reduced Culture Condition (RCC) and Standard Culture Condition (SCC) plants showed good survivability during the entire after three months of conservation period. MS medium supplemented with various combinations and concentrations of growth regulators showed maximum of 100 per cent survivability in most of the treatments except T_3 and T_{13} in *in-vitro* conserved plantlets (80 %). Plantlets kept under normal conditions also showed maximum of 100 per cent survivability in most of the treatments except T_2, T_3, T_5 and T_{11} which showed 80 per cent survivability.

4.3.7.2 Number of shoots

MS medium supplemented with Kinetin (3.0 mg/l) + NAA (0.5 mg/l) shown the highest number of shoots both *in-vitro* conserved plantlets (3.27±0.08) and plantlets kept in normal conditions (3.86±0.12) which was on par with MS medium supplemented with BAP (2.00) + NAA (1.00). Whereas MS medium supplemented

with Kinetin (2.0 mg/l) shown lowest number of shoots in both *in-vitro* conserved plantlets (1.39±0.03) and plantlets kept in normal condition (1.90±0.05).

4.3.7.3 Shoot length (cm)

MS medium supplemented with Kinetin (3.0 mg/l) + NAA (0.5 mg/l) shown significantly high shoot length in both *in-vitro* conserved plantlets (4.07±0.08) and plantlets kept in normal conditions (8.13±0.13). Whereas, MS medium supplemented with Kinetin (2.0 mg/l) shown lowest shoot length in *in-vitro* conserved plantlets (2.50±0.05) and MS medium supplemented with Kinetin (1.0 mg/l) shown the lowest shoot length in plantlets kept in normal conditions (4.60 ± 0.05).

4.3.7.4 Number of roots

As far as the number of roots is concerned, MS medium supplemented with BAP (3.0 mg/l) +NAA (0.5 mg/l) showed a significantly high number of roots per explants in both in *in-vitro* conserved plantlets (3.12 ± 0.08) and plantlets kept under normal conditions (4.07 ± 0.08). Whereas MS medium supplemented with Kinetin (1.0 mg/l) shown significantly less number of roots per explants in both *in-vitro* conserved plantlets (1.93 ± 0.08) and plantlets under normal condition (2.23 ± 0.06).

4.3.7.5 Root length (cm)

MS medium supplemented with BAP (3.0 mg/l) +NAA (0.5 mg/l) shown significantly high root length in both *in-vitro* conserved plantlets (1.27±0.04) and plantlets kept in normal conditions (6.13±0.12). Whereas, MS medium supplemented with Kinetin (1.0 mg/l) shown lowest root length in *in-vitro* conserved plantlets (0.67±0.03) and plantlets kept in normal conditions (4.17 ± 0.06) which was on par with BAP (3 mg/l) in both the condition

4.3.6 Number of leaves

As far as the number of leaves is concerned, MS medium supplemented with BAP (2.00 mg/l) + NAA (1.00 mg/l) shown a significantly high number of leaves in both *in-vitro* conserved plantlets (6.31±0.08) and plantlets kept in normal conditions (9.52±0.07). Whereas, MS medium supplemented with Kinetin (1.0 mg/l) shown the lowest number of leaves in *in-vitro* conserved plantlets (5.40±0.01) and MS medium supplemented with Kinetin (2.0 mg/l) shown the lowest number of leaves in plantlets kept in normal conditions (7.53±0.12).

DISCUSSION

V DISCUSSION

Western Ghat is a home for many Rare, Endangered, Threatened (RET) species. It harbours many medicinal plants with broad pharmacological significance. *Alpinia galanga* is one of the important medicinal plant which is gaining a lot of importance due to the presence of tremendous medicinal properties at the same time this plant is under threat because of overexploitation and destructive harvesting. To maintain a balanced ecosystem, the development of cultivation and conservation strategies are necessary (Shankar and Ved, 2003) because every plant species has a role in supporting sustainable biodiversity.

The present research was carried out during the year 2019-20 on *Alpinia galanga* (L.) Willd. It is one of the important RET medicinal plant. The data were recorded on the morphological characters, tissue culture and *in-vitro* conservation studies in *Alpinia galanga* and the results of the present investigation are discussed in this chapter with the available supporting literature under the following headings.

5.1 Morphological characterization of *Alpinia galanga* (L.)Willd.

In the present study, fourteen accessions of *Alpinia galanga* were evaluated to know the genetic variability and also to identify elite genotypes with desirable traits for further research. In any crop, morphological characterization is considered to be an essential first step in the description and classification of plant germplasm because a stipulated breeding programme in any crop mainly depends upon the magnitude of genetic variability (Smith and Smith, 1989).

5.1.1 Qualitative characters

There is much scope for improving the genetic variability by increasing the size of the sample population. Variability was observed among accessions in qualitative characters *viz*., leaf shape, leaf colour and rhizome colour.

