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Cyto-morphological and Biochemical Studies of Pointed Gourd (Trichosanthes dioica Roxb.

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CYTO-MORPHOLOGICAL AND BIOCHEMICAL STUDIES OF POINTED GOURD (*TRICHOSANTHES DIOICA* ROXB.)

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INSTIUTTE OF BIOLOGICAL SCIENCES
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RAJSHAHI, BANGLADESH

THIS DISSERTATION IS DEDICATED

TO MY

BELOVED MOTHER

AND

DEPARTED FATHER

University of Rajshahi

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CERTIFICATE

I feel pleasure in certifying the thesis entitled **"Cyto-morphological and Biochemical Studies of Pointed Gourd (*Trichosanthes dioica* Roxb.)"** submitted by Md. Alamgir Kabir to the Institute of Biological Sciences, University of Rajshahi, Bangladesh for the degree of Doctor of Philosophy.

I hereby certify that i) Md. Alamgir Kabir has fulfilled the required residential period, ii) the works included in this thesis were carried out by him and iii) to my knowledge the data are original and genuine.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This investigation was conducted to study the morphological, cytological and biochemical behaviours among eighteen varieties/lines of *Trichosanthes dioica* (Roxb.). Morphological variations in eighteen varieties/lines of *Trichosanthes dioica* were determined by Metroglyph method which might have reflected their genetic relationship.

Interphase nuclear phenotype of *Trichosanthes dioica* were studied in present investigation. Interphase nuclear volume (ICV) was measured. Percentage heterochromatin values were also calculated statistically by determining the area of nucleus and of chromocentres by planimetry. Then the heterochromatin values were expressed as percent nuclear area.

Lengths and ratios of representative complement of shoot tip chromosomes of *Trichosanthes dioica* were determined at the desirable stage of cells and were plotted in scatter diagrams. The average values of total length and arm ratio were calculated constituting the haploid complement of those cells. Then the chromosome of haploid complements were numbered in decreasing order of total chromatin length and increasing order of arm ratio within the same length. Total frequency was estimated and the values were expressed in percentage. Finally, the standard karyotype was derived based on centromeric formula and range of chromatin lengths per chromosome.

From extensive karyotypic analysis in the present study sex chromosomes were identified based on probabilistic inferences. The sex chromosomes were assumed completely based on inferential way and they were termed as sex regulatory. They varied from variety to variety/line and thus, it was also assumed that the sex in pointed gourd is governed by the particular gene.

Preliminary phytochemical analysis of metabolic extracts from leaves of pointed gourd (*Trichosanthes dioica* Roxb.) revealed the presence of alkaloids, flavonoids, glycosides, tannins and steroids in different density. Quantitatively two phytochemical compound i.e., soluble sugar and chlorophyll content were estimated from the extracts of leaves. Estimation of protein, phenol, proline and antioxidant capacity (%) were made from the extracts of fresh fruit of pointed gourd. The antioxidant capacities of methanolic fruit extracts of *Trichosanthes dioica* was determined by *in vitro* assay models such as DPPH free radical assay.

Polyacrylamide gel electrophoresis was used for determining protein banding pattern in ten varieties/lines of *T. dioica*. Variations were observed in number and intensity of bands. A total of 47 bands were found and highest similarity was found to be 80% between BARI 1 and BARI 2. Lowest index was found to be 14.28% between BARI 1 and PG006 of pointed gourd.

CHAPTER ONE

INTRODUCTION

INTRODUCTION

Trichosanthes dioica Roxb. (Family: Cucurbitaceae), commonly known as pointed gourd in English and potol in Bengali is a dioecious perennial herbaceous vegetable. It is widely grown throughout India (Chakravarthy, 1982) and fruits of this plant are used as vegetable in Indian traditional food system from time immemorial. It is one of the popular cucurbitaceous vegetable crops cultivated in Bangladesh. The Bengal-Assam area is the primary centre of origin of pointed gourd (Nath and Subramanyam, 1972) and to a lesser extent in other parts of South Asia (Mythili and Pious, 1999). Besides fruits, other parts of the plant such as the leaves and tender shoots are also being used in the traditional system of medicine since ancient times (Sharma and Pant, 1988 and Singh, 1989).

The plant of pointed gourd is creeper and grows as vine. Roots are tuberous with long taproot system. Vines are pencil thick in size with dark green cordate simple leaves. Flowers are tubular white with 16–19 days initiation to anthesis time for pistillate flowers and 10–14 days for staminate flowers. Stigma remains viable for receptive approximately 14 hours and 40–70% of flowers set fruit. Based on shape, size and striation, fruits can be grouped into 4 categories (Singh and Whitehead, 1999) viz. (1) Long, dark green with white stripes, 10–13cm long, (2) Thick, dark green with very pale green stripes, 10–16cm long, (3) Roundish, dark green with white stripe, 5–8cm long and (4) Tapering, green and striped, 5–8cm long (Singh, 1989). Traditionally *T. dioica* is multiplied through seeds, stem cuttings and root cuttings. Propagation through seeds is not desirable due to poor germination and imbalanced male-female ratio. Seed based populations have a tendency to give more male than female plants and in some cases the ratio goes up to 85:15 limiting their use as their utility ends with pollination (Som *et. al.*, 1993).

For study on genetic diversity using morphological characters 64 pointed gourd genotypes (PG001 to P064) were assessed through multivariate analysis from an experiment conducted in Regional Agricultural Research Station, Ishurdi, Pabna during the growing season 2002-2003 (Khan *et. al.*, 2008). The genotypes were grouped into twelve clusters. The genotypes of Jessore were distributed in different clusters. The highest inter genotype distance as 366.3 was observed between the genotypes P0022 and P0007, and the lowest 2.6 was observed between the genotypes P0043 and P0044. Cluster V had the highest cluster mean value for internode length, fruit weight per plant and yield. The highest inter-cluster distance was noticed between cluster III and II (45.71) and the lowest between cluster VII and VI (3.33). Fruit weight, seeds per fruit and fruit weight per plant contributed maximum to the total divergence.

An experiment was conducted on variability and estimation of genetic parameter, correlation, path analysis and genetic diversity of 24 accessions of pointed gourd *(Trichosanthes dioica)* at Regional Agricultural Research Station, BARI, Ishurdi, Pabna during the period from November 2005 to November 2006 and 2007 (Kabir, 2007). Significant variations were recorded among the pointed gourd accessions in respect of different parameters such as days to first flower, fruit length, fruit breadth, single fruit weight, pulp seed ratio, number of fruits per plant, weight of fruit per plant and yield of fruit. Correlation coefficient indicated that fruit yield per plant was highly significant and there was a positive association with weight of fruit per plant, number of fruits per plant and single fruit weight.

Trichosanthes dioica Roxb. is extensively propagated through vegetative means and as a result of continuous vegetative propagation and judicious selection, a large number of varieties and forms having restricted distribution have been accumulated (Sarker *et. al.*, 1987). These cultivars are commercially available in the market under different local names without any uniformity and standardization in nomenclature. Moreover, no agronomic information is available which can be used as basis for delineating and standardizing different available cultivars. These cultivars have remained completely uninvestigated from the cytological aspects. The importance of karyotype study in establishing phyletic relationship and evolutionary trends is well recognized (Sharma and Sharma, 1959). Lately, with the aid of improved chromosome techniques it has been possible to work with the chromosomal basis of intervarietal and even interstrain differences in a large number of cruciferous taxa such a *Cheiranthus, Hesperis, Iberis, Matthiola, Raphanus* etc. (Datta, 1971 and 1974; Sharma and Datta, 1961). A numerical uniformity in the chromosome complements is represented in the cytologically investigated members of *Trichosanthes.* A somewhat detailed morphological and chromosomal analysis of the mentioned cultivated varieties and related taxa may throw light to the matters of cytomorphological and phylogenetic interest. With these views the present investigation was conducted in a part considering conventional cytological aspects.

However, remarkable advances have been made in techniques for the study of linear differentiation of chromosomes which have revolutionized the cytogenetic research (Sarker and Datta, 1987). The extreme usefulness of the banding techniques has been proved in its application in precise identification of individual chromosomes. Developed techniques for visualization of constitutive heterochromatin provide means for studying the distribution pattern of the same in plant materials. Since nineties, RAPD marker has been used for gender determination successfully in many economically and ecologically important dioecious plant species such as *M. dioica* (Baratakke *et. al*., 2013), *Populus tremuloides* (Hou *et. al*., 2009), *Trichosanthes dioica*

(Kumar *et. al.*, 2008) etc. But this method has also some disadvantages, like poor reproducibility. There have been relatively few studies so far which have attempted to exploit Giemsa C-banding techniques also to investigate the relatedness between taxa.

Five commercial varieties of *Trichosanthes anguina* (Snake Gourd), namely Turag, Dhaka Green, Super Long Green, Anika and Apurbo were investigated cytogenetically at the molecular level for authentic characterization (Alam *et. al*., 2011). The variety Super Long Green was found to possess 2*n* = 23 chromosomes and considered as a primary trisomic. 2*n* = 22 chromosomes were found in other 4 varieties. The 5 varieties showed distinct RAPD bands with 6 different primer combinations. In addition, the Apurbo and Turag, varieties showed unique DNA fragments with the primer combinations OPA-3 and OPA-4 respectively. A combination of cluster and karyotype analysis clearly indicated that Turag and Apurbo were distinctly related from the other 3 varieties. Therefore, with the help of cytogenetical and RAPD analysis, it was possible to characterize each variety.

Fifty two progenies of pointed gourd raised through embryo culture, were used for identification of RAPD markers associated with sex expression traits (Kumar *et. al.*, 2012). Genomic DNA from male and female progeny plants and from a parthenocarpic clone (IIVRPG-105) were extracted individually and bulked by sex type. Forty one random decamer primers were screened with the three bulks and a total of 509 amplification products were obtained, of which five were found to be associated with sex expression. The five markers were then tested with individual plant DNA samples, and two sex-associated RAPD markers were identified. A 1000bp amplification product from the primer OPC05 was found to be present only in males and absent in both the female and parthenocarpic plants. Similarly, a 400bp amplification product from the primer OPC14 was found to be present only in female individuals.

Knowledge on magnitude and nature of genetic variation helps in formulating breeding programme for improvement of a crop. Objective of a study was to use a diverse set of pointed gourd germplasm to estimate the extent of genetic diversity with respect to yield and yield components by Bharathi and Vishalnath (2010-2011). Another study on genetic variation in 64 pointed gourd accessions was conducted using the Randomly Amplified Polymorphic DNA (RAPD). Out of 45 random primers screened five were selected, which gave 38 clear and bright fragments, out of which 30 (79.5%) fragments were considered polymorphic (Khan *et. al.*, 2009).

Chromosome studies and *in situ* estimation of 4C nuclear DNA content were carried out on dioecious *Coccinia indica* and *Trichosanthes dioica* to understand the differential condensation of mitotic chromosome in relation to sex (Guha *et. al*., 2004). The somatic chromosome number for each sex form of *C. indica* and *T. dioica* was

found to be 2n = 24 and 22, respectively. The karyotypes of the sex forms of both species show high homogeneity, though a distinct hetermorphic pair of sex chromosomes is found in male plants of *C. indica*. Interrelationship between the 4C nuclear content and the chromosome length has been explained in terms of average packing ratio. The data suggests that average packing ratio is a determinant of distinction between two sexes and therefore could be used as a parameter for analyzing the karyotypes of dioecious plants.

Pointed gourd strictly maintains the sexual phenotypes of male and female indicating clear genetic difference between both sexes (Adhikari *et. al*., 2014). This clear differentiation of sexual phenotype, combined with its perennial nature, an increasing economic importance of the crop, and recent interest in breeding improved cultivars, makes the species attractive for the study of different aspects of sex determination.

Cytological and immunochemical studies were carried out on dioecious *Coccinia indica* to understand the genetic control of sex expression (Guha *et. al*., 2014). The somatic chromosome number of both the sex forms of *C. indica* was found to be 2*n* = 24 and the karyotype shows high homogeneity, though a distinct heteromorphic pair of sex chromosomes is found in male sex forms of *C. Indica*. Soluble protein profile from the tuberous roots of the male and female plants of *C. indica* did not show any marked distinction, only a variation in the staining pattern was observed.

Cucurbitaceae is one of the most genetically diverse groups of plants approximately having 130 genera and 800 species (Renner and Pandey, 2013; Jeffrey, 2005) many of which are economically important.

Classical studies have established that, during meiosis, the X and Y chromosomes of the model dioecious plant *Silene latifolia* pair over a region at the ends of their q arms (Lengerova *et. al*., 2003). They used fluorescence *in situ* hybridization of two molecular markers to demonstrate that this widely accepted model is incorrect. From their data they concluded that the homologous arm of the X chromosome is the p arm and that of the Y chromosome is the q arm. The establishment of the proper orientation of the pseudoautosomal region is essential for mapping and evolutionary studies.

Dioecism accompanied by sex chromosome dimorphism is common in animals but less prevalent in plants. In a minority of dioecious plants, sex determination depends on sex chromosomes, usually an XY system, in which males are heterogametic (XY) and females are homogametic (XX) (Charlesworth, 2002; Matsunaga and Kawano, 2001; Ainsworth, 2000). The presence of dioecious forms in a number of cucurbitaceous genera makes it an interesting model family to study sexual dimorphism, but the mechanism of sex determination has been studied in only a few such plant species as *Coccinia* and *Bryonia* (Roy and Roy, 1971; Correns, 1903).

In the plant kingdom dioecy is found only approximately in 4% of the angiosperm. Dioecism has arisen independently in different families and genera, and several distinct genetic mechanisms regulating dioecy have been found in different plant species (Durand and Durand, 1990; Irish and Nelson, 1989). The presence of sex chromosome has been claimed for some dioecious angiosperms, but only in few cases these claims have been documented (Parker and Clark, 1991; Lewis and John, 1968; Westergard, 1958). More often the sex ratio in dioeceous plant species is controlled by the expression of allele at one to several loci (Irish and Nelson, 1989). In *T. dioica*, the male and female plants strictly maintain their respective sexual phenotypes. This indicates clear genetic basis of difference between male and female individuals of this species. Pointed-gourd breeding program has recently been initiated to develop new cultivars.

Singh *et. al.* (2002) stated that dioecy represents an inconvenience in pointed-gourd breeding. Currently there is no method for distinguishing between male and female plantlets prior to flowering in *T. dioica*. A method to determine the gender of plants before flowering would facilitate breeding and selection, by enabling screening for gender at an early stage, thereby simplifying the breeding of male and female plants for different objectives, and saving time and economic resources.

Dioecism also exists in *T. dioica* and there is a record of polyploidy series from diploid to tetraploid with a basic number of $n = 11$ chromosomes (Bhaduri and Bose, 1947). Inter-specific and intra-specific variation, and constancy in the amount of nuclear DNA content of flowering plants have been reported by several authors (Sinha *et. al.*, 1997; Mukherjee and Sharma, 1986; Bennett, 1985; Riana and Rees, 1983; Bennett and Smith, 1976; Price, 1976).

Although dioecism exits in fairly large number of cucurbit genera including *Trichosanthes*, sex inheritance has been studied in only a few of them and the mechanism underlying sex expression is not well understood in most cases. Male heterogamety had been correlated with the presence of a heteromorphic pair of chromosomes in a few taxa such as *Coccinia grandis* (Chakravarty, 1959; Roy and Roy, 1971; Sen, 1976; Sen and Datta, 1977), *Trichosanthes cucumeroides, T. japonica* (Nakajima, 1937) and *T. palmata* (Ayyanger, 1949). However, the presence of specific sex chromosomes has not been confirmed in any of the taxa excepting in *Coccinia grandis*, where the existence of a XY-mechanism of sex determination is now well established.

In dioecious organisms presence of sex chromosomes are used for identification of gender and for this purpose somatic cells of plants are arrested with metaphase stage of mitosis by treating them with different types of fluids. Thereafter from stained scattered

metaphase chromosomes karyogram is prepared and the sex chromosomes (XX and XY) are detected.

Like animals, sex chromosomes have been successfully identified in dioecious *Rumex, Cannabis, Humulus* and *Silene* (Parker, 1990). In many other plant species it has been possible but the problem is that in most of the cases, sex chromosomes are not much different from autosomes (Michalik *et. al.*, 2009). Sometimes the size of sex chromosomes is too small (Shirkot *et. al.*, 2002) and therefore it becomes difficult for identification of sex chromosome.

Several dioecious plants have an active X-Y system of sex determination with heterogametic males (XY) and homogametic (XX) females. In some other plants e. g. *Rumex* and *Humulus* X: A ratio of sex determination operates. But in case of pointed gourd *(Trichosanthes dioica)* no such information at chromosome or DNA level is available (Kumar *et. al.*, 2008). Thus, the present study aims to identify the sex chromosomes in pointed gourd on inferential basis.

As it has been mentioned earlier in this chapter that fruits of pointed gourd are used as vegetable in this subcontinent along with other parts of South Asia, thus, biochemical properties are important subject matter to the consumers. On the other hand, vegetative parts of this plant are being used traditionally from ancient times and thus, from all the points of view the present study also deals the basic chemical constituents in relation to human health.

A study was designed by Akter *et. al.* (2011) to investigate the antioxidant, antidiarrhoeal and cytotoxic properties of the aerial parts of *Trichosanthes dioica*. The petroleum ether, ethyl acetate, methanol and water extracts were tested for antioxidant activity using nitric oxide scavenging assay, total antioxidant capacity and total flavonoid content determination; castor oil-induced and magnesium sulphate-induced diarrhoea in mice were used to evaluate antidiarrhoeal activity while Brine shrimp lethality bioassay was employed for cytotoxicity test. The extracts exhibited significant radical scavenging capacity against nitric oxide. The order of radical scavenging was ascorbic acid > water extract > ethyl acetate extract > methanol extract > petroleum ether extract. The assay also revealed significant total antioxidant activity and a good amount of flavonoids in the extracts. Results of antidiarrhoeal tests at the doses of 200 and 400mg/kg body weight significantly ($p<0.05$, 0.001) reduced the frequency and severity of diarrhoea in both animal models. Methanol extract showed the highest inhibition of defaecation. The extracts also showed moderate cytotoxicity against Brine shrimp. The results suggest that aerial parts of *Trichosanthes dioica* possess significant antioxidant, antidiarrhoeal and moderate cytotoxic activities.

The chlorophylls, Chl a and Chl b are virtually essential pigments for the conversion of light energy to stored chemical energy. The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content; thus, chlorophyll content can directly determine photosynthetic potential and primary production (Curran *et. al.*, 1990; Filella *et. al.*, 1995). In addition, Chl gives an indirect estimation of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll (Filella *et. al.*, 1995; Moran *et. al.*, 2000). Furthermore, leaf chlorophyll content is closely related to plant stress and senescence (Hendry, 1987; Merzlyak and Gitelson, 1995; Peñuelas and Filella, 1998; Merzlyak *et. al.*, 1999).

This plant *(T. dioica)* also serves as a rich source of minerals such as Mg, Na, K, Cu and S (Sharma *et. al.*, 1989) whose significant role in controlling and managing diabetes is well known and cannot be ignored as specific concentration of these minerals have been reported to take part in carbohydrate metabolism as well as insulin release (Elson and Haas, 2007; Yeh *et. al.*, 2003; Lopez-Ridaura *et. al.*, 2004; Kar *et. al.*, 1999).

Some specific medicinal properties have been identified, viz., hypocholesterolemic, hypoglyceridimic, and hypophospholipemic when shade-dried fruits were mixed in the food of non-diabetic animals (Sharma and Pant, 1988; Sharma *et. al.*, 1989; Singh, 1989; Mukharjee, 1996). It also serves as a rich source of vitamin C (Sharma and Pant, 1988). Sharmila *et. al.* (2007) and Chandrasekhar *et. al.* (1988) observed cholesterol lowering activity of the aqueous fruit extract of *Trichosanthes dioica* in normal and streptozotocin diabetic rats.

Rai *et. al.* (2008) showed the glycemic attributes of an aqueous extract of *Trichosanthes dioica* leaves in normal as well as various diabetic models. The variable doses of 250, 500 and 750mg kg^{-1} body weight of the extract were administered orally to normal and streptozotocin (STZ) induced sub- and mild-diabetic rats in order to define its glycemic potential. This evidence clearly indicates that the aqueous extract of *Trichosanthes dioica* leaves has good hypoglycemic potential along with a high antidiabetic profile. Rai *et. al.* (2010) reported the *in vitro* assessment of antimicrobial activity of different concentration of extract of different part of *Trichosanthes dioica*. Five clinical isolates of different bacterial strains were used and the disc diffusion method was adopted. The results revealed that leaves, fruits and seeds of *Trichosanthes dioica* plant may be used as antibacterial agents.

Shivhare *et. al.* (2010a) evaluated the antioxidant activity of fruits of *Trichosanthes dioica* (Cucurbitaceae) and compared with ascorbic acid (standard). Anti-oxidant activity of aqueous extract of *Trichosanthes dioica* (TSD) fruits was studied for its free radical scavenging property in different *in vitro* methods as 1, 1 diphenyl-2- picryl

hydrazyl, nitric oxide, reducing power assay and hydrogen peroxide radical method. The findings could justify the inclusion of this plant in the management of antioxidant activity. Shivhare *et. al*. (2010c) reported a scientific evaluation for the wound healing potential of methanolic (MeOH) extract of *T. dioica* fruits. Shivhare *et. al*. (2010b) studied methanolic extract of the plant *T. dioica* for assessment of healing potential in the form of simple ointment using full thickness burn wound model in rats. The effect produced by the extract ointment provides significant healing when compared with the control and standard groups.

Biochemical characterizations of 64 pointed gourds were done using three isozyme viz. acid phosphatase, peroxidase and glutamate oxaloacetate transaminase (Khan *et. al*., 2009). A wide range of diversity among the gremplasm based on their acid phosphatase, peroxidase and glutamate oxaloacetate transaminase isoenzyme banding patterns were observed. Earlier chemical study reveals that in addition to a number of tetra and pentacyclic triterpenes, the toxic bitter principles cucurbitacins (a group of often highly oxygenated tetracyclic compounds with a unique carbon skeleton and almost a carbonyl group in ring C) may be considered as a taxonomic character of Cucubitaceae. The various chemical constituents present in *T. dioica* are vitamin A, vitamin C, tannins, saponins (Chopra *et. al.*, 2002). Two main phytosterols present in *T. dioica* are namely, 24α- ethylcholest-7-enol & 24β-ethylcholest-7-enol (Kongton, 2003). Mainly numbers of tetra & pentacyclic triterpenes which are bitter and toxic in nature are reported earlier. Different types of cucurbitacins (Cucurbitacin-E, Cucurbitacin-J, Cucurbitacin-D) are reported to present in significant amount in leaves (Kumar, 2011). Leaves are rich in vitamins and contain 9.0mg Mg, 2.6mg Na, 83.0mg K, 1.1mg Cu and 17.0mg S per 100g (Singh, 1989). Phytochemical estimation of aqueous and ethanolic extracts of seeds has shown the presence of saponins, tannins and trichosanthin (Ghaisas *et. al.*, 2008). It is reported that methanolic seed extract contains 7-oxidihydrokarounidol-3-benzoate as the most predominant component (Toshihiro *et. al.*, 1997). Two main phytosterols present in *T. dioica* are namely 24α-ethylcholest-7-enol & 24β-ethylcholest-7-enol. Seeds of *T. dioica* were also reported to contain lectin which is a carbohydrate (specifically galactose) binding protein and homologous to Type-II ribosome inhibitory proteins (Ali *et. al.*, 2004). It is reported that seeds of *T. dioica* contain a large amount of peptides (Kabir *et. al.*, 2000).

The basic use of protein profile is to study the taxonomic and evolutionary relationship among plant species (Boulter and Turner, 1966; Johnson, 1969). Protein banding pattern is such an important parameter for finding out genetic diversity and affinity among different species and among cultivars or/and race of any species based on the similarity index. The usefulness of this parameter has been elaborated in biosystemic studies. This study may provide also useful information on the relationship of different

varieties/lines of pointed gourd from phylogenetic point of view, since no such information has been found on this crop plant.

In view of above-mentioned research attributes the present study on *Trichosanthes dioica* was conducted:

1. To determine the pattern of morphological variations along with scrutinization of different morphological parts of taxonomic importance.
2. To find out the organization of interphase nucleus and its heterochromatic nature.
3. To find out similarities and differences amongst the karyotype of different varieties/lines for providing diagnostic feature in the haploid complement.
4. To identify the sex chromosomes in both male and female plants.
5. To characterize different varieties/lines based on soluble young leaf sugar, protein and few other chemical constituents along with findings of genetic diversity and affinity based on protein profile.

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials

Eighteen varieties/lines of *Trichosanthes dioica* (Roxb.) were used as experimental material in the present study. One or more than one variety/line was used in different experiments. A brief account of these varieties is given in **Table 1.**

Table 1: Cultivated area and source of collection of eighteen varieties/lines of *Trichosanthes dioica*

Serial No.	Variety/line	Cultivated area	Source of collection
1	BARI 1	Pabna	BARI, Ishurdi
2	BARI 2	Pabna	BARI, Ishurdi
3	KALI BOMBAY	Rajshahi	Farmer of Rajshahi
4	MALE 1	Ishwardi	BARI, Ishurdi
5	PG003	Natore	BARI, Ishurdi
6	PG005	Kushtia	BARI, Ishurdi
7	PG006	Pabna	BARI, Ishurdi
8	PG008	Kushtia	BARI, Ishurdi
9	PG010	Pabna	BARI, Ishurdi
10	PG011	Pabna	BARI, Ishurdi
11	PG012	Kushtia	BARI, Ishurdi
12	PG015	Kushtia	BARI, Ishurdi
13	PG018	Rangpur	BARI, Ishurdi
14	PG019	Rangpur	BARI, Ishurdi
15	PG020	Natore	BARI, Ishurdi
16	PG022	Rangpur	BARI, Ishurdi
17	PG023	Pabna	BARI, Ishurdi
18	PG028	Jessore	BARI, Ishurdi

Methods

1. Study of morphological features

Plants of all the eighteen varieties/lines of *Trichosanthes dioica* were grown during the growing season of 2013-14 in the experimental field of IBSc, University of Rajshahi for their morphological studies. The plants were grown in rows of 1m×1m inter-row and intra-row, respectively. The vines were made ring-shaped and put down into soil at 3-4cm depth. The varieties/lines were investigated and described under two heads: qualitative and quantitative. The data on morphological characters were recorded sometimes from experimentation field of Bangladesh Agricultural Research Institute

(BARI), Ishurdi, Pabna about 50km away from Rajshahi. Diagnostic features were examined with keen interest.

Pattern of morphological variation: The material consisting of 18 varieties/lines from *Trichosanthes dioica* were grown under uniform conditions during 2013-14, with three replications of each. The plants were grown in rows at the distance as mentioned earlier for the study of morphological features and observations were made on five plants randomly selected for 12 morphological characters as follows:

Stem length: Length of five stems were measured in cm randomly and it was estimated at first flowering stage.

Length of inter-node: Inter-node length of five random plants were measured in cm and the mean value was estimated.

No. of inter-node: Total number of inter-node from five plants was calculated in cm randomly at first flowering stage.

Leaf area: Five randomly selected leaves per plant were measured in cm^2 and the mean was estimated as leaf area.

Fresh weight of leaf: Fresh weight of five randomly selected leaves were measured in gram and the average value for leaf weight was estimated.

Days to flower: Number of days from the date of sowing to the anthesis of the first flower in each plant was recorded.

Length of flower: Five flowers per plant were measured in cm and the mean was estimated.

Fresh weight of flower: Fresh weight of five flowers were measured in gram and the average flower weight was estimated.

Length of fruit: Length of five randomly selected fruits were measured in cm and the average stem length was estimated.

Circumference of fruit: Five randomly selected fruits per plant were measured in cm and the mean was estimated.

Weight of fruit: Weight of five randomly selected fruits per plant was determined in gram.

Seeds per fruit: Seeds from five randomly selected fruits were counted and the mean seed number per fruit was estimated.

From the data, a mean table was prepared where each value was the mean over replications. To study the pattern of morphological variations in different varieties/lines

Metroglyph and index score method (Anderson, 1957; Mehra and Anderson, 1969; Singh and Chaudhury, 1979) was followed.

Two most variable characters were selected. One of them, stem length was used on the X-axis and the other, leaf area on the Y-axis. The mean of Y-values were plotted against the mean of X-values for each variety/line. A particular variety/line was thus represented by a glyph on graph. For the purpose of distinction, the varieties/lines were represented by circular legend. Besides, the stem length and leaf area of plants, all other characters were represented by rays on the graph, the ray for the same character having the same position in each glyph. The range of variation in each character was represented by varying length of rays, i.e., a variety/line having low value with no ray, medium value with short ray and high value with long ray. Thus, the length of the ray was either nil, short or long depending on the index value. The index values were decided on the basis of range of variability and were divided into three classes, i.e., 1-no ray, 2-short ray and 3-long ray. The minimum and maximum score that an individual could get was n×1 and n×3, respectively, where 'n' was the total number of characters considered.

2. Interphase nuclear phenotype

For studying interphase nuclear structure and making karyological analysis the steps mentioned below were followed.

Fixation of shoot tips: In order to study the interphase nucleus and chromosome morphology, the shoot tips of 1.5-2cm in length, were treated with saturated solution of paradichlorobenzene (1-4 dichlorobenzene or PDB) in glass vials for 4-5 hours at 10^0C. The saturated solution of PBD was prepared by dissolving 750mg PDB in 50ml distilled water and incubated over night at 60^0C. The treated shoot tips were thoroughly washed with distilled water and fixed in 1:3 acetoalcohol for 48 hours at room temperature. Afterwards they were preserved in 70% ethanol and stored in refrigerator till used.

Staining of shoot tips: The preserved shoot tips were stained following the method of Haque *et. al.* (1976) with certain modifications.

i) The preserved shoot tips were washed thoroughly by distilled water for 5 minutes.

ii) After washing with distilled water the shoot tips were transferred to 50% HCl (by dilution) for about 30 minutes for dissolving the middle lamella of cells.

iii) The shoot tips were washed with distilled water for 8-10 minutes.

iv) Then the shoot tips were transferred in 2% iron alum (ferric ammonium sulphate) solution for 10-15 minutes.

v) Then the shoot tips were washed again with frequent change of distilled water for 8-10 minutes.

vi) The shoot tips were then stained with 0.5% haematoxylin for about 10-15 minutes and washed again with distilled water for 5-7minutes.

Preparation of slides: The meristematic zone of the stained shoot tips were squashed in 0.5% acetocarmine on a clean slide and the meristematic cells were covered with a cover glass. Then heat-cool and pressure technique was applied until all the cells as well as the chromosomes in the cells were scattered in all directions.

Photomicrography and observation: Prepared temporary slides for nuclear phenotype and the karyotypic study were examined under a compound microscope with 40×15 magnification. Photomicrographs were taken from the desired preparations for studying nuclear phenotype and chromosomes were measured from photomicrographs based on the times of magnification. The recorded values were then converted in micrometer (μm).

Interphase chromosome volume: In order to determine the interphase chromosome volume from shoot tip cells, nuclear volume of cells were measured first by oculometer and the values were converted into micron (μ) with the help of a stage micrometer. The nuclear volume (NV) was calculated using the formula of sphere, $NV = 4/3\pi r^3$ (Nayer *et. al.*, 1971). The nuclear volume divided by the somatic chromosome number gave the Interphase chromosome volume (ICV) as mentioned bellow:

$$ICV = \frac{\text{Nuclear volume}}{\text{Somatic chromosome number}}$$

Interphase nuclear structure and heterochromatin: To study the nuclear phenotype, the same slide was used which had been used for studying ICV.

To determine the nuclear structure, 10 nuclei from each preparation were examined. From each nucleus number of chromocentres (dark positions) was counted and as chromocentres correspond to heterochromatin (Nagl and Fusening, 1979), percentage heterochromatin values were obtained statistically by determining the area of nucleus and of chromocentres by planimetry. Then the heterochromatin values were expressed as percent nuclear area using the flowing formula:

$$\text{Heterochromatin\%} = \frac{\text{Chromocentre area}}{\text{Nuclear area}} \times 100$$

3. Somatic karyotype

Classification of chromosome: The chromosomes were classified primarily based on arm ratios according to Kutarekar and Wanjari (1983). Arm ratios per haploid set were determined according to position of centromere and were calculated adopting the following formula:

$$\text{Arm ratio (AR)} = \frac{\text{Short arm length (SA)}}{\text{Long arm length (LA)}}$$

The chromosomes having the ratios 0.76 and above were termed as metacentric (m), 0.51 to 0.75 as sub-metacentric (s^m) and less than 0.51 as sub-terminal (s^t) chromosome.

Depending upon the range and general average of chromatin length per chromosome they were grouped as follows:

i) **Large (A):** Chromatin length above 4.28µm
ii) **Medium (B):**Chromatin length between 3.42 to 4.27µm
iii) **Relatively short (C):** Chromatin length between 2.56 to 3.41µm
iv) **Short (D):** Chromatin length 2.55µm and less.

Then the chromosome formula for each variety/line was derived.

Scatter diagram: Chromosome morphology was studied in each of three cells, selected on the basis of similar degree of contraction of the chromosomes. For the analysis of quantitative karyotype scatter diagram was prepared following the method proposed by Ahmed *et. al*. (1983). Briefly a scatter diagram of the arm ratios and lengths of chromosome in each cell was used to determine homologous pairs of chromosomes and haploid values of the genome.

For identification of the chromosomes in different varieties, the morphological categories on the basis of total length and arm ratio were adopted and the steps are as follows:

Centromeric formula: Centromeric formula was prepared for each variety/line based on its arm ratio.

Proposed standard karyotype: Finally the standard karyotype was derived based on centromeric formula and range of chromatin length per chromosome.

Ideogram: Ideograms were also prepared for all of the chromosome pairs side by side according to their length (from longer to shorter) keeping the short arm in each case pointing upwards and the centromere at the same plane.

Total frequency: The total frequency percentage (TF%) was calculated using the formula of Huziwara (1962). The formula is given below:

$$\text{TF\%} = \frac{\text{Total length of all short arm}}{\text{Total length of cromosome complement}} \times 100$$

4. Identification of sex regulatory chromosomes

In higher plants, individual are classified as males, females or hermaphrodites, on the basis of whether the flower possesses only anther, or only ovary or both (Gupta, 1997). A range of types from the hermaphrodites to those having unisexual flowers with trace of other sex are known in higher plants. These variations in higher plants are listed in **Table 2.**

Table 2: Different types of sex expression in higher plants

Type of sex expression	Flowers of different types
1. Hermaphrodites	All perfect (⚥) flowers
2. Monoecious	Separate ♀ and ♂ flowers, but on the same plant
3. Dioecious	Separate ♀ and ♂ flowers on different plants
4. Andromonoecious	Perfect (⚥) and ♂ flowers on the same plant
5. Gynomonoecious	Perfect (⚥) and ♀ flowers on the same plant
6. Trimonoecious	Perfect (⚥), ♀ and ♂ flowers on the same plant
7. Androdioecious	Perfect (⚥) and ♂ flowers on the separate plant
8. Gynodioecious	Perfect (⚥) and ♀ flowers on the separate plant

Extensive karyotyping of metaphase chromosomes has revealed that both sex chromosomes, X and Y are metacentric (Ciupercescu *et. al.*, 1990); Grabowska-Joachimiak and Joachimiak, 2002). The Y chromosome possesses nearly equal arms (arm ratio, r = 1.09), whereas it is possible upon visual inspection to identify the p and q arms of the X chromosome (r = 1.44) as suggested by Ciupercescu *et. al.* (1990).

In the present study arm ratio of the chromosomes of pointed gourd was calculated by using the formula, SA/LA and thus, the value obtained was always below 1.00. The value 0.75 and above was considered for metacentric chromosome.

