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Estimation of Genetic Diversity among Five Cultivars of *Zingiberofficinale*Rosc.(Ginger) of Sikkim Himalaya using RAPD Marker

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Abstract

Problems: Molecular information of ginger germplasm of Sikkim varieties is essentialfor characterization and protection of Sikkim elite ginger germplasm.
Experimental approach: The present investigation was carried out for analysis of genetic diversity among five varieties of ginger through RAPD markers.
Finding: Total 104 clear, reproducible and scorable fragments ranging from 150-13000 bp were generated from 21 primers and 99% were found polymorphic. Cluster analysis using UPGMA algorithm placed the 40 accessions into three main clusters,the genetic dissimilarity matrix between genotypes ranged from (0.74994-5.33209) between the 5 cultivars studied**.**The extent of genetic diversity among these cultivars was computed through parameters of gene diversity, sum of allele numbers per locus and Shannon's information indices.Dendrogram was constructed using NTSYSpc, indicating the genetic diversity between five cultivars of ginger using a Jaccard's coefficient. Cluster analysis of data using 40 accessions into four main clusters the genetic with a range (0.35-0.87), the principal component analysis placed into four groups.**Conclusion:** The prevalence of a relatively high level of polymorphism in the cultivars of ginger will help to breeders for ginger development breeding program.
Keywords: Ginger**,**Cultivar**,** Sikkim, RAPD, Majhauley**.**

Introduction

*Zingiberofficinale*Roscoe (Ginger) is an economically important tropical cash crop of the world, which has no exception for Sikkim, India. It is monocotyledon and belongs to familyZingiberaceae, having chromosome number 2n=22 (Raghavan and Venkatasubban, 1943). Ginger is a rhizomatous and subterranean stems and prefers to grow on red soils having pH from 5.5 to 6.5 an altitude ranging upto to 5000ft. Cultivation of ginger was started since ancient times but its origin has remained obscure yet. Dahlgren et al., (1985) considered it to be originated in eastern India. However, there are many countries like Australia, Bangladesh Jamaica, Nigeria, Sierraleone, Brazil, China and Indonesia have been cultivating ginger as a commercial cash crop for a long period of time. In India, ginger is cultivated in almost all the tropical and subtropical parts, especially in Kerala, Tamil Nadu, West Bengal, Bihar, Sikkim Himalchal Pradesh, Uttar Pradesh and Maharashtra.

Gingers have high medicinal properties because of its ginger oil, which contains 72 components in the volatile oil (Miyazawa and Kameoka, 1988). Major components are zingiberene, bisabolene, sesquiphellandrene, curcumene etc. (Ghorab et al., 2010). It is used in a natural dietary component which shows high antioxidant and anti-carcinogenic properties (Manju and Nalini, 2005), rich in secondary metabolite such as oleoresin (Sakamura et al., 1996),gingerol has anti inflammatory and chemopreventive properties (Kim et al., 2005). In Asian countries, it has been used for relieving from arthritis, rheumatism, coughs, fever and infectious diseases and bio pesticide as an anti-fungal activity (Okigbo, 2005**).**

In Sikkim, ginger is cultivated since ancient period for religious practices and culinary and medicinal purposes by LepchaBungthing, Raj Bijuwas and LimbooPhedangma (folk healer) indicating its attachment with the indigenous people of Sikkim (Rai et al., 2009). It is grown as the third important cash crop of Sikkim Himalaya region followed by *Ammommumsubalatum* and *Citrus reticulatae,* as it is annual crop and required very less concern with high economic return. It is widely cultivated in all the region of Sikkimupto the altitude of 4500 ft. and has readymade market in the village level. Five cultivars varieties of *Zingiberofficinale*Rosc are *Bhaisay, Gorubthangey, Jorethangey, Charinangrey, Majhauley* reported from Sikkim and Darjeeling hills (J.R. Subba, 1984***)***.

The most important role of conservation is to preserve the genetic variation and evolution process in viable populations of ecologically and commercially viable varieties genotypes in order to prevent potential extension (Palai and Rout, 2007). Identification and characterization of ginger germplasm (varieties) are very important for the conservation and utilization of plant genetic resource. Certain amount of work is available on identification of different varieties of ginger in Orissa and Coimbatore, India (Rout 2007, Harisaranraj, 2009).

The germplasm of Sikkim varieties of ginger are also seriously affected by fungal and bacterial disease and breeding of ginger is badly affected by poor flowering and seed set, the conservation of ginger varieties and building up molecular data is very necessary for improvement of ginger varieties of Sikkim.

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 thus reflecting the base of the gene pool with no true genetic relatedness (Green, 1971). The breeding of experimental planting material of a perennial, heterozygous crop like ginger is difficult. However, like any other crop, the main objective of ginger breeding is to improve the quantity and quality of the end product. The methods of introduction, selection, and hybridization have been used with success for ginger improvement. The different varieties have been developed to suit the requirements of the various agroclimatic regions. However, proper selection criteria have not yet been established. This apart, the prediction of the performance of mature ginger based on their evaluation in the early years has not been perfected. Application of modern techniques, as has been done in other crops, is a greater challenge to ginger breeders and ginger biotechnologists. In order to stop further reduction in its 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, and improvement of the existing varieties through molecular biology and biotechnological techniques is a pre-requisite in ginger research.

Morphologicalcharactersarenotreliabletoclassify thetypes,although some of the typescanbedistinguishedtoa certainextentfromrhizome charactersNybeand Nair(1982).Biochemical markers suchas total gingerol, shogaol, polyphenolcontent etc. areused to identifythesuperiorgingerplant. (Ravindranet al.,(1994); Manmohan das et al., (2000). However,gingerbreedersareoftenunabletousethesemarkerseffectively becausethey aregreatly influencedby environmentalfactorsandshow a continuousvariationwitha highdegree of plasticity. Hence,toovercomethese problems,researchhasshiftedtousing moresensitive DNAmarkertechnology.Molecularmarkersveryefficiently enhancemorphological,cytological,and biochemicalcharactersin germplasmcharacterization,varietalidentification,clonalfidelity testing,assessmentofgenetic diversity, validation ofgenetic relationship, phylogenetic andevolutionarystudies etc. (Williamset al.,1990). It is reported that molecular marker is the best approach for identification of plant genotypes than seems to be more effective as it allows direct access to the hereditary material unlike the morphological marker (Paterson et al., 1991). RAPD (Random amplified polymorphism of DNA) marker guide on useful information at various levels include as population structure, gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding level of gene phylogenetic relationship and identification of plant genotypes of clonal organism (Bardakei et al., 2001). RAPD is a very easy, reliable and economic tool to study intraspecific polymorphism in plants. Few scientific reports are available using molecular markers in germplasm characterization of ginger (Nayak et al., 2005;Palai and Rout, 2007). RAPD markers are also used in identification of resistant varieties of crops or fruit plant Williams et al., 1990.