Among the fourteen accessions evaluated, ACC-8 and ACC-10 showed higher rhizome yield per plant in comparison to the other accessions. Also, it can be noted that the accessions ACC-8 and ACC- 10 have similar traits (Qualitative characteristics) *viz.,* yellow-green leaf colour, lanceolate leaf shape, white colour flower and greyed yellow colour rhizome. Whereas all the other accessions showed varied qualitative characteristics (Table 4 and 5). Hence, the results indicate that ACC-8 and ACC-10 can be selected for any further improvement in areas of research or cultivation, as they are superior genotypes for yield.

5.1.2 Quantitative characters

The mean performances of fourteen accessions were computed and the significant differences were observed among the accession for all the characters studied *viz.,* plant height, leaf length, leaf width, leaf area, petiole length,

inflorescence length, flower bud size, pedicel length, relative height between androecium and gynoecium and yield per tiller. The results indicate that for all the component characters studied, there is a lot of variability among the accessions. It also creates scope for selection for the majority of the traits in the progenies because variability serves as an essential prerequisite for all crop improvement programmes and the source material for any crop improvement would be selected by studying the amount of variability available in the germplasm.

To study growth and yield characters, the genetic potential of yield must be examined through the study of its component characters by employing useful biometrical tools as yield is dependent upon different growth and yield contributing traits. Some of these parameters include genotypic (GCV) and phenotypic (PCV) coefficient of variation, heritability, genetic advance over mean. Genotypic and phenotypic correlation help to base selection procedure to a required balance when two opposite desirable characters affecting the principle characters which are being considered. It is also useful to improve different characters simultaneously (Falconer, 1981). The results obtained during the course of research work are discussed below in detail.

5.1.2.1 Variability studies

To improve the genetic makeup of any plant species, evaluation of genotypic and phenotypic characters is essential and the genotypes performing better over the existing types for yield and other yield attributing traits are selected for further studies. The degree of crop improvement depends on beneficial and usable variability.

Analysis of phenotypic characters alone would not be reliable as it always has an influence of environmental factors on it (Moller and Thornhill., 1997) and also meagre knowledge of the existence of variability for different characters may not reveal accurately that which particular character is showing the highest degree of variability. Hence, estimates of both genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were carried out.

Estimation of the genotypic coefficient of variation is essential to know the amount of genetic variation present for different desirable traits. Genetic variability is considered as one of the important breeding components for the improvement of a plant species (Govindaraj *et al*., 2015). Such information helps in locating suitable parental lines for crop improvement. Hence, to make the selection better, it is essential to study and partition the total variability existing in germplasm into genetic, phenotypic and environmental variability.

The phenotypic variance was found to be higher than genotypic variances for all the characters taken into consideration. But, the phenotypic variance is not very

reliable, since it includes both genetic and environmental effects. Thus, it is essential to split the total variance into genetic and non-genetic components.

In the present research work, high GCV and PCV were observed for petiole length, inflorescence length and leaf area. Similar results were obtained by Tuppad *et al*. (2017) in *Holostemma ada-kodien* and Shruthi *et al*. (2018) in *Embelia ribes*. The results indicated that the genetic component in total variation is more and environmental influence is less in these traits. Thus, the selection scheme planned based on these characters will have a great selection response. Moderate GCV and PCV were observed for yield per tiller, pedicel length and leaf length. Similar findings were obtained by Yadav *et al*. (2007) in *Chlorophytum borivilianum*, Tuppad *et al*. (2017) in *Holostemma ada-kodien* and Shruthi *et al*. (2018) in *Embelia ribes*, whereas low GCV and PCV were observed for plant height, leaf width and flower bud size. The results are in conformity with the results of Tuppad *et al*. (2017) in *Holostemma ada-kodien,* Mastiholi *et al*. (2018) in *Salacia chinensis* and Sandesh *et al*. (2018) in *Oroxylum indicum*.

The above findings indicate that the character with moderate and high magnitude of GCV and PCV suggests the existence of some variability in the population. Therefore, selection for the above traits can also be beneficial for crop improvement.

The observed data on phenotypic coefficient of variation and genotypic coefficient of variation was found to be closer to each other for most of the traits and this indicates the influence of the environment for these characters is negligible and the role of the genotypic performance for the full expression of the phenotype.

5.1.2.2 Heritability and genetic advance

Heritability is a key to the measure, how well differences in genes account for differences in their traits. Burton and Devane (1953) suggested that the estimation of the genotypic coefficient of variation and heritability would give the amount of progress to be expected by selection. The estimation of heritability gives an idea about the amount of observed variability, which is inherent and attributed to the genetic difference (Tuppad *et al*., 2017). The heritability helps the breeder to create the base for selection, improve the characters and also used to estimate the genetic advances (Patel and Desai, 2017). Even though the estimation of broad sense heritability may give criteria for selection but to arrive at a reliable conclusion, heritability and genetic advance should be considered jointly (Jhonson *et al*., 1955).

In the present study, heritability ranged from 44.24 per cent in flower bud size to 83.35 per cent in the petiole length. It is found to be high for characters like petiole length (83.35%), plant height (74.47%), leaf length (68.95 %) which was on par with leaf area and inflorescence length (68.03 %) and pedicel length (64.87%). Similarly,

high heritability estimates were also reported by Chitra and Rajamani (2010) in *Gloriosa superba*, Tuppad *et al*. (2017) in *Holostemma ada-kodien*, Shruthi *et al*. (2018) in *Embelia ribes* and Sandesh *et al*. (2018) in *Oroxylum indicum*. From the heritability estimates, it is clear that these characters are less influenced by the environmental factors and controlled by additive gene effect and selection based on this character will improve the yield.