So the value nearest to 1.00 indicates mostly equal arms p and q particularly for X chromosome. Since Y chromosome possesses also equal arms, so in this case also highest value for arm ratio nearest to 1.00 was considered. In case of similar and nearest arm ratio, the formula LA/SA was also used and the highest value was considered for identifying the sex chromosome. In this way the sex chromosomes were identified based on probabilistic inferences. It was made completely in inferential way.

5. Biochemical studies

I. Screening test:

Phytochemical screening was performed using the standard procedures (Deka *et. al.*, 2015) as follows:

Preparation of leaf extracts: The fresh and mature leaves were dried in electric oven at 60°C for 24 hours. Then it was grinded with mortar and pestle. Mixed in methanol

and it was shaken vigorously. Then the extract obtained was filtered and concentrated at 70°C. Dried extracts were kept in a refrigerator at 4°C and used for further study.

Test for alkaloids:

a. Mayers test: The test solution was treated with Mayers reagent. Mayers reagent solution was prepared with potassium iodide and mercuric chloride.

b. Wagners test: The test solution was treated with some acidic solution and Wagners reagent.

Test for flavonoids:

a. Ferric chloride test: The test solution was treated with ferric chloride solution.

b. Shinoda test: The test solution was treated with few fragments of Mg ribbon and conc. HCl.

Test for glycosides:

a. Keller killiani test: Test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added.

b. Bromine water test: Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result.

Test for steroids:

Salkowaski test: The test solution was treated with a few drops of conc. H_2SO_4, shaked well and allowed to stand.

Test for tannins:

a. Ferric chloride test: The test solution was treated with ferric chloride solution.

b. Gelatin Test: The test solution was treated with 1% gelatin solution containing 10% NaCl.

II. Quantitative test:

Chlorophylls determination in leaves: A chlorophyll concentration of young leaves (2-week old after treatment was imposed) was determined spectrophotometrically as previously described with some modifications (Lichtenthaler and Wellburn, 1985). 250mg leaves of pointed gourd form each of the varieties/lines.

Preparation of reagent

i. 80% acetone

It was prepared by mixing 80ml of pure acetone and 20ml of double distilled water (DDW).

Procedure

1. The tissues were grinded with a pestle and 3ml of 80% acetone were added.
2. The solutions were then centrifuged at 5000 rpm for 5 minutes and the supernatants were transferred to 10ml volumetric flasks.

3. The process was repeated till the pellet became colourless.
4. The mortar and pestles were washed thoroughly with 80% acetone and the washings were added to the volumetric flask, the volumes were adjusted to 5ml.
5. Absorbances of the solutions were read at 645 and 663nm respectively against 80% acetone blank.
6. The amount of chlorophyll present in the extract was calculated in terms of mg chlorophyll per gm of tissue using the following equation.

mg chlorophyll a/gm of tissue = [12.7×(A663)-2.69×(A645)]×V/1000×W
mg chlorophyll b/gm of tissue = [22.9×(A645) -4.68×(A663)]×V/1000×W
mg total chlorophyll (a+b)/gm of tissue = [20.2×(A645)+8.02×(A663)]×V/1000×W

Soluble sugar content in leaves: The soluble sugar was estimated by the method of Dey (1990).

Preparation of reagent

i. 5% phenol; Phenol solution was prepared by mixing 5gm of phenol in 95ml of DDW.
ii. 90% ethanol; It was prepared by mixing 90ml of pure ethanol and 10ml of DDW.

Procedure

1. Certain gram fresh leaf materials were kept in 5ml of alcohol for 1 hour at 60^0C in incubator.
2. The extract was then decanted into a 25ml volumetric flask and the residue was re-extracted.
3. Final volume was made up to 15ml by adding alcohol.
4. 1ml aliquot was transferred to a thick walled test tube and 1.0ml of 5% phenol was added to it and mixed thoroughly.
5. 5ml of analytical grade sulphuric acid was then added to it and mixed thoroughly by vertical agitation with a glass rod.
6. For exothermic reaction the test tube was cooled in the air. Absorbance was recorded at 485nm on Beckman DU 640 spectrophotometer.
7. The corresponding concentration was determined against a standard curve **(Fig. 1)** prepared by using a glucose solution. The amount of sugar was expressed as mg g^{-1} FW^{-1}.

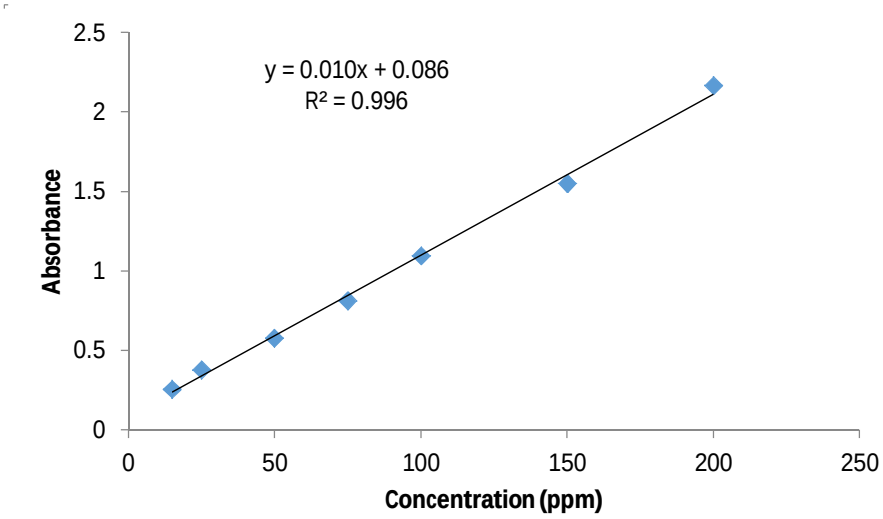


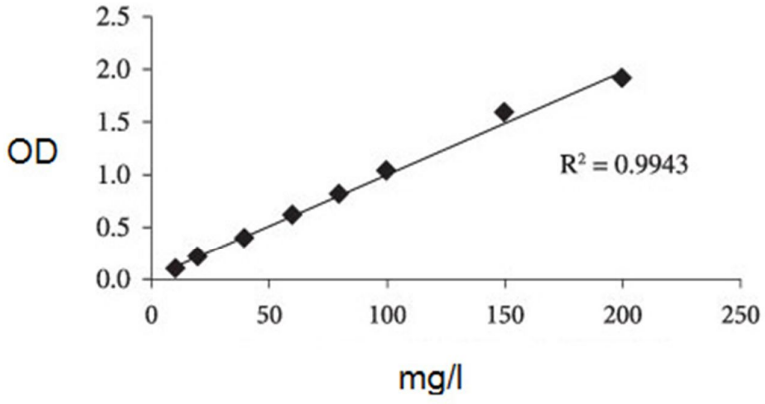
Fig. 1: Standard curve of glucose in leaves of *Trichosanthes dioica*

Antioxidant capacity in fruits: The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. The measurement of the DPPH radical scavenging activity was performed according to a published method (Brand-Williams *et. al.*, 1995). Antioxidants scavenged DPPH radical by through the donation of proton forming the reduced DPPH. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discolouration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour depending on the number of electrons taken up. The antioxidant capacity (%) in fruits were calculated adopting the following formula:

$$AA\% = 100 - [\frac{\{Abs\ (sample) - Abs\ (blank)\}}{Abs\ (control)} \times 100]$$

Procedure

1. The zero reading of spectrophotometer at 517nm was noted by using methanol.
2. Only 1ml DPPH (0.0006 M) was added in methanol at 517nm for read control.
3. Then 50µL of sample was mixed in 1ml DPPH (0.0006 M) and added to methanol.
4. Absorbance was recorded at 517nm.
5. Then observed the decrease of absorbance after 16 minutes.
6. The antioxidant activity was determined from a standard curve and calculated on a fresh weight basis of **Fig. 2.**



Absorbance of DPPH radical solutions prepared in methanol.

Fig. 2: Standard curve of antioxidant capacity (%) in fruits of *Trichosanthes dioica*

Estimation of phenol in fruits: Total phenol content in matured fruit was measured using Folin–Ciocalteu's phenol reagent and gallic acid standard with some modifications (Kogure *et. al.*, 2004).

Preparation of reagent

i. Na_2CO_3 (14.3gm/500ml or1 N)

ii. Then 20ml Na_2CO_3 + 80ml water was mixed well.

Procedure

1. 100mg fruit sample was grinded in methanol and stored at -20°C before analysis.
2. 250µL of Folin- Ciocalteu's phenol reagents was added with 50µL of the extract + 500µL of 20% water solution of Na_2CO_3 and then vortex.
3. 4.2ml water was added to make it 5ml.
4. As control, reagent without adding extract was used.
5. Then it was incubated for 30 minutes at room temperature.
6. The absorbance measured at 765nm.
7. The total phenolics were measured using the gallic acid calibration curve **(Fig. 3)** and were expressed as mg L^{-1} gallic acid g^{-1} extract (GAE).

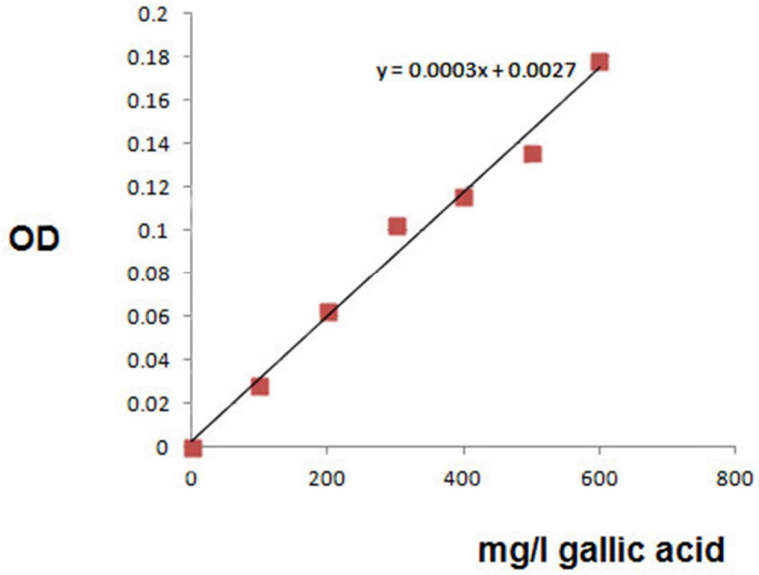


Fig. 3: Standard curve of phenol estimation in fruits of *Trichosanthes dioica*

Estimation of proline in fruits by colorimetric method: Proline was determined from fruit samples as described by Bates *et. al.* (1973).

Procedure

1. 3% sulfosalicylic acid (5µL/mg fresh weight) was added to the sample and the sample was grinded. The tubes on ice were kept until finishing with all samples.
2. Sample was centrifuged for 5 minutes at room temperature using centrifuge with maximum speed.
3. The reaction mixtures were prepared in separate tube: 100µL of 3% sulfosalicylic acid, 200µL of glacial acetic acid and 200µL of acidic nindydrin.
4. 100µL from the supernatant of the plant extract was added and mixed well in the tubes.
5. The tubes were incubated at 96°C for 60 minutes.
6. Then the reaction on ice was terminated.
7. Readings were taken immediately at a wave length of 520nm in spectrophotometer. The proline concentration was determined from a standard curve **(Fig. 4)** and calculated on a fresh weight basis mmol proline (gm FW)$^{-1}$.

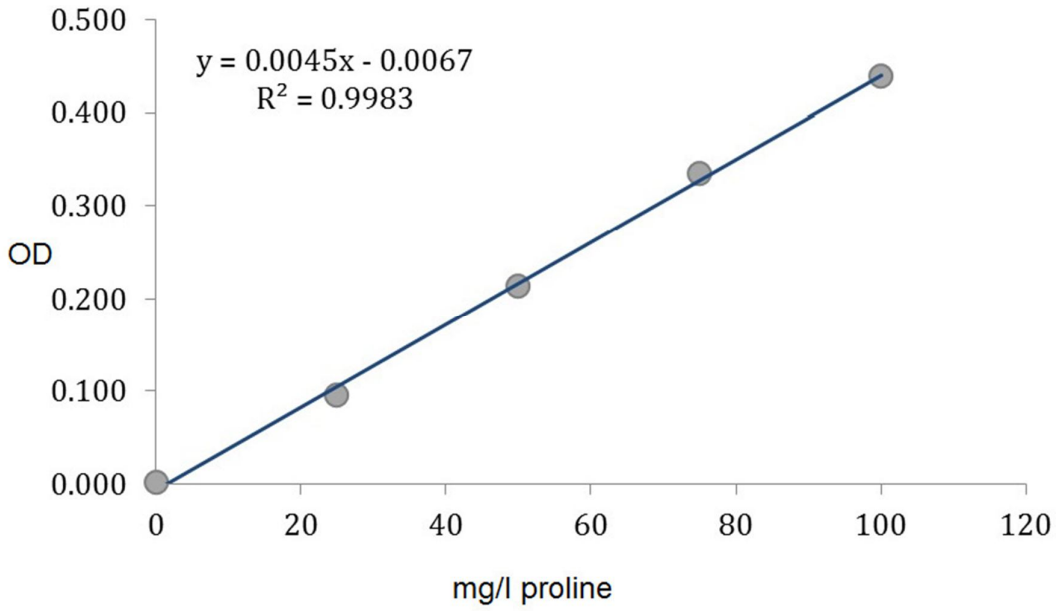


Fig. 4 Standard curve of proline estimation in fruits of *Trichosanthes dioica*

Estimation of protein in fruits: Total soluble proteins were extracted from the fruits with a modification of the method by Guy *et. al.* (1992).

Extraction of protein

1. This consisted of homogenization with a chilled mortar and pestle using a buffer containing ice-cold 50 mM Tris-HCl, p^H 7.5: 2 mM EDTA (ethylenediaminetetra-acetic acid) and 0.04 % (v/v) 2-mercaptoethanol.
2. The homogenate was centrifuged at 4000 rpm for 30 minutes at room temperature (Nejad Masoodi and Yazdi-Samadi, 1992).
3. Supernatant was re-centrifuged for 20 minutes and stored at -20°C for analysis (Hames and Rickwood, 1990).

Measurement of sample

1. Pipetted 100µL of each sample to microcentrifuge tube.
2. To each tube 1ml of Coomasie Brilliant Blue G 250 was added and mixed gently by vortexing.
3. Transferred the BSA (bowin serum albumin) samples to disposable cuvetts and the absorbance was measured at 595nm.
4. The protein was calculated with the help of a standard curve **(Fig. 5)** on fresh weight basis of fruit.

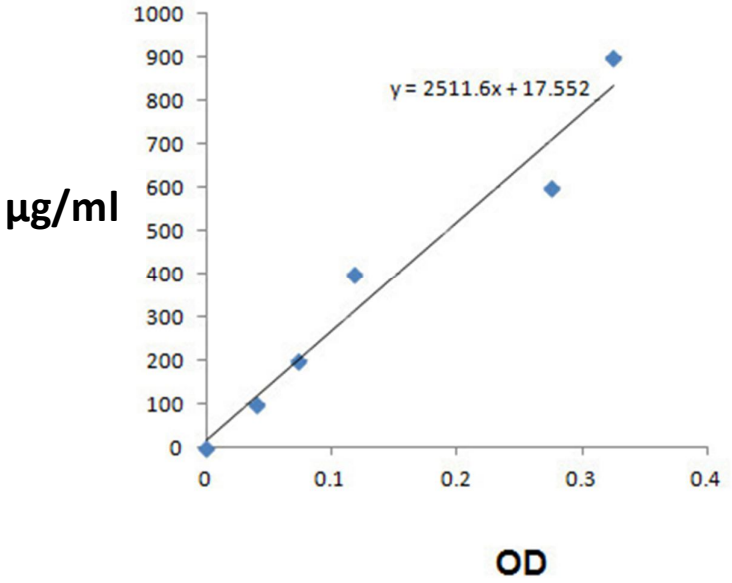


Fig. 5 Standard curve of protein estimation in fruits of *Trichosanthes dioica*

III. Protein banding pattern: Polyacrylamide gel electrophoresis method is most commonly used for separating the protein molecules as well as checking the purity and determining their molecular weight also. Sodium Dodecyl Sulphate (SDS) is an inorganic detergent, which binds to most proteins in amounts roughly proportional to molecular weight of protein about one molecule of SDS for every two molecules of amino acid residues. The bound SDS contributes large negative charge, rendering the intrinsic charge of protein insignificant. In addition, native conformation of the protein is altered when SDS is bound and most protein assumes similar shape and thus similar ratio change to mass. Slab gel electrophoresis in the presence of SDS therefore, separates proteins almost exclusively on the basis of mass, with smaller polypeptides migrating more rapidly towards the anode.

Protein patterns of the selected ten varieties/lines of *T. dioica* were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). The adopted procedure is described below:

i. Preparation of crude protein extract: Fresh leaves of ten varieties/lines of *T. dioica* were crushed into powder and made paste by using a homogenizer with 0.1mM phosphate buffer, pH 7.0. The temperature was maintained at 4°C by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheese cloth in the cold room. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge machine at 6000 rpm for 15 minutes at 4°C.

The clear supernatant was collected and saturated to 100% saturation by adding solid ammonium sulphate with gentle stirring. The precipitate was collected by centrifugation at 7000 rpm for 10 minutes at 4°C. Then the precipitate was dissolved in minimum volume of pre-cooled distilled water, and dialyzed against water for 12 hours and then against 0.1mM phosphate buffer, pH 7.0 for overnight at 4°C. It was again centrifuged at 7000 rpm for 6 minutes at 4°C to remove any insoluble material present in the solution and the clear supernatant was used as crude protein extract.

Reagents and solutions

ii. Preparation of 30% acrylamide solution: 33.30gm of acrylamide and 0.9gm of N, N-methylene-bis-acrylamide were dissolved in 70ml of distilled water in a 100ml of volumetric flask by heating in a hot water bath and the final volume was made up to the mark by adding distilled water. The solution was filtered and stored in a dark bottle at room temperature. The solution was discarded after 30 days, since acrylamide was gradually hydrolyzed to acrylic acid and ammonia. Acrylamide monomer is neurotoxic, so mask and gloves were worn while handling the powder and solution and it was not pipetted by mouth.

iii. Preparation of 1.5 M Tris-HCl buffer (pH 8.8): 18.70ml of Tris base was dissolved in 90ml of distilled water in a conical flask and mixed well. The pH of the solution was adjusted to 8.8 by adding diluted HC1 drop by drop. The final volume was made up to 100ml with distilled water.

iv. Preparation of 0.5M Tris-HCI buffer (pH 6.8): 6.00gm of Tris base was dissolved in 90ml of distilled water in a conical flask and mixed well. The pH of the solution was adjusted to 6.8 by adding diluted HC1. The final volume was made up to 100ml with distilled water.

v. Preparation of 10% sodium dodecyl sulphate (SDS) solution: 10% SDS solution was prepared by dissolving 5gm of SDS in 40ml distilled water in a 50ml volumetric flask. After dissolving, the final volume was made up to 50ml with distilled water.

vi. Preparation of 10% ammonium per sulphate (APS) solution: 10% APS solution was prepared by dissolving 0.50gm of APS in 4ml of distilled water. The final volume was made up to 5ml with distilled water. The solution was stored in eppendorf tubes (500μL in each tube) at 20°C.

vii. TEMED (N, N, N′, N′-Tetramethyl ethyl diamine): The commercially available preparation of TEMED from Sigma Chemicals Co., USA. was used without modification.

viii. Preparation of 4% sodium dodecyl sulphate (SDS) solution: 4% SDS solution was prepared by dissolving 2gm of SDS in 40ml distilled water in a 50ml volumetric flask. After dissolving, the final volume was made up to 50ml with distilled water.

ix. Preparation of Bromophenol Blue (BPB) solution: Bromophenol Blue solution was prepared by mixing the components as given below and was stored at 4°C.

Components	Amount
Bromophenol Blue	10mg
Glycerol	2ml
0.5 M Tris-HCl buffer	0.20ml
Distilled water	10ml

x. Preparation of sample buffer: The sample buffer was prepared by mixing the following components and was stored at 4°C.

Components	Amount
4% SDS	13ml
Glycerol	5ml
0.5 M Tris-HCl buffer (pH 6.8)	7ml

xi. Preparation of Coomassie Brilliant Blue (CBB) staining solution: It was prepared by mixing the following components.

Components	Amount
CBB R250	0.25gm
Glacial acetic acid	15ml
Methanol/Ethanol	100ml
Distilled water	85ml

xii. Preparation of CBB destaining solution-1: The CBB destaining solution-1 was prepared by mixing the following components, dissolved in distilled water and the final volume was made up to 3 liters with distilled water.

Components	Amount
Glacial acetic acid	10ml
Methanol/Ethanol	10ml
Distilled water	10ml

xiii. Preparation of destaining solution-2: 100ml of destaining solution-2 was prepared by mixing the following components.

Components	Amount
Glacial acetic acid	7ml
Methanol/Ethanol	25ml
Distilled water	68ml

xiv. Preparation of electrophoretic buffer (Chamber buffer): Electrophoretic buffer was prepared by the following components.

Components	Amount
Tris base	9.09gm
Glycine	43.20gm
SDS	3.00gm

These components were dissolved in distilled water and the final volume was made up to 3 litres with distilled water and was preserved in a fridge.

xv. Preparation of sample: 100µL of the protein sample was mixed with 100µL of sample buffer in an eppendorf tube and heated for 2-3 minutes at 100℃ in a water bath. The sample was then used for SDS-polyacrylamide gel electrophoresis.

xvi. Procedure: Clean and dry plates (7cm × 10cm) were assembled with a spacer (1.5cm thick) and were hold together on a gel casting stand. The assembly was checked for leakage.

xvii. Preparation of separating or running gel for slab gel-electrophoresis: The following solutions were taken in a conical flask. Then the flask was swirled gently to mix. To avoid instantaneous polymerization, the flask containing the solution was kept in an ice bath. The solution was used immediately.

Components	Amount
30% acrylamide solution	6.50ml
1.5 M Tris-HCl buffer, pH 8.8	4.05ml
Deionized water	2.50ml
10% SDS solution	75µL
TEMED	6.25µL
10% APS solution	75µL

xviii. The separating gel solution was applied to the sandwich.

xix. The top of the gel was covered slowly with a layer of water. It was then allowed to polymerize the gel solution for about one hour at room temperature.

xx. The layer of water was then soaked by using absorbent papers.

xxi. Preparation of stacking gel: The following solutions were taken in a conical flask. Then the flask was swirled gently to mix. To avoid instantaneous polymerization, the flask containing the solution was kept in an ice bath. The solution was used immediately.

Components	Amount
30% acrylamide solution	450µL
0.5 M Tris-HCl buffer, pH 6.8	375µL
Deionized water	2.11ml
10% SDS solution	30µL
TEMED	5µL
10% APS solution	30µL

xxii. The stacking gel was poured on the separating gel. Then the Teflon comb was inserted immediately into the layer of the stacking gel solution. Additional stacking gel was added to fill completely the space in the comb. Measure was taken not to trap air bubbles. The gel solution was allowed to polymerize for about 30 minutes.

xxiii. The Teflon comb was carefully removed without tearing the edges of the polyacrylamide wells. After the comb was removed, the wells were rinsed with electrophoretic buffer to remove unpolymerized monomer. The gel wells were filled with electrophoretic buffer.

xxiv. The gel sandwich was then attached to upper buffer chamber and the lower buffer chamber was filled with the recommended amount of electrophoresis buffer. The upper buffer chamber was partially filled with the electrophoresis buffer so that the top of the gel sandwich was sunk into the electrophoresis buffer.

xxv. 20µL of different sample protein solutions were carefully applied at the bottom of the different wells. The remainder of the upper buffer chamber was filled with electrophoresis buffer. Electrophoresis was carried out by applying electric power supply at a current of 30mA. The power supply was disconnected when BPB dye reached near the bottom of the gel.

xxvi. Recovery of the gel: The gel sandwich was removed from the upper buffer chamber and was laid on a sheet of absorbent paper or paper towels. Slide was removed carefully. Then the gel was removed from the lower plate.

xxvii. Staining of the gel: After recovery, the gel was stained with the staining solution for two hours at room temperature.

xxviii. Destaining of the gel: After two hours, the gel was removed from the staining solution and destaining was done by soaking the gel in destaining solution. When the gel became transparent, it was taken out and rinsed with water. On the transparent gel various blue coloured bands were visible indicating the separated polypeptides.

After destaining the location of protein bands were recorded. Rf (Relative front) values were calculated according to the following formula:

Rf (for the particular protein band) value = $\frac{\text{Distance travelled by band}}{\text{Distance travelled by tracking dye}}$

xxix. Similarity index: It was calculated according to the method suggested by Sheen (1972) as follows:

Similarities index (Sl) = $\frac{\text{Similarities}}{\text{Similarities+Dissimilarities}} \times 100$

Where, similarity was taken as the number of pairs of similar bands on the basis of their Rf values in the varieties.

A dissimilarity was indicated by the number of different bands.

Statistical analysis: Data recorded from different experiment were analyzed statistically as follows:
Mean, standard error and standard deviation (Analysis of Variance; one way) and DMRT were also determined using the following formulae:

Mean:

$\overline{X} = \Sigma X/N$

Where, $\overline{X}$ = Arithmetic mean
Σ = Sign of summation
X = Value per variable
N = Number of variable

Standard error:

SE = SD/N

Where, SE = Standard Error
SD = Standard Deviation

Standard Deviation:

$SD = \{\Sigma X^2 - (\Sigma X)^2/N\}/N-1$

CHAPTER THREE

RESULTS

RESULTS

The results obtained in this investigation are presented under the following heads:

1. Morphological features
2. Interphase nuclear phenotype
3. Somatic karyotype
4. Identification of sex regulatory chromosomes
5. Biochemical studies:
    I. Screening test
    II. Quantitative test
    III. Protein banding pattern

1. Morphological features

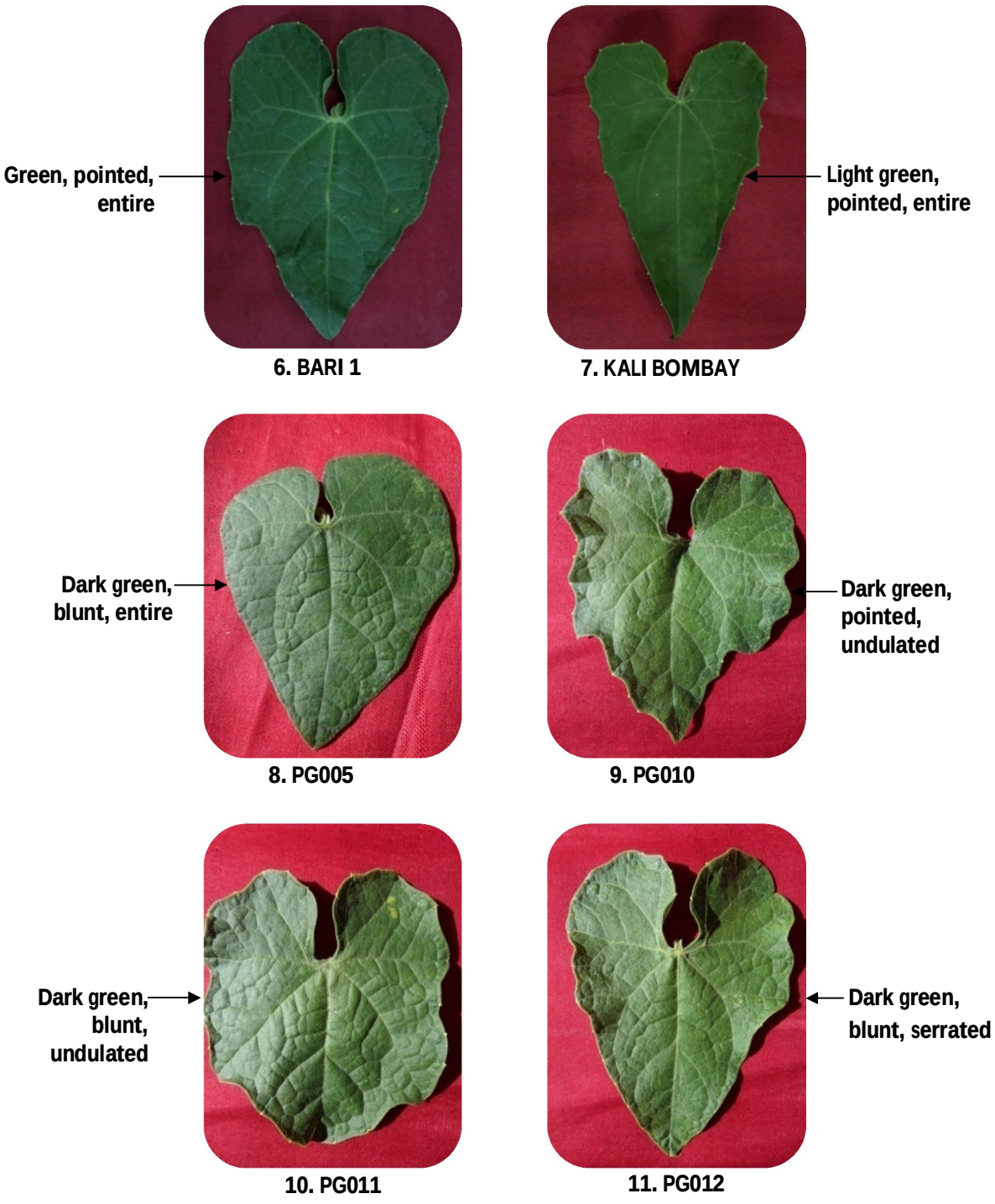
The flower of pointed gourd is white in colour. Each flower is comprised of five sepals and five petals. Staminate flower comprises three stamens in long pedicel and borne singly. Pistillate flowers are also solitary (single) in short peduncles with five carpels, usually three, thick and short style, terminated by three bi-lobed or divided papillate stigma. The male flowers of pointed gourd were found to be bigger in size than female flowers except the line PG003 which had a long base. The female flowers were found to be ended with a swollen ovary covered with fine white pubescence. Some morphological parts of different varieties/lines of *Trichosanthes dioica* (Roxb.) are shown in **Figs. 6-23.** The morphological characteristics are described into two parts; qualitative characteristics and quantitative characteristics.

Qualitative characteristics

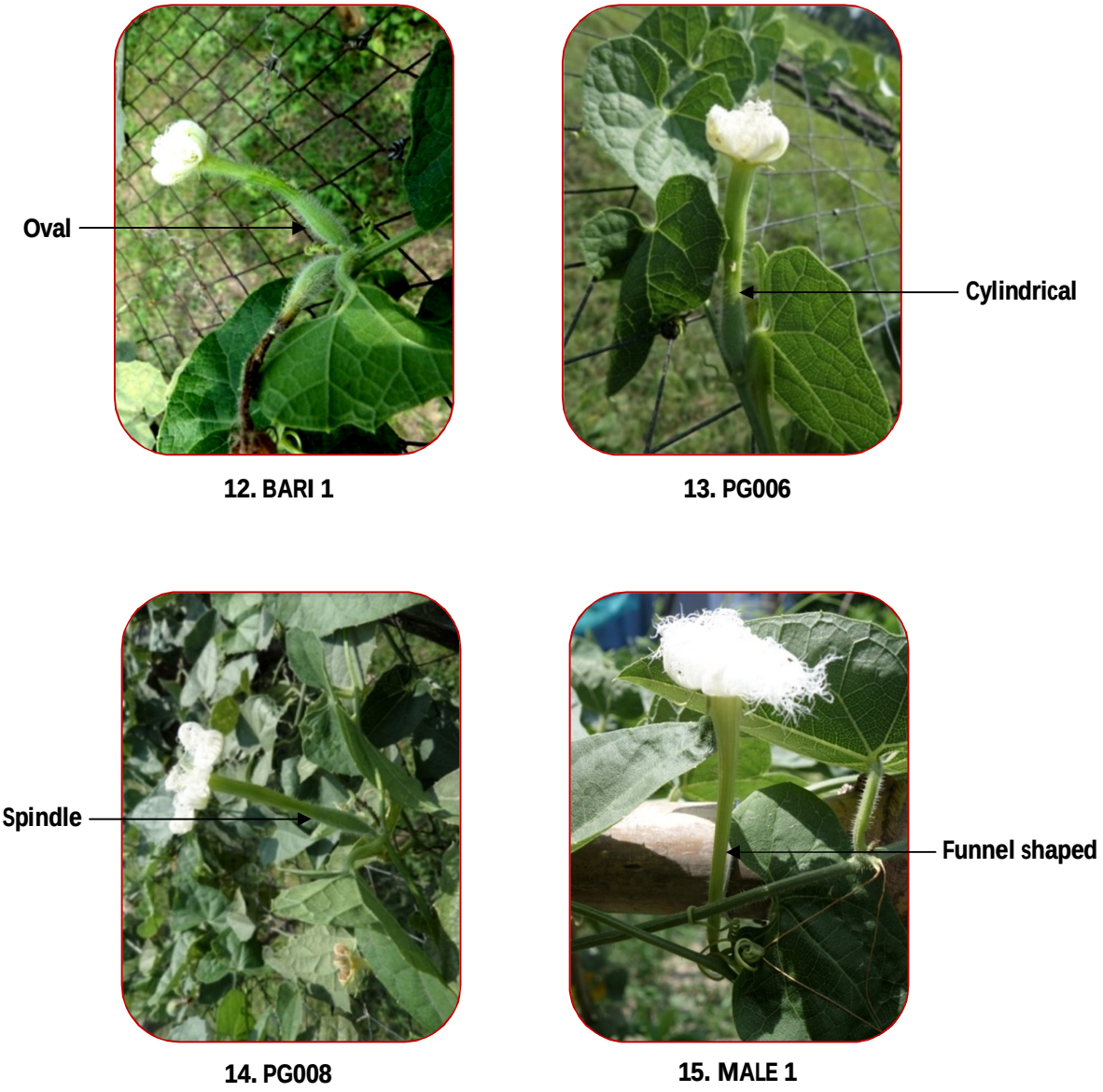
For determination the qualitative characteristics of morphological parts carrying taxonomic importance in all the varieties/lines of *Trichosanthes dioica* were scrutinized and presented in **Tables 3 and 4.** A concise account of these characters is given below.

In case of stem colour eighteen varieties of pointed gourd showed green, dark green and light green colour. Most of them exhibited green coloured stem. Almost similarly the stem structure was of two types and they were either pentagonal notched or roundish. Almost all the varieties/lines were of notched type, although in case of two lines it was observed not prominently.