In the present communication RAPD was used to study the genetic variation among 5 ginger varieties grown in 40 different places including all districts of Sikkim. It is the first time in Sikkim to characterize a sample of ginger used for the study from all the district ofSikkim. Since

cultivation of the same varieties of ginger for long period of time may lead to the genetic erosion, it is necessary to study the available diversity and introduce new variability as well for planning breeding programs.

Material and Methods

Plant material

The investigation was conducted at the Biotechnology Department, Sikkim State Council of Science and Technology, Sikkim. The experimental material comprised of five differentgenotypes of ginger mainly Bhaisay, Gorubthangey, Jorethangey, CharinangreyandMajhauley of ginger maintained in the field gene bank of Sikkim State Council of Science and Technology, RumtekSajong.

DNA extraction

Fresh ginger rhizomes leaves were used for the isolation of DNA. The genomic DNA was isolated by CTAB method (Pradhan et al., 2012). The extraction buffer contains 3% CTAB, 2 M NaCl, 100 mM Tris, 20 mM EDTA and 0.1% ß mercaptoethanol, 70% ethanol and 10mM ammonium acetate.

PCR Amplification

RAPD PCR reactions were generated using 63 different decamers (Clonitech technologies)for screening of primer using five clones of micropropagated ginger which were randomly picked, to determine the suitability of each primer for the study. The best conditions used for 45 cycles for single primer were asfollows: 94ºC for 4 min, 35º C for 1 min and 72ºC for 2 minuteswith an initial denaturation temperature at 94ºC for 1 min and anfinal extension at 72ºC for 5 minutes. All reactions wereperformed in a thermal cycler (Applied Biosystem 2070)asdiscussed by Williams et al.,1990. The total reaction mixturecontained 12.5µ l master mix (Promega), 10µ M primer, (DNAsample was diluted at 1:10 ratio), 1.0 units of TaqDNAPolymerase (Promega), 20mM $MgCl_2$and adjusted to finalvolume of 25µ l with nuclease free water (Thermo scientific,2D8611).

Electrophoresis of PCR products

All the amplified DNA were analyzed by gelelectrophoresis taking 5µ l of PCR product in a 1.5% agarose gelat 8V/cm for 90 min with 1X TBE buffer, visualized afterethidium bromide staining and photographed using gel documentation system (Gel Logic 200 imaging system)

Amplified DNA Marker Scoring

Amplified products in gels images were scored in thebasis of presence (1)and absence (0)of bands images visualizedunder UV light (Kodak Molecular Imaging V. 45.1) individually.The amplified bands were named according to the number ofprimer and size (base pair)of the band was measuredapproximately with standard DNA marker. The bands showinglow visual strength/ intensity regarded as negative control and they are not included for analysis.All PCR reactions were run at least thrice and only reproducible and clear bands were scored and aligned by diversity data base software (NTSYSpc and POPGENE freeware).The POPGENE freeware

(Yeh et al., 1997) was used to partition genetic diversity among the 40 sample. The same software was used to calculate genetic distances and similarities between populations and to draw a dendrogram based on Nei's (1972) genetic distances using the UPGMA to show the genetic relations between the different cultivars. Jaccard's coefficient similarity wasmeasured and a dendrogram based on similaritycoefficients generated by the n-j method was obtained.The relationship among the individual plants was further assessed by Principal Component Analysis (PCA).

Results and Discussion

Study of diversity of ginger in Sikkim using RAPD analysis
Genetic variation and its relevance to agronomy

Molecular study using universal markers, such as, RAPD can help in characterization and identification of plant germplasm. To assess the genetic variation in five (5) cultivars of *Zingiberofficinale*(ginger), (**Table 1**) a total of 63 RAPD primers were screened, out of which 21 primers showed consistent bands. These 21 primers were used for further amplification. A total of 104 clear and reproducible and scorable RAPD fragments ranging from 150–13000 bp were generated from 21 primers. Of the 104 scorable RAPD bands, 99 were found to be polymorphic. Each primer was tried thrice and the results were reproducible.

The number of polymorphic bands per primer (Clonitech Technologies) ranged from two in CL-80 and Cl-75 to thirteen in CL-28, with an average of 4.95 polymorphic bands per primer (**Table 2).** Selection of polymorphic alleles was carried out in a careful manner and only the clear, repeatable and polymorphic bands were scored and used in for statistical analyses (**Figure 1, A-D**). The percentage of polymorphic bands ranged from 50% to 100%. Cultivars, such as, CL-182, CL-192, CL-80, CL-23, CL-1, CL-11, CL-25, CL-40 etc. (**Table 2**) revealed 100% polymorphism, while CL-76 showed 75% polymorphism. Cultivar (CL-105) showed 50% polymorphism, followed by CL-75, CL- 54 with 66% polymorphism (**Table 2**). Cultivar (CL-28) showed the highest number of bands (13) and the CL-188 produced only one band (Table 2). For all primers, the average polymorphism was recorded 95.20 %, however, a few primers (**Figure 2 and 3**) also showed 100% polymorphism among the varieties owing to their self incompatibility, which suggests a highly heterogeneous plants and high genetic variation within *Zingiberofficinale*.

