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STUDY ON GENETIC DIVERSITY OF POINTED GOURD (*Trichosanthes dioica* Roxb.) BASED ON ISSR MARKERS

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ABSTRACT

The genetic diversity studies were conducted during 2012-13 and 2013-14 on pointed gourd are based on morphological and growth habit, which can cause problems in identification of genetic relatedness. Identification of genetic relationships in 24 genotypes of pointed gourd was investigated using Inter Simple Sequence Repeat (ISSR) primers. Analysis started by using twelve markers that allowed us to distinguish 24 genotypes. Out of twelve, four ISSR primers were used for identification and for establishing a profiling system to estimate genetic diversity, which generated a total of 426 bands. The similarity coefficient ranged from 0.78 to 0.95. The dendrogram indicated that all genotypes are grouped into 2 major clusters namely; A and B. Major cluster 'A' consisting of only 23 genotypes whereas major cluster 'B' consisting of only 1 genotype that was IPG-16 variety having 0.80 similarities coefficient with cluster 'A'. Genetic diversity analysis based on molecular markers in pointed gourd genotypes showed higher similarity at genetic level amongst the genotypes of point gourd. Only one genotype IPG-16 showed genetic diversity as compared to other twenty three genotypes.

(Key words: Pointed gourd, ISSR, genetic diversity)

INTRODUCTION

Pointed Gourd *(Trichosanthes dioica* Roxb.) is a perennial, dioecious, tropical cucurbitaceous vegetable crop which gives continuous production for about 7-8 months of the year except winter season when the plants goes under dormancy. It is known by the various vernacular names like *parwal*, *palwal*, *parmal, parora, patal, patola* or *potal* in different parts of India. Pointed gourd is believed to have originated in Indian subcontinent or Indo-Malayan region is as its original home (Seshadri,1986). It is one of the important cucurbitaceous vegetable of northern India. This crop is extensively cultivated in eastern Uttar Pradesh, Bihar, West Bengal and Assam and to a lesser extent in small pockets in Odisha, Chhattisgarh, Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu and Gujarat. Recently, it has been introduced in and around Hyderabad and Bangalore region (Bhardwaj, 2011).

In the gangetic plains, it is mostly grown on sandy soils in river beds. In Chhattisgarh state, its cultivation is mostly confined to the parts of Raigarh, Sarguja, Surajpur and Jashpur districts. Pointed gourd is coveted vegetable during summer and rainy seasons. It is considered to be highly remunerative vegetable crop by the farmers as it is one of the choicest cucurbit which is liked by the consumers. Owing to comparatively low cost of production, high return and suitability for riverbed cultivation, pointed gourd is mostly grown by small and marginal farmers. This vine vegetable has better keeping and handling quality and can be transported to long distance to other states where it is not under cultivation.

Recent molecular phylogenetic data have indicated that genus, *Trichosanthes*, is the largest genus in the Cucurbitaceae family, with over 90 species (Boer and Thulin, 2012) of which 22 are found in India (Singh *et al.,* 2008). Although, the Bengal-Assam area in India is considered as its primary centre of origin, the species and wild forms of *Trichosanthes* are found in many parts of India. The related species are *Trichosanthes japonica* and *Trichosanthes multiloba* which occur in the north-eastern region; along with semi-wild *Trichosanthes dioica*. A widely distributed species is *Trichosanthes bracteates* which occurs in eastern

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India, extending to the south, and sporadically in the Himalaya. *Trichosanthes cordata* (related to *Trichosanthes anguina)*, occurs in the peninsular region extending to north-eastern plains and hills. The green tender fruit is the edible part of the plant, which is cooked in various ways either alone or in combination with other vegetables. The fruit is also utilized to make special sweets. Its tender shoots and leaves are also used as pot herbs particularly in West Bengal. The fruits of pointed gourd are rich in neutra-ceutical properties. They are rich in protein and vitamin A. Fruits also contains ample quantities of various nutrients and mineral elements. It contains protein 2.0 g, fat 0.3 g, carbohydrate 2.2 g, fiber 3.1 g, Mg, 9.0 mg, Na 2.6 mg, K 83.0 mg, Cu 1.1 mg, S 17.0 mg, phosphorus 40.0 mg, calcium 30.0 mg, thiamine 0.05 mg, nicotinic acid 0.5 mg and oxalic acid 7.0 mg 100^{-1} grams of edible portions.

