MORPHO-AGRONOMIC CHARACTERIZATION AND GENETIC VARIABILITY ASSESSMENT IN MANGO-GINGER (*CURCUMA AMADA*; ZINGIBERACEAE)

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ABSTRACT

The present study was undertaken at University of Tsukuba, Japan to investigate the genetic variability pattern in mango-ginger acquired from Myanmar, which is home to many species of the Zingiberaceous taxa. Mango-ginger (Curuma amada) is a valuable spice and aromatic crop having medicinal significance. A high to moderate variance for plant height, rhizome weight, finger rhizome thickness, sheath length, and leaf length was observed in the germplasm assayed during the two years. Principal component analysis yielded a varying distribution pattern of the genotypes and explained 94.09% and 93.55% of the total variation by the first four PCs during the year 2005 and 2006, respectively. During 2005 gene bank accessions appeared as a distinct and divergent group on the plot whereas accessions belonging to rest of collection sources remained undistinguished from each other.

Keywords: *Curcuma amada, mango-ginger, characterization, morphology, Myanmar*

INTRODUCTION

Curcuma amada Roxb. is an important member of the genus *Curcuma* and is commonly known as mango-ginger due to the raw mango-like aroma of the rhizome. Mango-gingeris known by different common names, including *amahaldi, amra haridra, amargandhi, amragandhi haridra, amad*, *ke-a-sanga, sarabasa (Sa), taldiha, talia, sarbanaghati,* and*ban-haldi*. It is found in wild (Srivastava et al. 2006), as well as in cultivation. Its distribution is confined to South-east Asia mainly India, Myanmarand Bangladesh.

C. amada is an herbaceous perennial with erect to semi-erect plant stature. The rootstock or radical bulb is ovoid/conical. The rhizome is large and branched, with a buff-colored external surface. The flesh color is light to pale yellow, with a

ISSN: 1019-8180

fragrance of green mango. Sessile/palmate tubers are thick, cylindrical, fleshy, fingered, and arise from the base of the rootstock. Pendulous tubers are present. The leaves are large, oblong–lanceolate shaped with an acuminate leaf apex, and are petiolated. The lower side of the leaf is puberulous, whereas the upper side is glabrous. *C. amada* has a lateral or central inflorescence on a long erect peduncle, covered with 5–6 sheaths, and hidden by the sheathing bases of the leaves. The inflorescence is spike/scape with a succession of strong, imbricated, pale-green or straw-colored fertile bracts. These bracts are terminated with a coma or tuft of pale-purple or rose-colored barren bracts, or leaves. Flowers are large and long, with 4–5 flowers in each bract. However, in herbarium specimens, it is difficult to distinguish *C. amada* from *C. longa* (Baker 1894; Roscoe 1828; Srivastava et al. 2006).

The present attempt is focused to delineate the diversity status of the mango-ginger mainly from Myanmar, which is home to many tropical and subtropical plant species including many of the Zingiberaceous taxa. The landscape and echo-diversity coupled with geographic variation in Myanmar resulted in existence of diverse plant genetic resources (San San Yi et al. 2007; Thaing-Swe 2007). The specific objectives included to study variability patterns based on different morphological traits, clustering patterns of the genotypes under study, association of the different plant traits, and to identify different groups based on yield components. Moreover, variability in mango-ginger was also studied with reference to the acquisition sources, i.e genebank, farm and local market.

MATERIALS AND METHODS

Plant Material: The plant material assayed in this study comprised 9 accessions of mango-ginger (*C. amada*) and one accession of turmeric (*C. longa*) which was included as reference genotype. Mango-ginger was acquired mainly from Myanmar, however, one accession was obtained from the village market in Thailand which is situated near the border of Myanmar. The only accession of *C. longa* included in the studies was acquired from Japan (Table 1). The germplasm acquired from Myanmar contained 4 accessions from genebank, 3 from rural farmers and 1 from local market. The collection was made mainly from central Myanmar (Shan state and Mandalay division) where mango-ginger and other members of Zingiberaceae are found. The germplasm under investigation were the local landraces, and under cultivation in the forming community for long time as backyard plantation.

