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Characterization of the genetic structure of mango ginger (*Curcuma amada* Roxb.) from Myanmar in farm and genebank collection by the neutral and functional genomic markers

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Abstract A preliminary characterization was undertaken to describe genetic structure of mango ginger (*Curcuma amada*) acquired from farmers and *ex situ* genebank in Myanmar using neutral (rice SSR based RAPDs) and functional genomic (P450 based analog) markers. The high polymorphism (> 91%) depicted has displayed existence of genetic variability in the germplasm investigated. Large number of source-specific alleles (neutral-markers = 78, functional-markers = 63) was amplified which revealed that neutral regions of the mango ginger were more variable compared with the functional regions. The major fraction of the molecular variance (neutral-markers = 85%, functional-markers = 93%) was explained within germplasm acquisition sources and this tendency was also supported by the estimate of gene diversity. The genebank accessions have shown comparatively more genetic variability than farmers' accessions. The variability observed in mango ginger may possibly be associated with the long history of its cultivation under diverse ecological conditions. The two marker systems elucidated their high resolving power which detected variability even in fewer genotypes assayed. As the target sites of these markers are different, therefore, the variability detected is believed to cover diverse part of the genome together with neutral and functional regions. We found the concurrent use of the different types of molecular markers valuable to comprehend a dependable variability pattern in the germplasm assayed.

Keywords: *Curcuma amada*, genetic diversity, mango-ginger, Myanmar, on-farm

INTRODUCTION

Mango ginger (*C. amada* Roxb.) is an important member of the family Zingiberaceae and famous due to its raw mango like aroma of the rhizomes. It is found wild (Srivastava et al. 2006), as well as in cultivation (Sasikumar, 2005) with a long history of traditional use in folk medicine in the sub-continent. It has been used for healing of wounds, cuts, and itching, for sprains and skin diseases, carminative properties as well as being useful as a stomachic, decoction of rhizome effective for colds and coughs (Jatoi et al. 2007). *C. amada* has pharmacological significance for a variety of ailments for example effective in skin allergies, effects on blood cholesterol and possess antioxidant properties as well as antibacterial activity. More than 130 chemical constituents including curcuminoids, the bioactive compounds of *Curcuma*, have been reported in *C. amada* rhizomes, of which 121 have been identified (Jatoi et al. 2007). Its peculiar raw mango like aroma valued it to be used in salad, culinary preparations and pickles making since long time (Nayak, 2002; Sasikumar, 2005).

Despite its importance due to its multiple usages a very few studies have been conducted. Sasikumar (2005) reviewed the genetic resources of *Curcuma* and stressed the need to initiate studies on molecular level. In an attempt random amplified polymorphic DNA (RAPD) analysis was performed on regenerated plantlets of *C. amada* to assess clonal fidelity, which revealed 103 scorable bands using 10 primers (Prakash et al. 2004). Although the observed genetic diversity was low, most of the

regenerated plantlets were similar to the mother plants (Prakash et al. 2004). While testing the applicability of rice microsatellite markers as RAPD markers in different species of Zingiberaceae, including *C. amada*, we found these markers useful in genetic diversity analyses (Jatoi et al. 2006). Although these studies are useful, they do not describe the overall diversity of *C. amada*.

To molecular markers P450-Based Analog (PBA), also known as functional genomic markers, is a recent addition (Yamanaka et al. 2003). Most of the molecular markers like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphhism (AFLP), Simple Sequence Repeats (SSR) and Intersimple Sequence Repeat (ISSR) usually record diversity in genetically neutral regions, while PBA markers have the ability of functional genomic analysis of multi-gene families, such as cytochrome P450. PBA markers are based on a specific multi-gene family that can assess genome wide diversity in a range of plant species. In higher plants, cytochrome P450 mono- oxygenases play important roles in the oxidative detoxification and the biosynthesis of secondary metabolites in higher plants (Yamanaka et al. 2003), and many P450 gene families have been found in various plant species. In the *Arabidopsis thaliana* genome about 0.9% of 29,000 genes (272 genes and 26 pseudogenes) could be categorised as cytochrome P450 genes (Riechmann et al. 2000). P450 genes characterized so far are very diverse and variable in their gene alignments. These markers were initially designed for the poorly studied genomes. The utility of this marker system for the diversity assessment has been tested successfully in different crop species like banana (Wan et al. 2005) and *Withania coagulans* (Gilani et al. 2009). They produce RAPD like banding pattern. High reproducibility and power of polymorphism detection in the functional regions are the main features making PBA distinguished from others markers (Yamanaka et al. 2003).

Previously we demonstrated that rice-SSR primer sets can be used as RAPD markers for the polymorphism detection and diversity assessment of different Zingiberaceae taxa (Jatoi et al. 2006). Keeping in view the monocot model of the rice and gingers, these primers sets were tested on trial basis in gingers that gave successful results. By the use of these markers we were able to classify the different members of the Zingiberaceae and it was in accordance with the current taxonomic classification. Moreover, genetic relationships among representative accessions of ginger (*Z. officinale*) also revealed useful information. The longer primer sequences and high annealing temperature were the features that made these markers more reproducible than the traditional RAPD markers.