In the present investigation,high genetic advance over mean coupled with high heritability was observed in characters like petiole length, inflorescence length, leaf area and pedicel length. Similar results are reported by Yadav *et al*. (2007) in *Chlorophytum borivilianum* and Tuppad *et al*. (2017) in *Holostemma ada-kodien*. Moderate genetic advance as per cent of mean was recorded in yield per tiller, leaf length, plant height and leaf width. Similar findings were obtained by Mastiholi *et al*. (2018) in *Salacia chinensis* and Shruthi *et al*. (2018) in *Embelia ribes*. Hence, the higher heritability coupled with moderate to high genetic advance values observed for these traits in the present investigation suggests that the existing variability among the accessions with respect to the traits were governed by additive genes (Panse, 1957) and selection will be beneficial for the improvement of such traits.

5.1.2.3 Correlation studies

Although variability estimates provide an idea about the performance of different characters, they do not reveal the extent and nature of the relationship prevalent between the yield and other component characters. The phenotypic performance of any plant species results from the interaction of a large number of factors. Therefore the final yield of any plant is the sum total of the effects of several component characters and is polygenetically controlled quantitative character.The influence of these characters on yield can be well understood through the correlation studies. In any plant species, assessment of the correlation study reveals the degree of association of plant characters for improvement of yield. The correlations studies are considered as one of the important component in plant breeding, as it reflects the association between two or more traits and also helpful in establishing the direction and magnitude of the relationship between yield and yield attributing characters. (Chitralekha *et al*., 2018). In correlation studies, the genotypic correlation coefficient is more important than the phenotypic correlationcoefficient, because, in the genotypic correlation coefficient, the effect of external andenvironmental factors is removed or minimized. In contrast, the phenotypic correlation includes a part of environmental correlation influencing variation in two characters (Ghannadha and Naghavi, 2002). Falconer (1981) stated that phenotypic correlation could exceed genotypic correlation only if the heritability of two characters were low and environmental correlations were high. Hence, genotypic correlation coefficients give an accurate measure of the genotypic associationsince it is an inherited relationship

between the traits. The greater the magnitude ofthe correlation coefficient, the stronger is the association.In other words, high positive correlations between two traits make simultaneous improvement in two or more attributes, whereas negative association indicates the compromise between desirable characters. Hence, correlation is an important strategy designed to break the genetic barriers of yield and to study the association of various characters.

In the present study, the genotypic correlations were higher than the phenotypic correlations indicating high heritable nature of the traits. The plant characters like plant height, petiole length and leaf width had a positive and highly significant correlation with rhizome yield per tiller while leaf area exhibited positive and significant association and relative height between androecium and gynoeciumhad a negative and significant association with rhizome yield per tiller at a genotypic level, while plant height and petiole length showed high positive significant correlation with rhizome yield per tiller at phenotypic levels. Yield per plant is a combination of many characters and polygenically controlled. For such traits, the direct selection is difficult. Hence, selection based on these highly associated traits with yield per plant will indirectly help in selecting the accession with high yield. Therefore, it is worthwhile to have accessions with high plant height, petiole length and leaf width to get higher yields. The results are similar to the findings of Mastiholi *et al*. (2018) in *Salacia chinensis* and Madhushree *et al*. (2018) in *Saraca asoca*. They also noticed positive association of plant height, petiole length and leaf width on yield per plant.

Among the various traits studied, Plant height showed a strong association with the yield at both genotypic and phenotypic level. Hence, selection based on this trait will improve the rhizome yield per plant in a further crop improvement programme.

5.2 Development of multiplication protocol in *Alpinia galanga* using tissue culture

Galanga is usually propagated through vegetative means by using rhizome pieces for commercial multiplication. Seed propagation is not commercially feasible in galanga due to low germination rate, which is a major constraint for large-scale cultivation through seeds. As a practice it is propagated through rhizomes and it requires huge planting material per hectare, *i.e.,* 5-5.5 tons. Therefore, there is a need to find out an alternative and faster method to multiply for re-introduction into its natural habitat. *In-vitro* propagation is one such tool that fulfills the conservation as well large scale multiplication of plants for efficient and fast propagation (Borthakur *et al.,* 1999*).* The efficiency of different concentrations and combinations of plant growth regulators has been evaluated for direct shoot regeneration. The frequency and number of shoots varied with PGRs and their concentration amended in the culture

medium. The shoot regeneration response was not observed when explants were grown on PGR free MS medium.