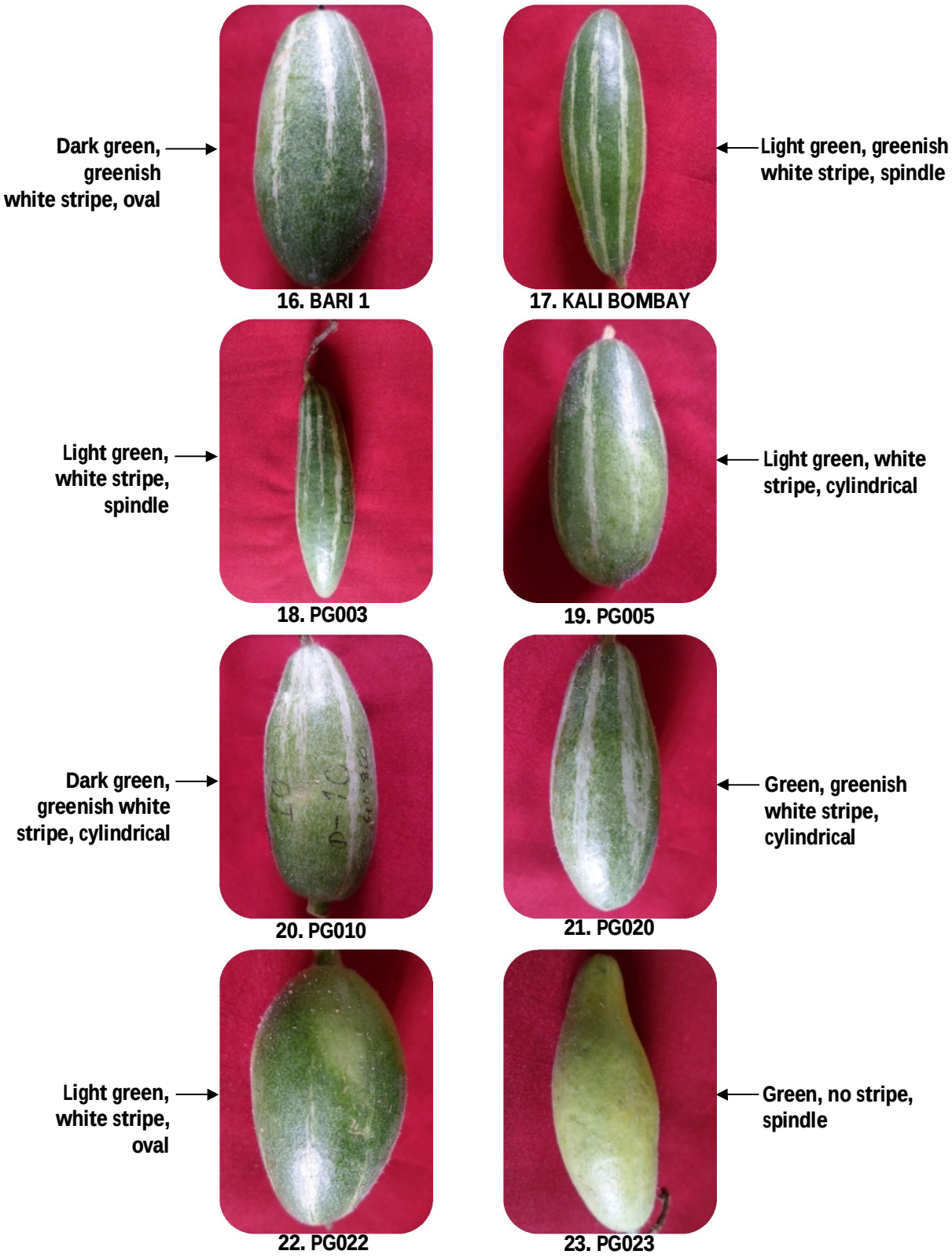
Leaf characters in eighteen varieties/lines of pointed gourd were found differentially. Six characters **(Figs. 6-11)** were found to be common in all of them. Leaf margin were



Figs. 6-11: Photographs showing marked leaf characters in different varieties/lines of *Trichosanthes dioica*



Figs. 12-14: Photographs showing marked flower and ovary characters in different varieties/lines of *Trichosanthes dioica*. Fig. 15: Male flower of *Trichosanthes dioica*



Figs. 16-23: Photographs showing marked fruit characters in different varieties/lines of *Trichosanthes dioica*

Table 3: Stem colour, stem structure, leaf colour, leaf type and leaf margin of eighteen varieties/lines of *T. dioica*

Variety/line	Stem colour	Stem structure	Leaf colour	Leaf type	Leaf margin
BARI 1	Dark green	Pentagonal, notched	Green	Pointed	Entire
BARI 2	Green	Pentagonal, roundish	Green	Pointed	Entire
KALI BOMBAY	Green	Pentagonal, notched	Light green	Pointed	Entire
MALE 1	Light green	Pentagonal, notched	Light green	Pointed	Entire
PG003	Green	Pentagonal, roundish	Light green	Pointed	Entire
PG005	Dark green	Pentagonal, notched	Dark green	Blunt	Entire
PG006	Green	Pentagonal, notched	Dark green	Blunt	Entire
PG008	Light green	Pentagonal, roundish	Light green	Pointed	Entire
PG010	Green	Pentagonal, notched	Dark green	Pointed	Undulated
PG011	Green	Pentagonal, roundish	Dark green	Blunt	Undulated
PG012	Green	Pentagonal, light notched	Light green	Blunt	Serrated
PG015	Green	Pentagonal, notched	Light green	Pointed	Entire
PG018	Green	Pentagonal, notched	Green	Pointed	Entire
PG019	Light green	Pentagonal, notched	Dark green	Blunt	Entire
PG020	Dark green	Pentagonal, notched	Dark green	Pointed	Undulated
PG022	Green	Pentagonal, notched	Green	Pointed	Entire
PG023	Light green	Pentagonal, notched	Green	Pointed	Entire
PG028	Light green	Pentagonal, light notched	Green	Pointed	Entire

Table 4: Shape of ovary, fruit colour, fruit stripe, fruit shape and fruit curvature of seventeen varieties/lines of *Trichosanthes dioica*

Variety/line	Shape of ovary	Fruit colour at marketable stage	Fruit stripe	Fruit shape	Fruit curvature
BARI 1	Oval	Dark green	Greenish white stripe	Oval	Straight
BARI 2	Cylindrical	Light green	White stripe	Cylindrical	Straight
KALI BOMBAY	Spindle	Light green	Greenish white stripe	Spindle	Straight
PG003	Spindle	Light green	White stripe	Spindle	Straight
PG005	Cylindrical	Light green	White stripe	Cylindrical	Straight
PG006	Cylindrical	Dark green	Greenish white stripe	Cylindrical	Straight
PG008	Spindle	Light green	Greenish white stripe	Spindle	Straight
PG010	Cylindrical	Dark green	Greenish white stripe	Cylindrical	Straight
PG011	Oval	Dark green	Greenish white stripe	Oval	Straight
PG012	Oval	Light green	White stripe	Oval	Straight
PG015	Oval	Light green	White stripe	Spindle	Straight
PG018	Oval	Light green	Greenish white stripe	Oval	Straight
PG019	Oval	Dark green	Greenish white stripe	Oval	Straight
PG020	Cylindrical	Green	Greenish white stripe	Cylindrical	Straight
PG022	Oval	Light green	White stripe	Oval	Straight
PG023	Spindle	Green	No stripe	Spindle	Straight
PG028	Cylindrical	Green	Greenish white stripe	Cylindrical	Straight

mostly entire but type and colour varied. Like stem colour leaf colours were green, dark green and light green.

Three types of ovary shape were observed and these were oval, cylindrical and spindle **(Figs. 12-14).** In case of male plant the flower stalk was funnel shaped **(Fig. 15).** Male flower was found to be bigger in size compared to that of female.

In case of fruit characters their colour were found to be green, dark green and light green with different patterns of stripes. The stripes were usually greenish white and white except a line PG023, which showed no stripes. Fruit curvatures in all the varieties were found to be straight. The fruit shapes were three types viz. oval, cylindrical and spindle and these were almost directly related to shape of ovary except one line (PG015) of pointed gourd. However, eight fruit characters **(Figs. 16-23)** were found to be common in the studied plant materials.

Quantitative characteristics

Data on twelve morphological characters were recorded quantitatively and they were analyzed statistically **(Table 5).** It was observed that length of inter-node ranged more or less from 4.50 to 6.00cm in most of the varieties/lines. Only the varieties/lines i.e., BARI 1, PG005, PG019 and PG028 showed the maximum values for length of inter-node which reached above 6.00. It was also observed that the length of stem was minimum, when the length of inter-node was minimum. PG015 showed minimum values for both the characters. So, it can be said that there is a positive relation between length of stem and length of inter-node. This variety also showed the highest values for circumference of fruit, weight of fruit and No. of seeds per fruit. On the other hand, the maximum value for inter-node length was shown by PG028. At the same time the variety showed the second highest value for stem length. The highest value of stem length and No. of inter-node were observed in BARI 1. So, there was also a positive relation between stem length and No. of inter-node. The values of leaf area revealed a wide range where the lowest value was recorded in PG023 and the highest in PG019. In PG023 the value of fresh leaf weight was observed as lowest. So, there was a relation between leaf area and fresh weight of leaf. As the minimum values of fresh weight of flower, length of fruit, circumference of fruit and weight of fruit were recorded in PG023, so there is a strong relationship among the characters specially length of fruit, circumference of fruit and weight of fruit.

Pattern of morphological variation

The same values for the same twelve characters described above were used for determining the genotypic diversity among eighteen varieties/lines of *Trichosanthes dioica*. Mean data with scores for different characters, and the range of variation in each character represented by varying length of rays are given in **Tables 6** and **7,**

Table 5: Mean data for different morphological characters of eighteen varieties/lines of *Trichosanthes dioica*

Variety/line	Length of stem (cm)	Length of inter-node (cm)	No. of inter-node	Leaf area (cm^2)	Fresh weight of leaf (gm)	Days to flowering	Length of flower (cm)	Fresh weight of flower (gm)	Length of fruit (cm)	Circumference of fruit (cm)	Weight of fruit (gm)	No. of seeds per fruit
BARI 1	122.81a	6.09ab	21.11a	70.92c	2.732b	105.06g	6.45d	0.821c	10.34ab	12.15b	44.81c	22.08c
BARI 2	85.34de	5.39e	12.89e	41.41f	1.102e	109.08f	7.91ab	0.914b	8.21c	11.02c	35.43de	23.91c
Kali Bombay	91.04cd	5.77c	14.85d	63.41d	1.523d	111.12ef	6.72cd	0.729cd	10.55ab	10.31d	37.91d	22.34c
Male 1	95.17c	5.46e	14.64d	65.24d	1.968cd	119.09c	8.33a	1.043a	-	-	-	-
PG03	97.64c	5.91b	16.47c	49.31e	1.852cd	115.73d	8.85a	0.982ab	11.50a	9.17e	34.96de	20.01cd
PG05	98.72c	6.21ab	15.97c	80.07b	2.658b	117.27d	7.15bc	1.005a	7.51d	10.35d	30.05ef	24.03c
PG06	84.61de	5.41e	12.31e	43.08f	1.565d	122.84bc	7.23bc	0.781c	7.52d	9.41e	23.98g	22.01c
PG08	73.32ef	4.76g	13.58de	31.95g	0.966ef	133.91a	7.85ab	0.697d	8.81c	9.42e	27.57f	27.11b
PG10	88.01d	5.59d	15.33cd	83.39b	3.396a	112.05e	6.72cd	0.827c	8.01c	11.25c	40.86cd	19.23cd
PG11	74.53ef	4.81g	15.13cd	59.23de	2.093cd	122.95bc	6.61cd	0.791c	8.69c	12.49b	47.02bc	27.51b
PG12	71.03f	4.62gh	14.98cd	62.57d	2.261c	113.27e	7.01c	0.993ab	8.29c	12.01b	41.96cd	24.61c
PG15	68.38f	4.47h	15.01cd	62.85d	2.268c	116.45d	7.61b	0.753cd	9.81b	13.48a	62.48a	33.29a
PG18	83.06de	5.35e	12.27e	81.73b	2.836b	120.67c	7.33bc	0.768cd	7.72d	10.51d	32.58e	18.09d
PG19	98.48c	6.17ab	16.25c	95.05a	3.025ab	125.58b	7.51b	0.975ab	9.48b	12.23b	50.04b	26.47b
PG20	93.78cd	5.89b	15.74cd	62.51d	2.382c	132.78a	6.62cd	1.028a	8.53c	11.25c	36.63d	27.12b
PG22	90.08cd	5.75c	15.18cd	51.48e	1.656d	130.56ab	6.55cd	0.713d	6.33e	10.13d	24.76g	16.79e
PG23	78.81e	5.05f	14.37d	27.13h	0.897f	110.23ef	7.11bc	0.695d	6.01e	7.09f	10.55h	20.23cd
PG28	113.71b	6.81a	18.02b	36.12g	2.046cd	120.34c	7.95ab	0.905b	11.22a	11.24c	50.48b	20.45cd

The mean values in a column the different letters are significantly different at $P < 0.05$ in LSD test.

Table 6: Mean data and the scores (in parenthesis) for twelve characters of eighteen varieties/lines of *T. dioica*

Variety/line	**Length of stem (cm) X-axis**	Length of inter-node (cm)	No. of inter-node	**Leaf area (cm^2) Y-axis**	Fresh weight of leaf (gm)	Days to flowering	Length of flower (cm)	Fresh weight of flower (gm)	Length of fruit (cm)	Circumference of fruit (cm)	Weight of fruit (gm)	No. of seeds per fruit	Total scores
BARI 1	122.81(3)	6.09(2)	21.11(3)	70.92(2)	2.732(2)	105.06(1)	6.45(1)	0.821(1)	10.34(2)	12.15(2)	44.81(2)	22.08(1)	17
BARI 2	85.34(1)	5.39(1)	12.89(1)	41.41(1)	1.102(1)	109.08(1)	7.91(2)	0.914(2)	8.21(1)	11.02(2)	35.43(1)	23.91(2)	14
Kali Bombay	91.04(2)	5.77(2)	14.85(1)	63.41(2)	1.523(1)	111.12(1)	6.72(1)	0.729(1)	10.55(2)	10.31(1)	37.91(2)	22.34(1)	13
Male 1	95.17(2)	5.46(1)	14.64(1)	65.24(2)	1.968(1)	119.09(2)	8.33(3)	1.043(3)	-	-	-	-	11
PG003	97.64(2)	5.91(2)	16.47(2)	49.31(1)	1.852(1)	115.73(1)	8.85(3)	0.982(2)	11.50(3)	9.17(1)	34.96(1)	20.01(1)	17
PG005	98.72(2)	6.21(2)	15.97(2)	80.07(2)	2.658(2)	117.27(1)	7.15(1)	1.005(2)	7.51(1)	10.35(1)	30.05(1)	24.03(2)	15
PG006	84.61(1)	5.41(1)	12.31(1)	43.08(1)	1.565(1)	122.84(2)	7.23(1)	0.781(1)	7.52(1)	9.41(1)	23.98(1)	22.01(1)	11
PG008	73.32(1)	4.76(1)	13.58(1)	31.95(1)	0.966(1)	133.91(3)	7.85(2)	0.697(1)	8.81(2)	9.42(1)	27.57(1)	27.11(2)	15
PG010	88.01(1)	5.59(2)	15.33(2)	83.39(2)	3.396(3)	112.05(1)	6.72(1)	0.827(1)	8.01(1)	11.25(2)	40.86(2)	19.23(1)	16
PG011	74.53(1)	4.81(1)	15.13(1)	59.23(1)	2.093(2)	122.95(2)	6.61(1)	0.791(1)	8.69(1)	12.49(2)	47.02(2)	27.51(2)	15
PG012	71.03(1)	4.62(1)	14.98(1)	62.57(2)	2.261(2)	113.27(1)	7.01(1)	0.993(2)	8.29(1)	12.01(2)	41.96(2)	24.61(2)	15
PG015	68.38(1)	4.47(1)	15.01(1)	62.85(2)	2.268(2)	116.45(1)	7.61(2)	0.753(1)	9.81(2)	13.48(2)	62.48(2)	33.29(3)	17
PG018	83.06(1)	5.35(1)	12.27(1)	81.73(2)	2.836(2)	120.67(2)	7.33(2)	0.768(1)	7.72(1)	10.51(1)	32.58(1)	18.09(1)	13
PG019	98.48(2)	6.17(2)	16.25(2)	95.05(2)	3.025(2)	125.58(2)	7.51(2)	0.975(2)	9.48(2)	12.23(2)	50.04(2)	26.47(2)	20
PG020	93.78(2)	5.89(2)	15.74(2)	62.51(2)	2.382(2)	132.78(3)	6.62(1)	1.028(3)	8.53(1)	11.25(2)	36.63(1)	27.12(2)	19
PG022	90.08(2)	5.75(2)	15.18(1)	51.48(1)	1.656(1)	130.56(2)	6.55(1)	0.713(1)	6.33(1)	10.13(1)	24.76(1)	16.79(1)	12
PG023	78.81(1)	5.05(1)	14.37(1)	27.13(1)	0.897(1)	110.23(1)	7.11(1)	0.695(1)	6.01(1)	7.09(1)	10.55(1)	20.23(1)	10
PG028	113.71(3)	6.81(3)	18.02(2)	36.12(1)	2.046(1)	120.34(2)	7.95(2)	0.905(2)	11.22(2)	11.24(2)	50.48(2)	20.45(1)	19

Table 7: Class intervals, index values and distribution of scores of *Trichosanthes dioica* under different intensities

Characters	Range of means	Index values					
		1		2		3	
		Range	Sign	Range	Sign	Range	Sign
Length of stem (cm) **(X-axis)**	68.38-122.81	89.36 (9)		89.37-110.34 (7)		110.35 (2)	
Length of inter-node (cm)	4.47-6.81	5.52 (9)		5.53-6.57 (8)		6.58 (1)	
Number of inter-node	12.27-21.11	15.22 (11)		15.23-18.17 (6)		18.18 (1)	
Leaf area (cm^2) **(Y-axis)**	27.13-95.05	59.30 (8)		59.31-91.47 (9)		91.48 (1)	
Fresh weight of leaf (gm)	0.897-3.396	2.06 (9)		2.07-3.22 (8)		3.23 (1)	
Days to flowering (first time)	105.06-133.91	118.83 (9)		118.84-132.60 (7)		132.61 (2)	
Length of flower (cm)	6.45-8.85	7.30 (10)		7.31-8.15 (6)		8.16 (2)	
Fresh weight of flower (gm)	0.695-1.043	0.856 (10)		0.857-1.00 (6)		1.01 (2)	
Length of fruit (cm)	6.01-11.50	8.73 (10)		8.74-11.45 (6)		11.46 (1)	
Circumference of fruit (cm)	7.09-13.48	10.79 (8)		10.80-14.49 (9)		14.50 (0)	
Weight of fruit (gm)	10.55-62.48	37.18 (9)		37.19-63.81 (8)		63.82 (0)	
No. of seeds per fruit	16.79-33.29	23.25 (9)		23.26-29.71 (7)		29.72 (1)	

(Number of scores shown in parenthesis)

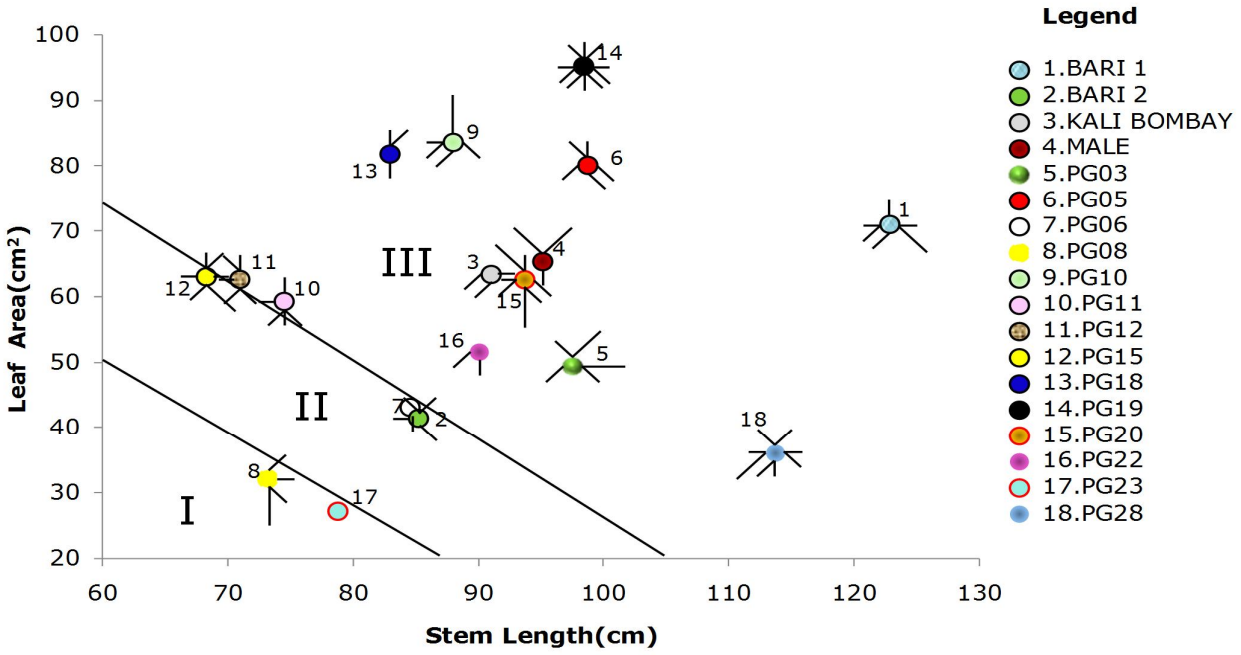


Fig. 24(A): Metroglyph diagram of various characters in eighteen varieties/lines of *T. dioica*

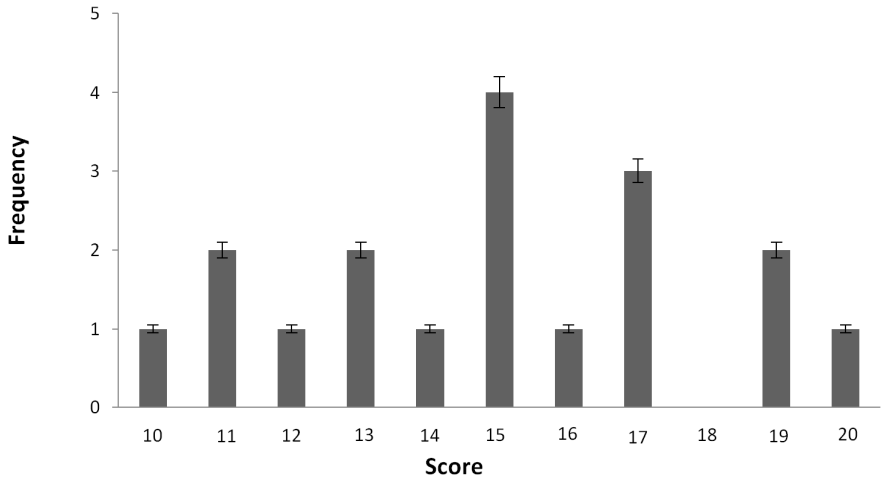


Fig. 24(B): Index score of eighteen varieties/lines of *Trichosanthes dioica*

respectively. Data are presented as metroglyph in **Figs. 24(A)** and **24(B).** From **Figure 24(B)** it was observed that the lowest score was 10 and it was obtained by PG023 (**Table 6)**. The highest score was found in PG019 and it was 20. It was also observed that the scoring value '15' was to be found as maximum times and the maximum frequency was 4. The performance of a genotype was denoted by the index score of that genotype and depending upon the score the length of ray varied. In the metroglyph the various varieties/lines used in the present study appeared to segregate in different clusters with the two most variable characters i.e., stem length and leaf area used for determining X and Y axis, respectively and thus for construction of metroglyph pattern. Apparently there were three clusters comprising of one or more varieties/lines out of eighteen which were studied. One of the varieties/lines namely BARI 1 represented by single status showed an isolation from rest of the clusters. Cluster I was represented by only two varieties/lines namely PG008 and PG023, although these were still distinguishable by their ray patterns. Cluster II was formed by three varieties/lines while cluster III was formed with the maximum number (thirteen) of varieties/lines. The ray's pattern on the glyph among the clusters revealed a marked variation for the presence or absence of rays and mostly inclined rays; horizontal rays were more or less common among the glyph. Rays pattern among clusters showed distinct variation which have been characterized as follows:

Cluster I: This cluster characteristically revealed low values for length of inter-node, number of inter-node, fresh weight of leaf, fresh weight of flower, circumference of fruit and weight of fruit. PG023 showed all the low values for all characters. However, PG008 showed the highest value for days to flowering. PG008 also showed medium values for length of flower, length of fruit and number of seeds per fruit.

Cluster II: Glyphs representing BARI 2, PG006 and PG015, where the last one possessed all of the low values except the value of days to flowering. PG006 showed the medium values of length of flower, fresh weight of flower, circumference of fruit and number of seeds per fruit. On the other hand, PG015 showed the highest value of number of seeds per fruit. PG015 also showed medium values of some characters such as fresh weight of leaf, length of flower, length of fruit, circumference of fruit and weight of fruit.

Cluster III: Most of the high values were observed in this cluster. PG028 showed the highest value of length of inter-node. The highest value for number of inter-node was shown by BARI 1. PG010 showed the highest value for fresh weight of leaf. PG020 showed two types of highest values i.e., days to flowering and fresh weight of flower. MALE 1 also showed the highest value for fresh weight of flower along with high value for length of flower. The highest value for length of flower was found in PG003 and this variety also showed the highest value for length of fruit. It was observed that all of the medium values were found in PG019. It was also observed that PG022 showed all the low values for all characters except two types of medium i.e., length of inter-node and days to flowering.

The three clusters, however, showed a distinct gradation for fresh weight of leaf and fresh weight of flower i.e., low (cluster I), intermediate (cluster II) and high (cluster III).

Frequency distribution of the highest and lowest quantitative values

The maximum and minimum values of twelve characters are shown in **Table 8** and in **Figure 25.** From the table it is clear that the high values for stem length and No. of inter-node were found in BARI 1. The highest values for length of flower and length of fruit were obtained by PG003 and that is why it can be said that there is a positive relationship between length of flower and length of fruit. Further it was observed that the highest values for circumference of fruit, weight of fruit and No. of seeds per fruit were recorded in PG015. So, there is also a positive relation among the characters. On the other hand, the lowest values of stem length and length of inter-node were possessed by PG015. It was also observed that the maximum number of lowest values i.e., leaf area, fresh weight of leaf, fresh weight of flower, length of fruit, circumference of fruit and weight of fruit were recorded in case of PG023. From the observation it may be said that there is a big portion of the variable characters which were found in case of PG015 and PG023.

Table 8: Highest and lowest quantitative values for twelve characters in the varieties/lines of *T. dioica*

Serial No.	Character	Highest	Variety/line	Lowest	Variety/line
1	Stem length (cm)	122.81	BARI 1	68.38	PG015
2	Length of inter-node (cm)	6.81	PG028	4.47	PG015
3	No. of inter-node	21.11	BARI 1	12.27	PG018
4	Leaf area (cm^2)	95.05	PG019	27.13	PG023
5	Fresh weight of leaf (gm)	3.39	PG010	0.89	PG023
6	Days to flowering	133.91	PG008	105.06	BARI 1
7	Length of flower (cm)	8.85	PG003	6.45	BARI 1
8	Fresh weight of flower (gm)	1.04	MALE 1	0.95	PG023
9	Length of fruit (cm)	11.50	PG003	6.01	PG023
10	Circumference of fruit (cm)	13.48	PG015	7.09	PG023
11	Weight of fruit (gm)	62.48	PG015	10.55	PG023
12	No. of seeds per fruit	33.29	PG015	16.79	PG022

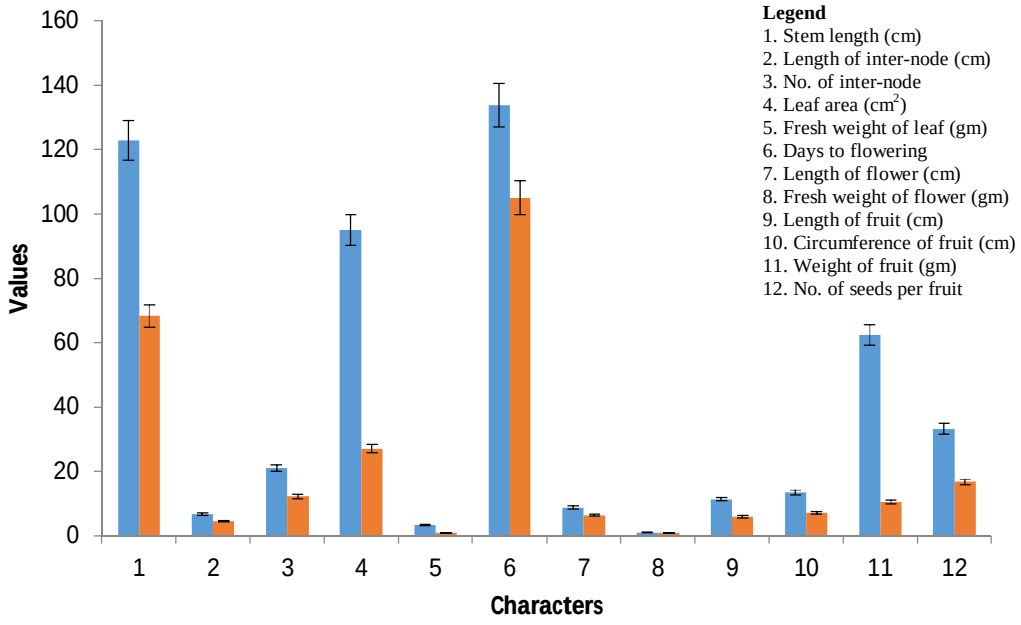


Fig. 25: Diagram showing the highest and lowest quantitative values in the varieties/lines of *Trichosanthes dioica*

2. Interphase nuclear phenotype

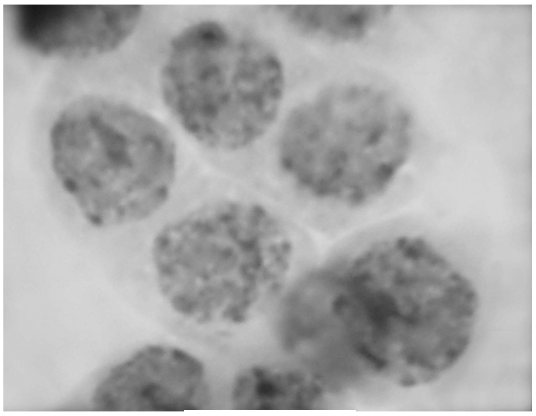
The interphase chromosome volume (ICV) in different varieties/lines of *Trichosanthes dioica* species varied differentially which are given in **Table 9**. The value $0.356\mu^3$ (PG023) was found to be higher than that of all varieties of pointed gourd. The value $0.111\mu^3$ (KALI BOMBAY) was lower compared to that of other varieties. The table reveals that there is more than three times difference between lower and higher mean values for interphase chromosome volume (ICV) of different varieties.

Interphase nuclear organization was chromocentric and the chromocentres were seen distinctly on a very high light background. As chromocentres correspond to heterochromatin (Nagl and Fusening, 1979) percentage heterochromatin values were obtained by determining the area of nucleus and chromocentres, and thereafter the values were expressed as percent nuclear area. The cells with well spread interphase chromosomes with chromocentres are shown in **Figs. 26-31.** Heterochromatin values in different varieties/lines of *Trichosanthes dioica* are given in **Table 9**.

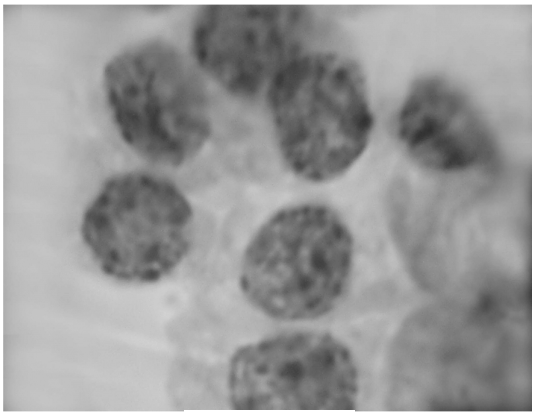
Table 9: Interphase chromosome volume (ICV), number of chromocentre and heterochromatin percentage in eighteen varieties/lines of *T. dioica*

Variety/line	ICV ($\bar{X}$±S.E)	Number of chromocentre ($\bar{X}$±S.E)	Heterochromatin % ($\bar{X}$±S.E)
BARI 1	0.162 ±0.014d	13.81±1.35bc	12.12±1.14c
BARI 2	0.246±0.011bc	15.85±0.87abc	14.55±0.88bc
KALI BOMBAY	0.111±0.006f	16.77±1.15abc	17.23±1.61ab
MALE 1	0.225±0.016c	14.81±1.31abc	14.30±1.30bc
PG003	0.280 ±0.018b	14.57±0.77abc	13.92±1.20bc
PG005	0.215±0.016c	18.04±1.33a	15.67±0.93bc
PG006	0.151±0.009de	17.01±1.13ab	16.52±1.01ab
PG008	0.124±0.010ef	16.64±0.86abc	17.42±1.46ab
PG010	0.181±0.014cd	15.78±1.42abc	16.66±1.03ab
PG011	0.137±0.009e	17.44±0.91ab	17.61±0.81ab
PG012	0.180±0.010cd	13.23±0.64c	13.58±0.99bc
PG015	0.146±0.013e	14.86±0.95abc	14.25±1.37bc
PG018	0.222±0.018c	17.06±1.22ab	16.80±1.37ab
PG019	0.194±0.013cd	17.09±0.93ab	19.98±1.98a
PG020	0.265±0.016b	16.12±1.04abc	15.39±1.02bc
PG022	0.172±0.013cd	16.32±0.98abc	16.78±0.96ab
PG023	0.356±0.028a	15.86±1.20abc	15.90±1.21bc
PG028	0.272±0.017b	16.32±0.86abc	16.14±0.98abc

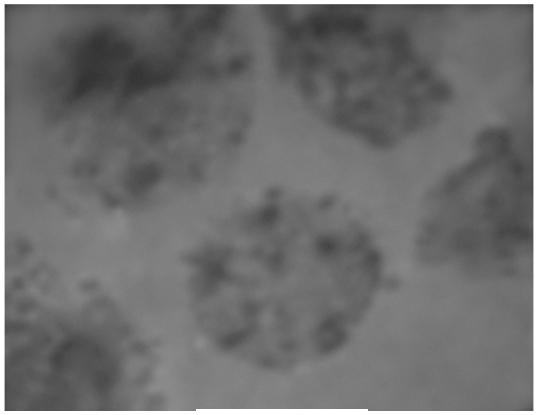
The mean values in a column the different letters are significantly different at $P < 0.05$ in LSD test.