The Union of Myanmar contains landscape diversity due to wide range of ecological conditions that resulted in the diverse agricultural systems. These factors coupled with existence of numerous ethnic communities have tremendous contribution to the diversity of plant genetic resources in Myanmar. Several attempts have been made for the diversity assessment of the major crops in Myanmar (Wan et al. 2005; San-San-Yi et al. 2008). However, the Zingiberaceae genetic resources of Myanmar have not been evaluated yet. The current study aimed to characterize genetic structure of mango ginger employing two different types of molecular markers. Accessions of *C. amada* acquired from farmers and *ex situ* genebank in Myanmar were compared for the occurrence of genetic diversity. In the present study two different types of molecular markers (functional and neutral) were used to get clear

Table 1. List of mango ginger (*C. amada)* accessions assayed along with their collection origins and acquisition sources.

Accession No.	State/Division (Township)	Collection source
ZO18-1	Shan	Genebank (VFRDC[1])
ZO21-1	Shan	Genebank (VFRDC)
ZO23-1	Shan	Genebank (VFRDC)
ZO43-1	Shan (Pyinoolwin)	Genebank (VFRDC)
ZO45-1	Shan	Genebank (VFRDC)
ZO48-1	Shan	Genebank (VFRDC)
ZO78-1	Mandalay (Ye Zin)	Farmer field
ZO107	Mandalay (Ye Zin)	Farmer field
ZO108	Mandalay (Ye Zin)	Farmer field
ZO112	Mandalay (Ye Zin)	Farmer field
ZO114	Mandalay (Pyinmana)	Farmer field
ZO128	Mandalay (Pyinmana)	Farmer field

[1]Vegetable and Fruit, Research and Development Center (VFRDC), Myanmar Agriculture Service (MAS), Ministry of Agriculture and Irrigation Union of Myanmar.

picture of diversity and genetic relationship in the germplasm under investigation. The concurrent use of different molecular techniques is believed to depict reliable picture of diversity (Wang et al. 2007). This is the first attempt that has been made for the delineation of the genetic diversity in *C. amada* in general, and from Myanmar in particular.

MATERIALS AND METHODS

Plant materials and DNA isolation

The germplasm investigated in this study comprised 12 genotypes of mango ginger (*C. amada* Roxb.) from Myanmar which represented genebank and farmers' collection (Table 1). Mango ginger acquired from the farmers was the landraces, which they use to grow as backyard plantation for the domestic use, and they were cultivating these landraces for a long time. Mango ginger accessions representing genebank were provided by the Vegetable and Fruit Research and Development Center (VFRDC), Myanmar Agricultural Services (MAS) Ministry of Agriculture and Irrigation, Union of Myanmar. These were the genotypes that were selected by the VFRDC for the conservation through *in vitro* culture. The genebank accessions represented central and eastern parts of the Shan state whereas landraces from farmers were collected in the Mandalay division. The acquired germplasm was grown into pots under glasshouse at University of Tsukuba, Japan. Young leaves of these accessions furnished the tissue for DNA extraction. Total DNA was extracted following the methods of Doyle and Doyle (1990) with minor modifications. Genotypes of the Zingiberaceae generally contain secondary metabolites that often hamper enzymatic reactions after long term storage. To avoid this problem, the use of fresh leaf samples and immediate commencement of experiments are strongly recommended. To minimize this problem, mercaptoethanol (1%) was added to the buffer.

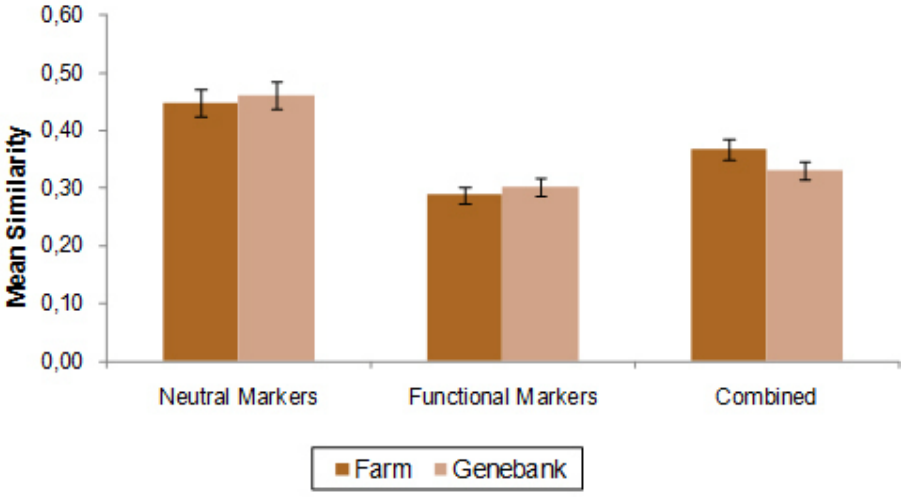

Fig. 1 Mean similarity observed for *C. amada* accessions acquired from genebank and farmers based on the Jaccard's similarity coefficients estimated by the functional and neutral markers independently as well as collectively.