The fruits are easily digestible and diuretic in nature and are also reported to have anti-ulcerous effects and many other medicinal properties. Apart from being a rich source of carbohydrates and vitamins, the stem and leaves of pointed gourd also contains many active bio-compounds with great medicinal properties (Sharma *et al.,* 1990; Rai *et al.,* 2010). It also invigorates the heart and brain. It is purported that pointed gourd possesses the medicinal property of lowering total cholesterol and blood sugar. These claims are supported by preliminary clinical trials with rats (Chandra Sekar *et al.,* 1988) and rabbits (Sharma and Pant, 1988; Sharma *et al.,* 1988). Pointed gourd is useful in the disorders of the circulatory system. The fruit shows some prospects in the control of certain cancer like conditions. According to Ayurveda, leaves of the plant are used as antipyretic, diuretic, cardio tonic, laxative, antiulcer, etc. Juice of leaves of *T. dioica* is used as tonic, febrifuge, in edema, alopecia, and in sub-acute cases of enlargement of liver (Nadkarni, 1982). In Charaka Samhita, leaves and fruits find mention for treating alcoholism and jaundice (Khare, 2004).

Although, well developed seeds are produced by the plants, seed propagation is not feasible mainly due to lower germination, slow growth rate of seedlings and segregating nature of the male and female plants (Kumar *et al.,* 2008 a). The dioecious nature of the plant has been the major constraint in initiating a substantial breeding programme in pointed gourd. It shows severe gender bias in favour of female plants and the ratio of female to male plant is normally 2.5:1. The plant strictly maintains the sexual phenotypes of male and female indicating clear genetic difference between both the sexes. However, there is little or no substantial morphological difference between male and female of *T. dioica* to identify the sex type before reproductive time. Genetic diversity and variability play a vital role for a successful breeding programme and it is essential to meet the diversified goals of plant breeding

Table 1. Details of genotypes of pointed Gourd (*Trichosanthes dioica* Roxb.)

Sr. No.	Collection No. and Name	Notation	Sources of Material
1.	Indira Pointed Gourd-1	IPG-1	Department of Horticulture, IGKV
2.	Indira Pointed Gourd-2	IPG-2	Department of Horticulture, IGKV
3.	Indira Pointed Gourd-3	IPG-3	Department of Horticulture, IGKV
4.	Indira Pointed Gourd-4	IPG-4	Department of Horticulture, IGKV
5.	Indira Pointed Gourd-5	IPG-5	Department of Horticulture, IGKV
6.	Indira Pointed Gourd-6	IPG-6	Department of Horticulture, IGKV
7.	Indira Pointed Gourd-7	IPG-7	Department of Horticulture, IGKV
8.	Indira Pointed Gourd-8	IPG-8	Department of Horticulture, IGKV
9.	Indira Pointed Gourd-9	IPG-9	Department of Horticulture, IGKV
10.	Indira Pointed Gourd-10	IPG-10	Department of Horticulture, IGKV
11.	Indira Pointed Gourd-11	IPG-11	Department of Horticulture, IGKV
12.	Indira Pointed Gourd-12	IPG-12	Department of Horticulture, IGKV
13.	Indira Pointed Gourd-13	IPG-13	Department of Horticulture, IGKV
14.	Indira Pointed Gourd-14	IPG-14	Department of Horticulture, IGKV
15.	Indira Pointed Gourd-15	IPG-15	Department of Horticulture, IGKV
16.	Indira Pointed Gourd-16	IPG-16	Department of Horticulture, IGKV
17.	Indira Pointed Gourd-17	IPG-17	Department of Horticulture, IGKV
18.	Indira Pointed Gourd-18	IPG-18	Department of Horticulture, IGKV
19.	Indira Pointed Gourd-19	IPG-19	Department of Horticulture, IGKV
20.	Indira Pointed Gourd-20	IPG-20	Department of Horticulture, IGKV
21.	Indira Pointed Gourd-21	IPG-21	Department of Horticulture, IGKV
22.	Indira Pointed Gourd-22	IPG-22	Department of Horticulture, IGKV
23.	Swarna Alaukik	Swarna Alaukik	HARP, Ranchi, Jharkhand
24.	Swarna Rekha	Swarna Rekha	HARP, Ranchi, Jharkhand