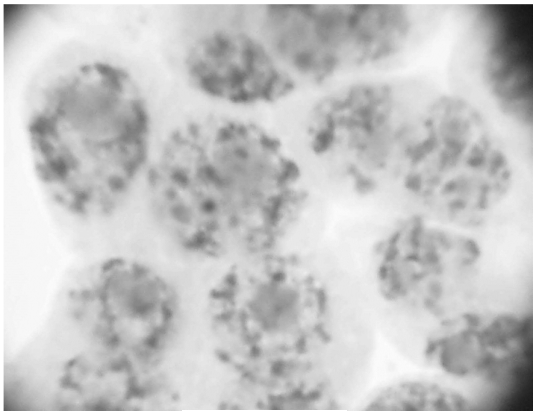
26. MALE I



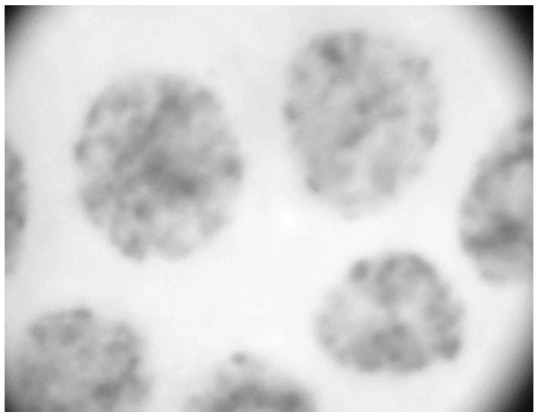
27. PG005



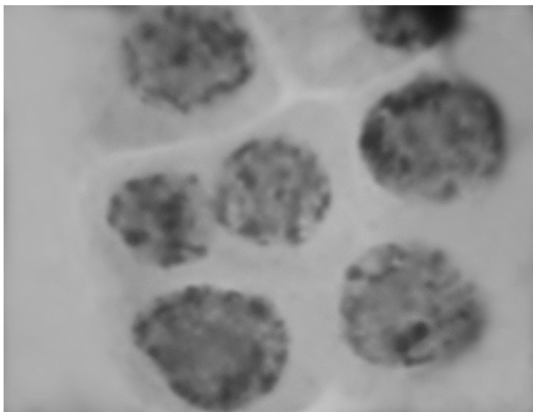
28. PG006



29. PG008



30. PG012



31. PG023

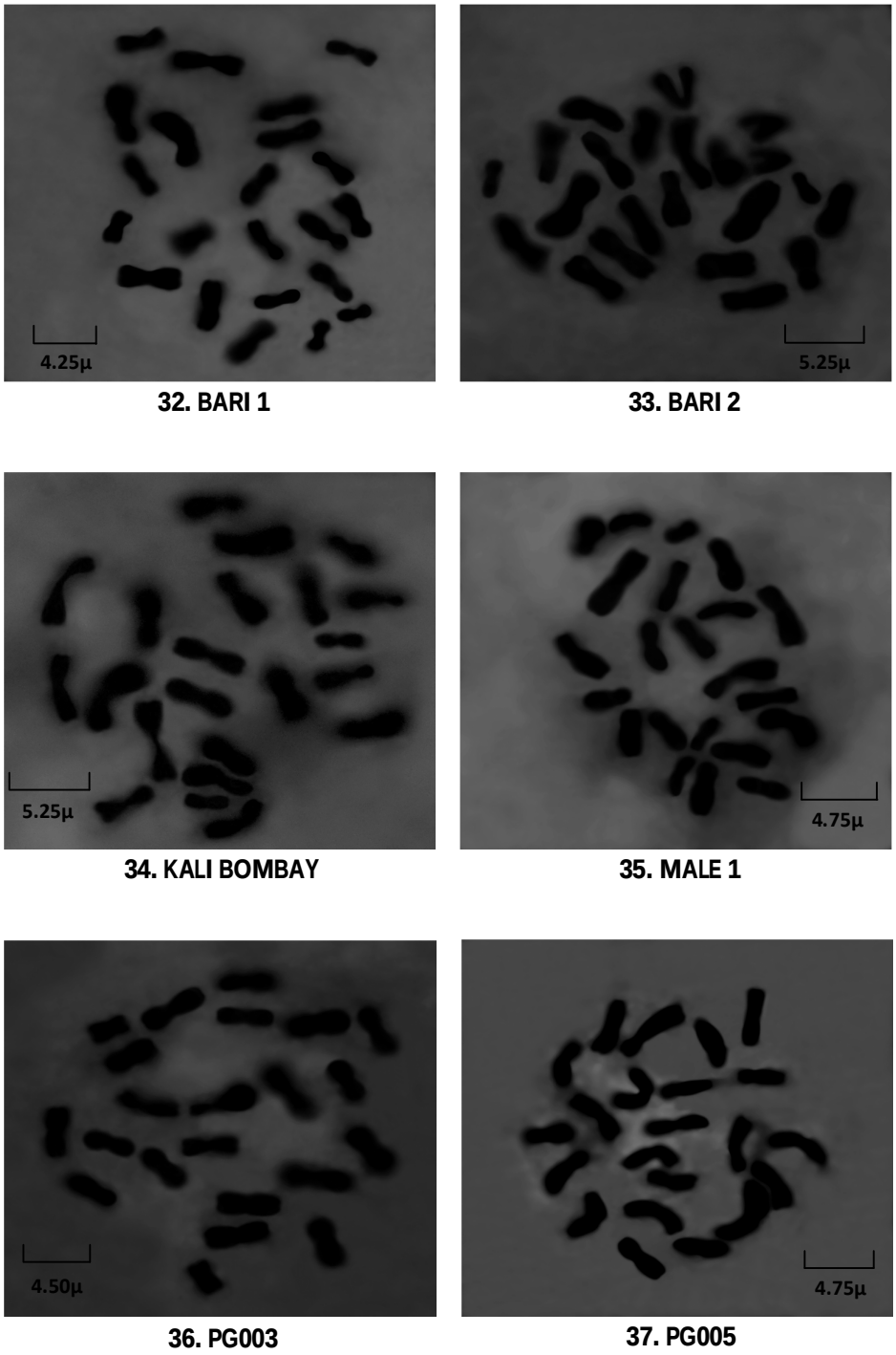
Figs. 26-31: Representative plate of interphase in six varieties/lines of *T. dioica*

Chromosome number in *Trichosanthes dioica* studied here was reported to be 2n = 22 (Bhaduri and Bose, 1947) which was confirmed in the present investigation. All the varieties of *Trichosanthes dioica* in the present study showed chromocentric nuclear organization and the chromocentres were seen distinctly on a very light background. The chromocentres became more clear and distinct after disruption of euchromatin by HCl indicating their heteromorphic nature. Chromocentres were dark and distinct and their number in all varieties/lines were more or less the same **(Table 9),** which were, however, less than the expected number 22. The highest value for chromocentre number was found in PG005 and the lowest value was found in PG012 having 18.04 and 13.23, respectively. Percentage of heterochromatin value obtained by PG019 having 19.98 was higher than those of other varieties/lines. The lowest percentage of heterochromatin value was recorded in BARI 1 having 12.12. It was observed that there is a close relationship with PG028 among other varieties.

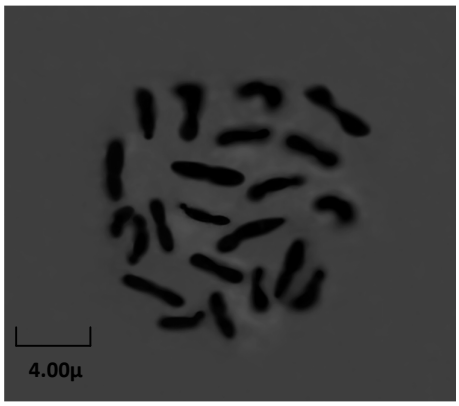
3. Somatic karyotype

To identify the chromosomes through somatic karyotype, shoot tips of all the varieties/lines of *Trichosanthes dioica* species were collected. Shoot tips of 1.5cm length were appropriate for obtaining maximum number of metaphase plates. At least three well spread metaphase plates were observed for this investigation. Somatic chromosomes were measured from photomicrographs. For making karyotipe analysis the method proposed by Ahmed *et.al.* (1983) was adopted on the basis of scatter diagram of total chromatin lengths (TCL) and arm ratios (AR) of all chromosomes in a number of cells. The cells with well spread metaphase chromosomes having more or less distinct morphology are presented in **Figs. 32-49.** The chromosome numbers for all the varieties/lines of *Trichosanthes dioica* species were found to be 2n=22. Chromosome morphology was determined quantitatively, and considerable points are described bellow for this study.

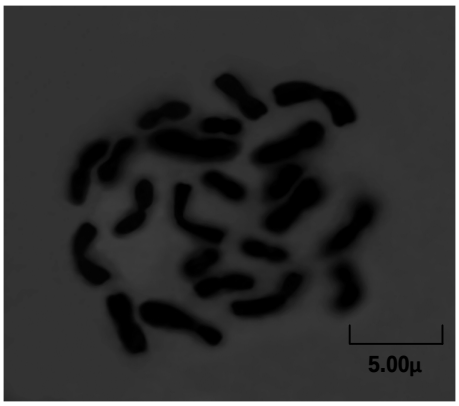
Chromosome morphology: Measurement for the lengths and ratios of representative complement of shoot tip chromosomes in the varieties/lines of *Trichosanthes dioica* are described. Data were taken at the desirable stage and were plotted in scatter diagrams **Figs. 50-67.** In all varieties/lines, pairs of adjacent points were considered to represent homologous chromosome and were marked with separate pairs of Roman alphabet on the separate scatter diagram. The average values of total length and arm ratio were calculated constituting the haploid complement of that cell. Then the chromosomes of haploid complements were numbered in decreasing order of total chromatin length and increasing order of arm ratio within the same length. The uniformity of the degree of contraction of chromosomes in the studied cells were determined by comparing haploid total lengths of all chromosomes and standardized haploid length and chromosomes



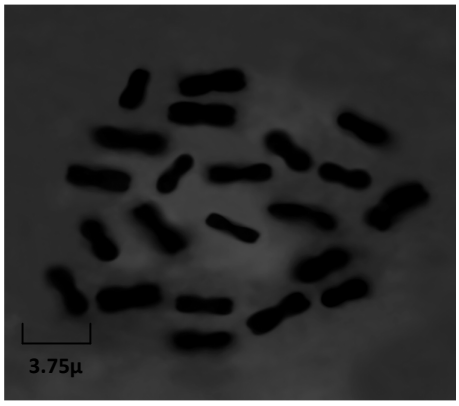
Figs. 32-37: Photomicrographs of metaphase chromosomes in six varieties/lines of *T. dioica*



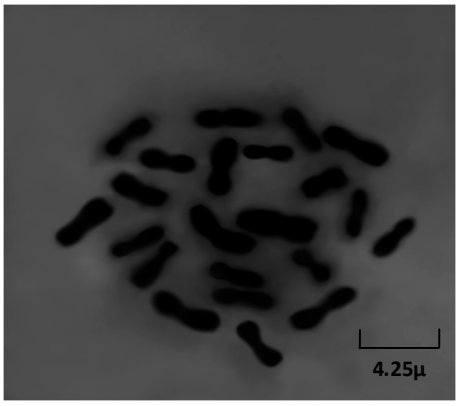
38. PG006



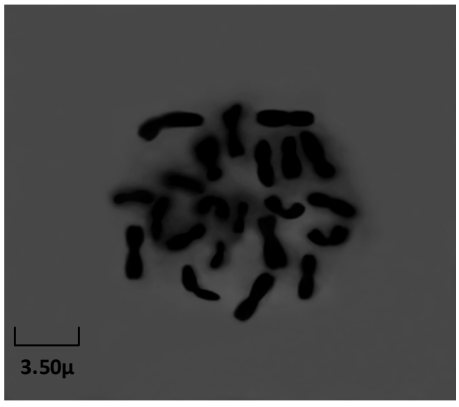
39. PG008



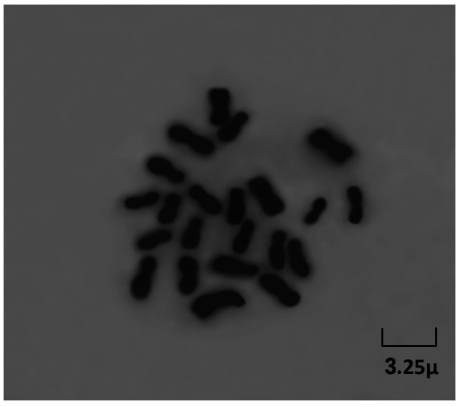
40. PG010



41. PG011

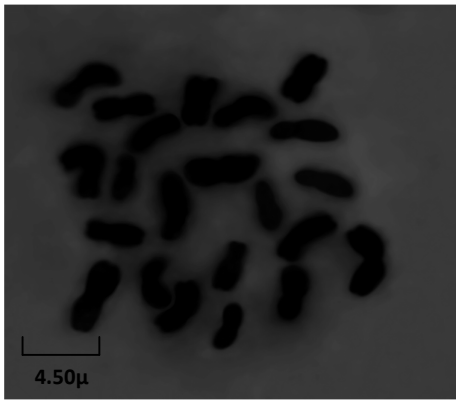


42. PG012

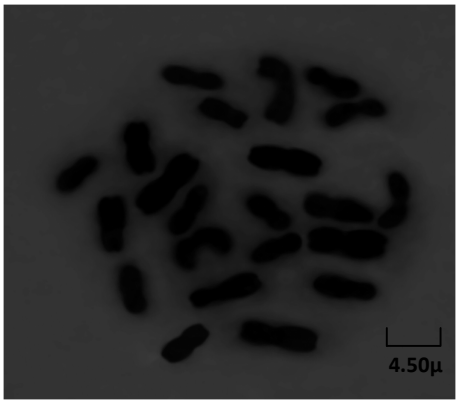


43. PG015

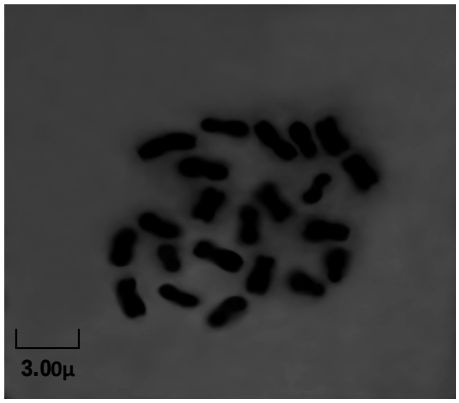
Figs. 38-43: Photomicrographs of metaphase chromosomes in six varieties/lines of *T. dioica*



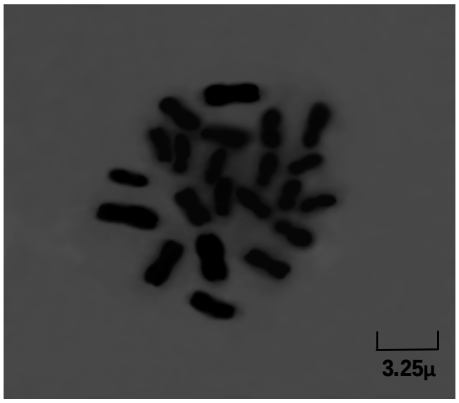
44. PG018



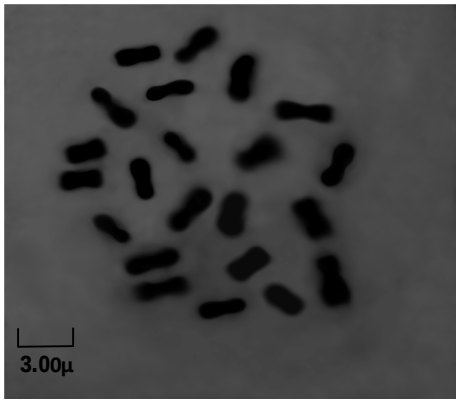
45. PG019



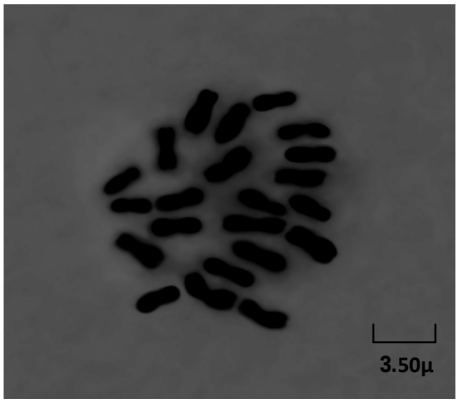
46. PG020



47. PG022

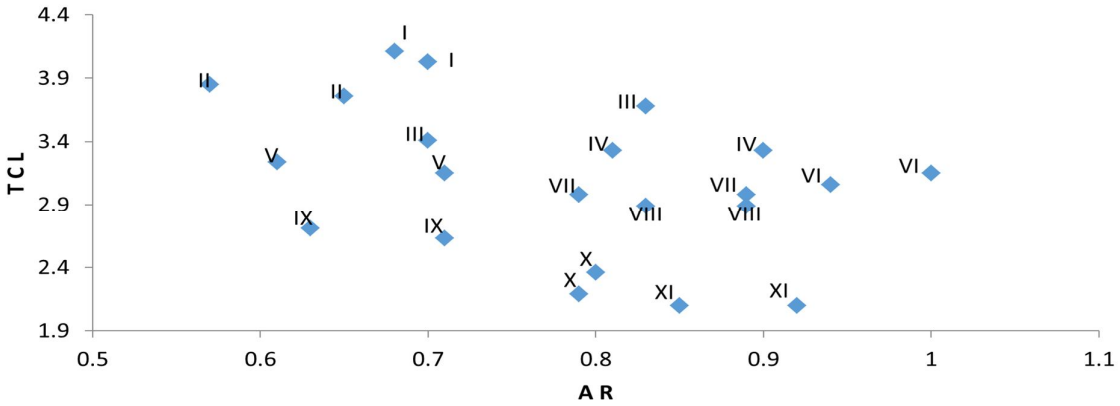


48. PG023

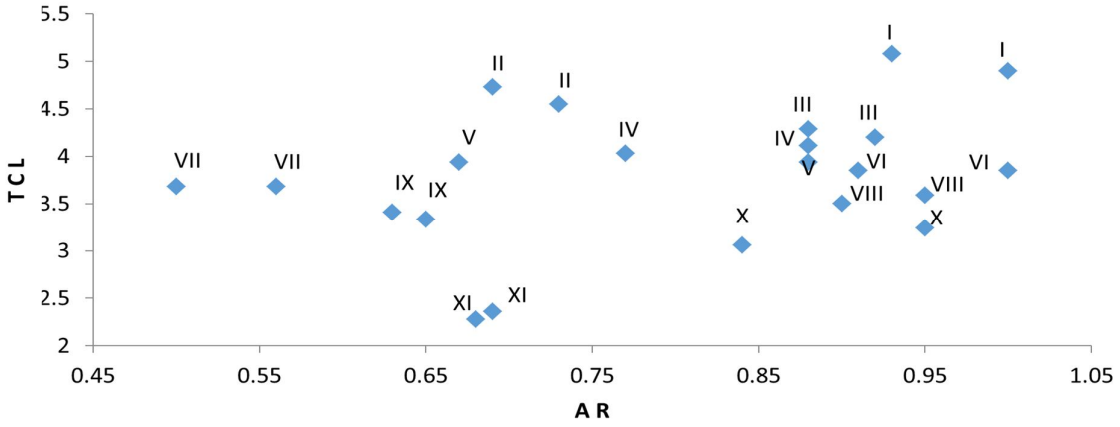


49. PG028

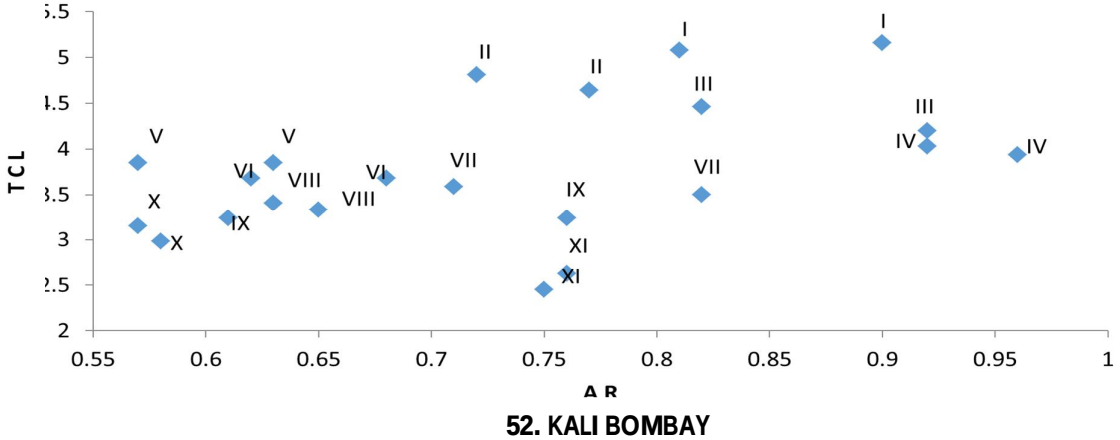
Figs. 44-49: Photomicrographs of metaphase chromosomes in six varieties/lines of *T. dioica*



50. BARI 1

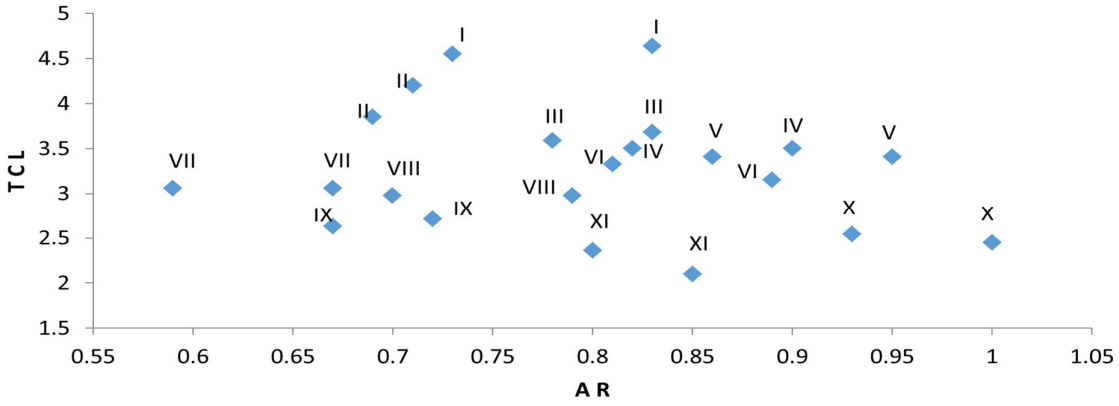


51. BARI 2

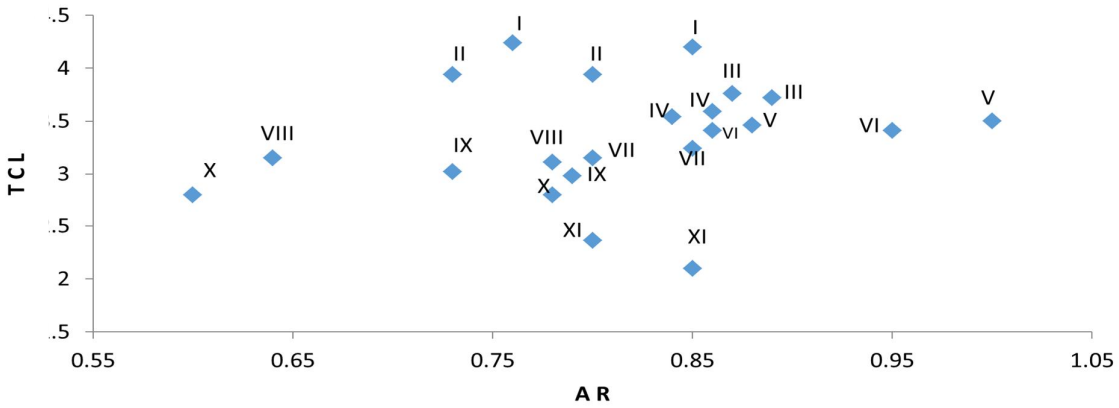


52. KALI BOMBAY

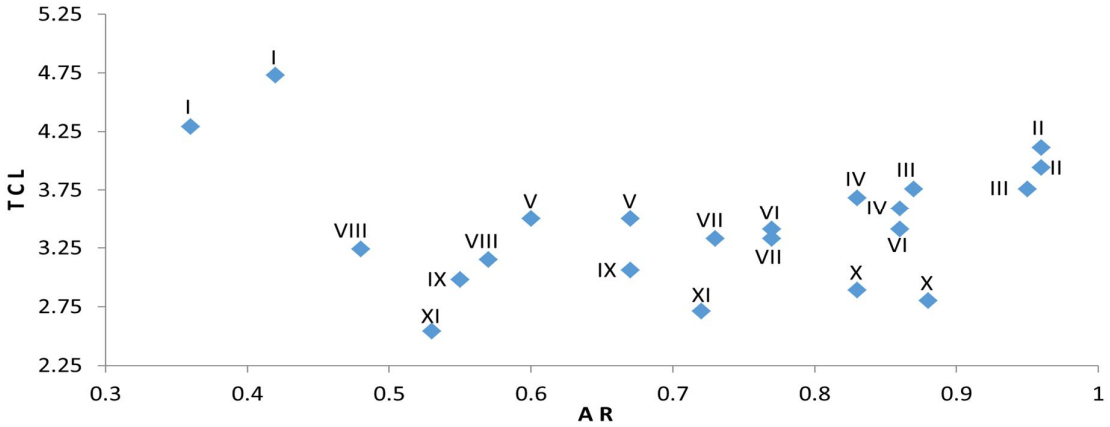
Figs. 50-52: Scatter diagram of the chromosomes in three varieties/lines of *T. dioica*



53. MALE 1

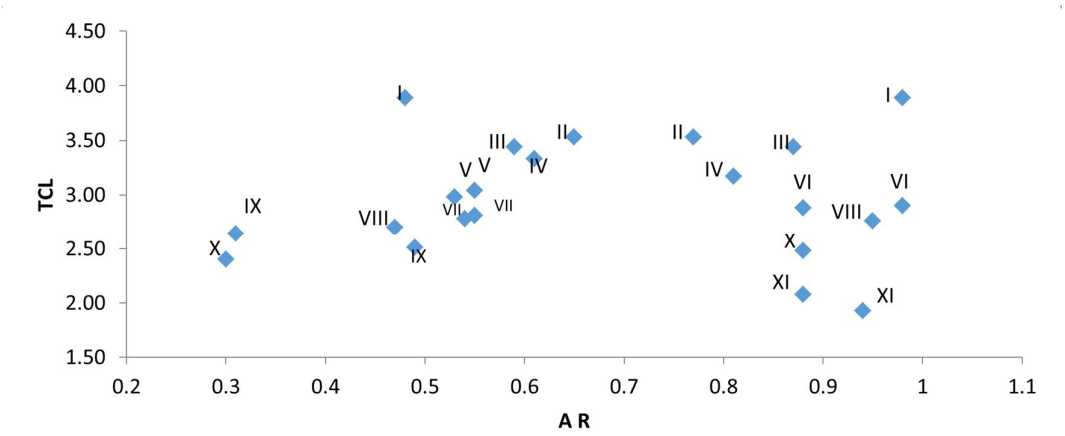


54. PG003

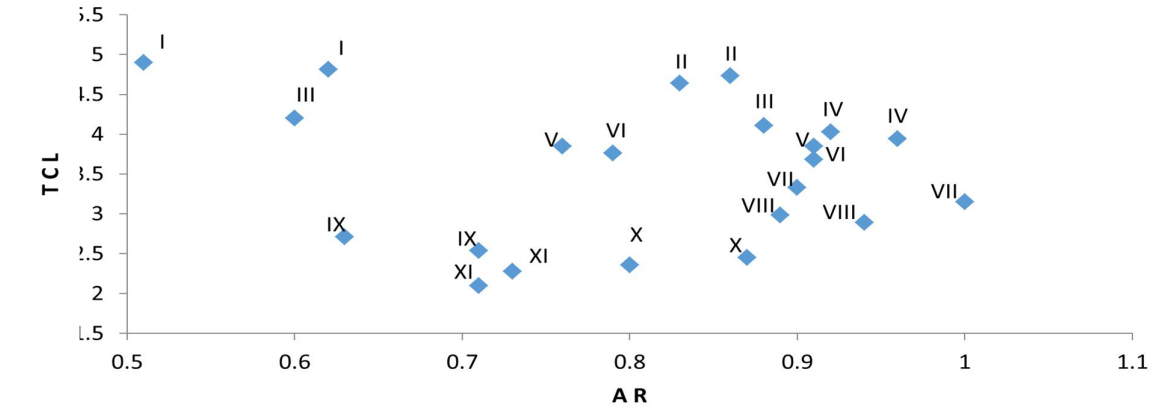


55. PG005

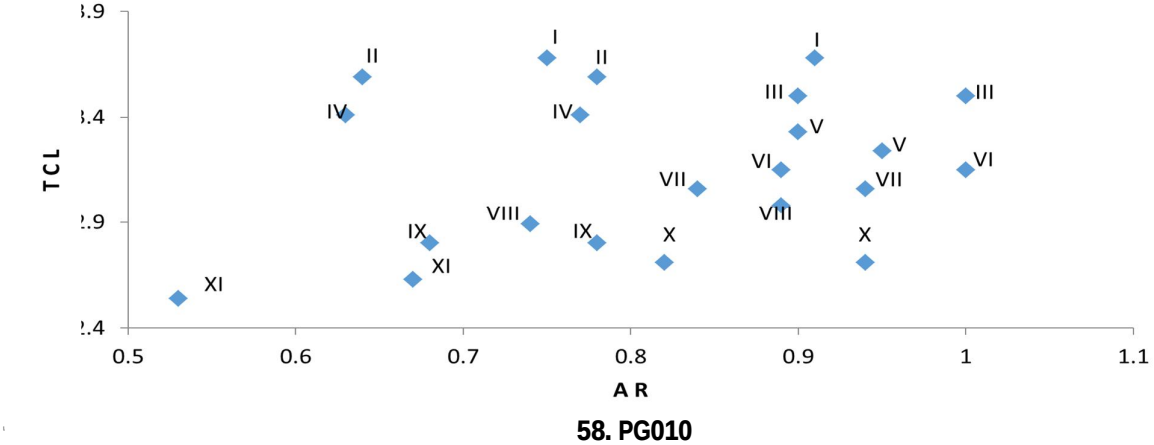
Figs. 53-55: Scatter diagram of the chromosomes in three varieties/lines of *T. dioica*



56. PG006

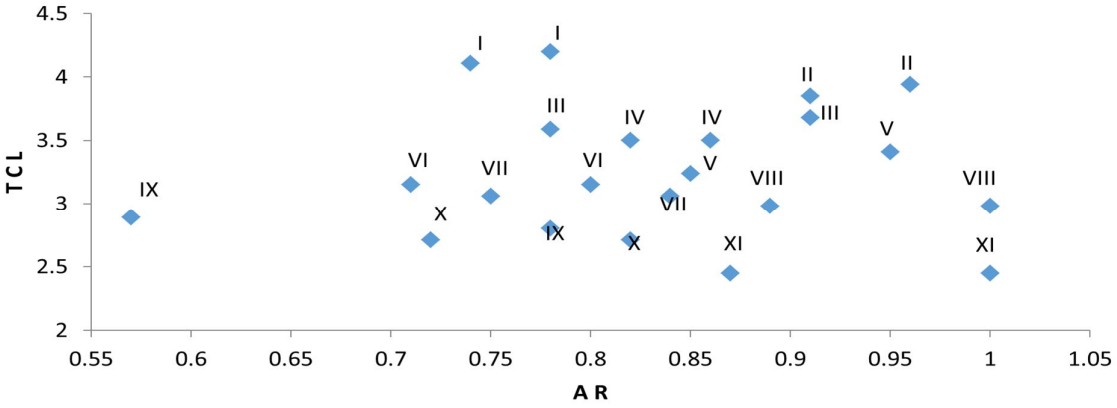


57. PG008

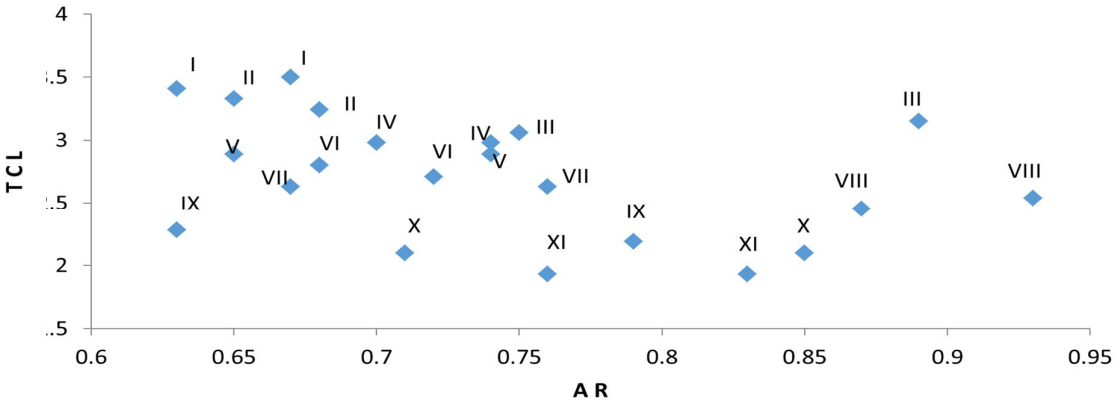


58. PG010

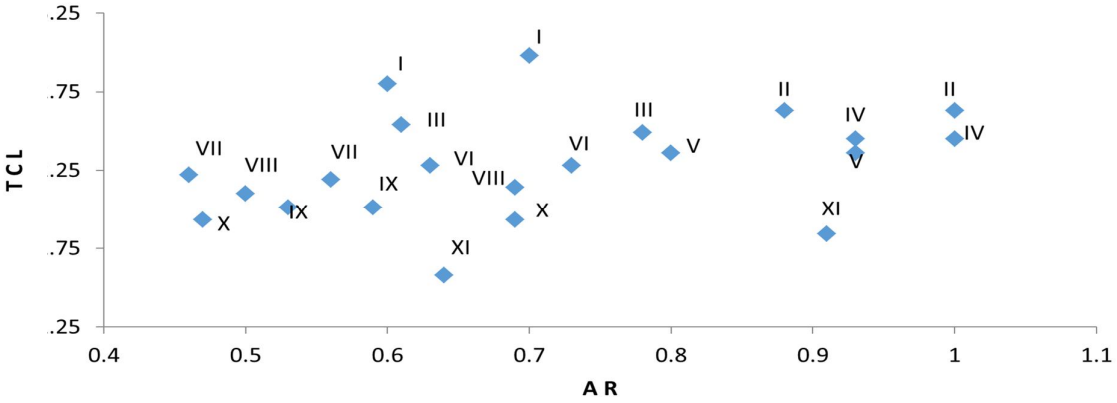
Figs. 56-58: Scatter diagram of the chromosomes in three varieties/lines of *T. dioica*



59. PG011

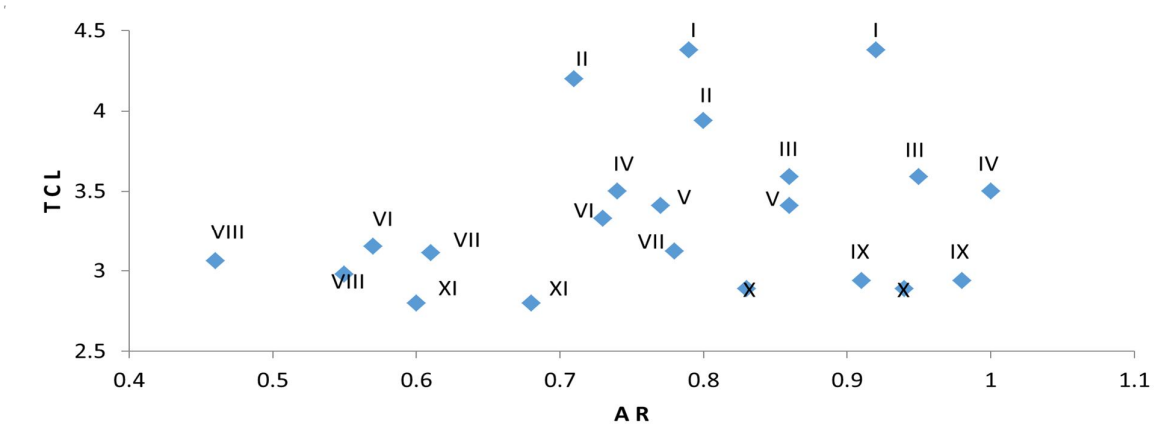


60. PG012

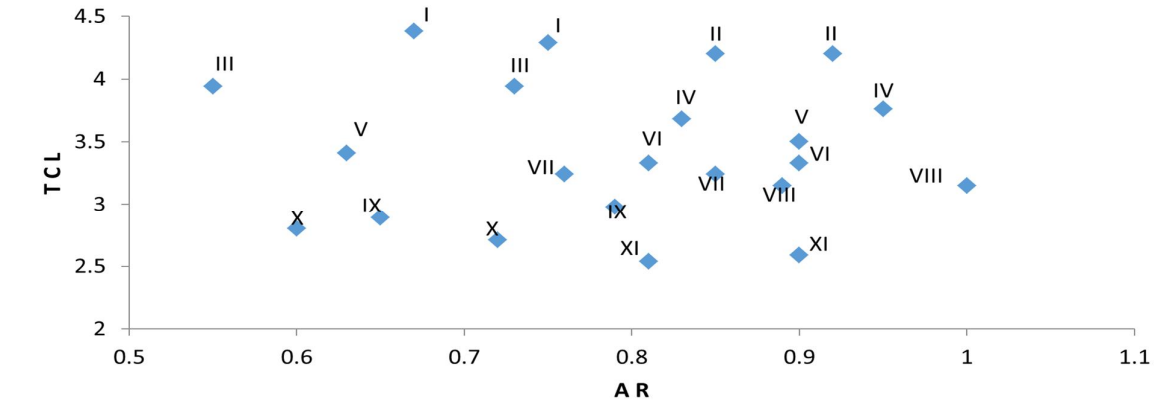


61. PG015

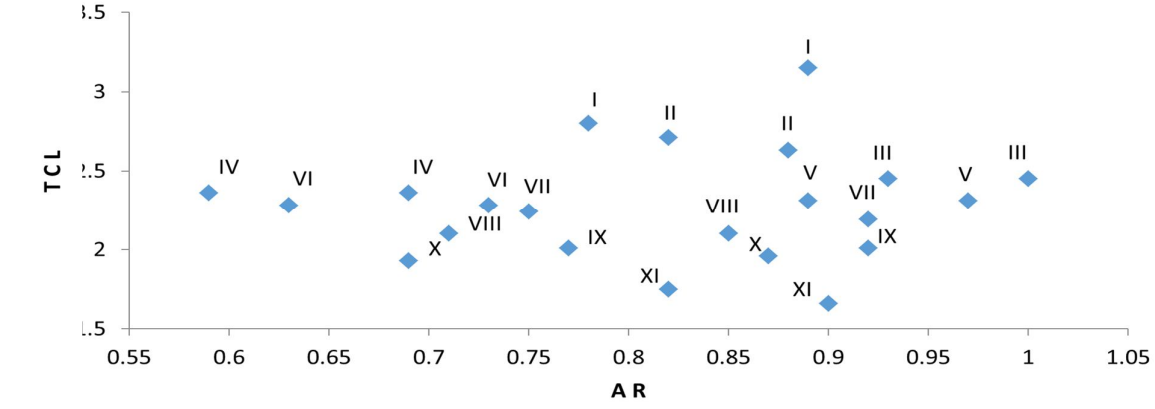
Figs. 59-61: Scatter diagram of the chromosomes in three varieties/lines of *T. dioica*