RSB-RAPD Assay (Rice SSR-based RAPD)

We previously screened eight rice SSR primer sets, based on their successful amplifications profiles and high polymorphisms across different genera in the family Zingiberaceae (Jatoi et al. 2006). The same primer sets were used here for larger assessment of the genetic diversity of mango ginger (*C. amada*). The total volume of the reaction mixture used for PCR analysis was 20 µL. Reaction mixture contained 1x Ex Taq buffer, 0.5 mM each of dNTPs, 1 unit of Ex Taq polymerase (TaKaRa), 0.5 µM each of forward and reverse primer, and 25 ng of DNA template. Amplification was carried out in a PCR thermal cycler (Mycycler, ver 1.065, BioRad). Thermal cycler was programmed to 1 cycle of 5 min

at 94ºC for initial strand separation. This was followed by 35 cycles of 1 min at 94ºC for denaturation, 1 min for annealing and 1.5 min at 72ºC for primer extension. Lastly, 1 cycle of 10 min at 72ºC was used for final extension, followed by cooling to 10ºC. The annealing temperature of each primer pair is given in Table 2. PCR products were electrophoresed using 8% polyacrylamide gels and ethidium bromide staining.

PBA assay

The PBA primer-sets comprised of three forward (CYP1A1F, CYP2B6F and CYP2C19F) and five reverse primers (CYP1A1R, CYP2B6R, CYP2C19R, heme2B6 and heme2C19). Fifteen combinations of these primer pairs were used in the study. Polymerase Chain Reaction (PCR) amplifications were performed using 20 ng of template DNA in a total reaction mixture of 25 µl containing 1 x PCR buffer (TaKaRa), 0.16 mM of dNTPs, 1 mM of each primer and 1 unit of *Taq* polymerase (Ex. Taq, TaKaRa). The PCR amplification reaction, carried out in a Thermal Cycler (Applied Biosystems), included an initial denaturation for 5 min at 94ºC followed by 32 cycles of 1 min at 94ºC, 2 min at 45ºC and 3 min at 72ºC. Annealing temperature varied for each primer set as given in Table 2. PCR products were electrophoresed using 1% agarose gels followed by ethidium bromide staining.

Table 2. DNA amplification in different mango ginger (*C. amada*) by neutral and functional markers.

Primer-pairs/combinations	Annealing temp.(°C)	Total bands (Polymorphic)	Polymorphism (%)	Average band (No.)
PBA markers[1]				
CYP1A1F/heme2B6	56	14 (14)	100	3.9
CYP2C19F/CYP1A1R	56	12(12)	100	3.6
CYP2C19F/heme2B6	52	9(9)	100	4.3
CYP2C19F/heme2C19	56	5(3)	60	3.1
CYP1A1F/CYP2B6R	56	18(18)	100	6.8
CYP2B6F/CYP1A1R	52	24(24)	100	7
CYP2B6F/CYP2B6R	52	22(22)	100	7.8
CYP2B6F/heme2B6	52	8(7)	87.5	3.6
CYP2B6Fheme2C19	52	14(14)	100	4.7
CYP1A1FCYP2C19R	46.5	10(10)	100	2.2
CYP2B6F/CYP2C19R	46.5	8(7)	87.5	2.3
CYP2C19F/CYP2C19R	46.5	15(15)	100	5.6
Average		**13.3(12.9)**	**94.58**	**4.6**
RSB-RAPD markers[2]				
RM1	55	14(11)	79	5.4
RM125	55	24(24)	100	10.4
RM171	55	17(14)	82	7.5
RM153	55	19(18)	95	6.6
RM154	60	17(17)	100	8
RM135	63	14(11)	79	7.1
RM131	60	16(16)	100	10.9
RM117	60	25(25)	100	8.8
Average		**18.3(17)**	**91.88**	**8.1**

[1]Yamanaka et al. (2003).
[2]Temnykh et al. (2000).

Data analysis

For statistical analyses, amplified DNA fragments were scored in a binary data matrix where presence of band was denoted as 1 and absence as 0. Genetic similarities among the genotypes were determined based on the Jaccard (1908) coefficient using the SIMQUAL program of the NTSYS-pc (Numerical Taxonomy System, version 2.0, Rohlf, 2000). The same binary data matrix was employed to perform Principal component analysis (PCA) based on correlation matrix to reveal genetic structure among the genotypes. Analysis of molecular variance (AMOVA) was carried out to partition the total genetic variance into within and among sources of collection. Gene diversity was calculated as outlined by Nei (1973). For AMOVA and gene diversity, the computer program GENALEX 6 (Peakall and Smouse, 2006) was used.