The present investigation showed that it was possible to successfully induce multiple shoots of *Alpinia galanga* from the rhizome bud cultures with an efficient *in-vitro* protocol and hence increasing field survival rate. The result was found to be consistent to the findings of Vincent *et al.* (1992), Borthakur *et al.* (1999), Parida *et al*. (2011), Mathew *et al.* (2014) and Singh *et al.* (2014) who also concluded that multiple shoots could be obtained using basal rhizome buds as explants in *Alpinia galanga.*

When the healthy rhizome buds were inoculated on the MS medium supplemented with various concentration and combination of growth regulators, new shoots began to proliferate within an average of eight weeks of inoculation. Direct organogenesis was found, *i.e*., shoots were directly induced from explants without producing callus. Similar findings were also obtained by Vincent *et al.* (1992); Borthakur *et al.* (1999); Parida *et al*. (2011); Mathew *et al.* (2014) and Singh *et al.* (2014) in *Alpinia galanga*. Cultured rhizome buds produced both shoots and roots simultaneously and these results are in agreement with the results obtained by Kuruvinashetty *et al.* (1982) in *Curcuma longa*, Balachandran *et al.* (1990) in *Curcuma spp*, Vincent *et al.* (1992) and Borthakur *et al.* (1999) in *Alpinia galanga*.

In the present study, after 12 weeks of inoculation, the maximum number of shoots induced per explant (2.07±0.08) and highest shoot length (3.40.±0.05) were found in the treatment combination of kinetin (3.00 mg/l) + NAA (0.5 mg/l) whereas kinetin (2.0 mg/l) alone showed less number of shoots (1.13±0.06) and minimum shoot length (2.60 ±0.05). Similar findings were also obtained by Vincent *et al.* (1992); Borthakur *et al.* (1999) in *Alpinia galanga* and Selvakkumar *et al*. (2007) in *Alpinia officinarum*. The maximum number of roots produced per explants (1.78±0.02) and the highest root length (3.63±0.03) were found in the treatment combination of BAP (3.00 mg/l) + NAA (0.5 mg/l). Similar results were obtained by Vincent *et al.* (1992) and Borthakur *et al.* (1999) in *Alpinia galanga* where the results are in contrast with the findings of Parida *et al*. (2011), Mathew *et al.* (2014) and Singh *et al.* (2014) in *Alpinia galanga*, where they observed maximum root induction and root length in the MS media with IAA as a supplemented along with BAP. The maximum number of leaves per explants (4.85±0.02) were found in the treatment combination of BAP (2.0 mg/l) + NAA (1 mg/l) while less number of leaves per explants (3.65±0.02) were observed in MS medium supplemented with Kinetin (1.0 mg/l) alone.The results are in conformity with the results of Borthakur *et al.* (1999); Parida *et al*. (2011) in *Alpinia galanga* and Shahinozzaman *et al*. (2013) in *Curcuma caesia*.

Plate 7. i. Healthy rhizome buds of *Alpinia galanga* (L.) Willd used for inoculation ii. *In-vitro* Cultured plants

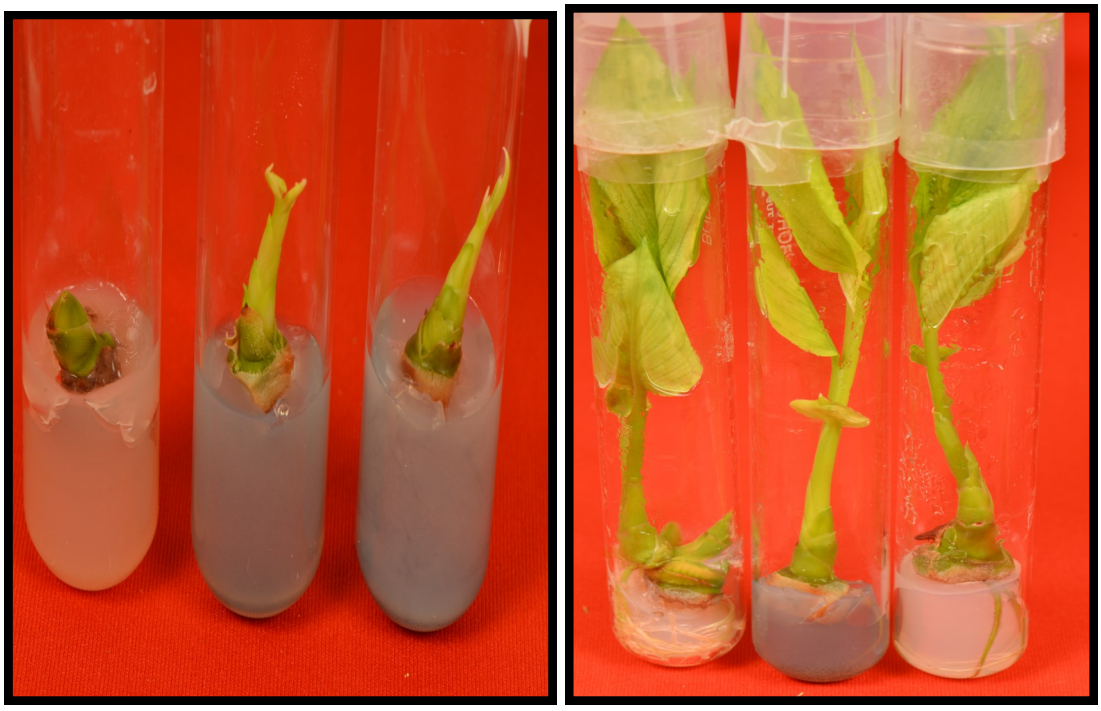

Plate 8. Response of rhizome bud explants inoculated on MS medium supplemented with different concentration of harmones at different intervals