62. PG018

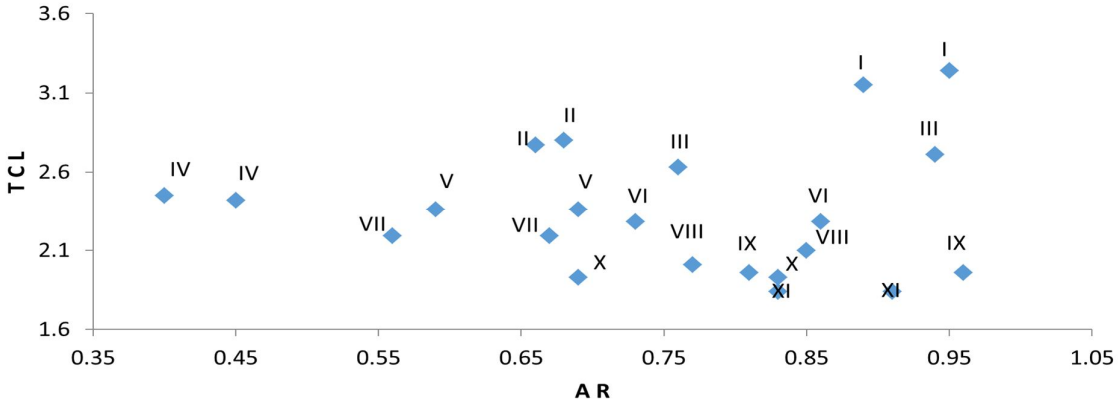


63. PG019

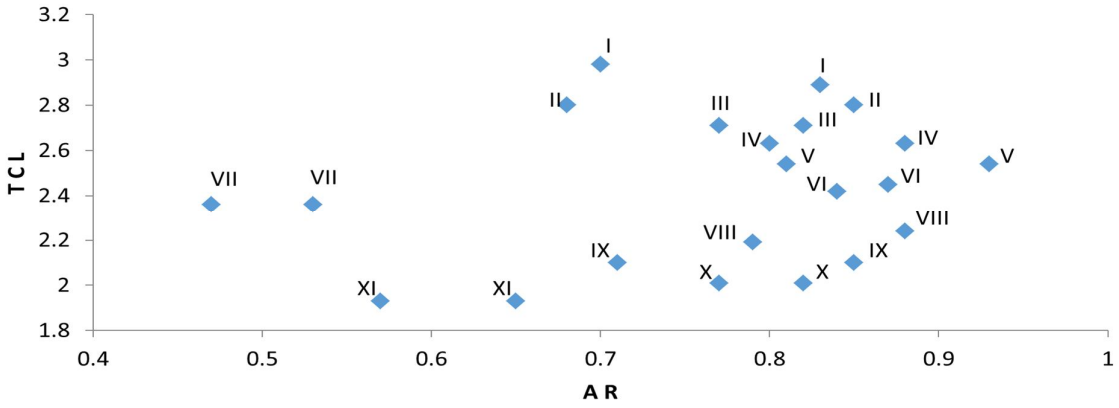


64. PG020

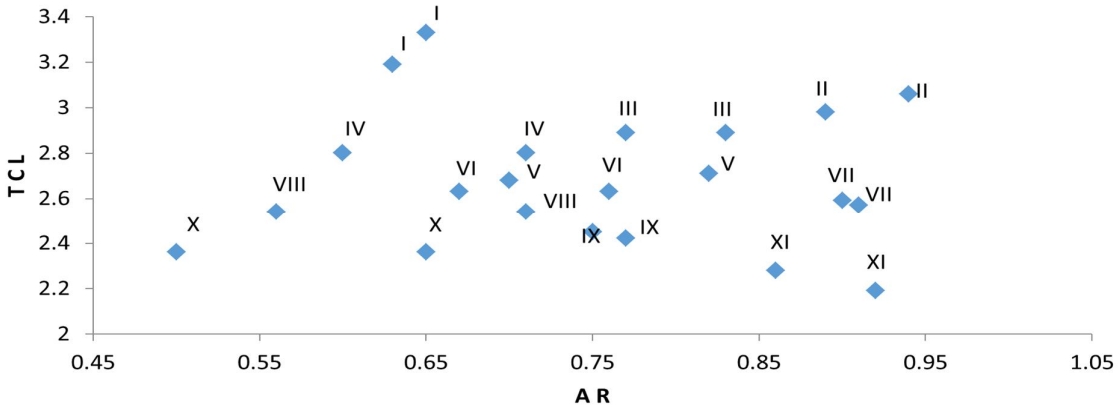
Figs.62-64: Scatter diagram of the chromosomes in three varieties/lines of *T. dioica*



65. PG022



66. PG023



67. PG028

Figs. 65-67: Scatter diagram of the chromosomes in three varieties/lines of *T. dioica*

distribution in this BARI 1 were determined in **Table 10(A).** The similarity and homogeneity of the distribution of chromosomal morphology in cell of BARI 1 were tested by the use of contingency table incorporating chromosome length and arm ratio classes in **Table 10(B).** Similarly in BARI 2, KALI BOMBAY, MALE 1 and up to the variety/line PG028 in **Tables 11AB-27AB** indicates the standardized haploid complement of chromosomes and the contingency table incorporating chromosome length and arm ratio classes.

Morphological feature of chromosome and proposed standard karyotype: The chromosomes were distributed to the various morphological categories using also probabilistic inferences, especially on the chromosome frequency in a given class per haploid set (A=Large, B=Medium, C=relatively short and D=short). The morphological features of the haploid complement in the varieties/lines of *Trichosanthes dioica* are given in **Tables 28-45.** The standard karyotypes were proposed for *Trichosanthes dioica* on the basis of centromeric formula, range and average of chromatin length per chromosome. Data on chromosome morphology, i.e., length, arm ratio, TCL%, TF% and chromosome type are also given in these tables. Ideograms for chromosome complement are shown in **Figs. 68-85** for BARI 1, BARI 2, KALI BOMBAY, MALE 1 and up to the variety/line PG028, respectively. Morphological features of the proposed standard karyotype are described below:

BARI 1: Seven pairs of chromosomes for this variety were found to be metacentic in III, IV, VI, VII, VIII, X, XI and four pairs sub-metacentic in I, II, V, IX **(Table 28).** The longest chromosome pair was 4.07µm in length and shortest chromosome pair was 2.10µm. The value of TCL was of 33.95µm, TF% was 43.36 and TCL% was highest as 11.98, the lowest was 6.19. The proposed karyotype formula was found to be $2B^{sm}+B^{m}+2C^{sm}+4C^{m}+2D^{m}$.

BARI 2: The chromosome complement in this variety was found with eight metacentric in I, III, IV, V, VI, VIII, X, XI and three sub-metacentric in II, VII, IX **(Table 29).** The longest chromosome pair was 4.99µm in length and shortest was 2.32µm with a TCL of 41.80µm. TF% was found to be 44.35 and TCL% was highest as 11.94 and lowest as 5.55. The proposed karyotypic formula was found to be $A^{sm}+A^{m}+B^{sm}+5B^{m}+C^{sm}+C^{m}+D^{m}$.

KALI BOMBAY: In this variety the chromosome complement was found with five metacentric in I, III, IV, VII, XI and six was sub-metacenrtic in II, V, VI, VIII, IX, X **(Table 30).** Among the chromosome pair's longest chromosome pair was found to be 5.12µm and shortest was 2.53µm with a TCL of 41.43µm. TF% was found to be 42.40 and TCL% was found to be highest as 12.35 and lowest as 6.12. The proposed karyotypic formula was found to be

$A^{sm}+2A^{m}+3B^{sm}+2B^{m}+2C^{sm}+D^{m}$.

Table 10(A): Standardized haploid length (X') of observed chromosomes in BARI 1

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.11	4.03	0.69	33.95
II	3.85	3.76	0.61	
III	3.68	3.41	0.76	
IV	3.33	3.33	0.85	
V	3.24	3.15	0.66	
VI	3.15	3.06	0.97	
VII	2.98	2.98	0.84	
VIII	2.89	2.89	0.86	
IX	2.71	2.63	0.67	
X	2.36	2.19	0.79	
XI	2.10	2.10	0.88	
∑x	34.40	33.53		

Table 10(B): The homogeneity in distribution of chromosomes among different haploid complements in BARI 1

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	2
	0.50 and less	-
Total		**2**
2.94 to 3.64 µ	0.76-1.00	4
	0.51-0.75	1
	0.50 and less	-
Total		**5**
2.23 to 2.93 µ	0.76-1.00	2
	0.51-0.75	1
	0.50 and less	-
Total		**3**
2.22 µ and less.	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
Grand total		**11**

Table 11(A): Standardized haploid length (X') of observed chromosomes in BARI 2

No of chromosome pairs	X_1	X_2	A R	Mean
I	5.08	4.90	0.97	41.80
II	4.73	4.55	0.71	
III	4.29	4.20	0.90	
IV	4.11	4.03	0.82	
V	3.94	3.94	0.77	
VI	3.85	3.85	0.96	
VII	3.68	3.68	0.53	
VIII	3.59	3.50	0.93	
IX	3.41	3.33	0.64	
X	3.24	3.06	0.89	
XI	2.36	2.28	0.68	
∑x	42.28	41.32		

Table 11(B): The homogeneity in distribution of chromosomes among different haploid complements in BARI 2

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	5
	0.51-0.75	2
	0.50 and less	-
Total		**7**
2.94 to 3.64 µ	0.76-1.00	2
	0.51-0.75	1
	0.50 and less	-
Total		**3**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 12(A): Standardized haploid length (X') of observed chromosomes in KALI BOMBAY

No of chromosome pairs	X_1	X_2	A R	Mean
I	5.16	5.08	0.86	41.43
II	4.81	4.64	0.74	
III	4.46	4.20	0.87	
IV	4.03	3.94	0.94	
V	3.85	3.85	0.60	
VI	3.68	3.68	0.65	
VII	3.59	3.5	0.76	
VIII	3.41	3.33	0.64	
IX	3.24	3.24	0.69	
X	3.15	2.98	0.57	
XI	2.63	2.45	0.76	
∑x	42.01	40.89		

Table 12(B): The homogeneity in distribution of chromosomes among different haploid complements in KALI BOMBAY

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	3
	0.51-0.75	3
	0.50 and less	-
Total		**6**
2.94 to 3.64 µ	0.76-1.00	1
	0.51-0.75	3
	0.50 and less	-
Total		**4**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 13(A): Standardized haploid length (X') of observed chromosomes in MALE I

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.64	4.55	0.78	35.84
II	4.20	3.85	0.70	
III	3.68	3.59	0.80	
IV	3.50	3.50	0.86	
V	3.41	3.41	0.90	
VI	3.33	3.15	0.85	
VII	3.06	3.06	0.63	
VIII	2.98	2.98	0.74	
IX	2.71	2.63	0.69	
X	2.54	2.45	0.97	
XI	2.36	2.10	0.82	
∑x	36.41	35.27		

Table 13(B): The homogeneity in distribution of chromosomes among different haploid complements in MALE 1

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	1
	0.51-0.75	1
	0.50 and less	-
Total		**2**
2.94 to 3.64 µ	0.76-1.00	4
	0.51-0.75	2
	0.50 and less	-
Total		**6**
2.23 to 2.93 µ	0.76-1.00	2
	0.51-0.75	1
	0.50 and less	-
Total		**3**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 14(A): Standardized haploid length (X') of observed chromosomes in PG003

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.24	4.20	0.80	36.71
II	3.94	3.94	0.77	
III	3.76	3.72	0.88	
IV	3.59	3.54	0.85	
V	3.50	3.46	0.94	
VI	3.41	3.41	0.90	
VII	3.24	3.15	0.83	
VIII	3.15	3.11	0.71	
IX	3.02	2.98	0.76	
X	2.80	2.80	0.69	
XI	2.36	2.10	0.82	
∑x	37.01	36.41		

Table 14(B): The homogeneity in distribution of chromosomes among different haploid complements in PG003

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	3
	0.51-0.75	-
	0.50 and less	-
Total		**3**
2.94 to 3.64 µ	0.76-1.00	5
	0.51-0.75	1
	0.50 and less	-
Total		**6**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	1
	0.50 and less	-
Total		**2**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 15(A): Standardized haploid length (X') of observed chromosomes in PG005

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.73	4.29	0.39	37.84
II	4.11	3.94	0.96	
III	3.76	3.76	0.91	
IV	3.68	3.59	0.84	
V	3.50	3.50	0.63	
VI	3.41	3.41	0.81	
VII	3.33	3.33	0.75	
VIII	3.24	3.15	0.52	
IX	3.06	2.98	0.61	
X	2.89	2.80	0.86	
XI	2.71	2.54	0.62	
∑x	38.42	37.29		

Table 15(B): The homogeneity in distribution of chromosomes among different haploid complements in PG005

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	2
	0.51-0.75	-
	0.50 and less	1
Total		**3**
2.94 to 3.64 µ	0.76-1.00	2
	0.51-0.75	4
	0.50 and less	-
Total		**6**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	1
	0.50 and less	-
Total		**2**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 16(A): Standardized haploid length (X') of observed chromosomes in PG006

No of chromosome pairs	X_1	X_2	A R	Mean
I	3.89	3.89	0.73	32.54
II	3.53	3.53	0.71	
III	3.44	3.44	0.73	
IV	3.33	3.17	0.71	
V	2.70+0.34*	2.64+0.34*	0.54	
VI	2.90	2.88	0.93	
VII	2.29+0.52*	2.26+0.52*	0.55	
VIII	2.76	2.70	0.71	
IX	2.64	2.51	0.40	
X	2.48	2.40	0.59	
XI	2.08	1.93	0.91	
∑x	32.90	32.21		

Table 16(B): The homogeneity in distribution of chromosomes among different haploid complements in PG006

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	1
	0.50 and less	-
Total		**1**
2.94 to 3.64 µ	0.76-1.00	-
	0.51-0.75	4
	0.50 and less	-
Total		**4**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	3
	0.50 and less	1
Total		**5**
2.22 µ and less.	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
Grand total		**11**

Table 17(A): Standardized haploid length (X') of observed chromosomes in PG008

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.90	4.81	0.57	38.65
II	4.73	4.64	0.84	
III	4.20	4.11	0.74	
IV	4.03	3.94	0.94	
V	3.85	3.85	0.84	
VI	3.76	3.68	0.85	
VII	3.33	3.15	0.95	
VIII	2.98	2.89	0.92	
IX	2.71	2.54	0.67	
X	2.45	2.36	0.83	
XI	2.28	2.10	0.72	
∑x	39.22	38.07		

Table 17(B): The homogeneity in distribution of chromosomes among different haploid complements in PG008

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	4
	0.51-0.75	2
	0.50 and less	-
Total		**6**
2.94 to 3.64 µ	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
2.23 to 2.93 µ	0.76-1.00	2
	0.51-0.75	1
	0.50 and less	-
Total		**3**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	1
	0.50 and less	-
Total		**1**
Grand total		**11**

Table 18(A): Standardized haploid length (X') of observed chromosomes in PG010

No of chromosome pairs	X_1	X_2	A R	Mean
I	3.68	3.68	0.83	34.69
II	3.59	3.59	0.71	
III	3.50	3.50	0.95	
IV	3.41	3.41	0.70	
V	3.33	3.24	0.92	
VI	3.15	3.15	0.95	
VII	3.06	3.06	0.89	
VIII	2.98	2.89	0.81	
IX	2.80	2.80	0.73	
X	2.71	2.71	0.88	
XI	2.63	2.54	0.60	
∑x	34.84	34.57		

Table 18(B): The homogeneity in distribution of chromosomes among different haploid complements in PG010

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
2.94 to 3.64 µ	0.76-1.00	4
	0.51-0.75	2
	0.50 and less	-
Total		**6**
2.23 to 2.93 µ	0.76-1.00	2
	0.51-0.75	2
	0.50 and less	-
Total		**4**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 19(A): Standardized haploid length (X') of observed chromosomes in PG011

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.20	4.11	0.76	35.70
II	3.94	3.85	0.93	
III	3.68	3.59	0.85	
IV	3.50	3.50	0.84	
V	3.41	3.24	0.90	
VI	3.15	3.15	0.76	
VII	3.06	3.06	0.80	
VIII	2.98	2.98	0.94	
IX	2.89	2.80	0.67	
X	2.71	2.71	0.77	
XI	2.45	2.45	0.93	
∑x	35.97	35.44		

Table 19(B): The homogeneity in distribution of chromosomes among different haploid complements in PG011

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	2
	0.51-0.75	-
	0.50 and less	-
Total		**2**
2.94 to 3.64 µ	0.76-1.00	6
	0.51-0.75	-
	0.50 and less	-
Total		**6**
2.23 to 2.93 µ	0.76-1.00	2
	0.51-0.75	1
	0.50 and less	-
Total		**3**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 20(A) : Standardized haploid length (X') of observed chromosomes in PG012

No of chromosome pairs	X_1	X_2	A R	Mean
I	3.50	3.41	0.65	29.84
II	3.33	3.24	0.67	
III	3.15	3.06	0.82	
IV	2.98	2.98	0.72	
V	2.89	2.89	0.69	
VI	2.80	2.71	0.70	
VII	2.63	2.63	0.72	
VIII	2.54	2.45	0.90	
IX	2.28	2.19	0.71	
X	2.10	2.10	0.78	
XI	1.93	1.93	0.80	
∑x	30.13	29.59		

Table 20(B): The homogeneity in distribution of chromosomes among different haploid complements in PG012

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
2.94 to 3.64 µ	0.76-1.00	1
	0.51-0.75	3
	0.50 and less	-
Total		**4**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	4
	0.50 and less	-
Total		**5**
2.22 µ and less.	0.76-1.00	2
	0.51-0.75	-
	0.50 and less	-
Total		**2**
Grand total		**11**

Table 21(A): Standardized haploid length (X') of observed chromosomes in PG015

No of chromosome pairs	X_1	X_2	A R	Mean
I	2.98	2.80	0.65	25.08
II	2.63	2.63	0.94	
III	2.54	2.49	0.69	
IV	2.45	2.45	0.97	
V	2.36	2.36	0.86	
VI	2.28	2.28	0.68	
VII	2.22	2.19	0.51	
VIII	2.14	2.10	0.60	
IX	2.01	2.01	0.56	
X	1.93	1.93	0.58	
XI	1.84	1.58	0.77	
∑x	25.38	24.82		

Table 21(B): The homogeneity in distribution of chromosomes among different haploid complements in PG015

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		-
2.94 to 3.64 µ	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		-
2.23 to 2.93 µ	0.76-1.00	3
	0.51-0.75	3
	0.50 and less	-
Total		**6**
2.22 µ and less.	0.76-1.00	1
	0.51-0.75	4
	0.50 and less	-
Total		**5**
Grand total		**11**

Table 22(A): Standardized haploid length (X') of observed chromosomes in PG018

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.38	4.38	0.85	36.94
II	4.20	3.94	0.76	
III	3.59	3.59	0.91	
IV	3.50	3.50	0.87	
V	3.41	3.41	0.81	
VI	3.33	3.15	0.65	
VII	3.12	3.11	0.70	
VIII	3.06	2.98	0.50	
IX	2.94	2.94	0.94	
X	2.89	2.89	0.89	
XI	2.80	2.80	0.64	
∑x	37.22	36.69		

Table 22(B): The homogeneity in distribution of chromosomes among different haploid complements in PG018

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	2
	0.51-0.75	-
	0.50 and less	-
Total		**2**
2.94 to 3.64 µ	0.76-1.00	4
	0.51-0.75	2
	0.50 and less	1
Total		**7**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	1
	0.50 and less	-
Total		**2**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 23(A): Standardized haploid length (X') of observed chromosomes in PG019

No of chromosome pairs	X_1	X_2	A R	Mean
I	4.38	4.29	0.71	37.60
II	4.20	4.20	0.88	
III	3.94	3.94	0.64	
IV	3.76	3.68	0.89	
V	3.50	3.41	0.76	
VI	3.33	3.33	0.85	
VII	3.24	3.24	0.81	
VIII	3.15	3.15	0.95	
IX	2.98	2.89	0.72	
X	2.80	2.71	0.66	
XI	2.59	2.54	0.85	
∑x	37.87	37.38		

Table 23(B): The homogeneity in distribution of chromosomes among different haploid complements in PG019

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	2
	0.51-0.75	2
	0.50 and less	-
Total		**4**
2.94 to 3.64 µ	0.76-1.00	4
	0.51-0.75	1
	0.50 and less	-
Total		**5**
2.23 to 2.93 µ	0.76-1.00	1
	0.51-0.75	1
	0.50 and less	-
Total		**2**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 24(A): Standardized haploid length (X') of observed chromosomes in PG020

No of chromosome pairs	X_1	X_2	A R	Mean
I	3.15	2.80	0.84	25.03
II	2.71	2.63	0.85	
III	2.45	2.45	0.97	
IV	2.36	2.36	0.64	
V	2.31	2.31	0.93	
VI	2.28	2.28	0.68	
VII	2.24	2.19	0.84	
VIII	2.10	2.10	0.78	
IX	2.01	2.01	0.84	
X	1.96	1.93	0.78	
XI	1.75	1.66	0.86	
∑x	25.32	24.72		

Table 24(B): The homogeneity in distribution of chromosomes among different haploid complements in PG020

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
2.94 to 3.64 µ	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
2.23 to 2.93 µ	0.76-1.00	3
	0.51-0.75	2
	0.50 and less	-
Total		**5**
2.22 µ and less.	0.76-1.00	5
	0.51-0.75	-
	0.50 and less	-
Total		**5**
Grand total		**11**

Table 25(A): Standardized haploid length (X') of observed chromosomes in PG022

No of chromosome pairs	X_1	X_2	A R	Mean
I	3.24	3.15	0.92	25.68
II	2.80	2.77	0.67	
III	2.71	2.63	0.85	
IV	2.45	2.42	0.43	
V	2.36	2.36	0.64	
VI	2.28	2.28	0.80	
VII	2.19	2.19	0.61	
VIII	2.10	2.01	0.81	
IX	1.96	1.96	0.89	
X	1.93	1.93	0.76	
XI	1.84	1.84	0.87	
∑x	25.86	25.54		

Table 25(B): The homogeneity in distribution of chromosomes among different haploid complements in PG022

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
2.94 to 3.64 µ	0.76-1.00	1
	0.51-0.75	-
	0.50 and less	-
Total		**1**
2.23 to 2.93 µ	0.76-1.00	2
	0.51-0.75	2
	0.50 and less	1
Total		**5**
2.22 µ and less.	0.76-1.00	4
	0.51-0.75	1
	0.50 and less	-
Total		**5**
Grand total		**11**

Table 26(A): Standardized haploid length (X') of observed chromosomes in PG023

No of chromosome pairs	X_1	X_2	A R	Mean
I	2.98	2.89	0.77	26.67
II	2.80	2.80	0.77	
III	2.71	2.71	0.80	
IV	2.63	2.63	0.84	
V	2.54	2.54	0.87	
VI	2.45	2.42	0.85	
VII	2.36	2.36	0.50	
VIII	2.24	2.19	0.83	
IX	2.10	2.10	0.78	
X	2.01	2.01	0.79	
XI	1.93	1.93	0.61	
∑x	26.75	26.58		

Table 26(B): The homogeneity in distribution of chromosomes among different haploid complements in PG023

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
2.94 to 3.64 µ	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
2.23 to 2.93 µ	0.76-1.00	6
	0.51-0.75	-
	0.50 and less	1
Total		**7**
2.22 µ and less.	0.76-1.00	3
	0.51-0.75	1
	0.50 and less	-
Total		**4**
Grand total		**11**

Table 27(A): Standardized haploid length (X') of observed chromosomes in PG028

No of chromosome pairs	X_1	X_2	A R	Mean
I	3.33	3.19	0.64	29.43
II	3.06	2.98	0.92	
III	2.89	2.89	0.80	
IV	2.80	2.80	0.66	
V	2.71	2.68	0.76	
VI	2.63	2.63	0.72	
VII	2.59	2.57	0.90	
VIII	2.54	2.54	0.63	
IX	2.45	2.42	0.76	
X	2.36	2.36	0.57	
XI	2.28	2.19	0.89	
∑x	29.64	29.25		

Table 27(B): The homogeneity in distribution of chromosomes among different haploid complements in PG028

Length classes X (µm)	Range of arm ratio	No. of chromosome
3.65 µ and above	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
2.94 to 3.64 µ	0.76-1.00	1
	0.51-0.75	1
	0.50 and less	-
Total		**2**
2.23 to 2.93 µ	0.76-1.00	5
	0.51-0.75	4
	0.50 and less	-
Total		**9**
2.22 µ and less.	0.76-1.00	-
	0.51-0.75	-
	0.50 and less	-
Total		**-**
Grand total		**11**

Table 28: Chromosome length, arm ratio, centromeric position and chromosome type in BARI 1 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
BARI 1	2n=22	Long arm (µm)	2.41	2.36	2.01	1.79	1.93	1.58	1.62	1.55	1.60	1.27	1.12	19.24
		Short arm (µm)	1.66	1.44	1.53	1.53	1.27	1.53	1.36	1.33	1.07	1.01	0.98	14.71
		Total length (µm)	4.07	3.80	3.54	3.32	3.20	3.11	2.98	2.88	2.67	2.28	2.10	33.95
		Arm ratio SA/LA	0.69	0.61	0.76	0.85	0.66	0.97	0.84	0.86	0.67	0.79	0.88	
		Centromeric position	s^m	s^m	m	m	s^m	m	m	m	s^m	m	m	
		Type	B	B	B	C	C	C	C	C	C	D	D	
		TCL %	11.98	11.21	10.44	9.79	9.41	9.15	8.76	8.51	7.86	6.70	6.19	

Karyotype formula: $2B^{sm}+B^{m}+2C^{sm}+4C^{m}+2D^{m}$
TF% = 43.36
(SA: Short arm; LA: Long arm)

Table 29: Chromosome length, arm ratio, centromeric position and chromosome type in BARI 2 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
BARI 2	2n=22	Long arm(µm)	2.54	2.71	2.23	2.23	2.23	1.97	2.41	1.84	2.06	1.66	1.38	23.26
		Short arm(µm)	2.45	1.93	2.01	1.84	1.71	1.88	1.27	1.71	1.31	1.49	0.94	18.54
		Total length (µm)	4.99	4.64	4.24	4.07	3.94	3.85	3.68	3.55	3.37	3.15	2.32	41.80
		Arm ratio SA/LA	0.97	0.71	0.90	0.82	0.77	0.96	0.53	0.93	0.64	0.90	0.96	
		Centromeric position	m	s^m	m	m	m	m	s^m	m	s^m	m	m	
		Type	A	A	B	B	B	B	B	B	C	C	D	
		TCL %	11.94	11.10	10.16	9.74	9.42	9.21	8.80	8.48	8.06	7.54	5.55	

Karyotype formula: $A^{sm}+A^{m}+B^{sm}+5B^{m}+C^{sm}+C^{m}+D^{m}$
TF% = 44.35
(SA: Short arm; LA: Long arm)

Table 30: Chromosome length, arm ratio, centromeric position and chromosome type in KALI BOMBAY of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
KALI BOMBAY	2n=22	Long arm (μm)	2.76	2.71	2.32	2.06	2.41	2.23	2.01	2.06	1.93	1.95	1.44	23.88
		Short arm (μm)	2.36	2.01	2.01	1.93	1.44	1.44	1.53	1.31	1.31	1.12	1.09	17.55
		Total length (μm)	5.12	4.72	4.33	3.99	3.85	3.67	3.54	3.37	3.24	3.07	2.53	41.43
		Arm ratio SA/LA	0.86	0.74	0.87	0.94	0.60	0.65	0.76	0.64	0.69	0.57	0.76	
		Centromeric position	m	s^m	m	m	s^m	s^m	m	s^m	s^m	s^m	m	
		Type	A	A	A	B	B	B	B	B	C	C	D	
		TCL %	12.35	11.40	10.45	9.61	9.29	8.87	8.55	8.13	7.81	7.39	6.12	

Karyotype formula: $A^{sm}+2A^{m}+3B^{sm}+2B^{m}+2C^{sm}+D^{m}$
TF% = 42.40
(SA: Short arm; LA: Long arm)

Table 31: Chromosome length, arm ratio, centromeric position and chromosome type in MALE 1 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X		XI	Grand total
												X	Y		
MALE 1	2n=22	Long arm (μm)	2.58	2.36	2.01	1.88	1.79	1.75	1.88	1.71	1.58	1.23	1.31	1.23	20.04
		Short arm (μm)	2.01	1.66	1.62	1.62	1.62	1.49	1.18	1.27	1.09	1.23	1.23	1.01	15.80
		Total length (μm)	4.59	4.02	3.63	3.50	3.41	3.24	3.06	2.98	2.67	2.46	2.54	2.24	35.84
		Arm ratio SA/LA	0.78	0.70	0.80	0.86	0.90	0.85	0.63	0.74	0.69	1.00	0.94	0.82	
		Centromeric position	m	s^m	m	m	m	m	s^m	s^m	s^m	m		m	
		Type	A	B	B	B	C	C	C	C	C	C		D	
		TCL %	12.82	11.23	10.13	9.77	9.52	9.04	8.55	8.30	7.45	6.86	7.08	6.23	

Karyotype formula: $A^{m}+B^{sm}+2B^{m}+3C^{sm}+3C^{m}+D^{m}$
TF% = 44.08
(SA: Short arm; LA: Long arm)

Table 32: Chromosome length, arm ratio, centromeric position and chromosome type in PG003 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG003	2n=22	Long arm (µm)	2.34	2.23	1.99	1.93	1.79	1.79	1.75	1.84	1.71	1.66	1.23	20.26
		Short arm (µm)	1.88	1.71	1.75	1.64	1.68	1.62	1.44	1.29	1.29	1.14	1.01	16.45
		Total length (µm)	4.22	3.94	3.74	3.57	3.48	3.41	3.19	3.13	3.00	2.80	2.23	36.71
		Arm ratio SA/LA	0.80	0.77	0.88	0.85	0.94	0.90	0.83	0.71	0.76	0.69	0.82	
		Centromeric position	m	m	m	m	m	m	m	s^m	m	s^m	m	
		Type	B	B	B	B	B	C	C	C	C	C	D	
		TCL %	11.50	10.73	10.19	9.71	9.48	9.30	8.70	8.52	8.16	7.63	6.08	

Karyotype formula: $5B^m+2C^{sm}+3C^m+D^m$
TF% = 44.82
(SA: Short arm; LA: Long arm)

Table 33: Chromosome length, arm ratio, centromeric position and chromosome type in PG005 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG005	2n=22	Long arm (µm)	3.24	2.06	1.97	1.97	2.14	1.88	1.90	2.10	1.88	1.53	1.62	22.29
		Short arm (µm)	1.27	1.97	1.79	1.66	1.36	1.53	1.42	1.09	1.14	1.31	1.01	15.55
		Total length (µm)	4.51	4.03	3.76	3.63	3.50	3.41	3.32	3.19	3.02	2.84	2.63	37.84
		Arm ratio SA/LA	0.39	0.96	0.91	0.84	0.63	0.81	0.75	0.52	0.61	0.86	0.62	
		Centromeric position	s^t	m	m	m	s^m	m	s^m	s^m	s^m	m	s^m	
		Type	A	B	B	B	B	C	C	C	C	C	C	
		TCL %	11.91	10.64	9.94	9.60	9.25	9.02	8.79	8.44	7.98	7.51	6.94	

Karyotype formula: $A^{st}+B^{sm}+3B^m+4C^{sm}+2C^m$
TF% = 41.10
(SA: Short arm; LA: Long arm)

Table 34: Chromosome length, arm ratio, centromeric position and chromosome type in PG006 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG006	2n=22	Long arm (µm)	2.29	2.07	2.00	1.91	1.73+0.34*	1.50	1.47+0.52*	1.62	1.85	1.58	1.05	19.93
		Short arm (µm)	1.60	1.46	1.44	1.34	0.94	1.39	0.81	1.10	0.72	0.86	0.95	12.61
		Total length (µm)	3.89	3.53	3.44	3.25	3.01	2.89	2.80	2.73	2.57	2.44	2.00	32.54
		Arm ratio SA/LA	0.73	0.71	0.73	0.71	0.54	0.93	0.55	0.71	0.40	0.59	0.91	
		Centromeric position	s^m	s^m	s^m	s^m	s^m	m	s^m	s^m	s^t	s^m	m	
		Type	B	B	B	C	C	C	C	C	C	D	D	
		TCL %	12.27	11.14	10.85	10.26	8.44	9.12	7.18	8.61	8.13	7.70	6.31	

Karyotype formula: $3B^{sm}+C^{st}+4C^{sm}+C^{m}+D^{sm}+D^{m}$
TF% = 38.75
(SA: Short arm; LA: Long arm, '*'=Length of sattelite chromosome)

Table 35: Chromosome length, arm ratio, centromeric position and chromosome type in PG008 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG008	2n=22	Long arm (µm)	3.11	2.54	2.41	2.06	2.10	2.01	1.66	1.53	1.58	1.31	1.27	21.58
		Short arm (µm)	1.75	2.14	1.75	1.93	1.75	1.71	1.58	1.40	1.05	1.09	0.92	17.07
		Total length (µm)	4.86	4.68	4.16	3.99	3.85	3.72	3.24	2.93	2.63	2.40	2.19	38.65
		Arm ratio SA/LA	0.57	0.84	0.74	0.94	0.84	0.85	0.95	0.92	0.67	0.83	0.72	
		Centromeric position	s^m	m	s^m	m	m	m	m	m	m	m	s^m	
		Type	A	A	B	B	B	B	C	C	C	D	D	
		TCL %	12.57	12.12	10.76	10.31	9.97	9.63	8.38	7.59	6.80	6.23	5.66	