Diversity measures were calculated using Nei's (1973) index, which ranged from h = 0.04 to h = 0.09 with a mean value of 0.08 (**Table 3**). The high matrix value indicated that the cultivars considered in the study are distantly related to each other. The percentage of polymorphic loci ranged from 38.46 % in Bhaisay to 82.69 % in Majhauley cultivars with an average (73.08%) polymorphic locus of per population (**Table 4**). The observed number of alleles (na) per locus varied from 1.3846 ± 0.4889 in Bhaisay to 1.8269 ± 0.3801 in Majhauley with an average value of 1.6731± 0.4714. The effective number of alleles (ne) per locus ranged from 1.2532 ± 0.3757 in Bhaisay to 1.4010 ± 0.3857 in Gorubthangey with an average of 1.3721± 0.3739 effective number of allele per cultivars. The lowest Nei's gene diversity (h) was recorded in Bhaisay

(0.1434 ± 0.1992), whereas the highest diversity was observed in Gorubthangey (0.2329 ± 0.1979) with an average gene diversity value of 0.2322 ± 0.1681 per cultivars.Bhaisay cultivars recorded the lowest Shannon's diversity index (0.2120 ±0.2849), while Majhauley cultivars recorded maximum (0.2120 ±0.2849) with a mean value (0.3329 ± 0.2849) per cultivar (**Table 5**).

Genetic differentiation within cultivars were observed based on Nei's original measures of genetic identity and genetic distance, in which genetic identity values measure genetic similarity between cultivars sample pairs. High degree of variability in genetic identity and genetic distance values were observed among the cultivars of gingers (**Table 5and Figure 4**).

In pair wise comparisons of Nei's original measures of genetic identity within cultivars, smaller values were observed between SKG 2 (Gorubthangey) and SKG 5 (Bhaisay) cultivars exhibited genetically most diverse cultivars (0.8978), whereas SKG 2 (Gorubthangey) and SKG 3 (Majhauley) cultivars recorded genetically most similar (0.9815).

The genetic variations using RAPD markers have been studied in a number of economically important plants (Rout et al., 1998; Pattanayak et al., 2011). The present investigation comprises the classification and genetic differentiation of five cultivars of *Zingiberofficinale*of Sikkim Himalaya. Based on the RAPD profile, measures of Shannon's index of diversity were from 0.21 to 0.36 with an average value of 0.33 (**Table 5**). The results show the prevalence of a relatively high level of polymorphism in the cultivars of ginger from Sikkim Himalaya. This is a fact that sample size and the total amount of bands can influence the percentage of polymorphic bands, other parameters, such as, Shannon's index of diversity and Simpson's index of diversity are more suitable in estimating genetic variability (Cruzan, 1998). The result is in agreement with the high percentage of polymorphism as recorded by Nei's genetic diversity index. The genetic relation using RAPD markers provides reliable methods for identification of varieties than morphological characters (Paterson et al., 1991). Routet al.,(2007) analyzedgeneticfingerprinting among eightvarietiesof*Zingiberofficinale*using RAPDmarkers,theinvestigationshowedthedistantvariationwithinthevarieties,similar result was obtained by(Harisaranrajet al.,2009) within the eight varieties ofgingerofOrissa. Similarly, Pattanayaket al.,(2011)assayedforty ninegingerclonescultivatedinNorthEastIndiausing RAPDmarkers reported highpolymorphismdetectedinacultivatedspecies.Nayaket al., (2005) reported significant genetic variations among 16 elitecultivarofgingers using cytological and RAPD markers.

Genetic relationships and the germplasm conservation

Genetic distance refers to the genetic deviation between species or between populations within a species. It is measured by a variety of parameters likeNei's standard genetic distance. This measure assumes that genetic differences arise due to mutations and genetic drift,Nei's D_A distance. This distance assumes that genetic differences arise due to mutations and genetic drift, but this distance measure is known to give more reliable population trees than other distances particularly for DNA data. Similarity indices measure the amount of closeness between

two individuals, the larger the value the more similar are the two individuals. There is a variety of alternative measures for expressing similarity, like Jaccard's coefficient of similarity which can be used for binary data and often is applied in RAPD-based studies. This coefficient is based on number of positive matches between two individuals whereas joint absences are excluded. Dissimilarity coefficients instead estimate the distance or unlikeness of two individuals, the larger the value the more different are the two individuals. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. Genetic distance can be used to compare the genetic similarity between different species. Genetic diversity studies helps in formulating proper conservation, preservation and selection planning which helps in interpreting the present status and future prognosis, particularly of the endangered and rare species.

The dendrogram (**Figure 4**)of the genetic relationships among the cultivars of *Zingiberofficinale*of Sikkim was constructed based on the Nei's genetic distances and the UPGMA method (**Figure 4**). All samples showed three main clusters, viz., (1) Cluster I representing Charinangrey, Gorubthangey and Majhauley, Cluster II representing Jorethangey and Cluster III representing Bhaisay. The clusters revealed the level of relatedness (0.74994 – 6.26042) between the 5 cultivars studied(**Figure 4**).

The first cluster further divided into two sub-groups. Sub-group one (1) showed only one cultivar "Charinangrey" and the sub-group two (2) clubbed two cultivars Gorubthangey and Majhauley, while other two Clusters II and III showed separate cultivars Jorethangey and Bhaisay (**Figure 4**).

In the first cluster, cultivar Charinangrey separated distinctly with cultivars Gorubthangey and Majhauley, which may be due to the fact that traditionally ginger is classified on the basis of morphological features; for example, Charinangrey (bird's nail) itself is small in size. These morphological features are subjected to substantial environment changes coupled with extensive cryptic mutation led to similarity in genetic makeup of the cultivars. The other two cultivars Gorubthangey (name of place)and Majhauley (medium in size) showed close similarity, which may be due to the reason that traditionally Majhauley is a subgroup of Gorubthangey and named according to the size of ginger. These cultivars possessed a high genetic identity (0.9815) and some degree of genetic differentiation (0.0488). Jorethangey and Bhaisay formed two distance clusters of their own and possessed a high genetic identity (0.9116) with some degree of genetic differentiation (0.0845) as recorded in (**Table 3**). The above information can play a very important role in conservation, selection and preservation of different cultivars of ginger in Sikkim Himalaya.

The same clusters were obtained in the dendrogram when the data were analyzed using NTSYSpc (**Figure 5**), which is in complete agreement with the earlier reports of ginger taxonomy. Dendrogram based on NTSYSpc analysis differentiated into five cultivars into four clusters with a Jaccard's coefficient of 0.35-0.87 **(Figure 5**). Cluster I represented the cultivar

Charinangrey, which was further divided into two sub-groups. Subgroup I (one) included Gorubthangey and sub-group II (two) included Majhauley.