such as breeding for increasing yield, wider adaptation and desirable quality (Khan *et al.,* 2016). Wide genetic variability of pointed gourd is available in Chhattisgarh (Panigrahi *et al.,* 2015). Since the genotypes collected are poorly characterized and at the beginning of any breeding programme, it is significant to discriminate among available genotypes to establish the level of genetic diversity so as to, identify the most suitable materials for utilization as cultivar for the farmers (Singh and Singh, 2015). No systematic efforts have been made till date to study the genetic diversity of this important vegetable crop in Chhattisgarh. Genetic diversity among individuals or populations can be determined using morphological and molecular markers. In contrast to morphological markers, molecular markers based on DNA sequence polymorphism, are independent of environmental conditions. Identification of genotypes based on morphological markers requires observations at all the stages of plant growth till flowering and fruiting and is not also very reliable because many traits of interest have low heritability and may be genetically very complex. Molecular markers provide a quick and reliable method for estimating genetic relationships among genotypes of any organism (Thormann *et al.,* 1994).

MATERIALS AND METHODS

The present investigation was conducted at Dr. R.H. Richharia lab, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during *kharif* 2013-14. Raipur is situated at the 21^0 16' N latitude and 81^0 36' E longitudes at an altitude of 289.56 meters above mean sea level. Raipur, the place of investigation, is a sub-humid region. It comes under the seventh agro-climatic zone of the country that is Eastern Plateau and Hills. In all, twenty four genotypes were collected from Department of Horticulture, IGKV, Raipur and HARP, Ranchi, Jharkhand (Table 1).

Genomic DNA isolation

The five to seven fresh leaves of each of the genotypes were collected in plastic bags under natural condition. These leaves samples were stored in refrigerator and used for isolation of genomic DNA. Genomic DNA was isolated by modified CTAB method of DNA Extraction as suggested by Jonathan and Wended (1990). Quantification of DNA samples isolated from each line was quantified on Nano Drop Spectroscopy (*NANODROP*, *2000c)* at 260 nm. For PCR amplification the concentrated DNA was diluted with sigma water such that the final concentration of DNA was maintained 40 g^{-1} µl for better amplification.

PCR amplification was performed for 12 ISSR markers in 24 genotypes in a reaction volume of 20 µl for confirmation of diversity analysis. Amplification was carried out in a thermal cycler (Applied Biosystems, 96 well veriti) with PCR buffer of 10X concentration with 15mM $MgCl_2$) (Genei make), 1mM dNTPS (Genei make), 5 ñmol ISSR primers (ILS make) and *Taq* DNA polymerase enzyme (Genei make 1U/ul) along with template DNA of 40 g^{-1}µl was taken. The initial denaturation cycle was of 2 min at 94°C followed by 35 cycles of denaturation at 95°C for 35 sec; annealing at 48-53°C for1 min and extension at 72°C for 90 sec along with final extension at 72°C for 15 min followed by storage at 4 for infinity. Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified products, since polyacrylamide gels have better resolution for amplified products. Gels were casted in *CBS-SCIENTIFIC* electrophoresis unit. At last, Bromophenol Blue loading dye (10X) was added to PCR products with 50bp Ladder (Genei make) was loaded in the first well. After electrophoresis, gels were stained with Ethidium bromide (10$µl^{-1}$100 ml) and visualized in BIORAD Gel Doc XR+.

Scoring of the bands as separated by gel was done in decreasing order of alleles for each of primers was done in decreasing order of allele's analyzed using NTSYS (Numerical Taxonomy System Biostatistics) PC Ver. 2.02e numerical software package. Only visually scorable and reproducible clear bands were considered for the construction of binary data matrix as one (1) for presence and as zero (0) for absence of band and those not amplified were designated as (NA). The bands which were very faint were not considered for scoring. The scores obtained using all the primers in the ISSR analysis were then pooled to create a binary data matrix and used to construct a dendogram using UPGMA (Unweighted Pair Group Method of Arithmetic Means) algorithm. The pair wise similarity between isolates and polymorphic bands were calculated using the genotypic data thus generated was used for studying molecular diversity analysis Jacquard's coefficient (Jaccard, 1908).