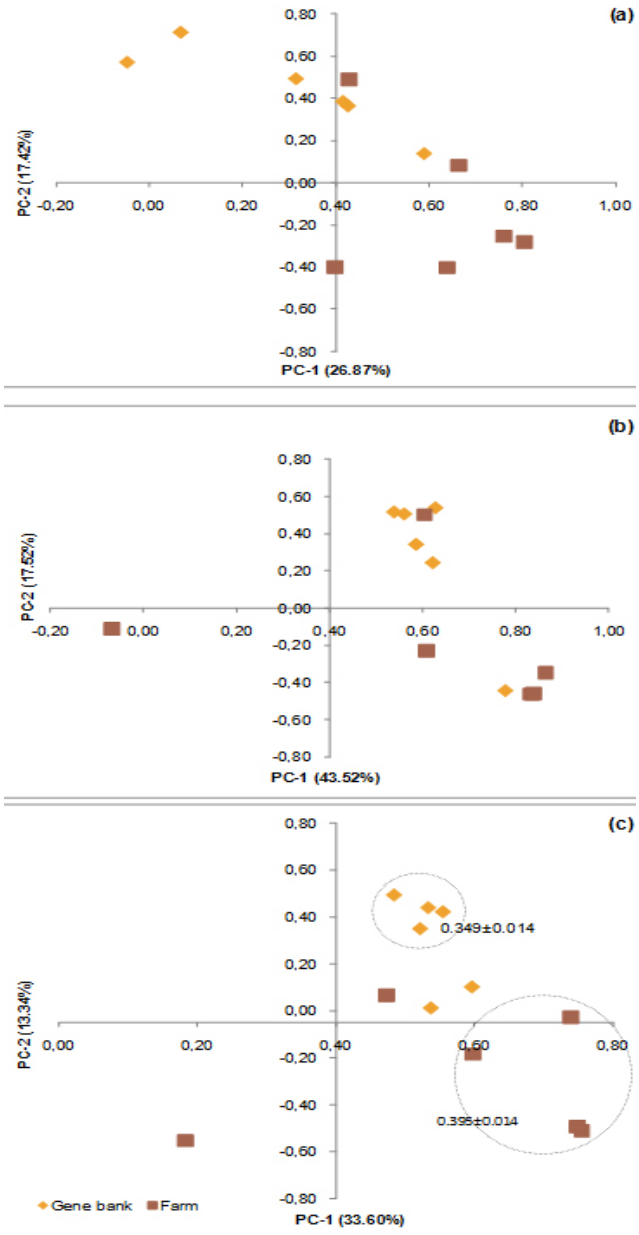

Fig. 2 Distribution of the *C. amada* accessions on the 2D scatter plot. The cumulative contribution of the first three principal components to total variation in case of PBA was 55.58%. For RSB-RAPD it was 69.7% whereas for combined data set it explained 56.57% of the total variation. (a) PBA markers. (b) RSB-RAPD markers. (c) Combined analysis of both markers.

RESULTS

In this study twelve landraces of mango ginger (*C. amada*) were investigated for diversity assessment using two different types of molecular markers. Out of 15 primer combinations of the PBA markers, 12 gave amplified products in the germplasm assayed whereas 3 primer combinations (CYP1A1F/CYP1A1R, CYP1A1F/heme2C19 and CYP2C19F/ CYP2B6R) did not yield PCR products and thus were not considered. The number of DNA fragments amplified in mango ginger (*C. amada*) by the PBA markers was 13.3 with a polymorphism rate of 94.6%, and average number of bands amplified per accession was 4.6 (Table 2). For the same set of genotypes, DNA fragments amplified by neutral markers were 18.3, and average bands per accession were 8.1 (Table 2). The mean similarity based on RSB-RAPDs was 0.441 and for PBA markers it was 0.367 in the whole set of mango ginger landraces.

Diversity profile in farmers' and genebank accessions

The current study deals with the germplasm which mainly comprised landraces, and acquired from the small scale subsistent farmers and genebank. Alleles specific to both collection sources were observed by the two markers in mango ginger accessions. The source-specific alleles amplified by PBA markers were 2.3 and 3 in farm and genebank, respectively (Table 3). The two primer combinations in farm accessions and one primer combination in genebank accessions did not amplify source-specific alleles. For the neutral markers alleles specific to farmers' accessions were 5.4 whereas 4.4 for genebank accessions (Table 3). Genetic similarities of the mango ginger accessions from the farmers' and

Table 3. Estimates of gene diversity and specificity of DNA fragments in genebank and farmers' accessions analyzed by the two markers.

Marker	Total No. of alleles	Private alleles[1]		Gene diversity ± SE[2]	
		Farm	Genebank	Farm	Genebank
PBA markers					
CYP1A1F/heme2B6	14	3	2	0.328 ± 0.072	0.269 ± 0.069
CYP2C19F/CYP1A1R	12	0	5	0.318 ± 0.065	0.301 ± 0.048
CYP2C19F/heme2B6	9	0	3	0.244 ± 0.109	0.362 ± 0.056
CYP2C19F/heme2C19	5	1	1	0.236 ± 0.106	0.486 ± 0.014
CYP1A1F/CYP2B6R	18	4	3	0.391 ± 0.017	0.384 ± 0.042
CYP2B6F/CYP1A1R	24	2	5	0.338 ± 0.013	0.272 ± 0.035
CYP2B6F/CYP2B6R	22	1	5	0.395 ± 0.028	0.335 ± 0.035
CYP2B6F/heme2B6	8	3	0	0.417 ± 0.029	0.451 ± 0.021
CYP2B6Fheme2C19	14	4	1	0.305 ± 0.054	0.393 ± 0.033
CYP1A1FCYP2C19R	10	3	4	0.343 ± 0.078	0.212 ± 0.060
CYP2B6F/CYP2C19R	8	3	2	0.370 ± 0.057	0.315 ± 0.044
CYP2C19F/CYP2C19R	15	3	5	0.423 ± 0.064	0.379 ± 0.038
Average	**13.3**	**2.3**	**3.0**	**0.342 ± 0.058**	**0.347 ± 0.041**
RSB-RAPD markers					
RM1	14	5	4	0.350 ± 0.041	0.422 ± 0.033
RM125	24	9	10	0.427 ± 0.029	0.334 ± 0.060
RM171	17	5	5	0.428 ± 0.014	0.391 ± 0.063
RM153	19	6	6	0.335 ± 0.018	0.389 ± 0.019
RM154	17	4	2	0.396 ± 0.012	0.476 ± 0.013
RM135	14	2	0	0.482 ± 0.010	0.475 ± 0.010
RM131	16	6	0	0.369 ± 0.030	0.441 ± 0.013
RM117	25	6	8	0.408 ± 0.015	0.371 ± 0.011
Average	**18.3**	**5.4**	**4.4**	**0.399 ± 0.021**	**0.412 ± 0.028**

[1]Alleles specific to farm or genebank accessions.
[2]As of Nei (1973): SE = Standard Error.

genebank collection in Myanmar were calculated using the data matrices generated by the two marker systems (Table 4). The mean similarity observed by the neutral markers was 0.45 and 0.46 in genebank and farmers' accessions, respectively, and in the same respective order for the functional markers it was 0.29 and 0.30 (Figure 1). The similarity coefficients complemented the gene diversity estimated in the mango ginger by the two marker types.