Based on NTSYSpc analysis differentiated into five cultivars into four clusters with a Jaccard's coefficient of 0.35-0.87 (**Figure 5**) and occurrence of three cultivars in the same group indicating that habitat homogeneity between three cultivars (Charinangrey, Majhauley and Gorubthangey), which is in agreement with the reports of Pattanayak*et al.,* (2011). Cluster II and III comprised of Jorethangey and Bhaisay. But in the case of Cluster IV included single cultivar Bhaisay (SGK 22). The possible for such separate group of the sample SKG 22 may be due to geographical bias or cryptic mutation. Other studies are also in agreement with the present results (Kizhakkayil and Sasikumar, 2010). Cultivars/genotypes that form different clusters are potential germplasm that may be exploited to broaden the genetic base (**Figure 5**).

Principal component analysis of five cultivars of gingers

As the individuals plants are collected from different region of Sikkim. PCA was performed to confirm similarity and diversity among the individual clones. The present finding of PCA **(Figure 6)** were similar to the cluster analysis showing comparable topology. High genetic diversity was recorded among the clones from Sikkim, India. Grouping pattern did not show any considerable dissimilarity except the clones SKG 13 and SKG 3 were classified as separate groups.Results of PCA showed high genetic diversity were recorded among the clones from Sikkim India. Similar investigation also reported by Jatoi et al., (2008) against different *Zingiberaceae*genus and observed higher diversity within *Z. officinale* and other genus. Sanjeev et al., (2011) also reported high diversity among the clones collected from India. Similarstudy wascarriedoutbyWatanabeet al*.,* (2006)usingriceSSRmarkerasRAPD markerforgeneticdiversityanalysisin*Zingiberaceae*.Theyreportedthathighvariationwas found among ginger, turmeric andgalangal species. Jainget al., (2006)reported high geneticdiversity usingphylogeneticanalysisandmetabolicprofiling among andwithingingerspeciesandresultfoundthatgingersvariantfromdifferentgeographical origins wereindistinguishable.

Conclusion

Ginger (*Zingiberofficinale*Roscoe) has been used since ancient time as a food, spices and medicinal purposes.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 Majhauleyshowed similarity. The cultivars Jorethangey and Bhaisayalso showed similarity.This present investigation showed that thedendrogram shows the distant variation within the five varieties. The genetic diversity in a gene pool is usually considered as being the major resource available to breeders for ginger improvement program.

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Table 1 Sources and population of the ginger clones used in the study.

Sl. No	*Cultivar variety* Zingiberofficinale	*Given code*	*Altitude*	*Latitude*	*Longitude*	*Location*
1.	Majhauley	SKG 1	2725 ft	N 27 20"00.9'	E088 27"01.5 '	Menglee
2.	Charinangrey	SKG 2	4106 ft	N 27 19"45.3'	E088 27"34.8 '	Mangzing
3.	Majhauley	SKG 5	3486 ft	N 27 14"25.6'	E088 26"23.4 '	TarkuTanak
4.	Charinangrey	SKG 6	1991ft	N 27 16"14.4'	E08830"35.6'	Sudhur1
5.	Charinangrey	SKG 8	1172 ft	N27 28" 00.0'	E08836"37.0'	Sudhur 2
6.	Charinangrey	SKG 9	1253 ft	N27 21"21.1'	E08830"0.5'	Mangten
7.	Gorubthangey	SKG 11	1470 ft	N2728"30.4'	E08830"20.7	Hegyathang1
8.	Majhauley	SKG 12	1620 ft	N27 28" 6.0'	E088 29" 55.9'	Hegyathang2
9.	Charinangrey	SKG 13	4793ft	N27 28"50.5'	E088 29"33.3'	Ravang1
10.	Charinangrey	SKG 14	4897ft	N27 28"50.5'	E088 29"33.3'	Ravang2
11.	Gorubthangey	SKG 15	2843 ft	N 27 09.273"	E 088 18.137"	Chisopani1
12.	Gorubthangey	SKG 16	3133 ft	N 27 09.026"	E 088 18.064"	Chisopani2
13.	Gorubthangey	SKG 17	3021 ft	N 27 05.732"	E 088 17. 970"	Babatey
14.	Majhauley	SKG 18	2933 ft	N 27 08.742"	E 088 17. 970"	Chisopani3
15.	Majhauley	SKG 19	2794 ft	N 27 09 .356"	E 088 18.224"	Sadam
16.	Majhauley	SKG 20	1151ft	N 27 11'3.74"	E 088 28'13.85"	Sadam
17.	Majhauley	SKG 21	4767 ft	N 27 07.223"	E 088 25.312"	Sadam
18.	Majhauley	SKG 22	4975 ft	N 27 07.018"	E 088 25.359"	Sadam
19.	Majhauley	SKG 23	3495 ft	N 27 9'38.23	E 88 24'28.62	Bhangjayang
20.	Majhauley	SKG 24	3544ft	N 27 10 38.67	E 88 20' 57.91	Kamrang
21.	Gorubthangey	SKG 25	3417 ft	N 27 11.939"	E 88 40.925"	Lower
22.	Bhaisay	SKG 26	3217 ft	N 27 12.284"	E 88 40.562"	Lower
23.	Gorubthangey	SKG 27	3825 ft	N 27 13.265"	E 88 40. 577"	Gangtok
24.	Gorubthangey	SKG 28	4274 ft	N 27 11.760"	E 88 40.760"	Middle
25.	Gorubthangey	SKG 29	4223 ft	N 27 11.902"	E 88 40.317"	Upper
26.	Gorubthangey	SKG 30	4138 ft	N 27.11.117"	E 88 40.382"	Aritar
27.	Gorubthangey	SKG 32	3875 ft	N 27 11 924"	E 88 40.385"	Middle
28.	Gorubthangey	SKG 33	3487 ft	N 27 11 .939"	E 88 40 924"	Pakyong
29.	Gorubthangey	SKG 34	3417 ft	N 2711.939"	E 88 40.925"	PakyongSudungl
30.	Jorethangey	SKG 35	3038 ft	N 27 12.248"	E 88 40.908"	Gangtok
31.	Jorethangey	SKG 36	5020ft	N 27°10'5.94"	E 88°11'52.42"	Sombarey
32.	Jorethangey	SKG 37	5094 ft	N 27°10'7.33"	E 88°11'44.56"	Tashiding
33.	Jorethangey	SKG 38	5618 ft	N 7°10'28.89"	E 88°11'46.47"	Tashiding
34.	Jorethangey	SKG 39	4850 ft	N 27° 9'57.65"	E 88°11'58.35"	Mangalbarey 1
35.	Jorethangey	SKG 40	4580 ft	N 27° 9'45.06"	E 88°11'57.45"	Gayshing
36.	Jorethangey	SKG 41	4586 ft	N 7°17'28.61"	E 88° 9'3.09"	Okhrey
37.	Bhaisay	SKG 42	4333 ft	N 7°17'21.24"	E 88° 9'12.36"	Mangalbarey 2
38.	Bhaisay	SKG 43	5046 ft	N 27°17'0.17"	E 88° 8'48.61"	Okhrey
39.	Bhaisay	SKG 44	5046 ft	N 27° 8'12.98"	E 88° 8'43.94"	Tashiding