ISSN: 1019-8180

Germplasm characterization: The study was conducted at Institute of Life and Environmental Sciences, University of Tsukuba, Japan (36º6'0 N latitude and 140º6'0 E longitude) during the year 2005 and 2006. The work reported here was carried out from March to December of the respective year. Mean maximum temperature at Tsukuba ranges from 30.9ºC (August) to 9.1ºC (December) and the mean minimum temperature varies from -3.8ºC (January) to -2.8ºC (December). This cold situation prolongs and even some time during April night temperature reaches freezing level. *C. amada* is basically long duration crop and remains in the field from April/May to Dec/Jan depending upon the agro-ecological conditions. In the current study rhizomes were sown in small pots under glasshouse conditions in the mid-March and transplanted into large pots and shifted in the open field during mid of May, 2005.

Data analysis: The data recorded for quantitative traits were subjected to different methods of multivariate statistics. Cluster and principal components analyses were performed to see the clustering and grouping patterns of mango-ginger genotypes. A distance matrix based on the Euclidean dissimilarity coefficients between all pairs of entries was constructed, which was then used to perform cluster analysis. A correlation matrix based on quantitative characters was used to perform principal component analysis. The two analyses were carried out using numerical taxonomy based software NTSYS-pc (Numerical Taxonomy System, version 2.0, Rohlf 2000). Descriptive statistics was employed to get means, standard deviations, standard errors and variances for the quantitative characters. To study the association of different traits with each other, correlation coefficients for all the quantitative traits were computed. To study the significance of the variability over the years, analysis of variance was conducted as outlined by Steel and Torrie (1980) using statistical software MSTATC (Anonymous 1989).Mean separation was done usingDuncan's multiple range test (DMRT) at $p<0.05$.

RESULTS

Variability in general: A high to moderate variance for plant height, rhizome weight, finger rhizome thickness, sheath length, and leaf length was observed in the germplasm assayed during the two years (Table3). The mean values observed for leaf length, leaves per tiller, petiole length, sheath length, plant height, tillers per plant, tillers width, finger rhizome and rhizome weight were comparatively

ISSN: 1019-8180

high in 2005 than 2006. However, leaf width, ligule length, sheath width and primary rhizome thickness observed in this study were relatively higher in the year 2006 as compared to the year 2005.

Distribution patterns of mango-ginger based on PCA: Principal component analysis yielded a varying distribution pattern of the genotypes in the two years with the minor differences in the contribution of the PCs towards total variation (Fig..1). The cumulative contribution of the first three PCs for the 10 genotypes analyzed for 16 quantitative traits was 94.09% and 93.55% in 2005 and 2006, respectively (Table 4). Among the various characters analyzed plant height, leaf length, sheath length, tiller width and primary rhizome thickness contributed more positively to PC1 during the 2005 and 2006. In contrast leaf width, leaves per tiller, ligule length, sheath width and finger rhizome and rhizome weight contributed negatively to PC1 for the two years. Petiole length contributed positively in 2005,while negatively in 2006. PC1 accounted for 76.77% during first year whereas its contribution was 76.61% in second year. Most of the characters contributing positively to first component were growth and yield components. PC2 accounted for 19.30% and 18.48% of the total variance explained by PCA in first and second year, respectively.

To view the distribution of germplasm accessions on the scatter plot, first two PCs were plotted (Fig.1). During 2005 genebank accessions appeared as a distinct and divergent group on the plot whereas accessions belonging to rest of collection sources remained undistinguished from each other. Mango-ginger representing three collection sources remained scattered and mixed on the plot when analyzed for the second year (Fig. 1).

Relationships among mango-ginger accessions: Dissimilarity coefficients based on Euclidean distance ranged from 0.027 to 3.350 with the mean distance of 0.614 in the first year, whereas it ranged from 0.026 to 2.696 leading to the mean value of 0.564 in the second year (Table 5). The lowest distance during 2005 was observed between ZO114 and ZO130, and the highest between ZO102 and ZO128. The lowest and highest distance during the second year was observed between ZO130 and ZO45-1, and ZO102 and ZO128, respectively. The mean values based on distance coefficients in mango-ginger collected from market were high (0.791) followed by farm (0.613) and genebank (0.260) during the year 2005 and similar trend also prevailed in 2006 also (Table 6).

