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ASSESSMENT OF MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY IN *CURCUMA LONGA* L. GERMPLASM BY SDS-PAGE

SUNITA KUMARI*[1], PRATIBHA SINGH[1] R. P. SINGH[1] AND N. A. KHAN[2]
[1]Department of Biochemistry,
[2]Department of Plant Molecular Biology and Genetic Engineering,
N.D. University of Agriculture and Technology, Kumarganj, Faizabad - 224 229 (U.P.) INDIA
e-mail: Sunitafzd@gmil.com

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***Corresponding author**

ABSTRACT
Turmeric belongs to Zingiberaceae family (Genus *Curcuma*) is most important medicinal plants that posses antimutagen, antiparasitic, antibacterial, antifungal and various other biological activities. For the assessment of morphological, biochemical and molecular (SDS-PAGE) diversity ten germplasm of turmeric was taken. In respect to morphological character plant height (cm), Yield of rhizome/plant (g) and length of finger rhizome (cm) varied from 79.86 cm to 108.43 cm, 198.40 g to 305.93 g and 5.33 to 12.23 cm, respectively. In the dried rhizome of *Curcuma longa* biochemical content *viz*. total mineral content, Carbohydrate content and Protein content varied from 4.43 to 5.21 per cent, 60.00 to 69.32 per cent and 3.33 to 6.66 per cent. On the basis of SDS-PAGE analysis protein were ranged from 43 kDa to 14 kDa. Germplasm were grouped in two major clusters which were further sub divided in 5 sub-clusters. Minimum similarity was found between NDH-88 and NDH-7 with 56 %. Combining all the results from morphological, biochemical and molecular data (SDS-PAGE) NDH-8, NDH-88, NDH-7 were most diverse among all the accessions used and these could be used for the choice for breeding experiments, crop production and development of gene pool with broad genetic base and new varieties of turmeric with high level of protein and other biochemical content.

INTRODUCTION

Turmeric (*Curcuma longa L.*) belongs to Zingiberaceae family is one of the most important medicinal plant. *Curcuma* is genus within Zingiberaceae family, and it is consists of about 80 species in the world (Hermann and Martin, 1991). Turmeric is a perennial herb distributed widely in tropical and subtropical regions of the world, being extensively cultivated in Asiatic countries, mainly in India and China. The rhizome is extensively used in Ayurveda and traditional medicine. The biochemical content in dried turmeric rhizomes was, protein content 3.6-6.8%, anthocyanins 18.9-37.0 μg/g, phenols 0.15-0.62%, tannins 0.32-0.76%, sugars 20.5-43.4%, oil 3.7-5.3%, curcumin 3.1-3.4%, ash 6.9-9.8% and moisture 90.2-91.3%, respectively (Niranjan *et al*., 2003). The presence of various metabolites such as curcuminoid , oil content, flavonoids, phenolics, some important amino acids, protein and high alkaloid content reveals that co-relation with its medicinal uses (Sarangthem and Haokip, 2010). Turmeric possess a broad spectrum of biological activities for instance anti-bacterial, anti-fungal, anti-parasitic, anti-mutagen, anti-inflammatory, hypolipidemic, hepatoprotective; lipoxygenase, cyclooxygenase and protease-inhibitory effects. Due to these biological activities turmeric widely used as medicinal plant. Being medicinal plant mistaken identification of turmeric will result in a serious problem for both manufacturers of traditional medicine products as well as for researchers. Most plant of Curcuma genus has very similar botanical characteristics due to which it very difficult to clearly identify each species. To know the extent of variation based on morphology alone is difficult because most of the morphological characters are greatly influenced by environmental factors as well as the developmental stage of the plant. This necessitates the assessment of diversity present in turmeric using the modern molecular approaches along with biochemical analysis. This would allow a more efficient utilization of plant characters in developing suitable varieties. Molecular approaches such as proein profiling by SDS-PAGE have importance in diversity assessments and this techniques has been frequently used in many species of Zingiberaceae. (Sadia *et al*., 2009; Netra and Prasad, 2007). Electrophoresis of protein is a powerful tool for detection of the genetic diversity and the SDS-PAGE of protein is particularly considered as a reliable technology because storage protein are highly independent of environmental fluctuation (Iqbal *et al*., 2005 and Javaid *et al*., 2004).SDS-PAGE analysis can be easily utilized for numerous purposes, such as categorization of germplasm, biosystematics study, varietal certification, and determination of phylogenetic association between diverse species (Iqbal *et al*., 2005). In present study we assess the genetic diversity of *Curcuma longa* L. germplasms on the basis of morphological, biochemical and SDS-PAGE protein profiling.