Karyotype formula: $A^{sm}+A^{m}+B^{sm}+3B^{m}+3C^{m}+D^{sm}+D^{m}$
TF% = 44.17
(SA: Short arm; LA: Long arm)

Table 36: Chromosome length, arm ratio, centromeric position and chromosome type in PG010 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG010	2n=22	Long arm (µm)	2.01	2.10	1.79	2.01	1.71	1.62	1.62	1.62	1.62	1.44	1.62	19.16
		Short arm (µm)	1.66	1.49	1.71	1.40	1.58	1.53	1.44	1.31	1.18	1.27	0.96	15.53
		Total length (µm)	3.67	3.59	3.50	3.41	3.29	3.15	3.06	2.93	2.80	2.71	2.58	34.69
		Arm ratio SA/LA	0.83	0.71	0.95	0.70	0.92	0.95	0.89	0.81	0.73	0.88	0.60	
		Centromeric position	m	s^m	m	s^m	m	m	m	m	s^m	m	s^m	
		Type	B	B	B	C	C	C	C	C	C	C	C	
		TCL %	10.59	10.34	10.09	9.84	9.46	9.08	8.83	8.45	8.07	7.82	7.44	

Karyotype formula: $B^{sm}+2B^{m}+3C^{sm}+5C^{m}$
TF% = 44.77
(SA: Short arm; LA: Long arm)

Table 37: Chromosome length, arm ratio, centromeric position and chromosome type in PG011 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG011	2n=22	Long arm (µm)	2.36	2.01	1.97	1.90	1.75	1.79	1.71	1.53	1.71	1.53	1.27	19.53
		Short arm (µm)	1.79	1.88	1.66	1.60	1.58	1.36	1.36	1.44	1.14	1.18	1.18	16.17
		Total length (µm)	4.15	3.89	3.63	3.50	3.33	3.15	3.07	2.97	2.85	2.71	2.45	35.70
		Arm ratio SA/LA	0.76	0.93	0.85	0.84	0.90	0.76	0.80	0.94	0.67	0.77	0.93	
		Centromeric position	m	m	m	m	m	m	m	m	s^m	m	m	
		Type	B	B	B	B	C	C	C	C	C	C	D	
		TCL %	11.64	10.91	10.17	9.80	9.31	8.82	8.58	8.33	7.97	7.60	6.86	

Karyotype formula: $4B^{m}+C^{sm}+5C^{m}+D^{m}$
TF% = 45.28
(SA: Short arm; LA: Long arm)

Table 38: Chromosome length, arm ratio, centromeric position and chromosome type in PG012 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG012	2n=22	Long arm (µm)	2.10	1.97	1.71	1.73	1.71	1.62	1.53	1.31	1.31	1.18	1.07	17.24
		Short arm (µm)	1.36	1.31	1.40	1.25	1.18	1.14	1.09	1.18	0.92	0.92	0.85	12.60
		Total length (µm)	3.46	3.28	3.11	2.98	2.89	2.76	2.62	2.49	2.23	2.10	1.92	29.84
		Arm ratio SA/LA	0.65	0.67	0.82	0.72	0.69	0.70	0.72	0.90	0.71	0.78	0.80	
		Centromeric position	s^m	s^m	m	s^m	s^m	s^m	s^m	m	s^m	m	m	
		Type	B	C	C	C	C	C	C	D	D	D	D	
		TCL %	11.58	11.00	10.41	9.97	9.68	9.24	8.80	8.36	7.48	7.04	6.45	

Karyotype formula: $B^{sm}+5C^{sm}+C^{m}+D^{sm}+3D^{m}$
TF% = 42.23
(SA: Short arm; LA: Long arm)

Table 39: Chromosome length, arm ratio, centromeric position and chromosome type in PG015 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG015	2n=22	Long arm (µm)	1.75	1.36	1.49	1.25	1.27	1.36	1.46	1.33	1.29	1.23	0.96	14.75
		Short arm (µm)	1.14	1.27	1.02	1.20	1.09	0.92	0.74	0.79	0.72	0.70	0.74	10.33
		Total length (µm)	2.89	2.63	2.51	2.45	2.36	2.28	2.20	2.12	2.01	1.93	1.70	25.08
		Arm ratio SA/LA	0.65	0.94	0.69	0.97	0.86	0.68	0.51	0.60	0.56	0.58	0.77	
		Centromeric position	s^m	m	s^m	m	m	s^m	s^m	s^m	s^m	s^m	m	
		Type	C	C	D	D	D	D	D	D	D	D	D	
		TCL %	11.51	10.47	10.01	9.77	9.42	9.07	8.79	8.44	8.03	7.68	6.80	

Karyotype formula: $C^{sm}+C^{m}+6D^{sm}+3D^{m}$
TF% = 41.24
(SA: Short arm; LA: Long arm)

Table 40: Chromosome length, arm ratio, centromeric position and chromosome type in PG018 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG018	2n=22	Long arm (µm)	2.36	2.32	1.88	1.88	1.88	1.97	1.84	2.01	1.51	1.53	1.71	20.89
		Short arm (µm)	2.01	1.75	1.71	1.62	1.53	1.27	1.27	1.01	1.43	1.36	1.09	16.05
		Total length (µm)	4.37	4.07	3.59	3.50	3.41	3.24	3.11	3.02	2.94	2.89	2.80	36.94
		Arm ratio SA/LA	0.85	0.76	0.91	0.87	0.81	0.65	0.70	0.50	0.94	0.89	0.64	
		Centromeric position	m	m	m	m	m	s^m	s^m	s^t	m	m	s^m	
		Type	A	B	B	B	C	C	C	C	C	C	C	
		TCL %	11.84	11.02	9.71	9.48	9.24	8.76	8.42	8.17	7.96	7.82	7.58	

Karyotype formula: $A^m+3B^m+C^{st}+3C^{sm}+3C^m$
TF% = 43.43
(SA: Short arm; LA: Long arm)

Table 41: Chromosome length, arm ratio, centromeric position and chromosome type in PG019 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG019	2n=22	Long arm (µm)	2.54	2.23	2.41	1.97	1.97	1.79	1.79	1.62	1.71	1.66	1.38	21.07
		Short arm (µm)	1.79	1.97	1.53	1.75	1.49	1.53	1.44	1.53	1.23	1.09	1.18	16.53
		Total length (µm)	4.33	4.20	3.94	3.72	3.46	3.32	3.23	3.15	2.94	2.75	2.56	37.60
		Arm ratio SA/LA	0.71	0.88	0.64	0.89	0.76	0.85	0.81	0.95	0.72	0.66	0.85	
		Centromeric position	s^m	m	s^m	m	m	m	m	m	s^m	s^m	m	
		Type	A	B	B	B	B	C	C	C	C	C	C	
		TCL %	11.52	11.17	10.47	9.89	9.19	8.84	8.61	8.38	7.79	7.33	6.82	

Karyotype formula: $A^{sm}+B^{sm}+3B^m+2C^{sm}+4C^m$
TF% = 43.97
(SA: Short arm; LA: Long arm)

Table 42: Chromosome length, arm ratio, centromeric position and chromosome type in PG020 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG020	2n=22	Long arm (µm)	1.62	1.44	1.25	1.44	1.20	1.36	1.21	1.18	1.09	1.09	0.92	13.80
		Short arm (µm)	1.36	1.23	1.20	0.92	1.11	0.92	1.01	0.92	0.92	0.85	0.79	11.23
		Total length (µm)	2.98	2.67	2.45	2.36	2.31	2.28	2.22	2.10	2.01	1.94	1.71	25.03
		Arm ratio SA/LA	0.84	0.85	0.97	0.64	0.93	0.68	0.84	0.78	0.84	0.78	0.86	
		Centromeric position	m	m	m	s^m	m	s^m	m	m	m	m	m	
		Type	C	C	D	D	D	D	D	D	D	D	D	
		TCL %	11.89	10.67	9.79	9.44	9.23	9.09	8.85	8.39	8.04	7.76	6.82	

Karyotype formula: $2C^m+2D^{sm}+7D^m$
TF% = 44.82
(SA: Short arm; LA: Long arm)

Table 43: Chromosome length, arm ratio, centromeric position and chromosome type in PG022 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG022	2n=22	Long arm (µm)	1.66	1.66	1.44	1.71	1.44	1.27	1.36	1.14	1.04	1.09	0.98	14.79
		Short arm (µm)	1.53	1.12	1.23	0.73	0.92	1.01	0.83	0.92	0.92	0.83	0.85	10.89
		Total length (µm)	3.19	2.78	2.67	2.44	2.36	2.28	2.19	2.06	1.96	1.92	1.83	25.68
		Arm ratio SA/LA	0.92	0.67	0.85	0.43	0.64	0.80	0.61	0.81	0.89	0.76	0.87	
		Centromeric position	m	s^m	m	s^t	s^m	m	s^m	m	m	m	m	
		Type	C	C	C	D	D	D	D	D	D	D	D	
		TCL %	12.44	10.83	10.39	9.47	9.20	8.86	8.52	8.01	7.63	7.50	7.16	

Karyotype formula: $C^{sm}+2C^m+D^{st}+2D^{sm}+5D^m$
TF% = 42.37
(SA: Short arm; LA: Long arm)

Table 44: Chromosome length, arm ratio, centromeric position and chromosome type in PG023 of *T. dioica*

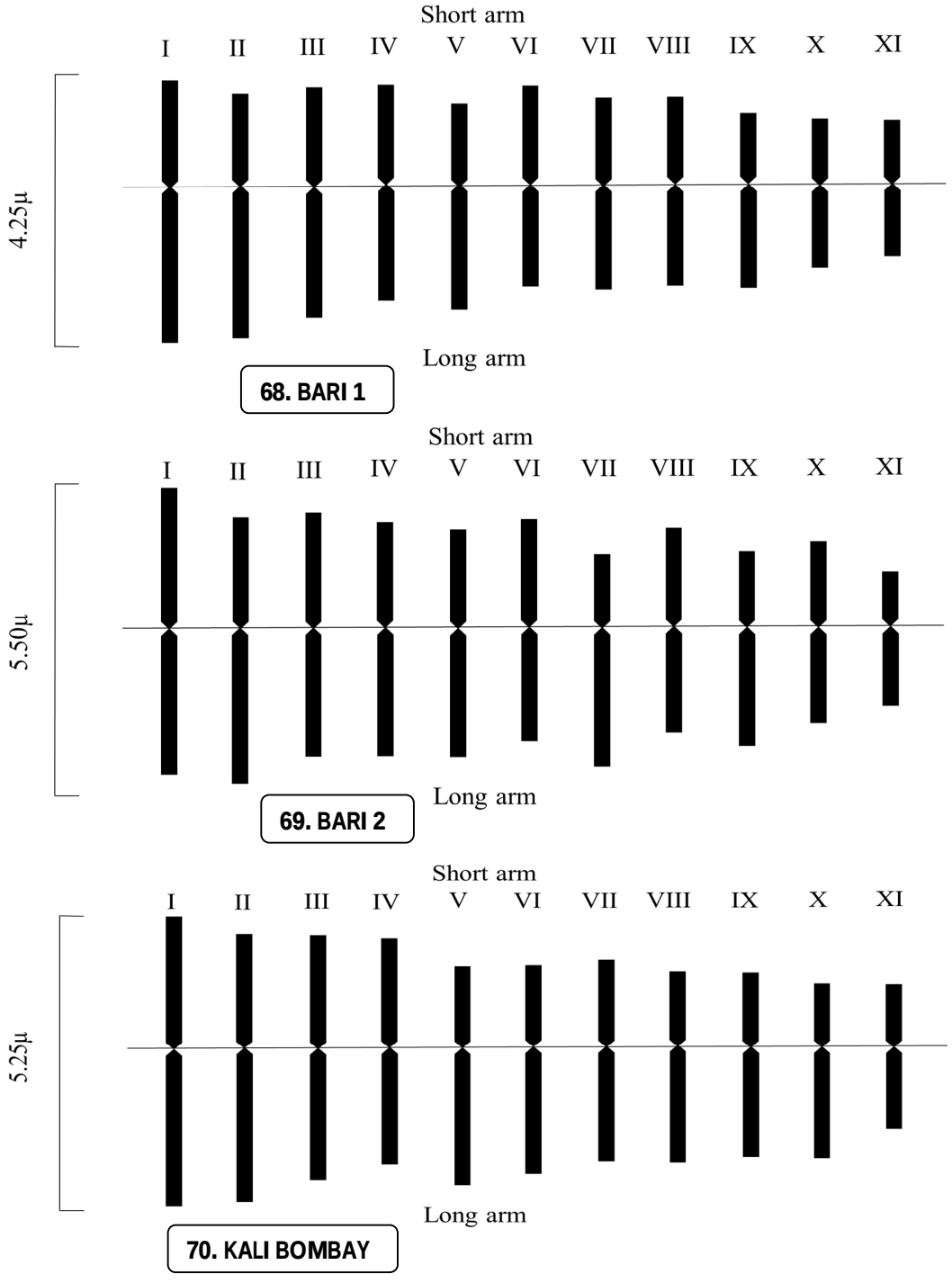
Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG023	2n=22	Long arm (µm)	1.66	1.59	1.51	1.43	1.36	1.31	1.58	1.21	1.18	1.12	1.20	15.15
		Short arm (µm)	1.27	1.21	1.20	1.20	1.18	1.12	0.79	1.01	0.92	0.89	0.73	11.52
		Total length (µm)	2.93	2.80	2.71	2.63	2.54	2.43	2.37	2.22	2.10	2.01	1.93	26.67
		Arm ratio SA/LA	0.77	0.77	0.80	0.84	0.87	0.85	0.50	0.83	0.78	0.79	0.61	
		Centromeric position	m	m	m	m	m	m	s^t	m	m	m	s^m	
		Type	C	C	C	C	D	D	D	D	D	D	D	
		TCL %	11.00	10.51	10.18	9.85	9.52	9.13	8.86	8.31	7.88	7.55	7.22	

Karyotype formula: $4C^m+D^{st}+D^{sm}+5D^m$
TF% = 43.19
(SA: Short arm; LA: Long arm)

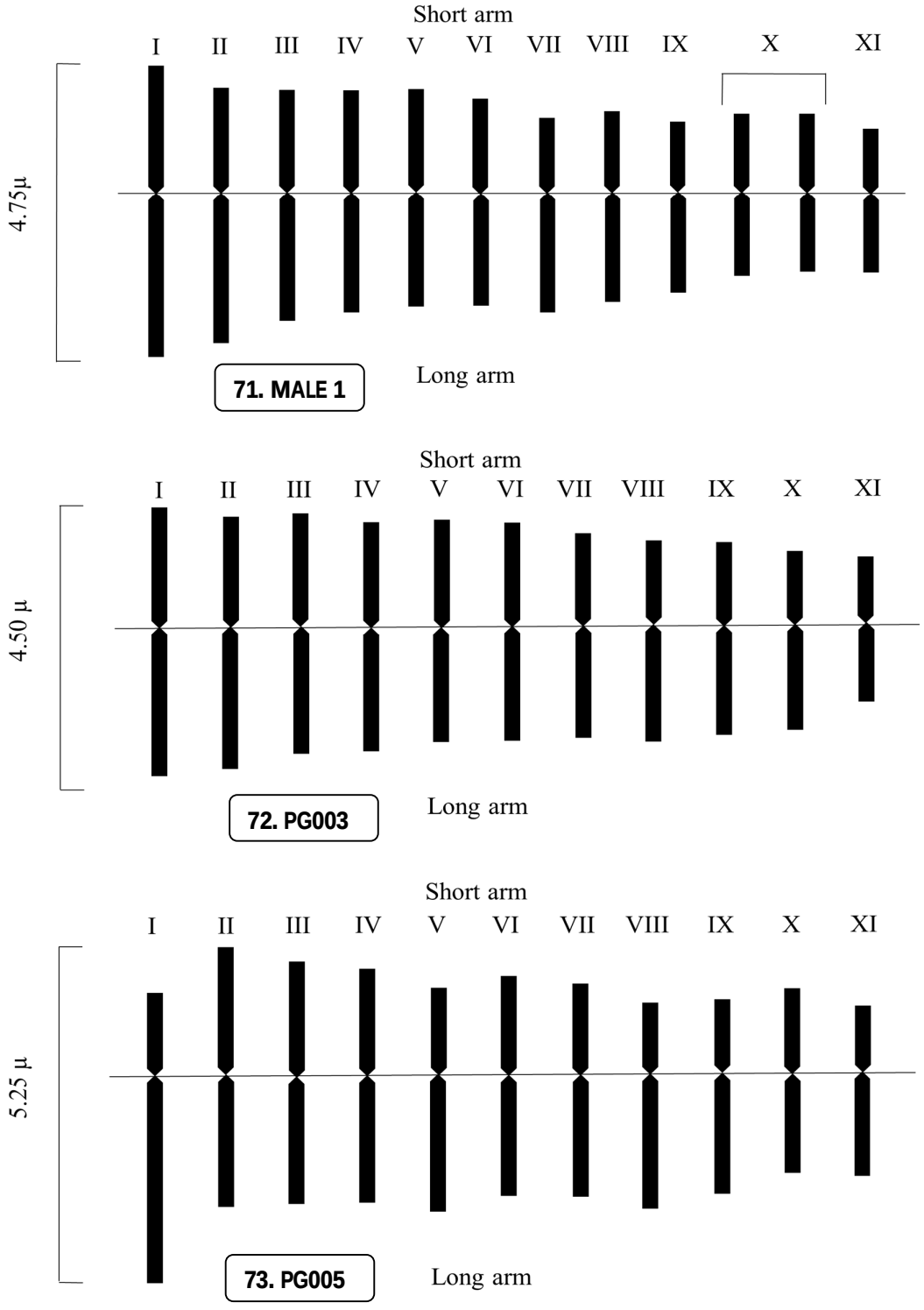
Table 45: Chromosome length, arm ratio, centromeric position and chromosome type in PG028 of *T. dioica*

Variety/ line	No. of chromosome	Chromosome pairs	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Grand total
PG028	2n=22	Long arm (µm)	1.99	1.58	1.60	1.69	1.53	1.53	1.36	1.56	1.38	1.51	1.18	16.91
		Short arm (µm)	1.27	1.44	1.28	1.11	1.16	1.09	1.23	0.98	1.05	0.86	1.05	12.52
		Total length (µm)	3.26	3.02	2.88	2.80	2.69	2.62	2.59	2.54	2.43	2.37	2.23	29.43
		Arm ratio SA/LA	0.64	0.92	0.80	0.66	0.76	0.72	0.90	0.63	0.76	0.57	0.89	
		Centromeric position	s^m	m	m	s^m	m	s^m	m	s^m	m	s^m	m	
		Type	C	C	C	C	C	C	C	D	D	D	D	
		TCL %	11.06	10.26	9.81	9.52	9.16	8.92	8.77	8.62	8.27	8.03	7.58	

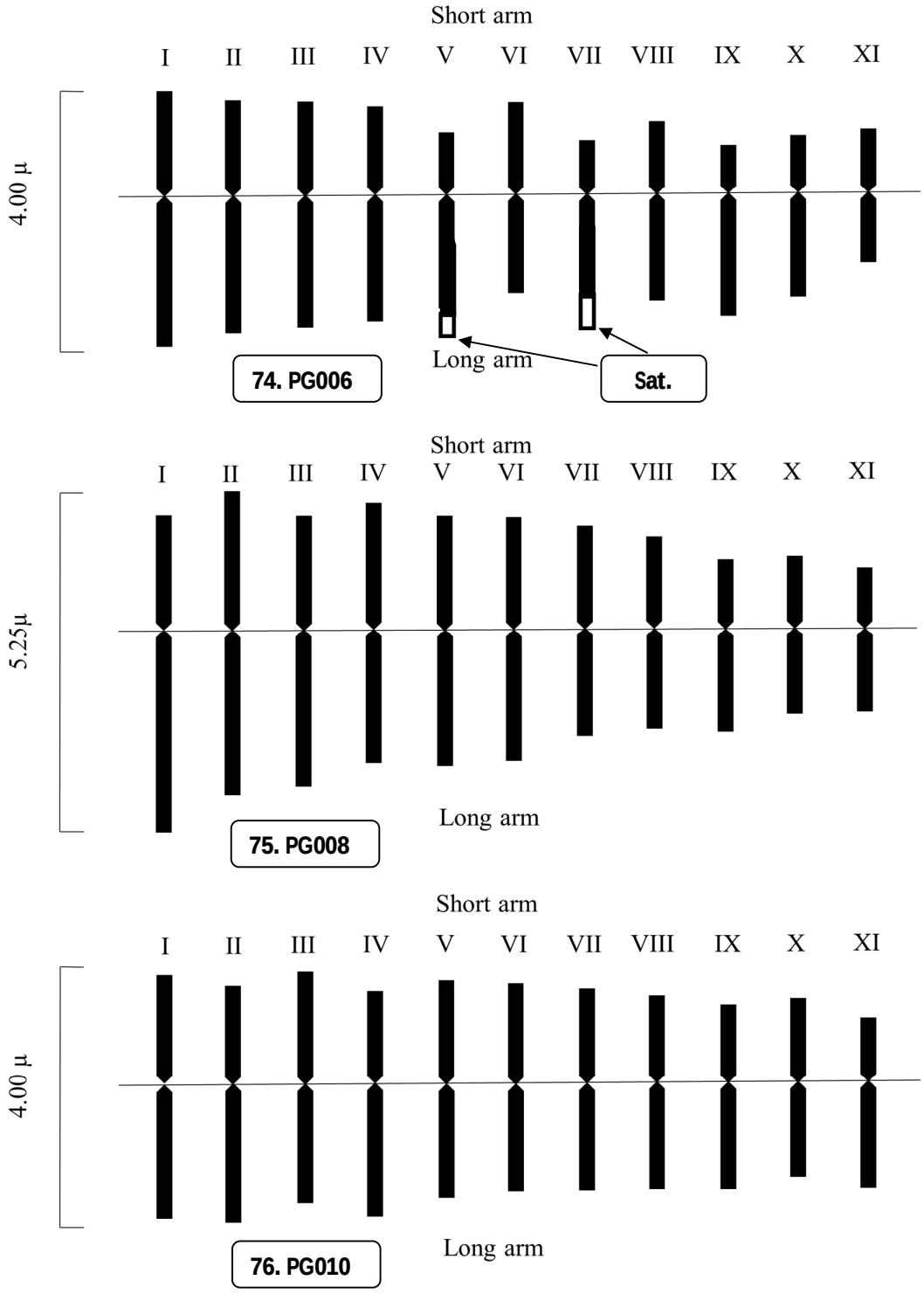
Karyotype formula: $3C^{sm}+4C^m+2D^{sm}+2D^m$
TF% = 42.56
(SA: Short arm; LA: Long arm)



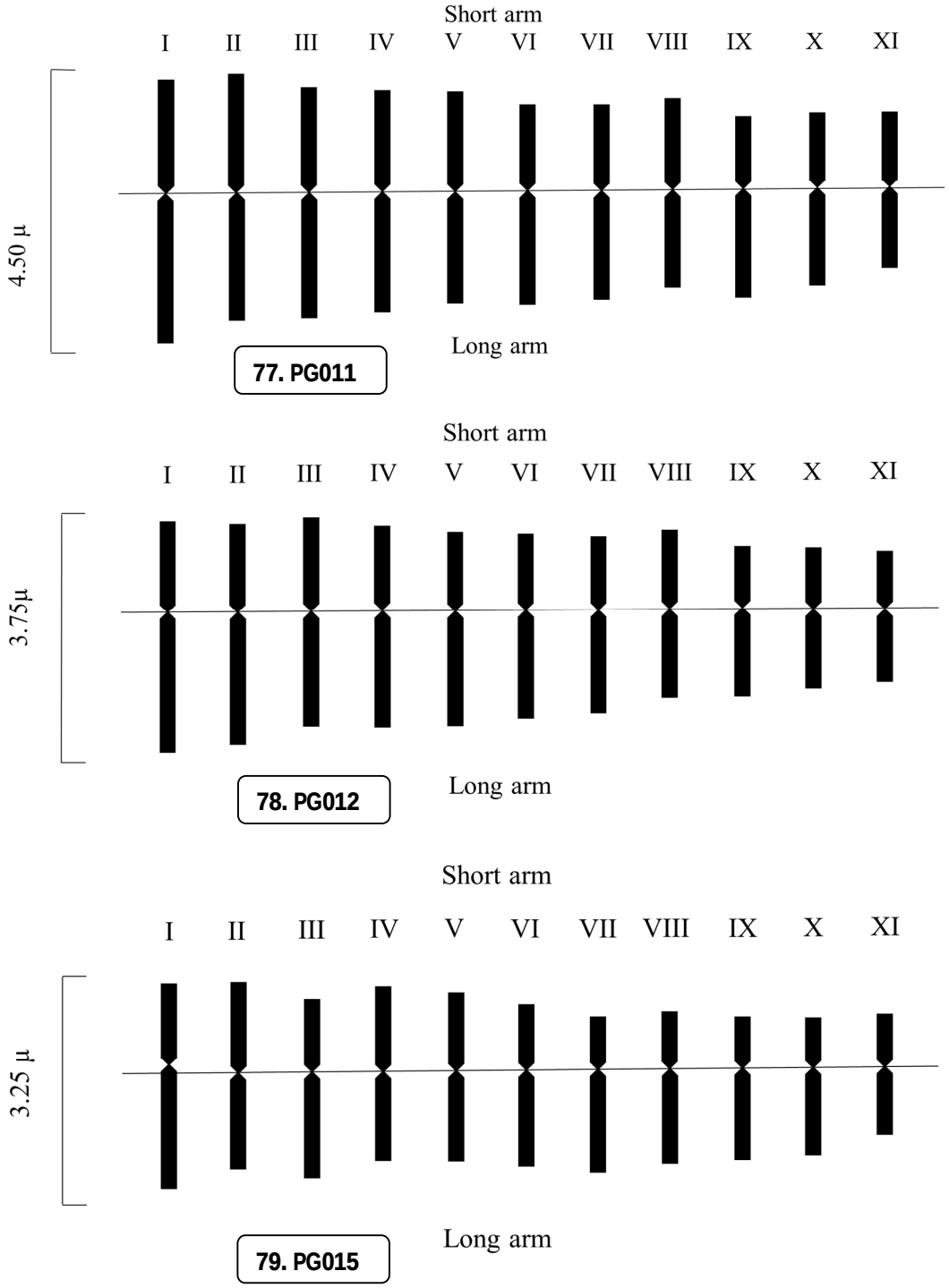
Figs. 68-70: Ideograms of the chromosomes in three varieties/lines of *T. dioica*



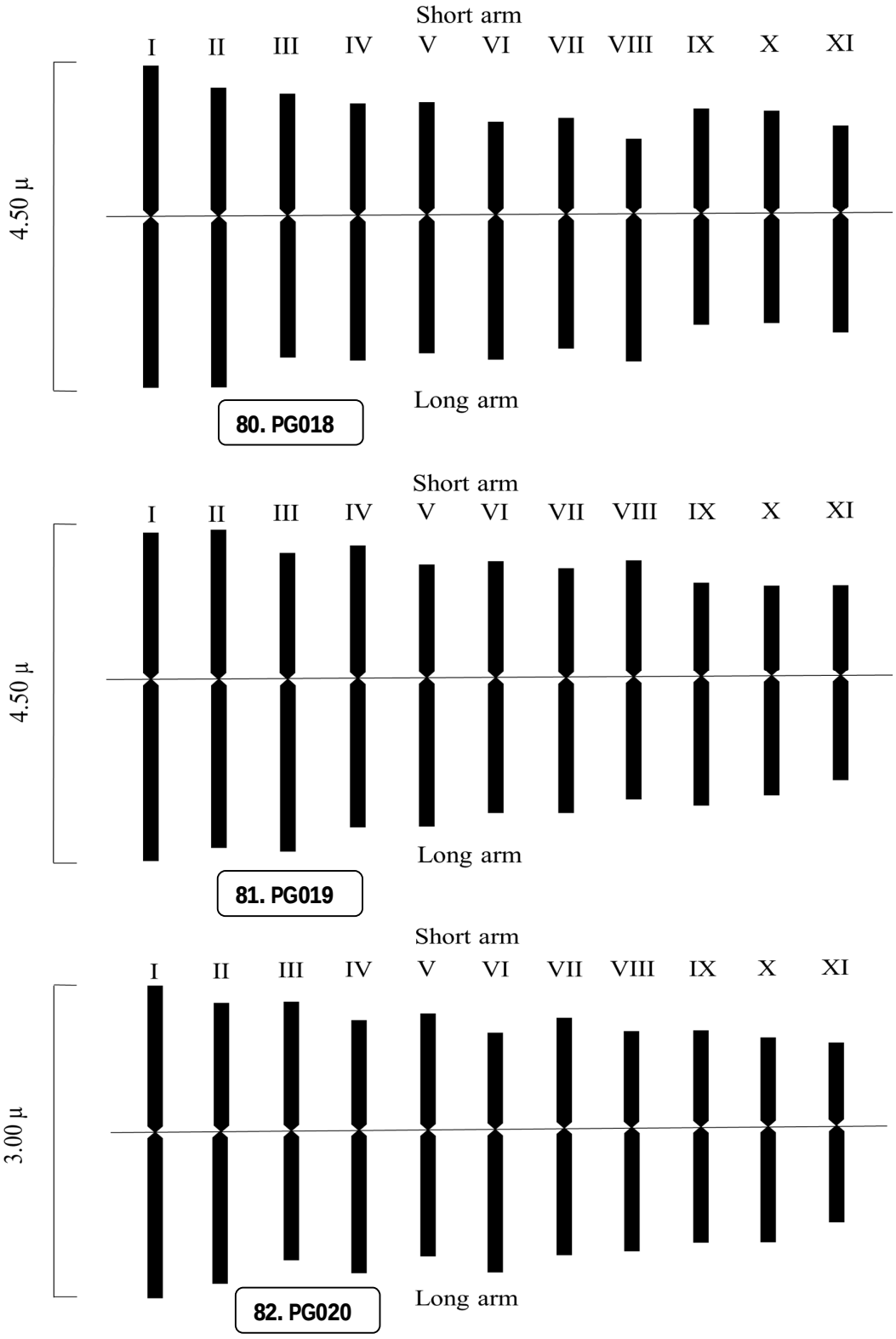
Figs. 71-73: Ideograms of the chromosomes in three varieties/lines of *T. dioica*



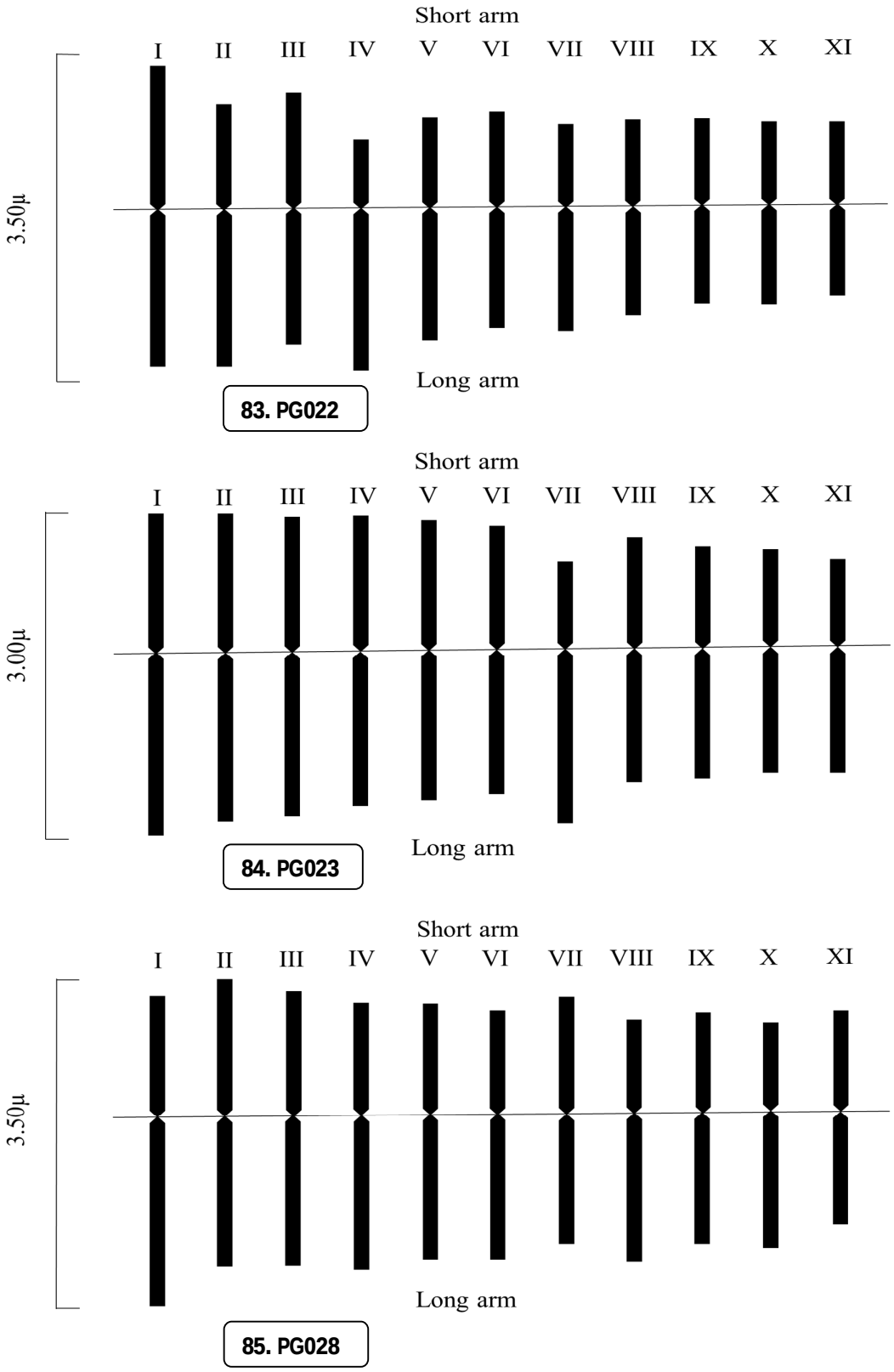
Figs. 74-76: Ideograms of the chromosomes in three varieties/lines of *T. dioica*



Figs. 77-79: Ideograms of the chromosomes in three varieties/lines of *T. dioica*



Figs. 80-82: Ideograms of the chromosomes in three varieties/lines of *T. dioica*



Figs. 83-85: Ideograms of the chromosomes in three varieties/lines of *T. dioica*

MALE 1: In this case, seven pairs were found to be metacentric in I, III, IV, V, VI, X, XI and sub-metacentric were four pairs such as II, VII, VIII, IX **(Table 31).** The longest chromosome pair was 4.59µm in length and the shortest was 2.24µm in length with a TCL of 35.84. TF% was determined to be 44.08. TCL% was found to be highest as 12.82 and lowest as 6.23.The proposed karyotype formula was as follows:
$A^{m}+B^{sm}+2B^{m}+3C^{sm}+3C^{m}+D^{m}$.

PG003: Nine pairs of chromosomes for this variety were found to be metacentric in I, II, III, IV, V, VI, VII, IX, XI and two pairs sub-metacentric in VIII, X **(Table 32).** The longest chromosome pair was 4.22µm in length and shortest chromosome pair was 2.23µm. The value of TCL was of 36.71µm, TF% was 44.82 and TCL% was highest as 11.50, the lowest was 6.08. The proposed karyotype formula was found to be
$5B^{m}+2C^{sm}+3C^{m}+D^{m}$.