ISSN: 1019-8180

Cluster analysis resolved mango-ginger accessions into 3 clusters during 2005 (Fig. 2). Cluster 1 comprised three accessions (ZO18-1, ZO23-1 and ZO89), cluster-II contained 5 accessions (ZO48-1, ZO78-1, ZO114, ZO130, ZO128) and cluster-III consisted of 2 accessions (ZO45-1 and ZO102). The variability estimates showing inter-cluster variation for various quantitative traits are given in Table 7. The genotypes representing genebank, farm and market remained dispersed into three clusters, and clustering patterns did not show association with collection source. Cluster-III showed the highest mean value based on Euclidean distance (1.434) followed by Cluster-II (0.363) and Cluster-I with 0.097 (Table 7). In the top down analysis for the year 2005, accessions with taller plants grouped in cluster-I followed by cluster-II and III, whereas accessions with high rhizome weight per plant were grouped into cluster-II followed by Cluster-I and III, respectively (Fig. 3).

DISCUSSION

The morpho-agronomical characterization of genotypes and knowledge on the genetic diversity among the accessions allow correct recommendations and serve as guidelines for actions to be taken in improvement programs (Cintra et al. 2005). The current study is the first report that deals with mango-ginger characterization particularly from Myanmar. The multitude of statistical methods used to analyze different aspects of mango-ginger provided a deep insight into variability indicating existence of considerable variability and diverse base which could be exploited for crop improvement as well as conservation of mango-ginger.

In the parallel studies, a high degree of variability was observed for different plant characters in *C. longa* (Pushkaran et al. 1985; Sasikumar and Sardana 1989; Jalgaonkar et al. 1990; Korla et al. 1992). Rao et al. (2005) assessed the genetic diversity among 54 turmeric genotypes and found wide diversity, and genotypes were grouped into six clusters showing considerable inter-cluster genetic divergence. In another attempt Chattopadhyay et al. (2004) observed significant variation in 10 promising *C. longa* genotypes for plant height, leaf length and leaf breadth and also with respect to different rhizome characters. Singh et al. (2003) found greatest variation for yield, plant height, weight of primary rhizome per plant and number of leaves. In another study comprising diversity analysis in 20 genotypes of turmeric appeared to have narrow genetic base which underwent high level of genetic erosion and selection pressure (Hikmat et al., 2012).Datta et al. (2001) evaluated the genetic variability in the morphological and yield

ISSN: 1019-8180

attributing traits of 11 turmeric germplasm and found no consistent variation in morphological traits among the germplasm.

In the present study a consistent trend was observed which prevailed during the two years that market accessions displayed more variability than farm and genebank accessions. This highlighted the importance of local markets in the rural communities as potential source of germplasm which may possibly contain rare genotypes. It is emphasized that for diversity studies and making crop improvement plans, one should not ignore local market for capturing diverse genotypes and local landraces. Variability displayed by the clonal species like *Curcuma* with sexual reproduction constraints could be due to the accumulation of mutational as well as micro-evolutionary changes that occur naturally. Sampling methods have considerable impact on diversity assessment and number of genotypes sampled may also affect variability patterns. In the current study, though the number of market samples was fewer, however, consistency in the variability patterns over the two years rendered a general trend-line that can be helpful while devising similar studies in future.

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Table 1. List of mango-ginger (*C. amada*) accessions, their collection origin and acquisition source used in the present study.

Code	Accession	Botanical Name	Source
1.	ZO18-1	*Curcuma amada*	Myanmar (Genebank)[1]
2.	ZO23-1	*C. amada*	Myanmar (Genebank)
3.	ZO45-1	*C. amada*	Myanmar (Genebank)
4.	ZO48-1	*C. amada*	Myanmar (Genebank)
5.	ZO78-1	*C. amada*	Myanmar (Local farm)
6.	ZO114	*C. amada*	Myanmar (Local farm)
7.	ZO128	*C. amada*	Myanmar (Local farm)
8.	ZO89	*C. amada*	Thailand (Market)
9.	ZO102	*C. amada*	Myanmar (Market)
10.	ZO130	*C. longa*	Japan (Market)

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ISSN: 1019-8180

Table 2. Different morphological traits and their description as recorded in mango-ginger (*C. amada*) accessions.