MATERIALS AND METHODS

The materials for the research work were collected from the field trial of Main experimental station, Vegetable Science field of N.D.U.A. & T., Kumarganj, Faizabad (U.P.) India. Freshly harvested turmeric rhizomes were sorted, cleaned to remove hairy roots, washed with potable water and air dried in open

space for about 10-15 days.

Morphological data

The plant height was measured in centimeters as the length was taken from pseudostem above the soil surface to the tip of the tallest leaf. After harvesting, the rhizome of each plant were cleaned by removing adhered soil particles, number of clump per plant was weighed. The average Length of finger/ mother rhizome was measured with the help of scale by randomly taking 3 finger from the three clump. Its mean value represented the length of finger/ mother rhizome.

Estimation of biochemical contents

The mineral content was determined by the standard procedure of Hart and Fisher (1971). Total carbohydrate was determined by Mc cready *et al*. (1950) and estimation of protein was done as per procedure described by Lowry *et al*. (1951) in seeds of ten germplasms of turmeric collected for the study. A standard curve of absorbance at 660 nm versus 1ìg of BSA was drawn and from this standard curve, the amount of protein in the sample tube was determined as protein per gram of the sample.

Preparation of sample extract

One gm of rhizome was crushed in 1ml of Extraction buffer (50 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$ and 1 mM $MnCl_2$, 1% (v/v) â-mercaptoethanol) buffer and the extract was centrifuged at 2,500 g for 3 min. at 4ºC. The pellet was washed in ice cold acetone and precipitated at the above speed. The final pellet was again dissolve in 0.5 ml above extraction buffer. Now, this is used as a source of sample protein.

SDS PAGE analysis

SDS-PAGE of turmeric rhizome protein was carried out in slab gels according to the method of Laemmli (1970). 80 μl protein samples were loaded onto the gel and the electrophoresis was carried out at 150V for 4% stacking gel and 90V for 15% separating gel. After electrophoresis the gel was stained with 0.05% (w/v) CBB-R250 dye in acetic acid:methanol:water (10:40:50,v/v) for 8 hrs. The gel was destained repeatedly in acid:methanol:water (10:45:45,v/v).

Data analysis and Dendrogram generation

Only clear and reproducible bands were score as 1 for present and 0 as absent and joint bands were not scored for SDS-PAGE. A binary marker protein bands were subjected to the SIMQUAL module of numerical software NTSYS-PC version 2.02 by using Jaccard's coffiecient of similarity to generate genetic distance matrix**.** The genetic distance matrix was subjected to the SHAN module to create a dendrogram based on an unweighted pair group method with arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Morphological analysis

The results of physical parameter of turmeric and turmeric rhizome are presented in Table 1. The data showed that plant height varied from 79.86 cm to 108.43 cm and highest was

Table I: Variation in morphological characteristic of turmeric:

Varieties/ Germplasm	Plant height (cm)	Yield of rhizome/Plant (g)	Length of fingers rhizome (cm)	Length of mother rhizome (cm)	Rhizome colour
NDH-7	104.36	284.56	5.50	7.20	Light orange yellow
NDH-8	108.43	305.93	11.50	10.20	Light orange yellow
NDH-45	79.53	295.16	6.13	5.13	Light yellow
NDH-68	85.70	249.63	9.26	6.80	Dark orange yellow
NDH-69	104.80	254.00	5.36	9.80	Light yellow
NDH-86	80.63	198.40	6.83	8.10	Light yellow
NDH-88	79.86	279.66	5.33	7.50	Light orange yellow
NDH-116	97.96	210.46	5.33	6.20	Light orange yellow
NDH-1	104.23	289.13	7.06	8.56	Dark orange yellow
Prabha	104.60	297.30	12.23	8.36	Dark orange yellow
CD at 5%	8.312	20.433	0.948	1.600	
SEm±	2.797	6.875	0.320	0.538	

Table II: Variation in biochemical content of turmeric rhizome

Varieties/ Germplasm	Carbohydrate content (%)	Total mineral Content (%)	Protein content (%)
NDH-7	64.50	5.12	6.38
NDH-8	69.50	5.21	6.66
NDH-45	60.00	4.83	3.88
NDH-68	68.07	4.89	3.33
NDH-69	67.85	4.95	4.99
NDH-86	64.89	4.43	4.16
NDH-88	68.74	4.72	3.60
NDH-116	66.15	4.79	5.27
NDH-1	69.23	4.96	5.55
Prabha	66.82	4.58	5.83
CD at 5%	NS	0.414	1.756
SEm±	-	0.135	0.568

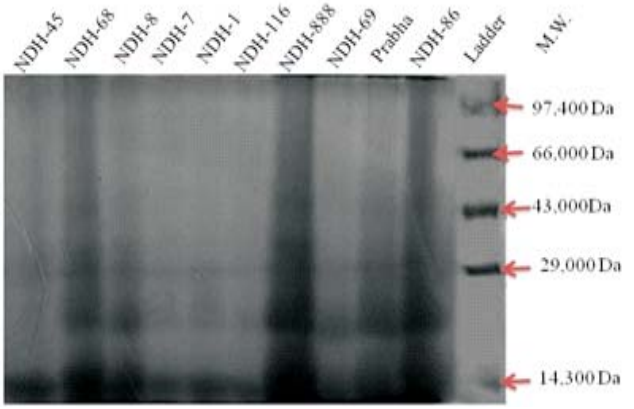

Figure 1: Rhizome protein profile of *Curcuma longa* L. on 15 % SDS PAGE (Lane 1 NDH-45, lane 2 NDH-68, lane 3 NDH-8, lane 4 NDH-7, lane 5 NDH-1, lane 6 NDH-116, lane 7 NDH-88, lane 8 NDH-69, lane 9 Prabha, lane 10 NDH-86, lane 11 Protein weight marker)

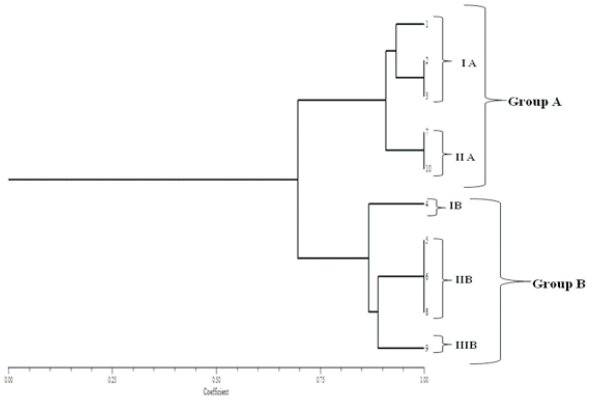

Figure 2: UPGMA cluster analysis showing the relationship among ten turmeric germplasm based on rhizome protein