RESULTS AND DISCUSSION

Identification of genetic relationships or genetic divergence in pointed gourd is very difficult because of the lack of morphological differences and erratic flowering. Authentic identification of taxa is necessary both for breeders to ensure protection of intellectual property right and also for propagators and consumers. The most traditional method of identifying species by phenotypic characters is now replaced by protein that is more reliable and authentic or DNA profiling largely because of several limitations of morphological data. In a current scenario, DNA profiling through ISSR technique has been used for the analysis of diversity and identification of duplicates within the large germplasm populations [22], phylogenetic relationship [23], rational designing of breeding programs [24] and management of genetic resources [25]. Evidently, ISSR technology is a rapid and sensitive technique, which can be used to estimate relationships between closely, and more distantly related species and groups of pointed gourd.

The genomic DNA was isolated from fresh young leaves of twenty-four pointed gourd accessions. The range of DNA quantity in the samples varied from 1250 ng to 2200 ng $µl^{-1}$. Having this wide range, the DNA of all the 24 samples

Table 2. Primer sequence of 4 polymorphic ISSR markers used for diversity analysis in 24 pointed gourd genotypes

Marker	Primer 5' to 3'	No. of bases	Tm (^{0}C)
UBC 807	AGAGAGAGAGAGAGAGT	17	42.5
UBC 810	GAGAGAGAGAGAGAGAT	17	42.9
UBC 812	GAGAGAGAGAGAGAGAA	17	44.3
UBC 815	CTCTCTCTCTCTCTCTG	17	44.9

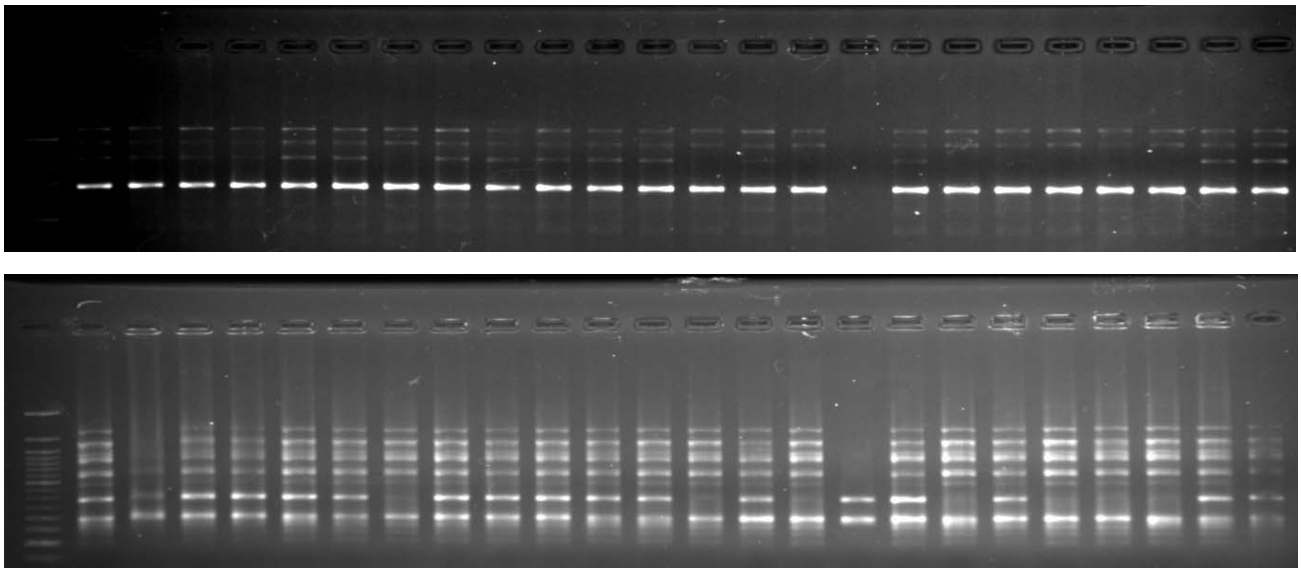


Fig. 1. ISSR profile generated by UBC 807 and UBC 812 primers of twenty four pointed gourd genotypes

