Chapter 6

Summary

*Zingiber officinale* (Family- Zingiberaceae), commonly called ‘ginger’, is an herbaceous rhizomatous perennial plant commonly cultivated in India. Plants are erect, semi erect, aerial shoots (pseudo-stems) with leaves, have numerous fibrous and fleshy roots that emerge from the branched rhizomes. Since time immemorial, ginger has been regarded to have numerous medicinal properties. Ginger has been cultivated since ancient times. Because of its long history of cultivation, many varieties/cultivars have been made or developed till date. Identification and characterization of ginger germplasm are very important for the conservation and utilization of plant genetic resource. Selection and breeding for the production of desirable traits in this crop have resulted in severe erosion of its genetic base over the time. Lack of proper conservation programmes have caused major reduction in its gene pool, as most of the breeding and conservation programs are still based on conventional morphological and agronomical descriptors, which are dependent on environmental and developmental factors. However, like any other crop, the main objective of ginger breeding is to improve the quantity and quality of the product. This apart, the prediction of the performance of mature ginger based on their evaluation in the early years has not been perfected.

In ginger research the previous era held major emphasis on standardizing parameters of *in-vitro* protocol, such as using a suitable explant, overcoming microbial
contamination, and optimizing media composition combined with growth regulation for better proliferation. Presently, attention is increasingly focused on evaluating field performance of the transformed in-vitro grown whole plantlets. However, there is no stable technique available for in-vitro multiplication of ginger in Sikkim.

In order to stop further reduction of ginger gene pool and to breed for new ginger types with more productiveness; less prone to natural calamities, diseases, as well as higher contain of ginger oil, a thorough knowledge of the existing genetic diversity, in-vitro culture studies and improvement of the existing varieties through various molecular biology, tissue culture and biotechnological techniques is a pre-requisite in ginger research.

Sikkim is known for its ginger production and different varieties. At present, many ethnic tribes inhabit in rural areas of Sikkim, and possibly these different tribes have transferred ginger cultivars to this place during the period of their settlement. Till date, rural people of Sikkim only know the ginger cultivars but lack scientific information and reports on it. However, systematic studies are not available particularly in the area of characterization of ginger germplasm, micropropagation and genetic fidelity study of tissue culture plants. Considering the importance of this cash crop, the present study was designed to address and resolve many problems that have cropped up in ginger cultivation.

6.1 Ecological studies

In order to assess the requirement of specific habitats, ecological studies of ginger in Sikkim Himalaya has been done using Geographic Information System (GIS)
with an overlay technique. The study revealed that the maximum ginger farmers of Sikkim preferred to cultivate *Majhauley* cultivar (35.86 %) followed by *Gorubthangey* (21.51 %), *Bhaisay* (18.14 %), *Charinangrey* (13.92 %) and *Jorethangay* cultivars (10.97 %) in all altitudinal ranges. The least cultivated *Charinangrey* in Sikkim at an altitude range from 4080 to 4790 ft. reflects that this cultivar prefers to specific habitats.

From 237 plots of ginger growing areas studied, 125 plots showed no diseases and 112 plots infected by fungal and bacterial diseases. According to district-wise, only north district of Sikkim showed no infection of diseases and resistant/less susceptible against pathogenic microorganism especially for *Charinangrey* and *Majhauley* cultivars.

Morphological characters on five cultivars of *Zingiber officinale* based on quantitative traits found that plant height, numbers of shoots, shoot diameter, canopy, leafs density, leaf length, numbers of tillers rhizome thickness and weight of rhizome, and qualitative traits like spike bract tip colour and shape of rhizome were important traits for differentiating among five ginger cultivars. Among all the morphological traits, characters, such as, rhizome thickness, no. of shoots and shoot diameter showed important contribution in differentiating different ginger cultivars. Further, many morphological characters are positively correlated, for examples, mean weight of the plant with the mean plant height per plant, mean canopy per plant, mean number of shoots per plant, mean shoot diameter per plant and mean number of leaf density per plant. Other morphological characters that have shown positive correlations are between mean no shoots vs. number of leaf density per plant, mean leaf length per plant vs. mean
spike length per plant. This information would play important role to directly improve rhizome character and yield in ginger.

Among the five cultivars of *Zingiber officinale*, based on ten quantitative and three qualitative morphological characters, cultivar *Charinangrey* differentiates distinctly from other four ginger cultivars. PCA analysis suggests that characters, such as plant height, canopy, numbers of shots, leaf density, spike length and shoot diameter, rhizome shape, spike length and leaf density and rhizome thickness size are important for differentiating five cultivars of *Zingiber officinale*.

**6.2 Tissue culture**

In the present tissue culture experiment, full and half strength MS Medium (Murashige and Skoog, 1962), Gambrog B5, SH (Schenk and Hildebrandt, 1972) and White (White, 1963) medium containing BAP in various concentrations was used for selection of the medium. Out of the five media tested, MS medium gave the best result.

Among the various explants tried, shoot tips explants gave the suitable response for initial growth and the highest number of multiple shoots as compared with root tip and leaf explants of ginger. Various concentrations of mercuric chloride (HgCl$_2$) and sodium hypochlorite (NaOCl) were attempt for aseptic sterilization. Among that concentration 0.1 % HgCl$_2$ and 6 % NaOCl emerged as the best treatment for sterilization.