found in NDH-8 (108.43 cm) followed by NDH-69 (104.80 cm), Prabha (104.60 cm) while lowest value was found in NDH-88 (79.86 cm). The data regarding to the plant height varied significantly in turmeric varieties/ germplasm. A similar observations were supported by Jalani *et al*. (2012) and Srivastava and Singh (2003). The major part of phenotypic variability in the physical characteristics was contributed by additive gene effects (Philips and Nair, 1986). Yield of rhizome/ plant varied from 198.40 g to 305.93 g. Maximum yield of rhizome/plant was recorded in NDH-8 (305.93 g) followed by Prabha (297.30 g) and NDH-86 (296.90 g) whereas, minimum yield of rhizome/plant was accounted in germplasm NDH-86 (198.40 g). The data regarding to the yield of rhizome/plant in turmeric varieties/ germplasm varied significantly during the year. The variation in the yield among the all turmeric cultivars grown under the same agro-climatic condition can be attributed to genetic factor. The results have been favourably supported by Chaudhary *et al*. (2006), however Srivastava and Singh (2003) reported that IC-212577 had highest yield per plant (333.03 g). The data showed that length of finger rhizome varied from 5.33 to 12.23 cm. Maximum length of finger rhizome was recorded in Prabha (12.23 cm) followed by NDH-8 (11.50 cm) and NDH- 68 (9.26 cm) whereas, minimum was accounted in germplasm NDH-116 and NDH-88 (5.33 cm). In respect of data on length of mother rhizome ranged from 5.13 to 10.20 cm. Maximum length of mother rhizome was found 10.20 cm in NDH-8 followed by 9.80 cm in NDH-69 and 8.56 cm in NDH-1 while minimum value was recorded in 5.13 cm in NDH-45. Chaudhary *et al*. (2006) reported that Krishna recorded maximum finger length (10.20 cm) followed by Rajendra Sonia. The results indicate close correlation with finding of Jalani *et al*. (2012). The variation in rhizome characters and fresh yield among various turmeric varieties could be due to genetic factors rather than the environmental condition as reported by Chaudhary *et al*. (2006). Ravishankar et al. (2013) found significant positive correlation of rhizome yield with length of primary finger, number of primary fingers, plant height and diameter of primary finger.

Biochemical analysis:

Data pertaining to biochemical content of turmeric rhizome has been shown in Table 2. Total mineral content varied from 4.43 to 5.21 per cent and highest total mineral content was recorded in NDH-8 (5.21%) followed by NDH-7 (5.12%) and NDH-1 (4.98 %) whereas, lowest value was recorded in NDH-86 (4.43%). These results are in close coherence with Niranjan *et al*. (2003) and Fattepurkar *et al*. (2009). Lokhande *et al*. (2013) also reported variation in mineral content from 6.27-6.81 per cent and highest value was found in Krishna cultivar. The variation observed on mineral content could be related to differences in variety, degree of maturity, agricultural practices, harvest time, plant location and use of fertilizers (Govindarajan, 1980). Some *Curcuma* species are important source of starch too besides its use as a spice and in medicine (Jyothi *et al*., 2003). According to Mangalakumari & Mathew (1986), turmeric is relatively rich in starch. *C. aromatica* Salisb. Or 'Kasturi manjal'(Malayalam-vernacular.) is used as source of starch. (Policegoudra *et al*., 2011). In reference to data on carbohydrate content in turmeric rhizome varieties/ germplasm varied non significantly. Carbohydrate content ranged between 60.00 to 69.50 per cent. It was noticed that maximum carbohydrate content was found in NDH-8 (69.50%) followed by NDH-1 (69.23%) and NDH-88 (68.74%) while minimum was noticed in NDH-68 (60.00%). The result is in close favour with Lokhande *et al*. (2013) who reported that carbohydrate content of all cultivars was in the range of 67.9 to 69.9 per cent. The Salem (67.9%) recorded minimum carbohydrate content while maximum was in Tekurpetha (69.9%). A similar observation was also recorded by Fatterpurkar *et al*. (2009) who found that carbohydrate content was varied from 73.47-78.31 per cent in two genotype of *Curcuma aromatic* L. and four genotype of *Curcuma longa* L. The variation observed on starch content could be related to differences in variety, degree of maturity, agricultural practices, harvest time, plant location and use of fertilizers (Govindarajan, 1980; Krishnamurthy *et al*., 1975). The variability in the carbohydrate content was also attributed to spacing between plants. The carbohydrate content increases with decreasing the plant distance and it was highest at 15 cm spacing between plant and the minimum value was observed at 35 cm distance Kamal and Yousuf (2012). The relatively high starch content in the present study