PG005: The chromosome complement in this variety was found with five metacentric in II, III, IV, VI, X, five sub-metacentric in V, VII, VIII, IX, XI and one sub-terminal in I **(Table 33).** The longest chromosome pair was 4.51µm in length and shortest was 2.63µm with a TCL of 37.84µm. TF% was found to be 41.10 and TCL% was highest as 11.91 and lowest as 6.94. The proposed karyotypic formula was found to be
$A^{st}+B^{sm}+3B^{m}+4C^{sm}+2C^{m}$.

PG006: In this variety the chromosome complement was found with two metacentric in VI, XI, eight sub-metacenrtic in I, II, III, IV, V, VII, VIII, X and one sub-terminal in IX **(Table 34).** Satellite chromosomes were found in two pairs i.e. V and VII. Among the chromosome pair's longest chromosome pair was found to be 3.89µm and shortest was 2.00µm with a TCL of 32.54µm. TF% was found to be 38.75 and TCL% was found to be highest as 12.27 and lowest as 6.31. The proposed karyotypic formula was found to be
$3B^{sm}+C^{st}+4C^{sm}+C^{m}+D^{sm}+D^{m}$.

PG008: In this case, eight pairs were found to be metacentric in II, IV, V, VI, VII, VIII, IX, X and sub-metacentric were three pairs such as I, III, XI **(Table 35).** The longest chromosome pair was 4.86µm in length and the shortest was 2.19µm with a TCL of 38.65. TF% was determined to be 44.17. TCL% was found to be highest as 12.57 and lowest as 5.66.The proposed karyotype formula was as follows:
$A^{sm}+A^{m}+B^{sm}+3B^{m}+3C^{m}+D^{sm}+D^{m}$.

PG010: Seven pairs of chromosomes for this variety were found to be metacentric in I, III, V, VI, VII, VIII, X and four pairs sub-metacentric in II, IV, IX, XI **(Table 36).** The longest chromosome pair was 3.67µm in length and shortest chromosome pair was 2.58µm. The value of TCL was of 34.69µm, TF% was 44.77 and TCL% was highest as 10.59, the lowest was 7.44. The proposed karyotype formula was found to be
$B^{sm}+2B^{m}+3C^{sm}+5C^{m}$.

PG011: The chromosome complement in this variety was found with ten metacentric in I, II, III, IV, V, VI, VII, VIII, X, XI and only one sub-metacentric in IX **(Table 37).** The longest chromosome pair was 4.15µm in length and shortest was 2.45µm with a TCL of 35.70µm. TF% was found to be 45.28 and TCL% was highest as 11.64 and lowest as 6.86. The proposed karyotypic formula was found to be
$4B^{m}+C^{sm}+5C^{m}+D^{m}$.

PG012: In this variety the chromosome complement was found with four metacentric in III, VIII, X, XI and seven was sub-metacentric in I, II, IV,V, VI, VII, IX **(Table 38).** Among the chromosome pair's longest chromosome pair was found to be 3.46µm and shortest was 1.92µm with a TCL of 29.84µm. TF% was found to be 42.23 and TCL% was found to be highest as 11.58 and lowest as 6.45. The proposed karyotypic formula was found to be
$B^{sm}+5C^{sm}+C^{m}+D^{sm}+3D^{m}$.

PG015: In this case, four pairs were found to be metacentric in II, IV, V, XI and sub-metacentric were seven pairs such as I, III, VI, VII, VIII, IX, X **(Table 39).** The longest chromosome pair was 2.89µm in length and the shortest was 1.70µm with a TCL of 25.08. TF% was determined to be 41.24. TCL% was found to be highest as 11.51 and lowest as 6.80. The proposed karyotype formula was as follows:
$C^{sm}+C^{m}+6D^{sm}+3D^{m}$.

PG018: The chromosome complement in this variety was found with seven metacentric in I, II, III, IV, VI, IX, X, three sub-metacentric in VI, VII, XI and one sub-terminal in VIII **(Table 40).** The longest chromosome pair was 4.37µm in length and shortest was 2.80µm with a TCL of 36.94µm. TF% was found to be 43.43 and TCL% was highest as 11.84 and lowest as 7.58. The proposed karyotypic formula was found to be
$A^{m}+3B^{m}+C^{st}+3C^{sm}+3C^{m}$.

PG019: Seven pairs of chromosomes for this variety were found to be metacentic in II, IV, V, VI, VII, VIII, XI and four pairs sub-metacentric in I, III, IX, X **(Table 41).** The longest chromosome pair was 4.33µm in length and shortest chromosome pair was 2.56µm. The value of TCL was of 37.60µm, TF% was 43.97 and TCL% was highest as 11.52, the lowest was 6.82. The proposed karyotype formula was found to be
$A^{sm}+B^{sm}+3B^{m}+2C^{sm}+4C^{m}$.

PG020: There were nine pairs of chromosomes found in this variety as metacentric in I, II, III, V, VII, VIII, IX, X, XI and two pairs were sub-metacentric in IV, VI **(Table 42).** Among the chromosome pair's longest chromosome pair was found to be 2.98µm and shortest was 1.71µm with a TCL of 25.03µm. TF% was found to be 44.82 and

TCL% was found to be highest as 11.89 and lowest as 6.82. The proposed karyotypic formula was found to be
$2C^{m}+2D^{sm}+7D^{m}$.

PG022: In this variety the chromosome complement was found with seven metacentric in I, III, VI, VIII, IX, X, XI, three sub-metacenrtic in II, V, VII and one sub-terminal IV **(Table 43).** The longest chromosome pair was 3.19µm in length and shortest chromosome pair was 1.83µm. The value of TCL was of 25.68µm, TF% was 42.37 and TCL% was highest as 12.44, the lowest was 7.16. The proposed karyotype formula was found to be
$C^{sm}+2C^{m}+D^{st}+2D^{sm}+5D^{m}$.

PG023: Nine pairs of chromosomes for this variety were found to be metacentric in I, II, III, IV, V, VI, VIII, IX, X, only one was sub-metacentric in XI and one was sub-terminal in VII **(Table 44).** Among the chromosome pair's longest chromosome pair was found to be 2.93µm and shortest was 1.93µm with a TCL of 26.67µm. TF% was found to be 43.19 and TCL% was found to be highest as 11.00 and lowest as 7.22. The proposed karyotypic formula was found to be
$4C^{m}+D^{st}+D^{sm}+5D^{m}$.

PG028: The chromosome complement in this variety was found with six metacentric in II, III, V, VII, IX, XI and five sub-metacentric in I, IV, VI, VIII, X **(Table 45).** The longest chromosome pair was 3.26µm in length and shortest was 2.23µm with a TCL of 29.43µm. TF% was found to be 42.56 and TCL% was highest as 11.06 and lowest as 7.58. The proposed karyotypic formula was found to be
$3C^{sm}+4C^{m}+2D^{sm}+2D^{m}$.

4. Identification of sex regulatory chromosomes

The present cytological investigation revealed the presence of a heteromorphic pair of sex chromosome (assumed) in male plants. Diploid male was, therefore, supposed to be heterogametic with 20+XY and the females were supposed to be homogametic with 20+XX, apparently. Types of sex chromosomes (assumed) and their arm ratios are shown in **Table 46.** In this investigation it was found that likely the heteromorphic pair of sex chromosomes (10^{th} pair), in which the first may be marked as X and the second as Y chromosome according to their arm ratio and of course both of them were metacentric based on centromeric position.

On the other hand, the arm ratio was 0.93 in X and 1.00 was in Y chromosome. The total length of X chromosome in MALE 1 was recorded as 2.46µm and Y chromosome was 2.54µm. The striking feature found in this study was that the identified (assumed) sex chromosomes varied from variety to variety instead of species specific. As a result the total length of X chromosome (sex regulatory) was recorded in BARI 1 as 3.11µm

Table 46: Determination of sex regulatory chromosomes based on arm ratio in *T. dioica*

Varieties/lines	Type*	Pairs (assumed)**	SA/LA	LA/SA
BARI 1	XX	VI	0.97	1.03
BARI 2	XX	I	0.97	1.04
KALI BOMBAY	XX	IV	0.94	1.07
MALE 1	XY	X	1.00 (X) 0.94 (Y)	1.00 (X) 1.06 (Y)
PG003	XX	V	0.94	1.06
PG005	XX	II	0.96	1.04
PG006	XX	VI	0.93	1.08
PG008	XX	VII	0.95	1.05
PG010	XX	VI	0.95	1.06
PG011	XX	VIII	0.94	1.06
PG012	XX	VIII	0.90	1.11
PG015	XX	IV	0.97	1.04
PG018	XX	IX	0.94	1.06
PG019	XX	VIII	0.95	1.06
PG020	XX	III	0.97	1.04
PG022	XX	I	0.92	1.08
PG023	XX	V	0.87	1.15
PG028	XX	II	0.92	1.10

* = Assumed sex regulatory (factor carrier)

** = Inferential basis

in the 6th pair. The value of total length in BARI 2 was 4.99µm in the 1st pair as X chromosome. In KALI BOMBAY the total length of X chromosome (sex regulatory) was 3.99µm in the 4th chromosome pair. The 5th pair of sex regulatory chromosome was X chromosome in PG003 and the value was 3.48µm. In PG005 the X chromosome (sex regulatory) was found in the 2nd pair as 4.03µm. In PG006 the value of X chromosome (sex regulatory) was 2.89µm in the 6th pair. In PG008 the total length of sex regulatory chromosome was 3.24µm in the 7th chromosome pair. In PG010 the same arm ratio (SA/LA) was found as 0.95 in the 3rd and 6th chromosome pairs at the same time, but the different arm ratio (LA/SA) was found as 1.04 and 1.06 in the 3rd and 6th chromosome pairs, respectively. So obviously the 6th chromosome pair was counted as sex regulatory chromosome because of the higher value of LA/SA arm ratio and the length was 3.15µm. The 8th pair of chromosome was X chromosome (sex regulatory) in PG011 and the value was 2.97µm. In PG012 the total length of sex regulatory chromosome was 2.49µm in the 8th chromosome pair. The value of total length in PG015 was 2.45µm in the 4th pair as sex regulatory chromosome. The total length of X chromosome (sex regulatory) was recorded in PG018 as 2.94µm in the 9th pair of chromosome. In PG019 the sex regulatory chromosome was possessed by 8th pair of chromosome and the value was 3.15µm. In PG020 the sex regulatory chromosome was found in the 3rd pair as 2.45µm. The 1st pair of chromosome was sex regulatory chromosome in PG022 and the value was 3.19µm. The sex regulatory chromosome was found in the 5th chromosome pair in PG023 having the value 2.54µm. The 2nd pair of chromosome was sex regulatory chromosome in PG028 and the length was 3.02µm.

In above mentioned all the female varieties/lines of pointed gourd all the chromosome pairs were found to be very much homomorphic and so called sex chromosomes were assumed to be carrier of sex controlled genes in functional state. In true sense, neither sex chromosome nor any authentic proof behind it was found in any of the varieties/lines of *T. dioica*. However, the present observations (inferential) have been discussed extensively in the next chapter.

5. Biochemical studies

Some basic but important biochemical experiments were conducted in the present investigation in three ways i.e., by screening test, quantitative test and studying protein profile. The findings are presented here as follows:

I. Screening test

Preliminary phytochemical analysis of metabolic extracts of leaves of pointed gourd (*Trichosanthes dioica*) revealed the presence of alkaloids, flavonoids, glycosides,

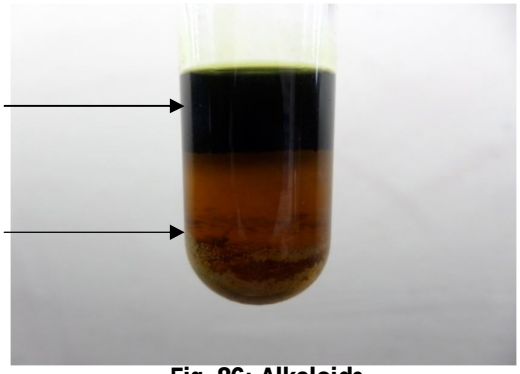


Fig. 86: Alkaloids

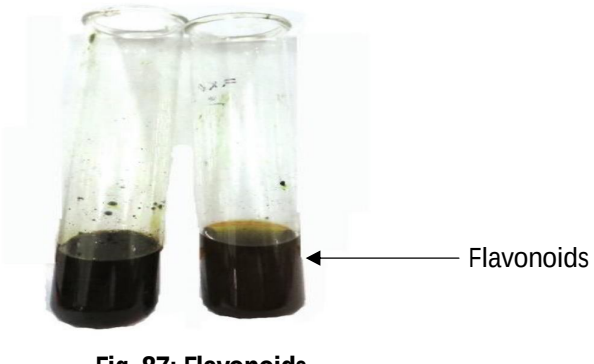


Fig. 87: Flavonoids

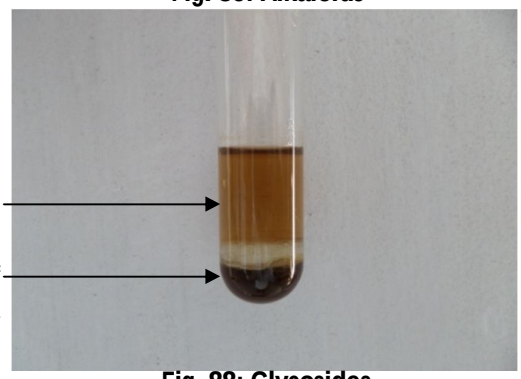


Fig. 88: Glycosides

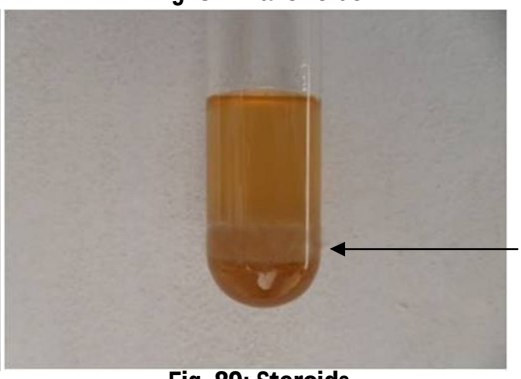


Fig. 89: Steroids

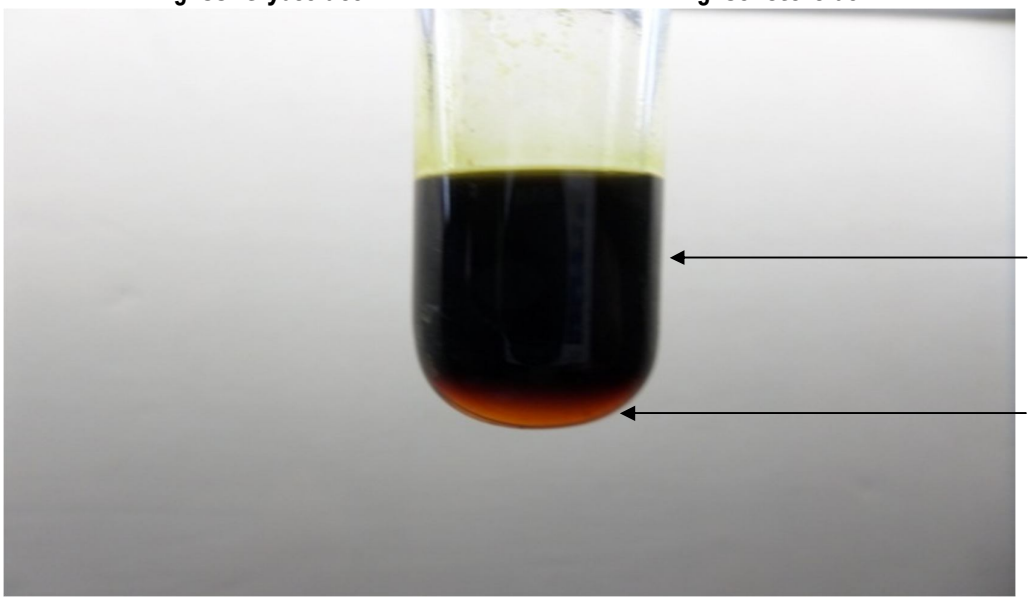


Fig. 90: Tannins

Figs. 86-90: Photographs showing different phytochemicals intensifying variously in *T. dioica*

steroids and tannins as constituents in extracts. All these are shown in **Figs. 86-90** and their abundance were determined on the basis of their density. It is notable that the photographs **(Figs. 86-90)** have shown the results for first test in each kind of experiment particularly. Their intensity is revealed in **Table 47.**

Table 47: Phytochemical screening from leaf extracts of *Trichosanthes dioica*

Serial No.	Phytochemicals	Intensity
1	Alkaloids	++
2	Flavonoids	+++
3	Glycosides	+
4	Steroids	+
5	Tannins	+++

+ = low, ++ = medium and +++ = high

Test for alkaloids

a. Mayers test: Cream colour appeared which showed the presence of alkaloids **(Fig.86).**

b. Wagners test: Brown precipitate would show the presence of alkaloids.

Test for flavonoids

a. Ferric chloride test: The intense green colour showed the presence of flavonoids **(Fig. 87).**

b. Shinoda test: Pink scarlet crimson colour occasionally cream to blue colour showed the presence of flavonoids.

Test for glycosides

a. Keller Killiani test: Two layers were observed. Lower reddish brown layer and upper acetic acid layer which turned bluish green would indicate a positive test for glycosides **(Fig. 88).**

b. Bromine water test: Observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Test for steroids

Salkowaski test: At the still position, lower layer turned red, indicated the presence the steroids **(Fig. 89).**

Test for tannins

a. Ferric chloride test: Dark colour appeared which showed the presence of tannins **(Fig. 90).**

b. Gelatin test: A white precipitate formed which showed the presence of tannins.

II. Quantitative test

A collection of eighteen varieties/lines of *Trichosanthes dioica* were studied for their chlorophyll-a, b and sugar content in leaves and also studied for quantitative estimation of antioxidant capacity (%), phenol, proline and protein in fruits of all the varieties.

Table 48: Chlorophyll-a and Chlorophyll-b content (mg/g) in leaves of *T. dioica*

Variety/line	Chlorophyll-a (Mean ± SE)	Chlorophyll-b (Mean ± SE)	Chlorophyll (a + b)
BARI 1	0.110±0.008e	0.061±0.004de	0.171
BARI 2	0.149±0.022cd	0.071±0.011d	0.220
KALI BOMBAY	0.203±0.005b	0.111±0.003c	0.313
MALE 1	0.179±0.013c	0.234±0.017a	0.413
PG003	0.366±0.066a	0.176±0.032b	0.543
PG005	0.173±0.013c	0.088±0.006cd	0.261
PG006	0.092±0.011ef	0.153±0.019bc	0.244
PG008	0.169±0.014cd	0.092±0.008cd	0.261
PG010	0.145±0.008cd	0.058±0.003de	0.203
PG011	0.056±0.003f	0.093±0.005cd	0.150
PG012	0.178±0.034c	0.039±0.007ef	0.217
PG015	0.163±0.024cd	0.147±0.021bc	0.311
PG018	0.190±0.026bc	0.186±0.025b	0.376
PG019	0.093±0.014ef	0.062±0.010de	0.154
PG020	0.174±0.026c	0.021±0.003f	0.196
PG022	0.188±0.023c	0.046±0.006e	0.234
PG023	0.112±0.007d	0.175±0.010b	0.287
PG028	0.119±0.008d	0.069±0.005d	0.188

The mean values in a column the different letters are significantly different at $P < 0.05$ in LSD test.

Chlorophyll-a and chlorophyll-b contents: The **Table 48** indicates that the highest value of chlorophyll-a was observed in PG003. The lowest value of chlorophyll-a was to be found in PG011. The highest value of chlorophyll-b was obtained by MALE 1 and the lowest value of chlorophyll-b was estimated in PG020. Total chlorophyll (a + b) was found in PG003 as a highest value and the lowest was found in PG011. It is somewhat clear that in which varieties/lines were observed the highest and lowest values of chlorophyll-a were observed in the same varieties/lines total chlorophyll (a + b) as the highest and lowest values were found respectively. DMRT estimation for chlorophyll-a and chlorophyll-b contents indicate same differences among the varieties/lines significantly.

Sugar contents in leaves: Soluble sugar contents in leaves of the varieties/lines of *Trichosanthes dioica* were determined showing in **Table 49(A).** The sugar

concentration was determined from a standard curve and calculated on a fresh weight basis (ppm). **Table 49(A)** reveals that the highest value of sugar content was found in PG023 and the lowest value was recorded in PG028. The second highest value was observed in PG008. Analysis of variance was estimated and the **Table 49(B)** indicates

Table 49(A): Sugar contents in leaves of *Trichosanthes dioica*

Variety/line	Sugar contents (mg/g) Mean ± SE
BARI 1	0.788±0.0583
BARI 2	0.569±0.0464
KALI BOMBAY	0.889±0.0814
MALE 1	0.619±0.0563
PG003	0.583±0.0496
PG005	0.835±0.0744
PG006	0.557±0.0366
PG008	1.044±0.0820
PG010	0.621±0.0358
PG011	0.775±0.0448
PG012	0.443±0.0433
PG015	0.873±0.0675
PG018	0.867±0.0459
PG019	0.817±0.0455
PG020	0.607±0.0489
PG022	0.677±0.0465
PG023	1.121±0.0967
PG028	0.376±0.0316

that there was a significant different at $P < 0.01$ level among the varieties, but non-significant different among the replications.

Table 49(B): Analysis of variances (ANOVA) for sugar contents in leaves of *Trichosanthes dioica*

Source	Sum of squares	df	Mean square	F-value
Variety	2.000	17	0.118	11.118**
Replication	0.0014	2	0.0007	0.065^{NS}
Error	0.360	34	0.0106	

** = Significant at $P < 0.01$.

Table 50(A): Estimation of antioxidant capacity (%), phenol, proline and protein in fruits of *Trichosanthes dioica*

Variety/line	Antioxidant capacity (%) (Mean ± SE)	Phenol mgl^{-1} gallic acid (Mean ± SE)	Proline µg/g (Mean ± SE)	Protein mg/g (Mean ± SE)
BARI 1	3.06±0.19	662.87±57.71	201.22±10.50	10.20±0.71
BARI 2	4.92±0.48	602.67±34.19	222.71±21.66	12.43±1.24
KALI BOMBAY	2.67±0.18	465.01±33.03	234.37±21.73	8.73±0.44
PG003	2.65±0.19	66.96±6.46	117.15±6.36	6.77±0.65
PG005	3.03±0.30	626.68±51.74	154.77±14.33	6.42±0.64
PG006	1.29±0.08	408.13±37.85	186.76±15.32	7.41±0.68
PG008	2.61±0.16	424.12±33.44	212.16±16.98	6.62±0.66
PG010	3.30±0.33	489.76±21.71	118.91±6.69	3.72±0.37
PG011	3.56±0.32	347.00±17.31	235.19±23.12	7.97±0.77
PG012	3.88±0.37	701.40±45.06	174.31±9.20	6.80±0.67
PG015	3.13±0.29	493.49±25.32	129.56±7.61	7.19±0.64
PG018	4.28±0.41	439.66±20.02	247.52±16.47	2.79±0.22
PG019	5.31±0.42	674.50±49.09	130.99±9.55	7.51±0.71
PG020	4.51±0.40	544.62±30.09	263.35±25.37	13.50±0.82
PG022	4.71±0.28	406.96±18.90	170.90±9.14	3.08±0.23
PG023	4.48±0.42	449.72±35.76	140.24±7.26	5.80±0.52
PG028	1.45±0.09	565.26±33.94	140.81±8.61	9.40±0.47

Table 50(B): Analysis of variance (ANOVA) for antioxidant capacity (%), phenol, proline and protein in fruits of *Trichosanthes dioica*

Subject of ANOVA (dependent variable)	Source of variation	Sum of squares	df	Mean square	F-value
Antioxidant capacity (%)	Variety	42.737	16	2.671	12.982**
	Replication	0.012	1	0.012	0.057 ^{NS}
	Error	3.292	16	0.206	
Phenol	Variety	1112065.153	16	69504.072	18.835**
	Replication	6755.223	2	3377.612	0.915 ^{NS}
	Error	118084.491	32	3690.140	
Proline	Variety	112005.762	16	7000.360	14.601**
	Replication	7213.454	2	3606.727	7.523**
	Error	15341.691	32	479.428	
Protein	Variety	404.143	16	25.259	21.602**
	Replication	6.561	2	3.281	2.806 ^{NS}
	Error	37.418	32	1.169	

** = Significant at $P < 0.01$, NS = Non-significant

Antioxidant capacity (%) in fruits: The highest value of antioxidant capacity (%) was measured in PG019 while the lowest value was found in PG006 and these are given in **Table 50(A).** The antioxidant capacity was determined from a standard curve and calculated on a fresh weight basis of fruits. Analysis of variance in **Table 50(B)** indicates that there was a significant difference at $P < 0.01$ level among the varieties, but the different was found to be non-significant among the replications.

Estimation of phenol in fruits: It was observed that the highest value of phenol was recorded in PG012 showing in **Table 50(A).** The lowest value was obtained by PG003. The estimation of phenol was measured from a standard curve and calculated on a fresh weight basis of fruits (mgL^{-1} gallic acid). Analysis of variance has been presented in **Table 50(B).** The table indicates that there is a significant difference at $P < 0.01$ level among the varieties, but the different is non-significant among the replications.

Estimation of proline in fruits: Estimation of proline in fruits of the varieties/lines of *Trichosanthes dioica* were determined and shown in **Table 50(A).** Proline was determined from a standard curve and calculated on a fresh weight basis (mg/L). The highest value was found in PG020 and the lowest value was recorded in PG003. The ANOVA **Table 50(B)** reveals that there was a significant difference at $P < 0.01$ level among the varieties, and also replications.

Estimation of protein in fruits: The highest value of protein was measured in PG020 while the lowest value was found in PG018 and these are shown in **Table 50(A).** The protein was determined from a standard curve and calculated on a fresh weight basis of fruits. Analysis of variance was made and presented in **Table 50(B).** The table indicates that there was a significant difference at $P < 0.01$ level among the varieties, but the difference was non-significant among the replications.

III. Protein banding pattern: A total of ten varieties/lines lines of *T. dioica* were studied for their banding pattern. Polyacrylamide gel electrophoresis was used for determining their protein profiles. Photograph of stained gels showing bands are shown in **Fig. 91.** Line diagrams of the electrophoresis patterns of the stained gels are shown in **Fig. 92.** The location of protein bands was recorded and the Rf values were calculated.

In general protein banding pattern showed variations in number and intensity of bands in different varieties/lines of *T. dioica*. A total of 47 bands were observed. These bands were numbered in 40 different positions along with increasing magnitude of Rf values. The types of bands were decided on the basis of their colour intensity like thin and distinct, diffused, thick and dense. The number and kinds of bands in different varieties/

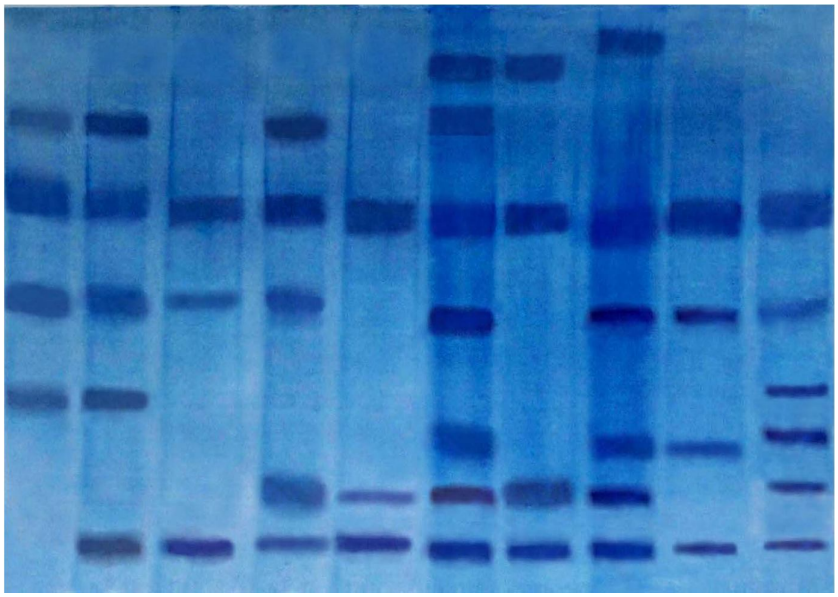


Fig. 91: Protein profiles in ten varieties/lines of *T. dioica*

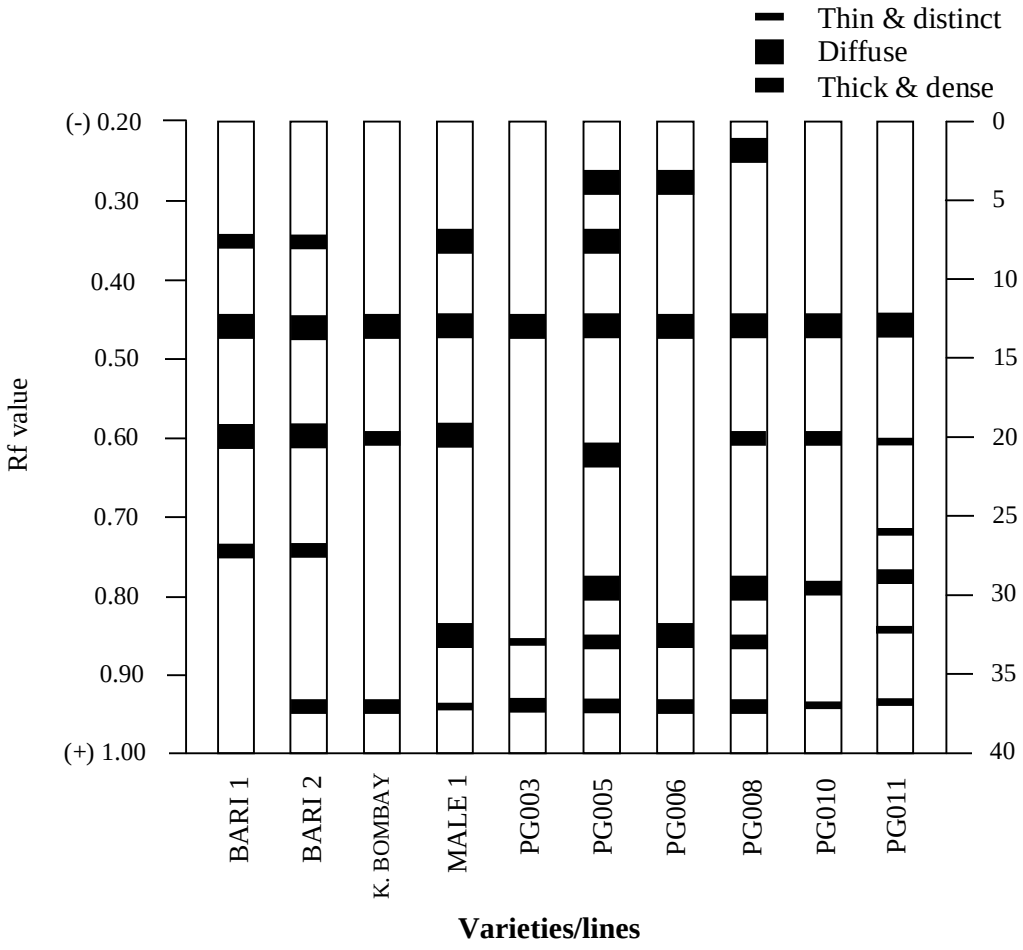


Fig. 92: Line diagram for visualizing the protein banding patterns in ten varieties/lines of *T. dioica*

lines are presented in **Table 51.** None of the varieties/lines exhibited all the bands as well as no band was found common to all the varieties/lines.

Percent similarity index was calculated in all possible ways which varied among the varieties/lines and these are shown in **Table 52.** Highest similarity index between BARI 1 and BARI 2 was found to be 80.00%. On the other hand, BARI 1 showed 40.00, 33.33, 16.66, 22.22, 14.28, 25.00, 33.33 and 42.85 percent similarity with protein banding pattern of KALI BOMBAY, MALE 1, PG003, PG005, PG006, PG008, PG010 and PG011, respectively. Highest similarity index between BARI 2 and MALE 1 was found to be 66.66%. On the other hand, BARI 2 showed 60.00, 33.33, 37.50, 28.57, 37.50, 50.00 and 57.14 percent similarity with protein banding pattern of KALI BOMBAY, PG003, PG005, PG006, PG008, PG010 and PG011, respectively. Highest similarity index between KALI BOMBAY and PG010 was found to be 75.00%. On the other hand, KALI BOMBAY showed 60.00, 50.00, 25.00, 40.00, 50.00 and 50.00 percent similarity with protein banding pattern of MALE 1, PG003, PG005, PG006, PG008 and PG011, respectively. Highest similarity index between MALE 1 and PG003 was found to be 60.00%. On the other hand, MALE 1 showed 50.00, 50.00, 57.14, 50.00 and 57.14 percent similarity with protein banding pattern of PG005, PG006, PG008, PG010 and PG011, respectively. Highest similarity index between PG003 and PG006 was found to be 75.00%. On the other hand, PG003 showed 42.85, 50.00, 40.00 and 42.85 percent similarity with protein banding pattern of PG005, PG008, PG010 and PG011, respectively. Highest similarity index between PG005 and PG011 was found to be 62.50%. On the other hand, PG005 showed 57.14, 44.44 and 37.50 percent similarity with protein banding pattern of PG006, PG008 and PG010, respectively. Highest similarity index among PG006, PG008 and PG011 was found to be 42.85%. On the other hand, PG006 showed 33.33 percent similarity with protein banding pattern of PG010. Highest similarity index between PG008 and PG011 was found to be 71.42%. On the other hand, PG008 showed 66.66 percent similarity with protein banding pattern of PG010. The similarity index between PG010 and PG011 was found to be 66.66%.

Results revealed that among ten varieties/lines of *T. dioica* the highest similarity index (80.00%) for protein banding pattern was observed between BARI 1 and BARI 2 and the lowest similarity index (14.28%) was found for BARI 1 with PG006.

Table 51: Number and kind of bands in ten varieties/lines of *Trichosanthes dioica*

Varieties/lines	Total number of bands	Kind of bands		
		Thin and distinct	Diffuse	Thick and dense
BARI 1	4	-	2	2
BARI 2	5	-	2	3
KALI BOMBAY	3	-	1	2
MALE 1	5	1	4	-
PG003	3	1	1	1
PG005	7	-	5	2
PG006	4	-	3	1
PG008	6	-	3	3
PG010	4	1	1	2
PG011	6	4	1	1

Table 52: Estimation of percent similarity index for protein bands in ten varieties/lines of *Trichosanthes dioica*

Varieties/lines	BARI 1	BARI 2	KALI BOMBAY	MALE 1	PG003	PG005	PG006	PG008	PG010	PG011
BARI 1		80.00	40.00	33.33	16.66	22.22	14.28	25.00	33.33	42.85
BARI 2			60.00	66.66	33.33	37.50	28.57	37.50	50.00	57.14
KALI BOMBAY				60.00	50.00	25.00	40.00	50.00	75.00	50.00
MALE 1					60.00	50.00	50.00	57.14	50.00	57.14
PG003						42.85	75.00	50.00	40.00	42.85
PG005							57.14	44.44	37.50	62.50
PG006								42.85	33.33	42.85
PG008									66.66	71.42
PG010										66.66
PG011										

CHAPTER FOUR

DISCUSSION

DISCUSSION

Trichosanthes L., one of the largest genera of Cucurbitaceae, is well represented in the eastern part of India and in Bangladesh. Clarke (1879) included the genus as a member of the tribe cucumerinae, while Müller and Pax (1889) considered it within the sub tribe trichosanthinae under the tribe cucurbitae. But no such agronomic information is available which can be used as basis for delineating and standardizing different available cultivars.