Gene diversity

The gene diversity was estimated for the two collection sources using data sets of both marker types separately. The gene diversity in farmers' accessions was 0.342 which ranged from 0.244 to 0.423, and for genebank accessions it ranged from 0.212 to 0.486 leading to an average gene diversity of 0.347 for PBA markers (Table 3). RSB-RAPD markers yielded a mean gene diversity of 0.399 and 0.412 for farmers and genebank accessions, respectively (Table 3). The mean gene diversity estimates obtained for the two collection sources slightly differed from each other, however, genebank accessions displayed relatively high gene diversity, and this trend prevailed in both the molecular markers used in this study. A major fraction of molecular variance (85%) in the current study was explained within farmers and genebank accessions, whereas between collection sources variance accounted for 15% only by the PBA markers (Table 5). For the RSB-RAPD markers a similar trend prevailed displaying high molecular variance of 93% within collection sources and 7% only between collection sources (Table 5).

Genetic relationships among mango ginger at farms and genebank

Principal component analysis displayed differential grouping patterns of the mango gingers by the two makers employed in this study. The PBA markers classified the major fraction from each collection-source into distinct groups (Figure 2a). However, some individual accessions from each source placed together. The cumulative contribution of the first three principal components to total variation was 55.58%. RSB-RAPD assay, with the exception of one accession from each source, grouped mango ginger distinctly representing each collection source separately (Figure 2b). The first three PCs explained 69.7% of the total variation. One accession from farmers' collections appeared as unique spot on the scatter plot. In case of analysis of the combined data set, major fraction from each source grouped together representing genetic divergence from each other (Figure 2c). The first three PCs contributed 56.6% of the total variation. A similar clustering pattern of the mango ginger accessions was observed in cluster analysis (Data not shown). However, clustering pattern did not correspond with the collection/acquisition source.

DISCUSSION

This is the first report that deals with the diversity analysis in mango ginger (*C. amada*) especially from Myanmar. Though, the genotypes investigated were less in number even then a high polymorphism was revealed in this study. The differential polymorphism in the germplasm assayed was dependant on the existence of variability in the functional and neutral regions that were targeted by the two marker types. Genetic variability depicted by the diversity profile elucidated the broad base of the mango ginger. In a parallel study in *C. zedoaria* using RAPD markers, Islam et al. (2005) recorded an average number of DNA fragments amplified as 14.5 ranging from 7 to 21, which were low as compared to our findings in *C. amada* using RSB-RAPDs. This also showed the relative efficiency of the RSB-RAPD markers over the RAPD markers for the detection of polymorphism in *Curcuma* accessions.

Generally polymorphism observed in the mango ginger was of two types; i) polymorphism due to amplification of DNA fragments in the large fraction of the genotypes, and it lead to high number of mean fragments generated per accession. This pattern of amplification was revealed by the RSB-RAPDs; ii) polymorphism due to band-presence in the small fraction of the germplasm under study which resulted in the less number of DNA fragments per accession, and this was the feature of the PBA markers (Table 2). These patterns not only helped to understand the genetic variability in the mango ginger landraces, but also reflected the efficiency of two marker systems.

The current trend of allele specificity showed that mango ginger under investigation had intra-specific variability more in neutral regions compared with the functional regions that resulted in the amplifications of relatively higher number of private alleles in farm and genebank accessions. The high

Table 4. Estimates of the Jaccard's similarity coefficients ± SE measured for mango ginger representing genebank and farmers' collections by the two molecular markers.
The upper diagonal represents neutral markers (RSB-RAPD) and lower diagonal shows functional markers (PBA).