Plate 9. *In-vitro* propagated plants of *Alpinia galanga* (L.) Willd. showing multiple shoots, well developed roots and leaves

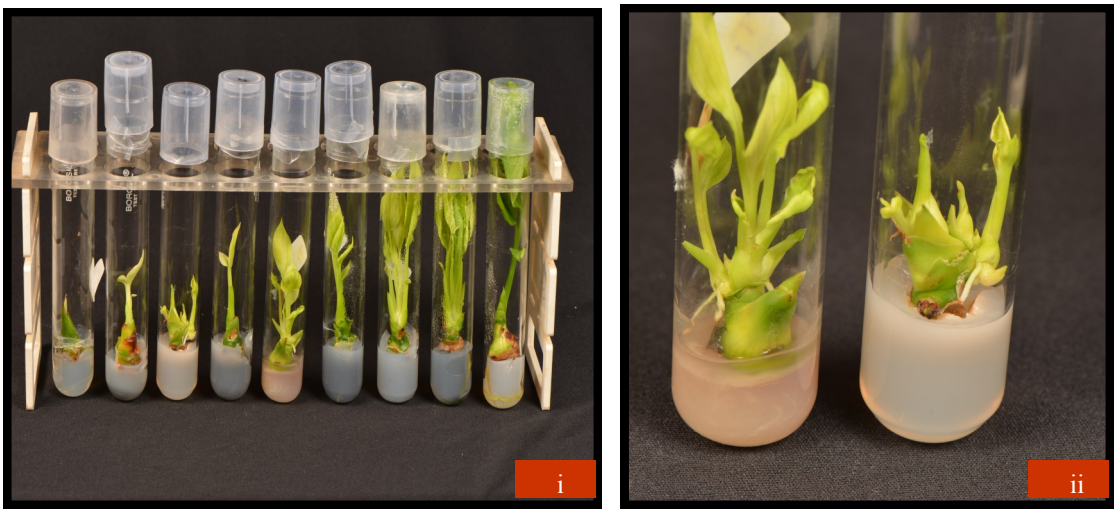

Plate 10. i. *In-vitro* cultured plants inoculated on MS medium with different concentration of growth regulator
ii. Rhizome bud explant showing multiple shoot growth