40.	Bhaisay	SKG 45	4732 ft	N 27° 8'13.72"	E 88° 8'53.43"	Okhrey

Table 2 Frequency of polymorphic bands generated by RAPD primers

Sl.No	Name of Primer	Sequence 5'-3'	Total No. of bands	No. of poly bands	Size range (Kb)	Polymorphic bands (%)
1.	GCC76	-GAGCACCAGT-	4	3	0.45-1.30	75
2.	GCC 182	-GTTCTCGTGT-	5	5	0.25-1.05	100
3.	GCC 192	-GCAAGTCACT-	3	3	0.25-0.65	100
4.	GCC 80	-GGGCCACGCT-	2	2	0.75-0.80	100
5.	GCC 23	-CCCGCCTTCC-	4	4	0.30-0.75	100
6.	GCC 1	-CCTGGGCTTC-	3	3	0.15-0.50	100
7.	GCC 11	-CCCCCCTTTA-	6	6	0.20-0.85	100
8.	GCC 25	-ACAGGGCTCA-	5	5	0.20-0.60	100
9.	GCC 40	-TTACCTGGGC-	5	5	0.10-0.85	100
10.	GCC 75	-GAGGTCCAGA-	3	2	0.15-0.40	66.66
11.	GCC 105	-CTCGGGTGGG-	2	1	0.15-0.30	50
12.	GCC 112	-GCTTGTGAAC-	7	7	0.15-0.65	100
13.	GCC 54	-GTCCCAGAGC-	6	4	0.30-0.90	66.66
14.	GCC 30	-CCGGCCTTAG-	5	5	0.15-0.50	100
15.	GCC 53	-CTCCCTGAGC-	9	9	0.05-0.85	100
16.	GCC 28	-CCGGCCTTAA-	13	13	0.05-0.85	100
17.	GCC 197	-TCCCCGTTCC-	7	7	0.20-0.85	100
18.	GCC 188	-GCTGGACATC-	1	1	0.125	100
19.	GCC 64	-GAGGGCGGGA-	3	3	0.30-0.60	100
20.	GCC 67	-GAGGGCGAGC-	4	4	0.25-0.55	100
21.	GCC 78	-GAGCACTAGC-	7	7	0.15-0.75	100
22.	Grand Total		104	99		95.19
			4.95 average band per primer			

Table 3 Nei's genetic distance and genetic identity among different cultivars of *Zingiberofficinale* of Sikkim Himalaya

No	SKG1	SKG2	SKG3	SKG4	SKG5
SKG1	****	0.9524	0.9514	0.9190	0.9121
SKG2	0.0488	****	0.9815	0.9142	0.8978
SKG3	0.0498	0.0186	****	0.9042	0.8744
SKG4	0.0845	0.0898	0.1007	****	0.9116
SKG5	0.0920	0.1078	0.1342	0.0925	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal). (SKG1, Charinangrey; SKG2, Gorubthangey; SKG3 Majhauley; SKG4 Jorethangey; SKG4 Bhaisay)

Table 4 Five different cultivar populations showing polymorphic loci and the percentage

Sl. No	Cultivars (*Zingiberofficinale*)	Polymorphic loci	Percentage of Polymorphic loci
1.	Charinangrey	54	51.92 %
2.	Gorubthangey	76	73.08 %
3.	Majhauley	86	82.69 %
4.	Jorethangey	70	67.31 %
5.	Bhaisay	40	38.46 %

Table 5 Estimation of genetic diversity parameter for the different cultivars of *Zingiberofficinale*from Sikkim Himalaya based on RAPD markers

Population	n	np*	%p*	na*	ne*	h*	I*
Charinangrey	6	54	51.92	1.5192 ± 0.5020	1.3416 ± 0.3943	0.1947± 0.2082	0.2878 ± 0.2966
Gorubthangey	12	76	73.08	1.7308 ±0.4457	1.4010 ± 0.3857	0.2329 ± 0.1979	0.3518 ± 0.2735
Majhauley	10	86	82.69	1.8269 ± 0.3801	1.3717± 0.3250	0.2322 ± 0.1681	0.3638 ± 0.2310
Jorethangey	7	70	67.31	1.6731 ±0.4714	1.3721± 0.3739	0.2195 ± 0.1943	0.3329 ±0.2727
Bhaisay	5	40	38.46	1.3846 ±0.4889	1.2532 ± 0.3757	0.1434 ± 0.1992	0.2120 ±0.2849

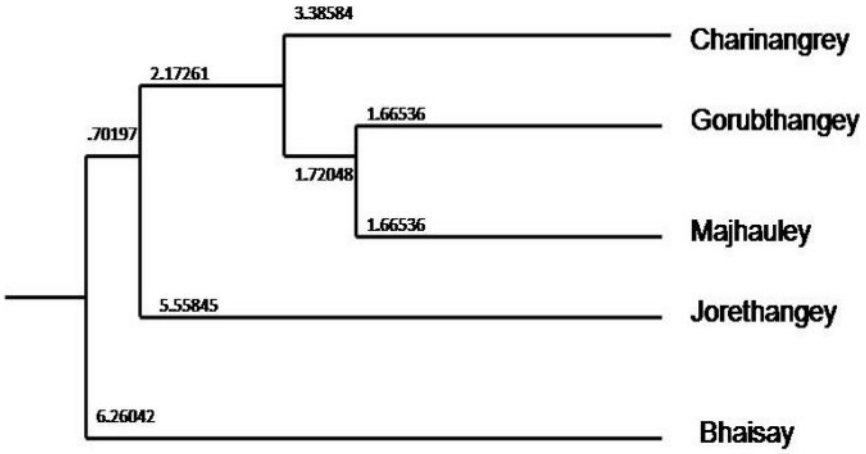

Figure 4 UPGMA dendrogram based on RAPD data showing relationships among different cultivars of the *Zingiberofficinale*. Numbers at branch points represent bootstrap values with 1000 replications.