	ZO18-1G	ZO21-1G	ZO23-1G	ZO43-1G	ZO45-1G	ZO48-1G	ZO78-1F	ZO107F	ZO108F	ZO112F	ZO114F	ZO128F
ZO18-1G		0.55 ± 0.041	0.51 ± 0.040	0.46 ± 0.040	0.44 ± 0.040	0.38 ± 0.040	0.23 ± 0.040	0.35 ± 0.040	0.47 ± 0.041	0.43 ± 0.041	0.43 ± 0.041	0.88 ± 0.040
ZO21-1G	0.29 ± 0.039		0.54 ± 0.041	0.47 ± 0.041	0.39 ± 0.040	0.45 ± 0.041	0.25 ± 0.041	0.42 ± 0.041	0.53 ± 0.041	0.48 ± 0.041	0.48 ± 0.041	0.48 ± 0.041
ZO23-1G	0.36 ± 0.038	0.34 ± 0.038		0.49 ± 0.040	0.42 ± 0.039	0.38 ± 0.040	0.21 ± 0.040	0.42 ± 0.040	0.47 ± 0.040	0.42 ± 0.040	0.41 ± 0.040	0.45 ± 0.040
ZO43-1G	0.28 ± 0.038	0.31 ± 0.038	0.23 ± 0.037		0.74 ± 0.039	0.36 ± 0.040	0.24 ± 0.040	0.36 ± 0.040	0.40 ± 0.040	0.40 ± 0.040	0.39 ± 0.040	0.44 ± 0.040
ZO45-1G	0.22 ± 0.037	0.25 ± 0.037	0.23 ± 0.036	0.46 ± 0.035		0.35 ± 0.040	0.18 ± 0.040	0.36 ± 0.040	0.37 ± 0.040	0.34 ± 0.040	0.35 ± 0.040	0.45 ± 0.040
ZO48-1G	0.49 ± 0.038	0.15 ± 0.038	0.22 ± 0.037	0.26 ± 0.037	0.24 ± 0.036		0.26 ± 0.040	0.52 ± 0.040	0.78 ± 0.040	0.80 ± 0.041	0.82 ± 0.040	0.41 ± 0.040
ZO78-1F	0.20 ± 0.039	0.32 ± 0.039	0.25 ± 0.038	0.20 ± 0.038	0.21 ± 0.037	0.16 ± 0.038		0.19 ± 0.040	0.26 ± 0.040	0.26 ± 0.041	0.27 ± 0.040	0.22 ± 0.040
ZO107F	0.30 ± 0.039	0.63 ± 0.039	0.33 ± 0.039	0.29 ± 0.038	0.25 ± 0.037	0.18 ± 0.038	0.40 ± 0.039		0.60 ± 0.040	0.56 ± 0.041	0.55 ± 0.040	0.34 ± 0.040
ZO108F	0.30 ± 0.037	0.31 ± 0.037	0.45 ± 0.036	0.27 ± 0.036	0.29 ± 0.034	0.21 ± 0.036	0.21 ± 0.037	0.31 ± 0.038		0.84 ± 0.041	0.84 ± 0.041	0.46 ± 0.040
ZO112F	0.21 ± 0.039	0.41 ± 0.039	0.25 ± 0.038	0.33 ± 0.038	0.27 ± 0.037	0.18 ± 0.038	0.61 ± 0.039	0.51 ± 0.039	0.24 ± 0.037		0.97 ± 0.041	0.41 ± 0.041
ZO114F	0.22 ± 0.039	0.37 ± 0.039	0.26 ± 0.038	0.33 ± 0.038	0.28 ± 0.037	0.20 ± 0.038	0.57 ± 0.039	0.42 ± 0.039	0.28 ± 0.037	0.71 ± 0.039		0.42 ± 0.040
ZO128F	0.13 ± 0.038	0.27 ± 0.038	0.18 ± 0.037	0.22 ± 0.037	0.17 ± 0.036	0.13 ± 0.037	0.41 ± 0.038	0.26 ± 0.038	0.21 ± 0.036	0.37 ± 0.038	0.40 ± 0.038	

diversity observed may possibly be associated with genetic changes due to the rapid evolution of non-coding regions (Nayak et al. 2006). While studying chloroplast DNA variation of different Zingiberaceae genera, Ahmad et al. (2009) found non coding chloroplast DNA regions more variant than the coding regions. Comparison of the mean private alleles elucidated minor differences at two acquisition sources by both the marker types. This pattern was also complemented by the estimates of gene diversity. This tendency indicated that diversity status in the mango gingers is almost similar in farm and genebank accessions. The genus *Curcuma* is believed to be at active stage of evolution (Sasikumar, 2005), and genetic diversity may possibly be associated with diverse ecological conditions (Paisooksantivatana et al. 2001).

The molecular variance analyzed by the two marker systems exhibited existence of variability at both the sources of germplasm acquisition. Occurrence of high genetic diversity within populations is a common trend in tropical plants (Hamrick and Loveless, 1989). The estimates of gene diversity observed for the genebank and farm accessions also supported this tendency. Nonetheless, in the current investigation genebank accessions have shown to display comparatively more divergence than farmers' accessions. In a parallel study in *C. zedoaria* using RAPD markers (Islam et al. 2005) and *C. alismatifolia* using allozyme markers (Paisooksantivatana et al. 2001), high levels of genetic diversity within a population was observed.

As an important implication, the comparative assessment of genetic diversity in this study highlighted the significance of using different types of molecular markers simultaneously. Nonetheless diversity patterns observed in mango ginger were more reliable as different portions of genome were targeted by the different markers for detection of variability. This study also supported the hypothesis of Crouch et al. (1999), which emphasizes the integration of the genetic estimates from different molecular techniques to get reliable picture of the genetic diversity. Moreover, in order to generate highly accurate estimates of genetic similarity in genetic diversity, the utilization of a range of marker systems is also necessary (Wang et al. 2007). RSB-RAPD has the additional benefit of having long primer sequences and high annealing temperatures which ensures its better reproducibility that has been a major concern in the regular RAPD markers with lower number of oligonucleotides. Another construe is the generation of considerable number of DNA fragments by the two marker types, which were specific to genebank and farmers' accessions. These highlights important attribute of these markers which can lead to develop SCAR markers to discriminate mango ginger at intra- as well as inter-specific level. Moreover, these studies should be extended to other species of *Curcuma*. In addition the curcuminoids content of these species should be determined. Combining the molecular data with curcuminoid profiles of the *Curcuma* species might pave a way to clear the taxonomic issues of the genus.