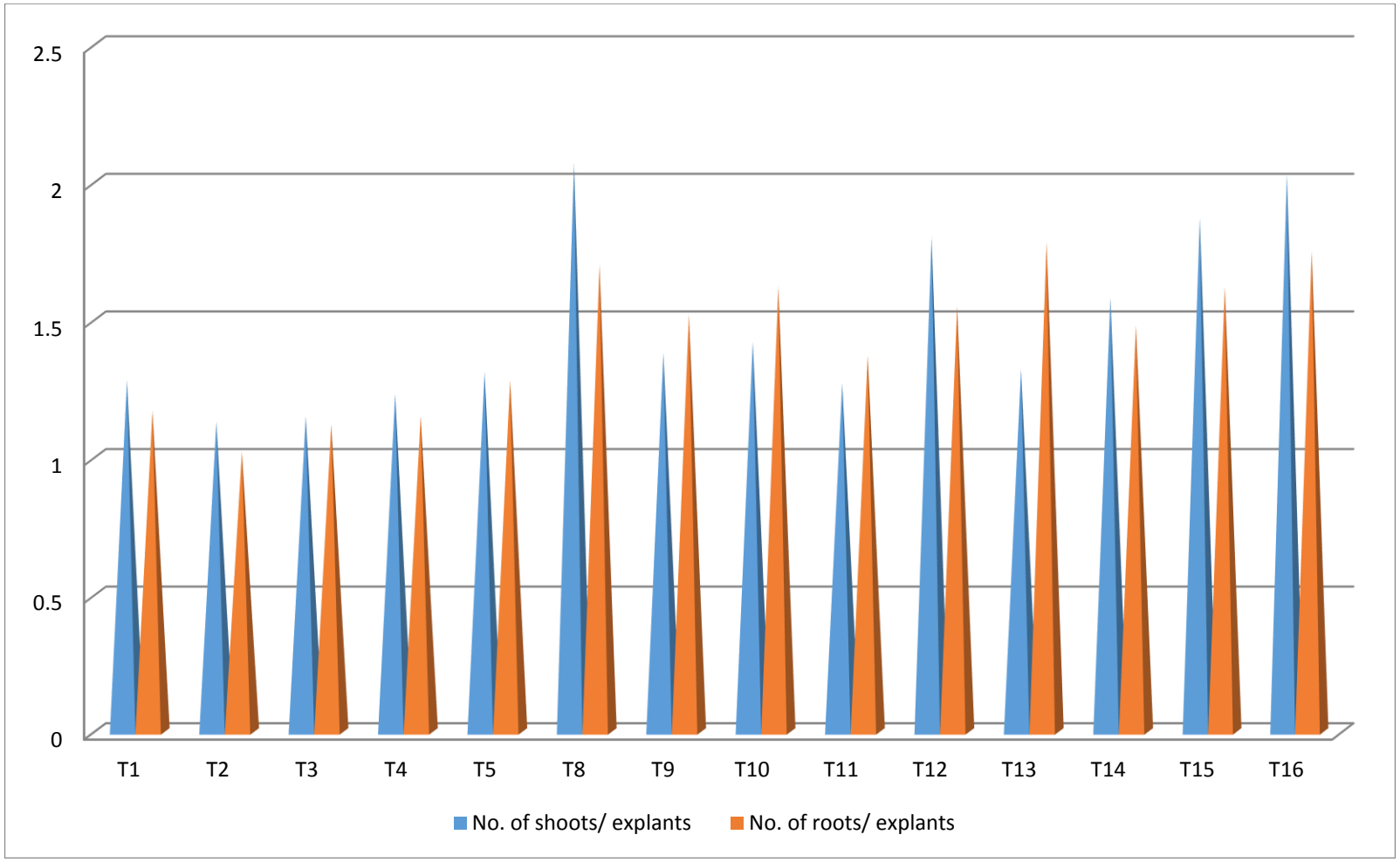

Fig 1. Growth of *Alpinia galanga* (L). Willd. using different plant growth regulators after 12 weeks of inoculation

Although the utilization of BAP or Kinetin individually with MS medium can initiate the shoots, but the shoot induction capacity can be improved by the addition of auxin. BAP and Kinetin alone could not provide satisfactory results, but in combination with NAA, it showed good results. This revealed that due to some interaction effects between two hormones, better results could be obtained. The same interaction effects were seen when BAP and Kinetin along with NAA were used for *in-vitro* propagation (Vincent *et al.*, 1992 in *Alpinia galanga*, Nair and Seeni, 2001 in *Celastrus paniculatus* and Mathew *et al.*, 2014 in *Alpinia calcarata*. They concluded that the manipulation in the levels of BAP, kinetin and NAA have an effect on the number of multiple shoots, shoot length, number of roots, root length and number of leaves. In this study, NAA is used for root induction and it was found to be best for *in-vitro* rooting and these results are in agreement with results obtained by Borthakur *et al.* (1999) in *Alpinia galanga*, Shahinozzaman *et al*. (2013) in *Curcuma caesia* and Mathew *et al.* (2014) in *Alpinia calcarata*.

The differential responses of various growth regulators on culture growth revealed that the type and concentration of PGRs and explant sources are the critical factors influencing organogenesis and different species respond differently to the PGRs combination and concentration. This is a demonstrated fact that the required amount of exogenous PGRs mainly depends on the endogenous level of the plant tissue and it varies with organ, genotype and the plant growth phase (Suresh and Ajay, 2004).

5.3 *In-vitro* conservation

The aim of the *in-vitro* conservation was to reduce the growth and also to conserve by providing slow growth condition in cultures. This technique also helps to check the survivability and success of *in-vitro* propagated plants. *In-vitro* conservation studies very are essential for the conservation of the plants, especially for plants that are figuring in the red list of medicinal plants of India (Rajasekharan *et al.*, 2009).

In the present study, *in-vitro* established plants were further kept for short term conservation for three months duration by providing low light intensity (2.97 μm^{-2} s^{-1}) and maintaining a temperature of 10 ^{0}C to reduce the growth and other growth-related traits and to analyze the survival per cent, shoot length, number of shoots, root length, number of roots and number of leaves after three months of conservation. Krishnan *et al*. (2011) reported the status of medicinal plants of Western Ghats of India and they concluded that not only *in-situ* methods but also *ex-situ* methods through biotechnological tools are required to conserve important medicinal plant species.

Even after three months of a conservation period, *in-vitro* conserved plants of galanga showed 100 per cent survivability in most of the treatments which indicate

that a particular concentration level of the combination of cytokinin and auxin is optimum for the cell division to occur leading to slow shoot growth for three months and similar results were also obtained by Parida *et al*. (2011), where the cultures of *Alpinia galanga* were maintained for two months with regular subculturing and they also continued *in-vitro* conservation further two years and noticed the same multiplication rate ever after two years of culture production. Mohanty *et al*. (2014) concluded that *Curcuma aromatica* and *Kaempferia galanga* were the best plant material for *in vitro* storage and cultures could be conserved even after 12 months.

MS medium supplemented with Kinetin (3.0 mg/l) + NAA (0.5 mg/l) showed significantly high shoot length and the number of shoots per explants and MS medium supplemented with BAP (3.0 mg/l) + NAA (0.5mg/l) showed high root length and number of roots per explants whereas BAP (2.0 mg/l) + NAA (1 mg/l) exhibited the highest number of leaves per explants when compared to other treatments indicating the occurrence of constant but slow cell division up to three months. Hence, MS medium supplemented with the hormonal combination cytokinins along with auxin could be used for short term *in-vitro* conservation of *Alpinia galanga*. Similarly, Pranaykumar *et al*. (2015) and Mastiholi *et al*. (2018) standardized the short term conservation protocol for *Decalepis hemiltonii* and *Salacia chinensis*, respectively for six months. In the same line, *in-vitro* conservation protocols have been standardised for few threatened medicinal plants.