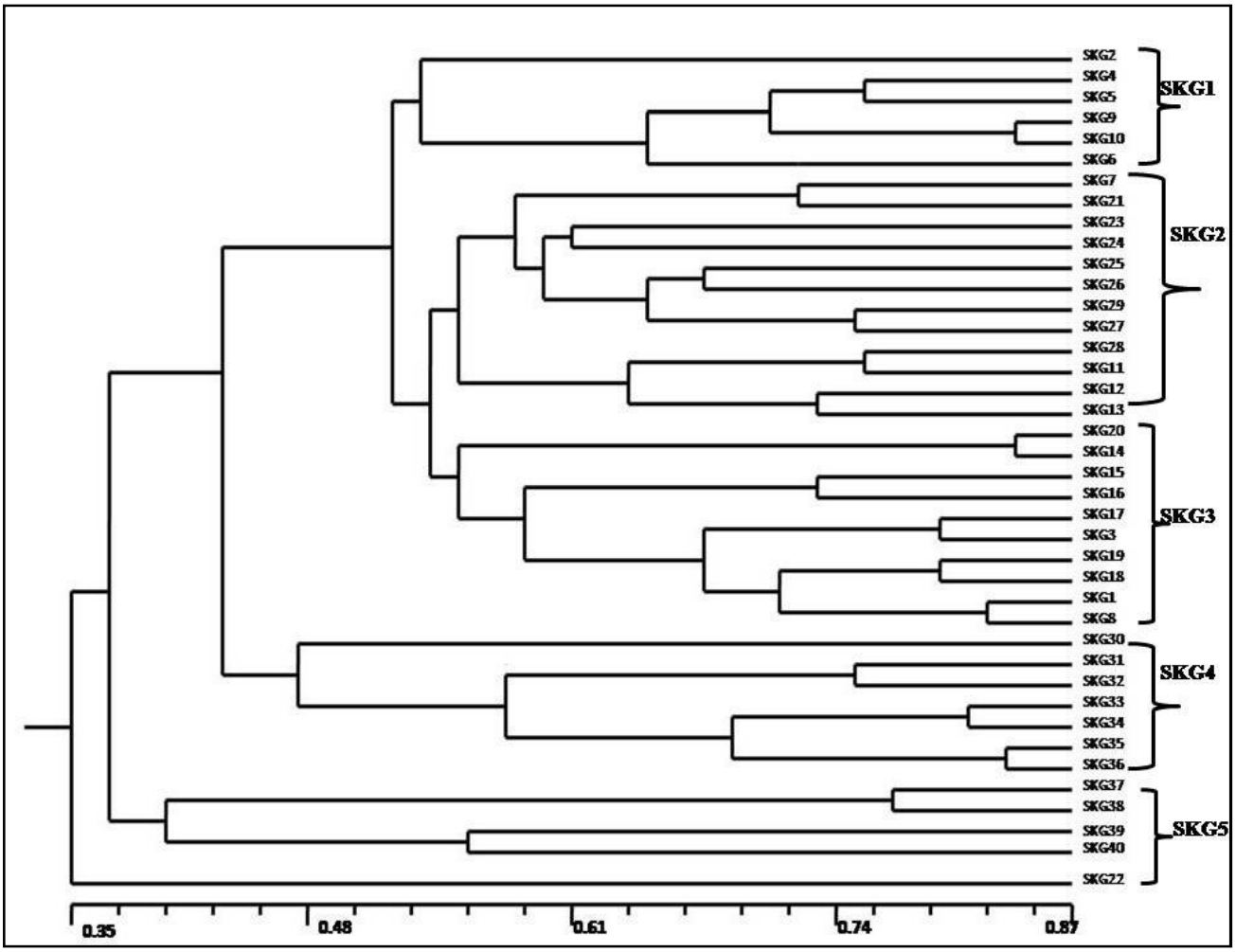

Figure 5 Dendrogram based on RAPD markers, indicating the genetic diversity between five cultivars of ginger (*Zingiberofficinale*) of Sikkim. The horizontal axis represent genetic similarity coefficient (Jaccard 1908). The codes (SKG 1– SKG 40) indicate an individual genotype and corresponds to those listed in Table 4. SKG1, Charinangrey; SKG2, Gorubthangey; SKG3 Majhauley; SKG4 Jorethangey; SKG4 Bhaisay)