Shoot initiation was good in MS medium fortified with both growth regulators (cytokinins and auxin) than MS medium fortified with only single growth regulator.
Early initiation of shoot was observed in MS medium containing BAP 2.5 mg/l and NAA 0.5 mg/l which took minimum time 8-10 days. When single growth regulator were used in MS medium the highest number of shoots were recorded in BAP showed as compared to kinetin.

In MS medium, Majhauley, Bhaisay and Charinangrey, Jorethangey and Gorubthangey cultivars showed maximum number of shoots, medium having combination of BAP 2.5 mg/l and NAA 0.5 mg/l with addition of 2 mg/l activated charcoal. This combination of growth hormones in the culture medium was able to produce dark leaf, optimum height and a good number of roots.

Various growth regulators were used in different concentration in the MS medium. Out of that BAP 2.5 mg/l plus NAA 0.5 mg/l gave the maximum number of shoots (19.98) per explant, as compared to Kinetin 2.0 mg/l plus NAA 0.5 mg/l gave (14.15) number of shoots per explant.

In MS medium, with the same hormonal concentration, BAP 2.5 mg/l and NAA 0.5 mg/l gave a good number of roots, this protocol eradicate a step of *in-vitro* rooting.

Three different combination were tried for best hardening media (A) perlite, soil and farmyard manure with ratio of 1:1: B) soil, farmyard manure, sand, perlite at the ratio 1:1:1: C) soil, farmyard manure and sand 1:2:1. Media B gave the maximum survival percentage with better growth resulting as a suitable medium for hardening.

In t-test analysis, single growth regulator medium gave significance difference between and within treatments ($P (T_{<=t})$ two tail = 0.01356997), whereas combination
of growth regulator showed no significance \( (P (T_{<}=t) \text{ two tail} = 0.321765267) \) but the number of shoots were recorded highest in this condition only.

Genetic fidelity of \textit{in-vitro} micropropagated clones of PCR-RAPD examination showed no variations in the micropropagated plants.

\textbf{6.3 Antimicrobial activity}

Total twenty five extracts were examined for antibacterial activity towards five microorganisms (\textit{Staphylococcus aureus} (MTCC 96), \textit{Bacillus subtilis} (MTCC 441), \textit{Klebsiella pneumoniae} (MTCC 432), and \textit{Pseudomonas aeruginosa} (MTCC 424). However, inhibitory activity was not recorded against \textit{Escherichia coli} (MTCC 739).

Chloroform extracts of all the cultivars showed inhibition against the growth of \textit{Staphylococcus aureus and Bacillus subtilis}. The chloroform extract of cultivar \textit{Majhauley} showed widest zone of inhibition of 26 mm and 24 mm.

Various solvents extracts of cultivars of ginger were examined against two gram negative bacteria \textit{Klebsiella pneumonia} and \textit{Pseudomonas aeruginosa}. The chloroform extracts of \textit{Majhauley} showed high inhibition against both the gram negative bacteria with 14 mm and 18 mm inhibition zone, respectively.

The statistically significant difference of one way ANOVA was achieved among all microorganism \( (P < 0.01790) \), gram positive bacteria \( (P < 7.69778E-15) \) and gram negative bacteria \( (P < 1.06101E-09) \). The maximum solvent significant found in acetone with significant value of \( (P < 1.44E-13) \), followed by methanol \( (P < 2.04231E-\)
Among various solvents extracts, acetone extracts was found as the highest significant, followed by methanol, ethanol, chloroform, and petroleum ether extracts. The present investigation showed that there was significant direct correlation between total phenol and total flavonoid content against antimicrobial activity towards gram positive and gram negative bacteria.

6.4 Antioxidant activity

Various solvents were used for estimation of total phenols and flavonoid content of five different cultivars of ginger. Chloroform proved to be the best solvent for extraction of phenol and flavonoid. The highest total flavonoid and total phenol content was found in the chloroform extract of Charinangrey and Majhauley cultivars. The differences in total phenolic content among five cultivars of gingers used were statistically significant (P < 0.0000661) whereas flavonoid contents were not significant (P < 0.348). Among all 25 extracts the highest DPPH scavenging activity found in acetone extracts of Majhauley cultivar, the highest reductive capability of the transformation of Fe$^{3+}$ to Fe$^{2+}$ in presence of the extract found in Chloroform extracts of Charinangrey, and highest H$_2$O$_2$ scavenging activity found in methanol extracts of Bhaisay.
6.5 Genetic diversity study of five cultivar gingers using RAPD

The results showed the prevalence of a relatively high level of polymorphism in the cultivars of ginger found in Sikkim Himalaya. A total of 104 clear, reproducible and scorable RAPD fragments ranging from 150–13000 bp were generated from 21 primers. Of the 104 scorable RAPD bands, 99 were found polymorphic. Among five cultivars the highest percentage of polymorphic loci, gene diversity and Shannon’s diversity index observed in Majhauley, Gorubthangey and Bhaisay cultivar respectively. Out of the five cultivars of ginger, Gorubthangey and Bhaisay found more diverse while Gorubthangey and Majhauley showed similarity. The cultivars Jorethangey and Bhaisay also showed similarity.