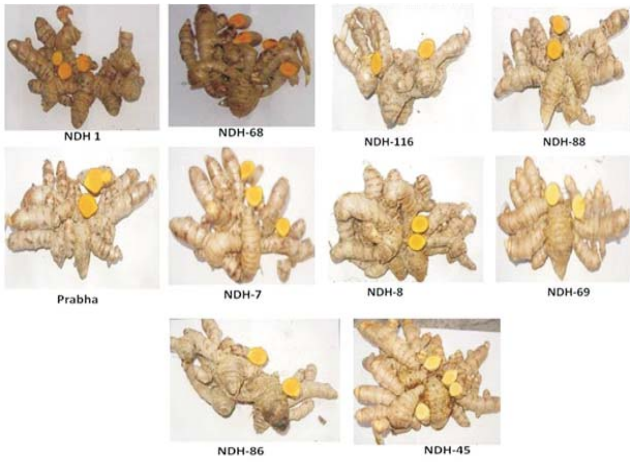

Figure 3: Morphological variability of different turmeric (*Curcuma longa* L.) rhizome

may be due to the genotype and the stage of maturity. Protein content in turmeric rhizome varied from 3.33 to 6.66 per cent. It was observed from the table that maximum protein content was recorded in NDH-8 (6.66 %) followed by NDH-7 (6.38 %) and Prabha (5.83%) whereas minimum was found in NDH-68 (3.33 %). Similar trends were reported by Niranjan *et al*. (2003) and Fattepurkar *et al*. (2009). Total protein content in *C. aromatica* ranged from 8.25 to 9.98 per cent (Sajitha *et al*., 2014). It was revealed from the data that total mineral content and protein content in turmeric varieties/ germplasm varied significantly unlike carbohydrate content which varied non significantly.

SDS-PAGE analysis

Variation in the presence or absence and the relative mobility of some electrophoretic bands was observed among ten turmeric germplasm on the basis of rhizome proteins. The highest variation in the electrophoretic profile of turmeric *(Curcuma longa)* germplasm was observed around 43 kDa to 14 kDa. All ten turmeric germplasm differ in intensity of bands as well as in number of protein bands. Maximum number of band was found in NDH-68 followed by NDH-88 and NDH-8, while NDH-7 and NDH-116 had less number of bands in comparison to other germplasm. A unique band of 43 kDa was observed in NDH-68 and NDH-88, while 32 kDa and 14 kDa protein band was observed in all the turmeric germplasm. In NDH-68 and NDH-88 35 kDa protein band was present.18 kDa protein band was observed in all the turmeric germplasm except in NDH-45. Electrophoretic profile as observed in rhizome storage protein NDH-68 and NDH-88 and NDH-86 had highest intensity of bands however in NDH-45, NDH-7, NDH-1, NDH-116 and NDH-69 had lowest intensity of bands while Prabha had medium intensity of protein bands in comparison to others. Most of the proteins were ranged from 43kDa to 14 kDa. Jayakumar *et al*. (2001) also studied the change in protein content and protein profile in the rhizome of turmeric (*Curcuma longa* L.) from the day of harvest the commencement of sprouting during different storage periods. The trend of total protein showed a gradual increase during the initial stage of storage period. As much as 35% increase was shown after 45 days. The rhizome of *Curcuma longa* L. exhibited about 90 % increase in protein content from the date of harvest to the end of dormancy. 66, 52 and 47 k Da protein have not been synthesized till 30 days of storage and prominently appear after 45 days of storage. In samples collected after 45, 60 and 75 days of storage, both 23 and 18 kDa protein have disappeared. Similar report with present findings was also reported by Moon-ai *et al*. (2011) in their study on the apparent purity (homogeneity) of the protein composition of the Superdex 75 fraction by reducing SDS-PAGE. The protein was comprised of only one detected band with an apparent molecular weight of approximately 32.5 kDa.