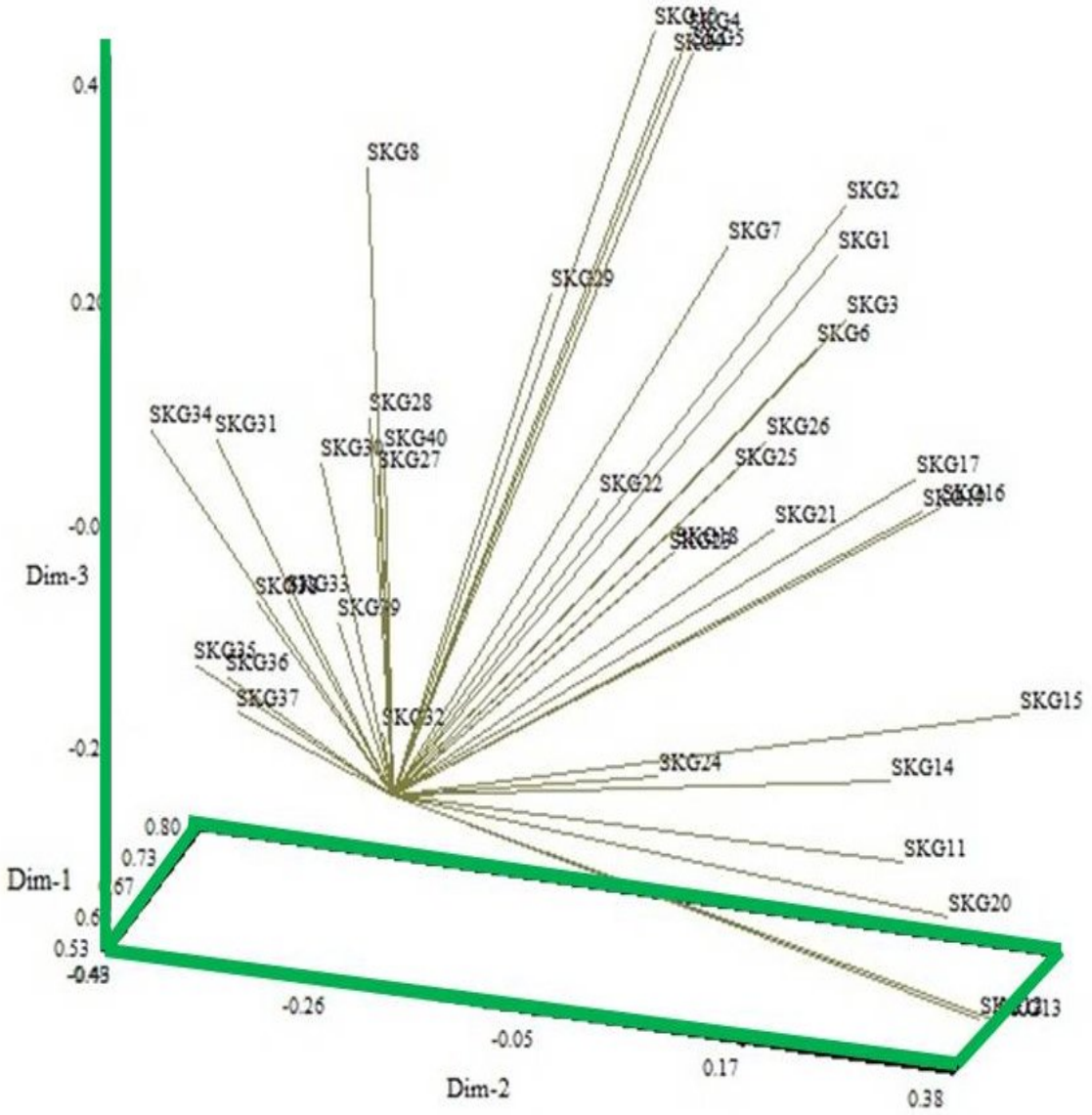

Figure 6 Principal Component Analysis showing grouping of ginger scatter plants individual on a three dimensional scatter plot**.**

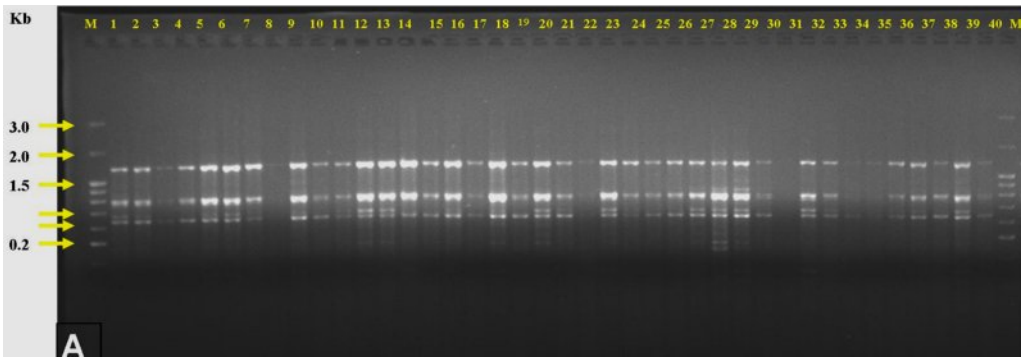

Figure 1 A Representative profiles amplified with CL 11 RAPD primer. M is the 100 bp molecular marker (Fermentas, Life Science, Germany with size in bp indicated on the left). 1- 40 indicate the fourty different genotypes of *Zingiberofficinale*of Sikkim Himalaya

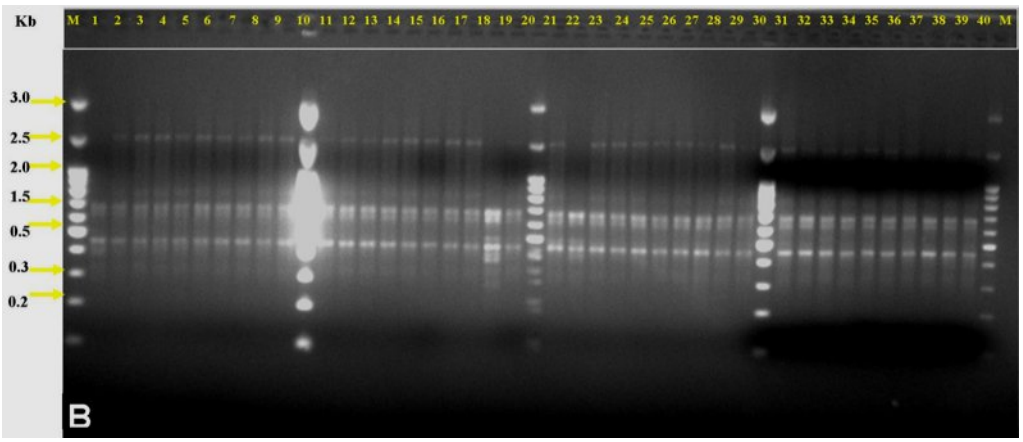

Figure 1 B Representative Profiles amplified with CL 53 RAPD primer. M is the 100 bp molecular marker (Fermentas, Life Science, Germany with size in bp indicated on the left). 1- 40 indicate the fourty different genotypes of *Zingiberofficinale*of Sikkim Himalaya.