Rajasekharan *et al.* (2009) standardized the *in-vitro* conservation in *Tylophora indica. In-vitro* cultured *plants* were subjected to conservation for six months without subculture with full potential to regenerate, producing viable shoots and nodes and noticed that root production remained unaffected during the conservation and also conserved cultures showed high rooting activity in mannitol and low-temperature treatments. Mohanty *et al.* (2014) carried *in-vitro* conservation studies on nine medicinally and economically important species of Zingiberaceae from Eastern India. *In-vitro* cultured plants were subjected to conservation period of eight to twelve months without subculturing for all species. They found that all the species of Zingiberaceae showed good response on culture media for a minimum period of eight months and they concluded that among all the species of Zingiberaceae, *Curcuma aromatica* and *Kaempferia galanga* were the best material for *in-vitro* conservation which could be conserved up to twelve months. Mastiholi *et al*. (2018) carried *in-vitro* conservation studies in *Salacia chinensis* and they found that, after six months of prolonged storage, *in-vitro* raised plants maintained at 10 ^{0}C showed slower growth in comparison to *in-vitro* plants kept at standard culture condition and concluded that short term conservation is more effective to maintain *in-vitro* cultured plants for prolonged periods without subculture.

Plate 11. Micropropagated plants of *Alpinia galanga* (L.) Willd. kept for *in-vitro* conservation

Plate 12. Comparison of growth for tissue cultured plants in RCC and SCC

5.4.1 Comparisons of growth for tissue cultured plantlets kept under Standard Culture Conditions (SCC) and Reduced Culture Condition (RCC) after three months.

A comparison study between *in-vitro* conserved plantlets in reduced culture condition (RCC) and tissue cultured plantlets in standard culture condition (SCC) in terms of survival per cent, shoot length, number of shoots, number of roots, root length and number of leaves were carried out and cultures exhibited a significant difference between SCC and RCC.

It was found that all the treatments exhibited good response towards the *in-vitro* conservation whereas MS medium supplemented with Kinetin (3.0 mg/l) + NAA (0.5 mg/l) showed highest shoot length and shoots number in both *in-vitro* conserved plantlets and plantlets kept in normal ambient conditions. MS medium supplemented withBAP (3.0 mg/l) + NAA (0.5 mg/l) showed high root length and the number of roots per explants while BAP (2.0 mg/l) + NAA (1 mg/l) exhibited the highest number of leaves per explants. This showed that this particular hormonal combination and concentration might be used for short term conservation as well as normal growth of tissue culture in *Alpinia galanga.*

The results revealed that, after three months of conservation, *in-vitro* raised plants maintained at 10 ^{0}C and low light intensity (2.97 $\mu m^{-2}s^{-1}$) showed slower growth in comparison to *in-vitro* plants maintained at standard culture condition. Reduction in storage temperature is feasible because it provides maximum conservation gain by way of optimal auxiliary bud and shoot formation and *in-vitro* conservation strategies also help in reducing the growth rate of cultures and avoiding frequent subculture (Rajasekharan *et al.,* 2009). Hence, it can be concluded that short term conservation is more useful to maintain *in-vitro* cultured plants for prolonged periods without subculture and these results are in conformity with the results of Mohanty *et al.* (2014) in Zingiberaceae and Mastiholi *et al*. (2018) in *Salacia chinensis* .

Conclusion

Based on results obtained in the present investigation, it can be concluded that among the fourteen accessions evaluated ACC-8 and ACC-10 showed better performance for all the traits studied and these accessions could be used for the further research programme. Healthy rhizome buds inoculated in MS medium supplemented with Kinetin (3.0 mg/l) + NAA (0.5 mg/l) and BAP (3.0 mg/l) + NAA (0.5 mg/l) might be an efficient protocol for *in-vitro* multiplication and *in-vitro* conservation.

Future line of work

1. The accessions can be further assessed in DUS trials.
2. Performance of promising accessions could be tested in mutli locations.
3. Further studies on *in-vitro* propagation and genetic fidelity assessment can be initiated.
4. Chemical and molecular studies may be taken up with molecular primers.

SUMMARY

VI SUMMARY

The present research work on "Morphological characterization, tissue culture and *in-vitro* conservation studies in *Alpinia galanga* (L.) Willd" a threatened medicinal plant was carried out at Division of Floriculture and Medicinal crops, ICAR- Indian Institute of Horticultural Research (IIHR), Hesarghatta, Bengaluru during the year 2019-2020. The salient findings of the investigation are summarized in this chapter.

Alpinia galanga (L.) Willd. plant accessions maintained at RET Field Gene Bank, Division of Floriculture and Medicinal crops, Indian Institute of Horticultural Research (IIHR) were selected to study the morphological characterization with three replication to document the variation present in the accessions for various qualitatitve and quantitative characters.