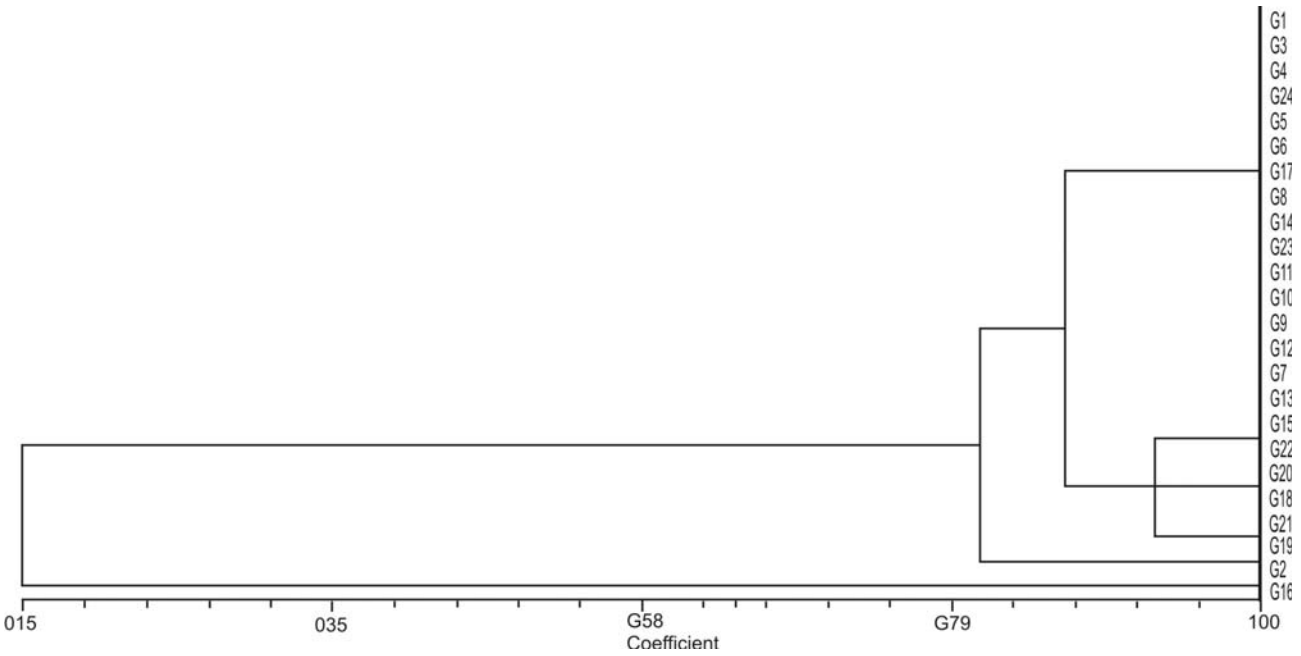
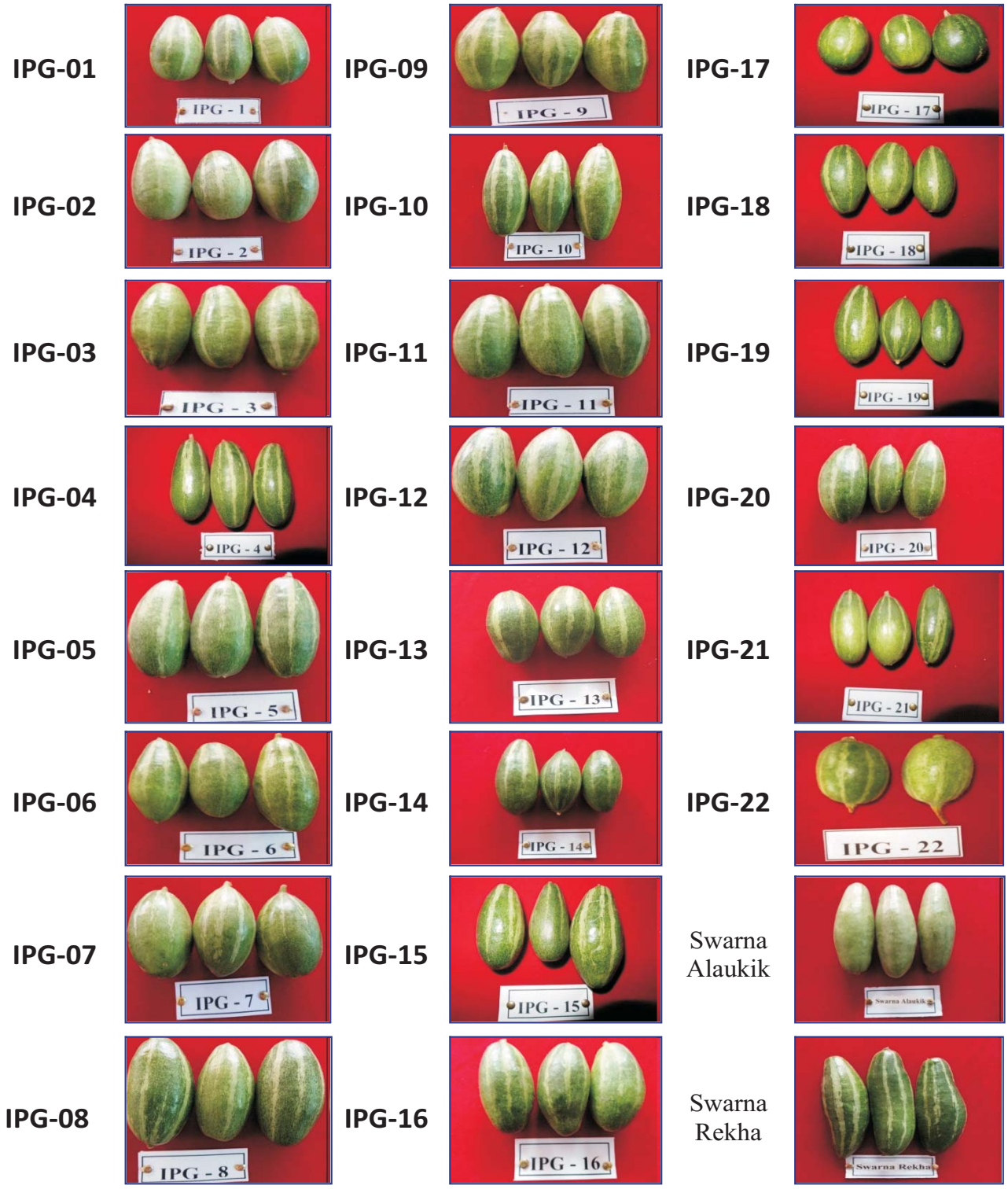