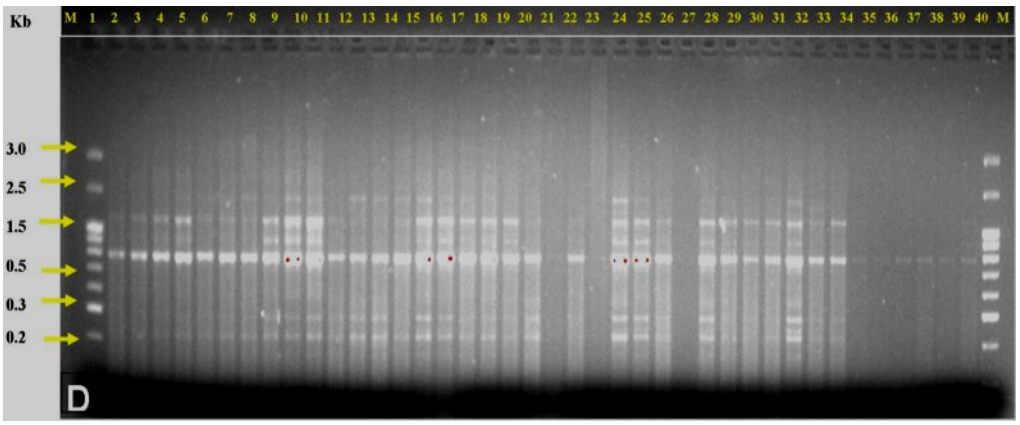

Figure 1 C Representative profiles amplified with CL 21 RAPD primer. M is the 100 bp molecular marker (Fermentas, Life Science, Germany with size in bp indicated on the left). 1- 40 indicate the fourty different genotypes of *Zingiberofficinale*of Sikkim Himalaya**.**

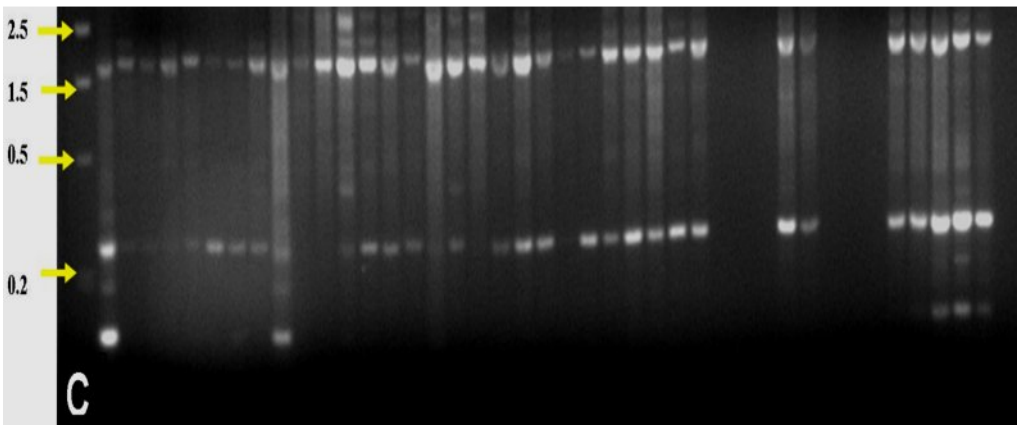

Figure 1 D Representative profiles amplified with CL 197 RAPD primer. M is the 100 bp molecular marker (Fermentas, Life Science, Germany with size in bp indicated on the left). 1- 40 indicate the fourty different genotypes of *Zingiberofficinale*of Sikkim Himalaya.

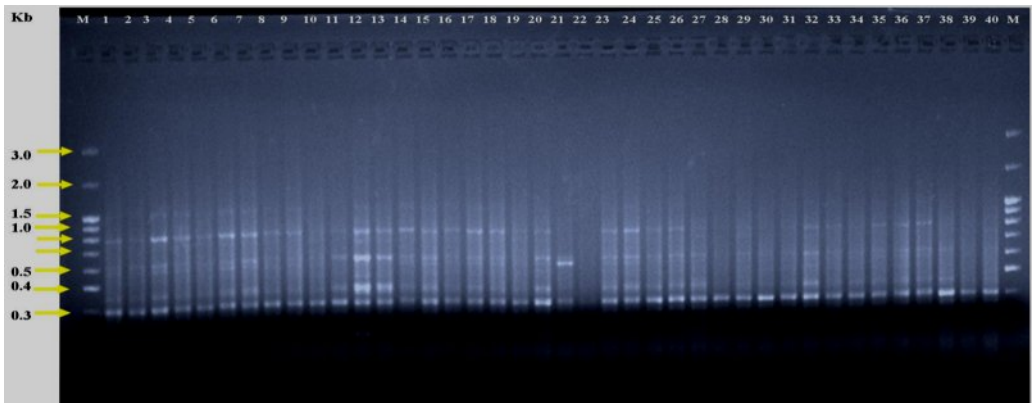

Figure 2 Representative profiles amplified with CL 25 RAPD primer. M is the 100 bp molecular marker (Fermentas, Life Science, Germany with size in bp indicated on the left). 1-40 indicate the fourty different genotypes of *Zingiberofficinale*of Sikkim Himalaya.

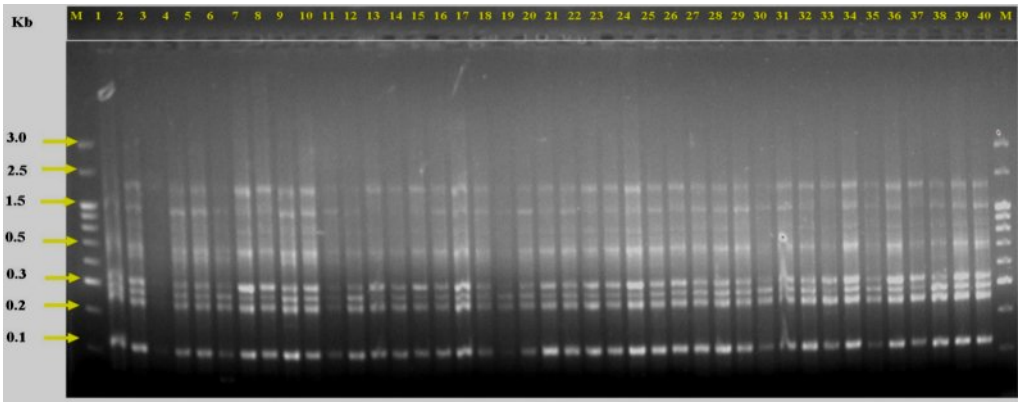

Figure 3 Representative profiles amplified with CL 53 RAPD primer. M is the 100 bp molecular marker (Fermentas, Life Science, Germany with size in bp indicated on the left). 1- 40 indicate the fourty different genotypes of *Zingiberofficinale*of Sikkim Himalaya.

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