Fourteen accessions of *Alpinia galanga* were characterized for growth parameters. The analysis of variance revealed highly significant difference among accessions for all the ten characters *viz*., plant height, leaf length, leaf width, leaf area, petiole length, inflorescence length, flower bud size, pedicel length, relative height between androecium and gynoecium and yield per tiller. Genotypic and phenotypic coefficients of variation were high for petiole length, inflorescence length and leaf area. Moderate genotypic and phenotypic coefficients of variation were found in yield per tiller, pedicel length, relative height between androecium and leaf length. This indicated a broad genetic base, less environmental influence and these traits are under the control of additive genes. The result obtained revealed that the estimates of the phenotypic coefficient of variation (PCV) were higher than the genotypic coefficient of variation (GCV) and high GCV and PCV were observed for petiole length, inflorescence length and leaf area. Broad sense heritability values revealed high heritability for characters like petiole length, plant height, leaf length, leaf area and inflorescence length and pedicel length. High genetic advance over mean coupled with high heritability was observed for characters like petiole length, plant height, leaf area and pedicel length. Hence, the present results showed the existing variability among the accessions with respect to these traits is mainly due to the additive type of genes.

The correlation study revealed that the genotypic correlations were higher than the phenotypic correlations indicating the high heritable nature of the traits. The plant characters like plant height, petiole length and leaf widthhad a positive and highly significant correlation with rhizome yield per tiller at the genotypic level, while plant height and petiole length showed a high positive significant correlation with rhizome yield per tiller at the phenotypic level.

Tissue culture was carried out to develop a multiplication protocol using rhizome buds as explants and explants were cultured on MS medium supplemented with various combinations and concentrations of hormones and noticed new shoots formation within an average of eight weeks of inoculation. Among different combinations, satisfactory results were obtained in the combination of Kinetin (3.00 mg/l) + NAA(0.5 mg/l) for shoot length and number of shoots, BAP (3.00 mg/l) + NAA (0.5 mg/l) for root length and number of roots and BAP (2.00 mg/l) + NAA (1 mg/l) for number of leaves.

In-vitro conservation was achieved by providing reduced light intensity (2.9 μm^{-2} s^{-1}) and maintaining a temperature of 10 ^{0}C to reduce the growth and other growth-related traits to conserve it for three months. Rhizome buds were inoculated on MS medium supplemented with various combinations of hormones and *in-vitro* established plants were further kept for short term conservation for three months duration under above mentioned ambient conditions. Among different combinations, satisfactory results were obtained in terms of survival per cent, shoot length, number of shoots and number of leaves by using the various combination and concentration of Cytokinins along with NAA. All the treatment exhibited good response towards the *in-vitro* conservation whereas MS medium supplemented with Kinetin (3.0 mg/l) + NAA (0.5 mg/l) showed the highest shoot length and the number of shoots while MS medium supplemented with BAP (3.0 mg/l) + NAA (0.5mg/l) showed high root length and the number of roots per explant while BAP (2.0 mg/l) +NAA (1 mg/l) exhibited the highest number of leaves per explant in both *in-vitro* conserved plantlets and plantlets kept in normal ambient conditions.

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APPENDICES

VIII APPENDICES

Appendix – I: Monthly meteorological data for the experimental year 2019-20 recorded at the ICAR-IIHR, Bengaluru

Month	Rainfall (mm)	Temperature (^{0} C)		Relative humidity (%)		Evaporation (mm)
		Maximum	Minimum	7 hrs	14 hrs	
June 2019	86.70	30.91	21.28	82.43	62.20	5.81
July	47.65	29.67	20.87	83.39	63.06	5.21
August	198.90	27.98	20.55	87.55	75.42	4.41
September	207.45	28.59	20.58	89.20	70.77	3.63
October	324.20	28.86	19.74	89.94	72.45	4.11
November	15.20	28.74	18.05	86.97	60.57	3.84
December	2.70	27.23	16.70	88.26	67.23	3.09
January 2020	0.00	30.17	14.19	84.42	46.55	4.38
February	0.00	31.46	15.56	77.14	38.83	5.69
March	6.45	33.65	18.18	73.10	37.97	6.79
April	104.00	34.36	19.36	75.60	43.67	6.77
May	74.30	34.52	21.30	81.77	50.48	6.12

Appendix– II: List of symbols and abbreviations

Symbols	Abbreviations
BAP	6 - Benzyl aminopurine
^{0}C	Degree celcius
CD (0.01)	Critical difference at 1 per cent level
CD (0.05)	Critical difference at 5 per cent level
Cm	Centimeter
cv.	Cultivar
et al.	And others
h^2	Heritability
G	Gram
GA_3	Gibberillic acid
GCV	Genotypic Coefficient of Variation
IAA	Indole - 3 - acetic acid
i.e.,	That is
kg	Kilogram
KIN	Kinetin
mM	Milimolar
µM	Micro molar
µl	Micro litre
NAA	Naphthalene Acetic Acid
No	Number
PCV	Phenotypic Coefficient of Variation
Ppm	Parts per million
RCC	Reduced Culture Condition
SCC	Standard Culture Condition
SEd	Standard Error of Deviation
S. Em	Standard error of mean
TDZ	Thiadiazuron
viz.	As follows
%	Per cent
&	And