Dendrogram analysis

The data obtained from SDS-PAGE was scored for the presence (1) and absence (0) of the bands and entered in a binary data matrix. Based on the results of electrophoretic band spectra, similarity index was calculated for all possible pair of electrophoregrams. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct the dendrogram by the unweighted pair group average method (UPGMA). Unweighted Pair Group Method with Arithematic Mean (UPGMA) dendrogram based on NTSYS-pc version 2.02 clearly separates all ten turmeric rhizome variety/ germplasm into two major groups (A and B) at 0.70 of similarity coefficient. Both major groups were separated with five germplasm in which Group A has five germplasm i.e. NDH-45, NDH-68, NDH-8, NDH88 and NDH-86 while group B has NDH-7, NDH-1, NDH-116, NDH-69 and Prabha. Major group A is again subdivided into two subgroups *i.e.* subgroup IA & II A. Subgroup IA has three germplasm i.e. NDH-45, NDH-68, NDH-8 in which NDH-68 and NDH-8 separated in the same subgroup and NDH-45 separated alone in another group. In subgroup IIA NDH88 and NDH-86 separated. Major group B is divided into three subgroup IB, II B, III B. IB has only one turmeric genotype i.e. NDH-7, NDH-1, NDH-116 and NDH-69 separated in the subgroup IIB while Prabha is separated alone in III B subgroup.

Maximum similarity among ten turmeric germplasm based on the rhizome protein were found in between NDH-88 and NDH-86, NDH-8 & NDH-68 and in between NDH-1, NDH-116 and NDH-69 with 100 % of similarity coefficient followed by Prabha & NDH-1 with 89 % of similarity coefficient. Minimum similarity was found between NDH-88 and NDH-7 with 56 % of similarity coefficient followed by Prabha and NDH-45 with 66 % 0f similarity coefficient. The results were in agreement with the findings of Erum *et al*. (2011) evaluated 28 accessions of *Trigonella* species collected from various ecological zones of Pakistan and to make a distinction of Kasuri methi from Kasur with other germplasm through slab type SDSPAGE using 12.25% polyacrylamide gel. Total three groups emerged on the dendrogram. Group I showed 100% similarity among themselves. Group II is also the representative of accessions of Methray. Third independent group represent the accession collected from Kasur commonly known as Kasuri Methi. Comparative analysis of protein profile of Kasuri Methi with Methray (Group I and II) showed that it is 60% dissimilar. Similar finding was also reported by Yatung, *et al*. (2014) on thirty chilli (*Capsicum annuum* L) germplasm collected from different part of the North eastern and other part of India and cultivated at research farm of Department of Vegetable Science,

College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India during March-October, 2011. The numerical analysis of SDS-PAGE of seed protein profiles showed that each cluster had slight discriminative protein banding profile. The cluster I includes1 of reference chilli germplasm (CHFC11 - 20), sharing many protein bands. The members of sub cluster Ia which were from East Siang, Arunachal Pradesh having highest intra-cluster similarities (99.51%). This finding confirmed that the genotype CHFC14 and CHFC15 may be very close at genetic level even though there was much difference at morphological traits. the germplasm CHFC9 and CHFC18 belongs to different sub cluster and maximum genetic distance (53.098 %) though they were collected from East Siang, Arunachal Pradesh which were having almost similar phenotypic traits.

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