Parameter/Character	**Description of the trait**
Leaf length (cm)	Measurement from leaf-tip to the leaf-base
Leaf width (cm)	Measured at point of maximum width
Leaf length/width ratio	Ratio of the leaf length to leaf width
Petiole length (cm)	Measured from ligule to start of lamina
Petiole/leaf-lamina ratio	Ratio of the petiole length to leaf-lamina length
No. of leaves per tiller	Total number of leaves on the tillers
Ligule length (mm)	Measured from ligule base to its top point
Sheath length (cm)	From soil surface to the ligule of top most opened leaf
Sheath diameter (cm)	Measured for the top most fully opened leaf
Sheath/petiole ratio	Ratio of leaf sheath length to petiole length
Plant height (cm)	Height of plant from soil surface to its highest point
No. of tillers per plant	Counting all those tillers that emerged out of soil
Tillers width (mm)	At the base of tillers
Primary rhizome thick. (mm)	Measured at the thickest point of primary/mother rhizome
Finger rhizome thick. (mm)	Measured at the thickest point of the finger rhizome
Weight (g/plant)	Weigh the rhizome on individual plant basis

ISSN: 1019-8180

Table 3. Variability in mango-ginger (*C. amada*)based on different morphological traits for the year 2005 and 2006.

Trait	Mean± SE[1]	Variance	Range	
2005				
Leaf length (cm)	54.73±2.58	66.45	44.00(ZO48-1)	70.12(ZO114)
Leaf width (cm)	14.97±0.57	3.30	11.88(ZO102)	17.42(ZO130)
Leaf length/width ratio	3.69±0.13	0.17	3.03(ZO89)	4.42(ZO18-1)
Leaves per tiller (No.)	7.55±0.28	0.79	6.57(ZO18-1)	9.20(ZO78-1)
Petiole length (cm)	16.58±1.09	11.81	13.98(ZO23-1)	25.75(ZO45-1)
Petiole/lamina ratio	0.31±0.02	0.00	0.24(ZO114)	0.48(ZO45-1)
Sheath/petiole ratio	2.22±0.17	0.30	1.28(ZO45-1)	2.99(ZO23-1)
Ligule length (mm)	1.80±0.13	0.16	1.32(ZO48-1)	2.70(ZO102)
Sheath length (cm)	35.15±2.14	45.60	27.88(ZO128)	48.25(ZO18-1)
Sheath width (mm)	8.18±0.62	3.86	4.88(ZO48-1)	12.62(ZO130)
Plant height (cm)	111.84±4.31	185.87	92.95(ZO48-1)	129.82(ZO114)
Tillers per plant (No.)	2.24±0.30	0.88	1.00(ZO45-1)	4.00(ZO114)
Tillers width (mm)	30.43±1.22	14.93	21.87(ZO18-1)	35.93(ZO114)
Primary rhizome thick. (mm)	35.11±0.99	9.84	31.70(ZO18-1)	41.65(ZO45-1)
Finger rhizome thick. (mm)	22.88±1.04	10.73	18.40(ZO102)	29.77(ZO114)
Rhizome weight (g)	233.39±25.05	6274.27	116.75(ZO102)	340.00(ZO128)
200 6				
Leaf length (cm)	50.08±3.20	102.64	38.95 (ZO18-1)	71.67 (ZO128)
Leaf width (cm)	15.07±0.94	8.89	10.88 (ZO102)	20.67 (ZO128)
Leaf length/width ratio	3.34±0.09	0.08	2.87 (ZO45-1)	3.87 (ZO23-1)
Leaves per tiller (No.)	5.94±0.44	1.90	4.22 (ZO45-1)	8.33 (ZO130)
Petiole length (cm)	13.93±0.86	7.36	9.42 (ZO18-1)	18.17 (ZO130)
Petiole/lamina ratio	0.28±0.01	0.00	0.21 (ZO128)	0.33 (ZO114)
Sheath/petiole ratio	1.64±0.21	0.42	0.98 (ZO114)	2.75 (ZO128)
Ligule length (mm)	1.85±0.09	0.07	1.23 (ZO45-1)	2.17 (ZO128)
Sheath length (cm)	31.05±2.00	39.93	21.83 (ZO18-1)	41.50 (ZO23-1)
Sheath width (mm)	9.72±0.76	5.70	8.07 (ZO114)	15.53 (ZO128)
Plant height (cm)	99.50±5.38	289.66	77.67 (ZO18-1)	129.33 (ZO128)
Tillers per plant (No.)	1.77±0.12	0.15	1.00 (ZO114)	2.33 (ZO78-1)
Tillers width (mm)	25.05±2.00	39.99	16.27 (ZO18-1)	37.33 (ZO128)
Primary rhizome thick. (mm)	35.96±0.83	6.97	32.27 (ZO114)	39.60 (ZO128)
Finger rhizome thick. (mm)	22.38±1.54	23.62	15.57 (ZO102)	30.50 (ZO128)