Mango ginger is grown widely at a back-yard garden in Myanmar by the diverse ethnic groups due to its significance as herbal medicine. The long history of cultivation in a range of geographic as well as climatic conditions might have accelerated the micro-evolutionary processes which have resulted in genetic changes. It was also reflected by the occurrence of private alleles specific to farm and genebank accessions. Paisooksantivatana et al. (2001) also ascribed the genetic diversity in *C. alismatofolia* with the existence of this in the diverse ecological conditions. In fact the germplasm representing farmers' accessions are the landraces that are being maintained and cultivated over

Table 5. Analysis of molecular variance (AMOVA) depicting partition of genetic variance attributed by genebank and farmers' collections on mango ginger.

Source	df	SS	MS	Estimated Variance	Percent (%)	F-value
PBA markers						
Among collection sources	1	56.667	56.667	4.894	15%	0.152^{ns}
Within collection sources	10	273.000	27.300	27.300	85%	
Total	11	329.667	83.967	32.194		
RSB-RAPD markers						
Among collection sources	1	33.417	33.417	1.733	7%	0.070^{ns}
Within collection sources	10	230.167	23.017	23.017	93%	
Total	11	263.583	56.433	24.750		

generations by the rural farmers. For the long-term survival and to tolerate environmental forces, existence of genetic variability is considered as a pre-requisite (Siddiqui et al. 2007a; Siddiqui et al. 2007b; Khan et al. 2008; Rabbani et al. 2008; Sultana and Ghafoor, 2008). For the vegetatively propagating species like *C. amada* having sexual reproduction constraints, this factor becomes more important and crucial. Backyard plantation or home gardens are the potential spots for the on-farm conservation of plant genetic resources (Trinh et al. 2003). Moreover, indigenous species and rare plant varieties can often be traced in home gardens which have disappeared from the larger ecosystem (Trinh et al. 2003). The suitable way to keep evolutionary processes to continue would also be to maintain populations of mango ginger on the farmers' fields.

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REFERENCES

AHMAD, D.; KIKUCHI, A.; JATOI, S.A.; MIMURA, M. and WATANABE, K.N. (2009). Genetic variation of chloroplast DNA in Zingiberaceae taxa from Myanmar assessed by PCR-restriction fragment length polymorphism analysis. *Annals of Applied Biology*, vol. 155, no. 1, p. 91-101. **[CrossRef]**

CROUCH, J.H.; CROUCH, H.K.; CONSTANDT, H.; VAN-GYSEL, A.; BREYNE, P.; VAN-MONTAGU, M.; JARRET, R.L. and ORTIZ, R. (1999). Comparison of PCR-based molecular marker analyses of *Musa* breeding populations. *Molecular Breeding,* vol. 5, no. 3, p. 233-244. **[CrossRef]**

DOYLE, J.J. and DOYLE, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, vol. 12, no. 1, p. 13-15.

GILANI, S.A.; KIKUCHI, A. and WATANABE, K.N. (2009). Genetic variation within and among fragmented populations of endangered medicinal plant, *Withania coagulans* (Solanaceae) from Pakistan and its implications for conservation. *African Journal of Biotechnology,* vol. 8, no. 13, p. 2948-2958.

HAMRICK, J.L. and LOVELESS, M.D. (1989). The genetic structure of tropical tree populations, association with reproductive biology. In: BOCK, J.H. and LINHAR, Y. eds. *The evolutionary ecology of plants*, Boulder, Westview Press, p. 129-146.

ISLAM, M.A.; MEISTER, A.; SCHUBERT, V.; KLOPPSTECH, K. and ESCH, E. (2005). Genetic diversity and cytogenetic analyses in *C. zedoaria* (Christm.) Roscoe from Bangladesh. *Genetic Ressources and Crop Evolution*, vol. 54, no. 1, p. 149-156. **[CrossRef]**

JACCARD, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletin Société Vaudoise des Sciences Naturelles*, 1908, vol. 44, p. 223-270.

JATOI, S.A.; KIKUCHI, A.; YI, S.-S.; NAING, K.-W.; YAMANAKA, S.; WATANABE, J.A. and WATANABE, K.N. (2006). Use of rice SSR markers as RAPD markers for genetic diversity analysis in *Zingiberaceae*. *Breeding Science*, vol. 56, no. 2, p. 107-111. **[CrossRef]**

JATOI, S.A.; KIKUCHI, A.; GILANI, S.A. and WATANABE, K.N. (2007). Phytochemical, pharmacological and ethnobotanical studies in mango ginger (*Curcuma amada* Roxb.; Zingiberaceae). *Phytotherapy Research*, vol. 21, no. 6, p. 507-516. **[CrossRef]**

KHAN, M.A.; RABBANI, M.A.; MUNIR, M.; AJMAL, S.K. and MALIK, M.A. (2008). Assessment of genetic variation within Indian mustard (*Brassica juncea*) germplasm using random amplified polymorphic DNA markers. *Journal of Integrative Plant Biology*, vol. 50, no. 4, p. 385-392. **[CrossRef]**

NAYAK, S. (2002). High-frequency *in vitro* production of microrhizomes of *Curcuma amada*. *Indian Journal of Experimental Biology*, vol. 40, no. 2, p. 230-232.