Fig. 2. Dendrogram constructed using UPGMA based on Jaccard's coefficient of 24 pointed gourd genotypes (ISSR Markers)

Fig. 3 Depicting Variability for leaf (IPG-01 to IPG-24)

Fig. 4 Depicting variability for Fruit (IPG-01 to IPG-24)



were brought to uniform concentration of $40ng^{-1}\mu l$. ISSR markers were taken for estimation of molecular diversity of pointed gourds. ISSR results were analyzed using NTSYS (Numerical Taxonomy System Biostatistics) PC Ver. 2.02e numerical software package. Out of 12, 4 ISSR primers (Fig. 1) showed typical examples of ISSR amplification patterns which generated a total of 426 bands which were scored as present (1) or absent (0) for determining the genetic relationship among the genotypes. Similarity matrices were calculated using NTSYS (Numerical Taxonomy System Biostatistics) computer programme. Cluster analysis was done within the SAHN program by using UPGMA (Unweighted pair-group method with arithmetic averages) method.

The similarity coefficient ranged from 0.78 to 0.95. The dendrogram indicated that all genotypes are grouped into 2 major clusters namely; A and B. Major cluster 'A' consisting of only 23 genotypes whereas major cluster 'B' consisting of only 1 genotype that is IPG16 variety having 0.80 similarities coefficient with cluster 'A' (Fig. 2).

Major cluster 'A' showed 2 sub clusters as 'A_1' and 'A_2' near the 0.75 similarity level which consist 22 and 1 genotype respectively. Sub-cluster 'A_1' showed further sub-clustering near the 0.68 similarity level as A_1 (a) and A_1 (b) which consisted 14 and 8 genotypes respectively. Sub-cluster A_1 (a) have 14 genotypes; whereas sub-cluster A_1 (b) showed further sub-clustering near the similarity level 0.70 as A1 (b1) and A1 (b2) which have 7 and 1 genotype(s) respectively.

Genetic diversity analysis based on molecular markers in pointed gourd genotypes showed higher similarity at genetic level amongst the genotypes of point gourd. Only one genotype IPG-16 showed genetic diversity as compared to other twenty three genotypes.

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