ISSN: 1019-8180

Rhizome weight (g)	216.77±26.84	7205.16	104.33(ZO102)	398.33 (ZO128)

[1]SE = Standard error

Table 4. Principal component analysis of the 16 quantitative traits in mango-ginger (*C. amada*) genotypes assayed during the year 2005 and 2006.

	2005			**2006**		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Eigen value	12.28	1.72	1.05	12.26	1.77	0.94
Percent	76.77	10.77	6.55	76.61	11.09	5.86
Cumulative	76.77	87.54	94.09	76.61	87.69	93.55

Table 5. Mean distance based on Euclidean coefficients between acquisition sources,and different clusters of mango-gingeraccessions observed during the two years.

	Mean distance			**Mean distance**	
Source	**2005**	**2006**	**Cluster**	**2005**	**2006**
Genebank	0.260	0.336	Cluster-1	0.097	0.103
Farm	0.613	0.470	Cluster-2	0.363	0.983
Market	0.791	0.810	Cluster-3	1.434	--
All	0.614	0.564	--	--	--

ISSN: 1019-8180

Table 6. Inter-cluster variation in mango-ginger (*C. amada*) based on different morphological traits observed during 2005 and 2006 (Mean±SE[1]).

Plant character	Clust-1	Clust-2	Clust-3
2005			
Leaf length (cm)	55.91±3.66	56.34±4.43	48.98±4.52
Leaf width (cm)	15.07±0.59	15.68±0.86	13.01±1.14
Leaf length/width ratio	3.80±0.41	3.58±0.15	3.77±0.001
Leaves per tiller (No.)	7.49±0.53	7.54±0.47	7.69±0.69
Petiole length (cm)	15.11±0.64	16.10±0.57	19.99±5.76
Petiole/lamina ratio	0.28±0.02	0.29±0.02	0.40±0.08
Sheath/petiole ratio	2.65±0.34	2.13±0.16	1.80±0.52
Ligule length (mm)	1.88±0.18	1.65±0.09	2.08±0.63
Sheath length (cm)	39.35±5.47	33.60±2.78	32.75±0.25
Sheath width (mm)	8.24±0.23	8.62±1.23	6.96±0.44
Plant height (cm)	114.10±8.26	113.42±7.01	104.50±8.50
Tillers per plant (No.)	2.72±0.36	2.14±0.49	1.75±0.75
Tillers width (mm)	27.52±3.09	31.88±1.40	31.20±0.70
Primary rhizome thickness (mm)	33.21±0.81	35.20±1.21	37.71±3.94
Finger rhizome thickness (mm)	21.11±0.74	25.04±1.40	20.13±1.73
Rhizome weight (g)	202.22±12.56	292.60±26.55	132.13±15.38
2006		**Cluster-1**	**Cluster-2**
Leaf length (cm)		51.27±5.19	48.29±2.80
Leaf width (cm)		15.76±1.38	14.02±1.15
Leaf length/width ratio		3.24±0.08	3.49±0.18
Leaves per tiller (No.)		6.24±0.58	5.49±0.68
Petiole length (cm)		14.09±1.28	13.69±1.18
Petiole/lamina ratio		0.28±0.02	0.28±0.02
Sheath/petiole ratio		1.72±0.28	1.52±0.33
Ligule length (mm)		1.82±0.14	1.91±0.04
Sheath length (cm)		30.27±2.79	32.22±3.12
Sheath width (mm)		10.37±1.20	8.74±0.39
Plant height (cm)		100.61±8.01	97.83±7.44
Tillers per plant (No.)		1.78±0.20	1.75±0.08
Tillers width (mm)		26.64±3.15	22.67±1.40
Primary rhizome thickness (mm)		36.06±1.21	35.80±1.24
Finger rhizome thickness (mm)		24.82±1.90	18.71±1.11
Rhizome weight (g)		263.06±31.68	147.33±14.73

ISSN: 1019-8180

[1]SE = Standard error