NAYAK, S.; NAIK, P.K.; ACHARYA, L.K. and PATTNAIK, A.K. (2006). Detection and evaluation of genetic variation in 17 promising cultivars of turmeric (*Curcuma longa* L.) using 4C nuclear DNA content and RAPD markers. *Cytologia*, vol. 71, no. 1, p. 49-55. **[CrossRef]**

NEI, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science of the United States of America*, vol. 70, no. 12, p. 3321-3323.

PAISOOKSANTIVATANA, Y.; KAKO, S. and SEKO, H. (2001). Genetic diversity of *Curcuma alismatifolia* Gagnep. (Zingiberaceae) in Thailand as revealed by allozyme polymorphism. *Genetic Resources and Crop Evolution*, vol. 48, no. 5, p. 459-465. **[CrossRef]**

PEAKALL, R. and SMOUSE, P.E. (2006). GENALEX 6: genetic analysis in Excel Population genetic software for teaching and research. *Molecular Ecology Notes*, vol. 6, no. 1, p. 288-295. **[CrossRef]**

PRAKASH, S.; ELANGOMATHAVAN, R.; SESHADRI, S.; KATHIRAVAN, K. and IGNACIMUTHU, S. (2004). Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. *Plant Cell Tissue and Organ Culture*, vol. 78, no. 2, p. 159-165. **[CrossRef]**

RABBANI, M.A.; PERVAIZ, Z.H. and MASOOD, M.S. (2008). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic Journal of Biotechnology*, vol. 11, no. 3. **[CrossRef]**

RIECHMANN, J.L.; HEARD, J.; MARTIN, G.; REUBER, L.; JIANG, C.Z.; KEDDIE, J.; ADAM, L.; PINEDA, O.; RATCLIFFE, O.J.; SAMAHA, R.R.; CREELMAN, R.; PILGRIM, M.; BROUN, P.; ZHANG, J.Z.; GHANDEHARI, D.; SHERMAN, B.K. and YU, G.L. (2000). *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science*, vol. 290, no. 5499, p. 2105-2110. **[CrossRef]**

ROHLF, J.F. (2000). TSYSpc 2.1. Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, New York.

SAN-SAN-Yi; JATOI, S.A.; FUJIMURA, T.; YAMANAKA, S.; WATANABE, J. and WATANABE, K.N. (2008). Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-center of origin. *Plant Breeding*, vol. 127, no. 2, p. 189-196. **[CrossRef]**

SASIKUMAR, B. (2005). Genetic resources of *Curcuma*: diversity, characterization and utilization. *Plant Genetic Resources*, vol. 3, no. 2, p. 230-251. **[CrossRef]**

SIDDIQUI, S.U.; KUMMAMARU, T. and SATOH, H. (2007a). Pakistan rice genetic resources-I: Grain morphological diversity and its distribution. *Pakistan Journal of Botany*, vol. 39, no. 3, p. 841-848.

SIDDIQUI, S.U.; KUMMAMARU, T. and SATOH, H. (2007b). Pakistan rice genetic resources-II: Distribution pattern of grain morphological diversity. *Pakistan Journal of Botany*, vol. 39, no. 5, p.1533-1538.

SRIVASTAVA, S.; SRIVASTAVA, M.; RAWAT, A.K.S. and MEHROTRA, S. (2006). Pharmacognostic evaluation of *Curcuma amada* Roxb. *Proceedings of National Academy of Science India*, vol. 76, no. B-II, p. 153-160.

SULTANA, T. and GHAFOOR, A. (2008). Genetic diversity in *ex-situ* conserved *Lens culinaris* for botanical descriptors, biochemical and molecular markers and identification of landraces from indigenous genetic resources of Pakistan. *Journal of Integrative Plant Biology*, vol. 50, no. 4, p. 484-490. **[CrossRef]**

TEMNYKH, S.; PARK, W.D.; AYRES, N.; CARTINHOUR, S.; HAUCK, N.; LIPOVICH, L.; CHO, Y.G.; ISHII, T. and MCCOUCH, S.R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L). *TAG Theoretical and Applied Genetics,* vol. 100, no. 5, p. 697-712.**[CrossRef]**

TRINH, L.N.; WATSON, J.W.; HUE, N.N.; DE, N.N.; MINH, N.V.; CHU, P.; STHAPIT, B.R. and EYZAGUIRRE, P.B. (2003). Agrobiodiversity conservation and development in Vietnamese home gardens. *Agriculture Ecosystems and Environment*, vol. 97, no. 1-3, p. 317-344. **[CrossRef]**

WAN, Y.; WATANABE, J.A.; YI, S.-S.; HTAIK, T.; WIN, K.; YAMANAKA, S.; NAKAMURA, I. and WATANABE, K.N. (2005). Assessment of genetic diversity among the major Myanmar banana landraces. *Breeding Science*, vol. 55, no. 3, p. 365-369. **[CrossRef]**

WANG, X.L.; CHIANG, T.Y.; ROUX, N.; HAO, G. and GE, X.J. (2007). Genetic diversity of wild banana (*Musa balbisiana* Colla) in China as revealed by AFLP markers. *Genetic Resources and Crop Evolution*, vol. 54, no. 5, p. 1125-1132. **[CrossRef]**

YAMANAKA, S.; SUZUKI, E.; TANAKA, M.; TAKEDA, Y.; WATANABE, J.A. and WATANABE, K.N. (2003). Assessment of cytochrome P450 sequence offers a useful tool for determining genetic diversity in higher plants. *TAG Theoretical and Applied Genetics*, vol. 108, no. 1, p. 1-9. **[CrossRef]**

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