Pointed-gourd breeding program has recently been initiated to develop new cultivars. Dioecy represents an inconvenience in pointed-gourd breeding. Currently there is no method for distinguishing male and female plantlets prior to flowering in *T. dioica*. A method to determine the gender of plants before flowering would facilitate breeding and selection, by enabling screening for gender at an early stage, thereby simplifying the breeding of male and female plants for different objectives, and saving time and economic resources. However, the findings obtained in this investigation are discussed considering respective characters as a whole studied.

1. Morphological features

In the present study eighteen varieties/lines of *Trichosanthes dioica* were found to be somewhat different from each other in respect of some morphological features which have been shown in **Tables 3-4** and **Figs. 6-23.** The recorded findings revealed variations in morphological features both qualitative and quantitatively. Stem colour in most of the varieties/lines were green and leaf characters were found differently Six characters were found to be common in all of them. Leaf colours were of three types. Three types of ovary shape were also observed. Male flower was found to be bigger in size compared to that of female. In case of fruit character the colours were green, dark green and light green with different patterns of stripes. Bharathi and Vishalnath (2010-2011) stated that a total of 22 varieties/land races of pointed gourd showed a considerable level of variability for qualitative traits such as fruit colour (light green to dark green), fruit striping (striped to without stripe), fruit shape (round to oblong) and leaf surface (smooth and rough). In the present study fruit shapes were three types viz. oval, cylindrical and spindle. However, the phenotypic variations observed in this study were in agreement with the findings of Bharathi and Vishalnath (2010-2011), and Sharma *et. al.* (1988). The fruit characters may be helpful for the selection of genotypes with desired quality.

The assessment of variability present in the crop helps for successful utilization of plant characters in developing suitable varieties for yield and stability. In present investigation length of stem was recorded from 68.38 to 122.81cm. The lengths were

measured at the first flowering time. Kumar and Brahmachari (1995) studied variability of Hilly, Dandali , Nimia and Santokhwa cultivars of pointed gourd. Santokhwa had the longest vines in two growing seasons, successively. Length of inter-node was recorded from 4.47 to 6.81cm. It is notable that length of inter-node and number of inter-node were also recorded at the first flowering stage. Number of inter-node was recorded from 12.27 to 21.11cm. Sarkar *et. al.* (1989) noted in pointed gourd that mean node number for the emergence of first female flower was 37.40. Days to flowering ranged from 105 to 133 in present investigation, whereas Yawalkar (1985) reported that in pointed gourd flowering started from 70-80 days after planting. Rahman (1988) also stated that it took 3 month for flowering/ fruiting after planting of vine or roots. Shanmugavelu (1989) reported in pointed gourd that it took 136 to 158 days for first flowering after planting. Prasad and Singh (1990) reported in pointed gourd that the first flower appeared at 5^{th} to 8^{th} node based on various varieties. In present investigation, length of flower and fresh weight of flower were recorded from 6.45 to 8.85cm and 0.695 to 1.043gm, respectively. It is notable that as dioecious plant there was no data recorded about fruit of male plant in pointed gourd. In present investigation, length of fruit, circumference of fruit, weight of fruit and number of seeds per fruit were recorded from 6.01 to 11.50cm, 7.09 to 13.48cm, 10.55 to 62.48gm and 16.79 to 33.29, respectively. Singh and Prasad (1989) reported that fruit length, width and weight were in the range of 4.95-9.8lcm, 2.98-3.56cm and 15.48-57.66gm, respectively depending on various genotypes. Shanmugavelu (1989) reported that pointed gourd fruits were 10 to 16cm long with very faint stripes and pale green in colour. Prasad and Singh (1990) investigated that fruits length and breadth were to 9.4cm and 3.0 to 3.4cm, respectively. The weight of a single fruit varied from 25.0 to 34.6gm. They also reported that the number of seeds per fruit varied from 14.5 to 19.9 in CHES-4 and CHES-14.

The way of analyzing the morphological variations among the eighteen varieties/lines of *Trichosanthes dioica* was found to follow the pattern of variations revealed by analyzing score to the variability's. The results of such metroglyph analysis were considered for the study **(Tables 5-8 and Figs. 24-25).** The performance of a genotype was denoted by the index score of the genotype and depending upon the score represented by the varying length of rays. The two most variable characters, i.e., stem length and leaf area used for determining X and Y axis, respectively to plot the graph. Beside, a few characters were found to increase with respect to the variable characters and in such cases the placement of glyphs would be different, if other characters are considered for the X and Y axis. Thus, metroglyph analysis has some limitations. However, it may be speculated from the study that variation for a number of characters among the varieties/lines of *Trichosanthes dioica* may be less due to conservation of

genes. Thus, the consideration of a pair of character is not always reasonable. However, maximum variability observed between these two characters could be due to high genetic relationship.

Nevertheless, the total variability of characters i.e., sumtotal indices for all the characters considered can be studied by metroglyph analysis. The pattern of ray on the glyph among the clusters and within a cluster showed marked variations for the presence or absence and the length of rays.

Cluster I represented by two lines were found to be distinguishable by their ray patterns. This cluster characteristically showed the lowest values for the length of inter-node, number of inter-node, fresh weight of leaf, fresh weight of flower, circumference of fruit and weight of fruit. This cluster also showed the lowest value for Y axis, which has directed for leaf area.

Cluster II was consisted of three lines where two lines namely BARI 2 and PG015 were comparatively related having genetic diversity for their ray patterns, but another line namely PG006 showed the lowest values for maximum characters except days to flowering.

Cluster III had represented for thirteen varieties/lines and most of the characters were found in the cluster having low, medium and highest values. By viewing this cluster it can be commented that there is a comparatively related genetic diversity present among the varieties/lines.

Findings discussed above suggested that despite the formation of different clusters among eighteen varieties/lines of *Trichosanthes dioica* they showed resemblance for many characters and a few diversity among them might have originated through introgression of genes. It may also be mentioned that the method of analysis despite of its limited application helped to ascertain the diversity among the eighteen genotypes of *T. dioica.*

However, based on D^2 values Bharathi and Vishalnath (2010-2011) grouped 22 accessions of *T. dioica* into five clusters. The pattern of group constellations indicated that genetic diversity was not directly correlated to the geographic diversity. Cluster 1 comprised 12 accessions, whereas cluster 5 contained a single accession. Almost similar result was obtained in the present study. This type of classificatory analysis may provide the foundation for designing an efficient pointed gourd breeding programme. Although the present study comprised only eighteen varieties/lines of pointed gourd, especially in the consideration of number of varieties/lines/races and perhaps by the use of hybtid index as suggested by Hatheway (1962).

To study the pattern of morphological variation in crop plants classificatory analysis (Metroglyph or D^2 statistics) are adopted. In this study metroglyph and index score

method as proposed by Anderson (1957) was used. This method has been suggested by several workers (Ramanujam and Kumar, 1964; Singh and Chowdhury, 1974; Harlan and De Wet, 1971).

2. Interphase nuclear phenotype

Interphase nuclear phenotypes in terms of interphase chromosome volume (ICV) in eighteen varieties/lines of *T. dioica* in the present investigation were found to vary among them. Mean value for ICV in KALI BOMBAY was lowest. The highest ICV was found in PG023. It was also observed that comparatively the lower values for ICV were found in the early marking varieties/lines of *T. dioica* such as BARI 1, BARI 2, KALI BOMBAY and so on. On the other hand comparatively the higher values for ICV were found in late marking varieties/lines except PG022.

Lafontaine (1974) stated that the structural organization in plant cell nuclei are two types, chromocentric and reticulate. In the present study interphase nuclei of shoot tip cells of *T. dioica* was found to be chromocentric. Chromocentric nuclear organization was reported in *Phaseolus* species by Patankar and Ranjekar (1984). The chromocentres became more clear and distinct after disruption of euchromatin by haematoxylin indicating their heteromorphic nature and the chromocentre numbers were countable. Similar types of results were reported in *Phaseolus* by Joshi and Ranjekar (1983). Chromocentre numbers in all the varieties/lines were more or less same which were, however, somewhat less than the expected number of 22. The reduction in number of chromocentres in all the varieties/lines might be due to fusion or overlapping of chromocentres indicating the somatic association of chromosomes. Dayal (1975) and Dayal and Prasad (1983) have mentioned that the number of chromocentres is considered to be controlled genetically and is, therefore, a species specific character. According to Lavania and Sharma (1983), there is a tendency to show heterochromatic segments during the course of evolution. Thus, the older plants may have more heterochromatin than the recent one. Based on this concept PG019 can be regarded as somewhat older and BARI 1 as recent one among eighteen varieties/lines studied. Different classes of heterochromatin have been reported among plants (Vosa, 1970; La Cour, 1978).

Interphase nuclear organizations of plants is species specific, however, its determinations are not clearly known (Nagl and Fusening, 1979). However, heterochromatin is found to vary from cell to cell and and particularly in meristematic and differentiated cells. No such observation was made in the present study, even there is no report in this regard earlier. Nagl (1979) has pointed out that in plants the variation in the proportion of heterochromatin between meristematic and differentiated cells can serve as an indication of differential DNA replications. Reduction in the

amount of heterochromatin in differentiated cells indicates the nuclear reduplication during differentiation (Kabir and Singh, 1989). In the present study heterochromatin percentage was determined from meristematic cells only. However, under replication of heterochromatin was reported in *Cucumis* species during leaf and fruit differentiation (Pearson *et. al.*, 1974).

Lafontaine (1974) stated that chromocentric nuclear organization is assumed to be governed by small size of chromosomes and low DNA content. He further suggested that repetitive DNA induces specific coiling and folding patterns, leading to chromatin condensation. Kabir (1993) said that in this type of study, cytogeneticists need to investigate interphase nuclear structure using various techniques of light, phase contrast, fluorescence and electron microscopy.

3. Somatic karyotype

First of all in order to identify the somatic chromosomes of the varieties/lines of *Trichosanthes dioica*, their shoot tip cells were treated with saturated solution of paradichlorobenzene (PDB) for 4 to 5 hours at 10°C and this treatment gave better results for spreading the metaphase chromosomes. The somatic chromosome numbers were found to be 2n=22. In the present investigation chromosome pairs were numbered from 1 to 11. The haploid chromosome complements were numbered from decreasing order of length following Rhoades (1955). The number was used as identification mark and thus each chromosome was allocated to a serial identification number. The chromosomes may not always have the same total length (Sindhu *et. al.*, 1982) because of variation from cell to cell and differences in fixation. The chromosome morphology varies from cell to cell and thus, major changes are associated with the cell division process also. The chromosome length of all varieties/lines of *Trichosanthes dioica* varied from cell to cell. The class interval 0.49µm for chromosome length chosen arbitrarily and the range for arm ratio as recommended by Kutarekar and Wanjari (1983) were followed. Munira *et. al*. (2010) reported that highest mean value (2.32µm) and lowest mean value (1.12µm) for identified chromosome were found in *Agave americana*. In present investigation the range of mean of the chromosome was 4.07-2.10µm in BARI 1, 4.99-2.32µm in BARI 2, 5.12-2.53µm in KALI BOMBAY, 4.59-2.24µm in MALE 1, 4.22-2.23µm in PG003, 4.51-2.63µm in PG005, 3.89-2.00µm in PG006, 4.86-2.19µm in PG008, 3.67-2.58µm in PG010, 4.15-2.45µm in PG011, 3.46-1.92µm in PG012, 2.89-1.70µm in PG015, 4.37-2.20µm in PG018, 4.33-2.56µm in PG019, 2.98-1.71µm in PG020, 3.19-1.83µm in PG022, 2.93-1.93µm in PG023 and 3.26-2.23µm in PG028. Present results reveal significant differences in terms of total chromatin length (TCL) 33.95µm and total frequency (TF%) 43.36 in BARI 1, TCL 41.80µm and TF% 44.35 in BARI 2, TCL 41.13µm and TF% 42.40 in KALI BOMBAY, TCL 35.84µm and TF% 44.08 in MALE 1, TCL 36.71µm and TF% 44.82

in PG003, TCL 37.84µm and TF% 41.10 in PG005, TCL 32.54µm and TF% 38.75 in PG006, TCL 38.65µm and TF% 44.17 in PG008, TCL 34.69µm and TF% 44.77 in PG010, TCL 35.70µm and TF% 45.28 in PG011, TCL 29.84µm and TF% 42.23 in PG012, TCL 25.08µm and TF% 41.24 in PG015, TCL 36.94µm and TF% 43.43 in PG018, TCL 37.60µm and TF% 43.97 in PG019, TCL 25.03µm and TF% 44.82 in PG020, TCL 25.68µm and TF% 42.37 in PG022, TCL 26.67µm and TF% 43.19 in PG023, and TCL 29.43µm, TF% 42.56 in PG028. The secondary constrictions were found only in PG006 at the 5^{th} and 7^{th} pairs of chromosomes at the end of long arms. The sub-terminal position of centromeres were found in PG005, PG006, PG018, PG022 and PG023 in the 1^{st} pair, 9^{th} pair, 8^{th} pair, 4^{th} pair and 7^{th} pair of chromosomes, respectively. According to Stebbins (1950), decrease in TCL is one of the factors indicating the trend for evolution. Thus PG020 may be considered as most advanced and BARI 2 as older among the varieties/lines of *T. dioica* studied. On the other hand, the species having asymetrical karyotype is supposed to be more advanced than symmetrical ones (Stebbins, 1950). From this point of view PG020 may not be considered as advanced one among eighteen varieties/lines of *T. dioica* studied since it possessed all metacentric chromosomes except two sub-metacentric **(Table 42).** In contrast, PG006 possessed maximum heterogenous karyotype in respect of centromeric position and hence may be considered as advanced **(Table 34).** On the basis of TF%, PG006 **(Table 34)** may be considered to be the most advanced while PG011 as the most older one **(Table 37).** However, this concept may be applicable particularly in case species. This is always species specific parameter.

Sarkar and Datta (1987) used three cultivars of *T. dioica* for chromosome analysis. They studied the distribution pattern of Giemsa C-heterochromatin in details and for that they followed the principle of BSG-technique as suggested by Vosa (1976). They made this study from adventitious roots using their apical meristems pretreating with saturated solution of alpha bromonapthalene. In the present study shoot tip instead of root tips was used using paradichlorobenzene as pretreating fluid and it gave well scattered metaphase chromosomes. However, Sarkar and Datta (1987) studied three female plants of pointed gourd and on the basis of chromosome length, number and position of bands they found long, medium and short chromosomes and grouped them into six groups. They found total chromosome length to vary from 68.55µm to 87.60µm. In the present study the total length of haploid complement was found to vary from 25.03µm to 41.48µm and the chromosomes were divided into long, medium, short and relatively short groups. The varieties/lines of pointed gourd in this study represented a homogenous assemblage of related taxa. They were apparently indistinguishable from each other on karyotypic features. Their close phyletic relatedness was found to be strongly reflected in chromosomal features as well except the male one.

Karyotype analysis plays significant role in determining the status of a taxon, because it signifies the very stable characters. Particularly, the problem arises in case of differentiating the taxa, when they posses same chromosome number and almost identical chromosome morphology. Now-a-days, conventional method of karyotype analysis is not considered generally for distinguishing the taxa of homomorphic nature. However, in this study somatic karyotype has been done following a quantitative method as suggested by Ahmed *et. al.* (1983). In this method a long schedule comprising many steps are followed and the unidentifiable chromosomes are allocated through standardization and homogeneity test. This method helps to characterize all the chromosomes separately and then the standard karyotypes are proposed. So it gives somewhat more accurate morphological features of the chromosomes.

Alam *et. al*. (2011) stated that comparatively a recent method for karyotype study is concerned with DNA-base specific banding with fluorochromes such as chromomycine A_3 (CMA). They stated also that fluorescent banding is quite satisfactory for detailed and critical chromosome analysis. They conducted such a study on five varieties of snake gourd *(Trichosanthes anguina).* They found no CMA in two varieties, but in other three varieties characteristic CMA bands were observed in respect of number, size, location and percentage of G-C rich area. This type of karyotypic study has not been done yet on pointed gourd.

Trichosanthes dioica has been characterized by a uniform number of 22 chromosomes in the present study and this finding has been supported by Bhaduri and Bose (1947) and Sen (1976). All the varieties/lines of *T. dioica* in this study were found to show their high homomorphic nature both morphologically and cytologically. Their close relationship was found to be reflected in chromosomal features.

However, karyotypic analysis at the molecular level may reveal a differential distribution of heterochromatin in the varieties/lines of *T. dioica,* and thereby demonstrating their active involvement in intraspecific diversification of *T. dioica* may be expected.

4. Identification of sex regulatory chromosomes

Whatever the findings on sex chromosome have been presented in the previous chapter **(Table 46)**, it is completely inferential basis. The present findings are not being supported by any strong arguments. Till today there is not a single work on the identification sex chromosomes of pointed gourd, based on what the present findings may be supported. Moreover, the most striking feature found in the present study was that the assumed sex chromosomes varied from variety to variety, which should be species specific, i.e., constant in a particular pair of chromosomes. On the other hand,

from the karyotypic study it was observed that chromosome complements in all the varieties/lines of pointed gourd revealed extreme homomorphic nature.

Nevertheless, this experiment of the present study was conducted to elucidate the sex expression in *T. dioica*, a dioecious cucurbit. Allen (1940) gave a list of plant species in which sex chromosomes had been reported. Subsequently Westergaard (1958) prepared a list of plant species where the presence of a pair of heteromorphic sex chromosomes was well established and also of those where it was not so well established. For some dioecious plant species Gupta (1997) made a list of different types of chromosome constitutions involving heteromorphic sex chromosomes in one sex and those are shown below.

Mechanism	Examples
1. Male heterogametic (♀XX; ♂XY)	*Humulus lupulus, Melandrium album, M. rubrum, Rumex angiocarpus, Populus* spp.*, *Salix**, *Smilax** and *Cannabis**
2. Male heterogametic (♀XX; ♂XO)	*Vallisneria spiralis, Dioscorea sinuata*
3. Male heterogametic (♂With an extra chromosome)	*Phoradendron flavescens, P. villosum*
4. Female heterogametic (♀XY; ♂XX)	*Fragaria elatior* and other *Fragaria* species
5. Compound chromosomes (e. g. ♀XX; ♂Y_1XY_2)	*Rumex acetosa, Humulus japonicus*
6. No sex chromosomes (perhaps gene controlled)	*Spinacia oleracea, Ribes alpinum, Vitis cinerea, V. rupestris, V. vinifera, Carica papaya, Asparagus officinalis, Bryonia dioica*
7. No sex chromosomes (in male, translocation heterozygosity)	*Viscum fischeri*

After Gupta (1997). *Less well established.

However, Singh (2002) stated that in higher plants, hermaphroditism condition is the original form of sexuality. Dioecism originated from the bisexual, or monoecious species through mutation and natural selection. The unisexual condition developed as a result of a trigger mechanism that suppresses the potentialities of the opposite sex in males and females. He also stated that the forward evolution is from bisexual to unisexual, and the reverse evolution is from dioecism to bisexuality. He said that several dioecious plants are without sex chromosomes and sex expression in those plants is under genetic control. For example he cited the name of the dioecious plants mentioned above in number 6 of the table and reported that sex determining gene in the spinach is on chromosome 1.

The family Cucurbitaceae provides interesting materials for study of cytogenetic mechanism of sex determination in a number of sex forms such as dioecious,

monoecious and hermaphroditic (Sarkar and Datta, 1988). Although dioecism exists in fairly large number of cucurbit genera including *Trichosanthes,* sex inheritance has been studied in a very less number and the mechanism of sex expression has not yet been well understood. They reported androecious representative of *Trichosanthes dioica* cv: Kalyani, characterized by the presence of a heteromorphic pair. This finding supports the findings on heteromorphic pair of an androecious line of the present study to a little extend, but not very much authentically. Sarkar and Datta (1988) also reported similar heteromorphic pair in androecious form of wild *T. dioica* and *T. anguina.* In their findings the gynoecious form of *T. dioica* showed diffused banding in a pair of chromosome, while the androecious sex could be characterized by one member of the pair without any band. However, in the present study no such study was made. One year back, Sarkar *et. al*. (1987) made a cytomorphological study on some wild and cultivated members of *Trichosanthes* and reported a heteromorphic pair of chromosome in eight cultivars of *T. dioica*. They stated that the heterozygous constitution of taxa as indicated in the occurrence of chromosomal heteromorphy has probably arisen during long cultivation and selection. They further stated that the significant role of structural alternations of chromosomes in inter- and intra-apecific diversification has been demonstrated in the genus *Trichosanthes* L.

In the present study chromosome morphology in all the varieties/lines of pointed gourd showed homogeneity at the extreme level. It was found to be somewhat supported by the findings of Karmakar *et. al.* (2014). They made an analysis of karyotype of the sex forms *Trichosanthes bracteata* and *Thladiantha dubia* and reported that in both the genera the karyotype of male and female plants showed high homogeneity and no heteromorphocity in relation to sex. However, in comparison to symmetrical nature of the chromosomes of *Thladiantha dubia,* they found agrogressive asymmetry in *Trichosanthes bracteata.* In the same year of 2014, Guha *et. al.* conducted a study on karyotype and sex expression in a dioecious cucurbit, *Coccinia indica.* They reported almost similar findings as it was found in the present study. The somatic chromosome number of both the sex forms of *C. indica* was found to be 2n = 24 and the karyotype showed high homogeneity, though a distinct heteromorphic pair of sex chromosomes was found in male sex form of *C. indica.*

Dioecism exists in a number of cucurbitaceous genera, but presence of the sex chromosomes are reported only in few species like *Coccinia indica* and *Bryonia dioica* (Chakraborty, 1948; Chattopadhyay and Sharma, 1991; Correns, 1903; D' Curz *et. al.*, 1972; Gala'n, 1946; Grant *et. al.*, 1994; Mayer and Charlesworth, 1992; Ming *et. al*., 2011; Oryma *et. al.*, 2009; Roy and Roy, 1971; Sen and Datta, 1977; Sinha *et. al*., 1997; Westergaard, 1958). The term sex chromosome generally means a pair of chromosomes where the sex determination locus resides regardless of whether they are

heteromorphic and non-recombining or homomorphic and recombining along their length (Kejnovsky *et. al.*, 2009). In the flowering plants, approximately 4-6% or 14000 species in 960 genera and 200 families are dioecious (Guttman and Charlesworth, 1998; Renner and Ricklefs, 1995). The family Cucurbitaceae includes quite a large number of dioecious species. However, Banerji and Das (1937), while studying the meiosis of pointed gourd *(Trichosanthes dioica),* did not observe any sex-chromosome pair nor did they obtain any evidence in that direction from a study of somatic mitosis. It is about 80 years now; findings of sex chromosomes in pointed gourd have not been reported authentically. From this point of view it may be said that the data on sex chromosome presented in this study is completely inferential basis and assuming. At the same time it may said that there is a definite mechanism, which is triggering for the expression of sex in pointed gourd.

Singh *et. al.* (2002) in a scientific correspondence described about female sex-associated RAPD marker in pointed gourd *(Trichosanthes dioica),* where they conducted the experiment in two stages, comprising 16 female and 4 female lines/entries of different geographical areas. In the first stage the DNA was pooled from all the male and female cultivars, separately and screening of primer was done on the pooled DNA's. Hundred decamer primers were screened for differences in male and female cultivars. In this way 5 primers were identified which produced probable female related bands. In the next stage, the 5 selected primers were used to confirm the presence and absence of bands in all the male and female entries, individually. In this stage 567bp band amplified by the OPC07 primer from the genomic DNA of all the female entries was absent in the PCR products of the DNA's from all the male entries. Thus, within the limits of male and female genotypes available in this study, the RAPD band $OPC07_{567}$ appeared to be the female sex-related DNA marker in *T. dioica*.

The members of the family Cucurbitaceae are interesting material for the study of genetic mechanisms of sex determination (Kumar *et. al.*, 2008). Several dioecious plants e. g. white campion *(Silene latifolia),* papaya *(Carica papaya)* and *Asparagus officinalis,* have an active X-Y system of sex determinations with heterogametic males (XY) and homogametic females (XX). In some other plants like sorrel *(Rumex)* and hop *(Humulus lupulus),* X: A ratio of sex determinations operates. But in case of *Trichosanthes dioica,* no such information at chromosome or DNA level is available (Kumar *et. al.*, 2008). Kumar *et. al.* (2012) had been able to identify sex-associated RAPD markers (the female specific $OPC05_{1000}$ and the female specific marker $OPC14_{400}$) which together can reliably differentiate male and female plants of *T. dioica* before maturity. Thus, the use of DNA markers to discriminate the two sexes has been advocated if the genetic mechanism of sex determination is not known (Xu *et. al.*, 2004). In 2014, Adhikari *et. al.* developed a genetic sex marker for the pointed gourd

(Trichosanthes dioica) to allow gender determaintion. Screening of genomic DNA with intersimple sequence repeat (ISSR) primer was used to discover sex- specific touch down polymerase chain reaction (Td-PCR) amplification products. Using pooled DNA from male and female genotypes and 42 ISSR primers, a putative male specific marker (~550bp) was identified. DNA marker specific to male is an indication of existence of nonepigenetic factors involved in gender development in pointed gourd. Thus, the ISSR technique has proved to be a reliable technique in gender determination of *Trichosanthes dioica.*

It is said that molecular methods provide valuable tool for good and easy identification of gender at any stage of growth and developments. Various works have been done with the help of molecular markers for the determination of sex in many dioecious plants. But cytologically the problem has remained same, as it was earlier in many dioecious plants, particularly in *Trichosanthes dioica*. In this plant species identification of the sex chromosome is still problematic because of high homomorphic nature of the whole chromosome complement. Some dioecious plants *Carica papaya* (Storey, 1953), *Pistacia vera* (Hormaza *et. al.*, 1994), *Ecballium elaterium* (Ainsworth, 2000) and *Asparagus officinalis* (Gao *et. al.*, 2007) sex is found to be controlled by single gene mechanism whereas, in *Mercurialis annua* (Louis, 1989) a multiple loci system is responsible for sex expression. So the identification of sex by chromosome has become difficult and that might be due to evolution of plant sex chromosome.The intrinsic interest in understanding such strange phenonema has led to studies in a diversity of dioecious plant, even though separate sexes are rare among flowering plants, and sex chromosomes are not present in all dioecious plants (Charlesworth, 2013), though they are common in liverworts (Westergaard 1958; Renner and Ricklefs, 1995; Ming *et. al.*, 2011).

The term ‘unisexuality’ applies to several distinct situations observed in plants, covering species with environmental sex determination (Policansky, 1981; Zimmerman, 1991; Pannell, 1997) and monoecious as well as dioecious species. In such case Charlesworth (2013) used the term ‘sex-determining gene’ only for dioecious species with genetic sex determination. In monoecious species and in environmental sex determination, genes are, of course, involved in the sex-determining developmental pathway, but there are no sex-determining loci, since all individuals are either monoecious, and capable of developing flowers of either sex, or can develop as either sex, depending on the environmental experienced in a given flowering season. In contrast, many dioecious species have a genetic polymorphism involving ‘sex-determining genes’ or ‘primary sex-determining genes’, which control whether a plant (or animal) as a whole develops as a male or female i.e., genes suppressing female functions on a Y chromosome, and loss of function of male fertility factors on the X

(Charlesworth, 2013). Does this phenomenon support the present findings in any way? Obviously it is a matter of keen study particularly on mutations, which at many loci in plant genomes can cause male sterility (Klekowski, 1988) and the same is likely for female sterility, since large number of genes are expressed during flower development (Wellmer *et. al.*, 2006).

However, the possibility of locating the sex determining regions in dioecious plant genomes by genetic mapping may make it possible to identify the sex determining genes in individual species and to be tested whether they are indeed different in different taxa, which would support independent evolution of dioecy in different plant taxa (Charlesworth, 2013). Is it the reason of variation of sex regulating chromosomes (indeed gene controlled) in different varieties/lines of *T. dioica* in the present study? If it is, then it may be said that above mentioned sex regulating genes may often be located within large non recombining genome regions. Charlesworth and Charlesworth (1978) stated that dioecy in flowering plants must often have evolved through at least two mutations, a male sterility mutation (creating females) and one or more female sterility mutation (creating males). Charlesworth and Charlesworth (1980) also stated that other kinds of chromosomal rearrangements, including translocations, can also suppress recombinations. Translocations involving sex chromosomes are known in plant species (No. 7 in the above mentioned table), and could be advantageous because they cause linkage between autosomal and sex-linked genes.

It is very much clear that the sex-determining system, particularly identification of sex chromosome in dioecious plant species still needs much genetic works, since identification of sex-determining gene(s) is much more difficult. Fine genetic mapping may help to identify the genome region involved, which often clarifies whether males or females are the heterogenetic sex for the region. Finding cluster of genes related to floral development in the region can provide support for a candidate region (Yano *et. al.*, 2012), but, ideally, many more detailed genetic studies are needed.

5. Biochemical studies

I. Screening test

Phytochemicals are non-nutritive chemical compounds, occur naturally in plants. They are termed as secondary metabolic compound. There are about 10,000 different phytochemicals having the curative effect on many diseases and metabolic syndrome.The plant pointed gourd (*Trichosanthes dioica*) reveals presence of tannins, saponins, triterpenoids, flavonoids and thus, leaves of the plants are used as antipyretic, diuretic, cardiotonic, laxative, antiulcer etc. (Toshniwal *et. al.*, 2013). Phytochemical investigation of *T. dioica,* using natural product technology, which correlates the presence of certain trace elements with its biological activities using laser induced

break down spectroscopy (Rai *et. al.,* 2010). Their study revealed the isolation of one known and two unknown flavonoids and the presence of certain glycemic elements responsible for the observed antidiabetic activity of *T. dioica*. Thus, the presence of isolated flavonoids and trace elements can be very well correlated with its medicinal value. However, in the present study five phytochemicals viz. alkaloids, flavonoids, glycosides, steroids and tannins were detected. Rai *et. al.* (2013) made a scientific validation of antihyperglycemic and antihyperlipidemic attributes of *T. dioica,* which may be traditionally used for managing diabetes mellitus.

Qualitative phytochemical analysis of *Trichosanthes dioica* methanol extract showed the presence of tannins, alkaloids, flavonoids, glycosides and steroids (Okwu, 1999). Medicinal plants have great significance in health of individuals and communities. The medicinal importance of plants lies in some chemical substances that produce a specific physiological action on the human body. The most essential of these bioactive compounds of plants are alkaloids, saponins, tannins, flavonoids and phenolic compounds and many more components.

Phytochemical from medicinal Plants serve as lead compound in drug discovery and design (Falodun and Agbakwuru, 2004). These results revealed that the plant has quite a number of chemical constituent, which may be responsible for many pharmacological action, although their specific roles were not investigated in this study. It has been reported that most active components in plants are mostly tannin, flavonoids, alkaloids and glycosides. An experiment was conducted by Deka *et. al.* (2015) in which they found the presence of alkaloids, flavonoids, glycosides, steroids and tannins in *T. dioica* leaf extracts. Another experiment was carried out by Shivhare *et. al.* (2010b) and they found the presence or absence of alkaloids, flavonoids, glycosides, steroids and tannins in petroleum ether extract, chloroform extract, methanolic extract and aqueous extract of *T. dioica* fruits, respectively.

The root of *T. dioica* is used as purgative and as tonic, febrifuge, in treatment of jaundice, anasarca and as cites (Sharma *et. al.*, 2002). In few other reports (Bhattacharya *et. al.*, 2009 and 2010; Bhattacharya and Haldar, 2010a, 2010b and 2011) anthelmintic effects of leaf and root; antibacterial, antimitotic and antitumour activities of the root of *T. dioica* were found. However, in the present study leaves of pointed gourd were used in place of root, so the phytochemicals may vary in their contents as well as the density along with some other physical properties. Deka *et. al.* (2015) stated that methanolic leaves extract of *T. dioica* was found to contain different phytochemical constituents and significant antioxidant activities in a dose of dependent manner.

II. Quantitative test

Total phenol content was found in the PG003 with 66.96 mgL^{-1} and PG012 with 701.40 mgL^{-1}. The presence of phenolic compound in the fruits of pointed gourd can act as antimicrobial to bind to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption and act as metal ion complication (Tiwari *et. al.*, 2011). Jakopic *et. al.* (2009) reported total phenolic content of 161.07 (mg GAE/100ml) from *Juglans regia* of cultivar Franqette. This difference in the result may be due to the part of the lime being extracted which is in this experiment peels were used and it also due to the different extraction method and different environmental condition. The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process (Perva-Uzulanic *et. al.*, 2006). So, it has been proven that when the plant material was extracted with an alcoholic solution, it posses the satisfactory result in determining the total phenolic content. Phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu (FC) reagent (Tiwari *et. al.*, 2011). However, it should be also noted that some chemical groups of proteins, organic acids, and sugars present in the extracts can also react with Folin-Ciocalteu reagent and therefore can interfere with the results (Huang *et. al.*, 2008). Lobo *et. al.* (2009) stated that in advance stages of oxidation, the increasing degree of polymerization results in a decreasing scavenging activity of polyphenols due to sterile hindrance. Likewise, many of the polyphenols are adsorbed on to the apple pulp, and therefore, they will not reach the must, explaining the subsequent decrease in its antioxidant activity, as compared with fresh apples (Van der Sluis *et. al.*, 2002).

It is known fact that chlorophyll as a type of plant pigment creates energy in the process of photosynthesis. So why is it important for human beyond sustaining plant life, that is not a question now. Rather it may be said that chlorophyll as a wole benefits human health, all of which help cleanse the body and allow it to function at an optimal level. The total chlorophyll content was measured using 663nm and 645nm with absorbance read. The highest value of total chlorophyll content was found in the PG003 with 0.543 and the lowest value was in PG011 with 0.150mg/g which has significant difference ($p<0.05$) between them. Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis. Earlier workers observed the leaves of lemon extract have high chlorophyll content. The method proposed is similar to that described (Vernon, 1960) and others reviewed (Holden, 1965), but absorbencies are measured at only two wavelengths, 663 and 645nm, before and after acidification of the sample, and only chlorophyll-a and chlorophyll-b are calculated.

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, specially degenerative diseases, and extensive lysis (Halliwell and Gutteridge, 1999). Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines (Yazdanparast and Ardestani, 2007; Yazdanparast *et. al*., 2008). Recently, many natural antioxidants have been isolated from different plant materials (Jovanovich and Simic, 2000; Packer and Ong, 1997). As reported in many studies, the activities of natural antioxidants in influencing diseases are closely related to their ability to reduce DNA damage, mutagenesis, carcinogenesis and inhibition of pathogenic bacterial growth (Roginsky and Lissi, 2005). Various antioxidant assay methods have been developed for food and biological smaples. Lipid peroxidation in biological systems has long been thought to be a toxicological phenomenon that can lead to various pathological consequences (Hochstein and Atallah, 1988). In addition reactive alde-hydes generated by lipid peroxidation can attack other cellular targets, such as proteins and DNA; thereby propagate the initial damage in cellular membranes to other macromolecules. Because lipid hydroperoxides formed in membranes important components of ROS generation *in vivo*. The detoxification appears therefore. Antioxidants play a vital role in inhibition of lipid peroxidation or in protection against cellular damage by free radicals. Shah and Seth (2010) found fruit extract of pointed gourd to lower the blood sugar, total cholesterol, low density lipoprotein cholesterol and triglyceride levels. This extract also increased the high density lipoprotein cholesterol, phospholipid and faecal sterol levels. It has been recognized that flavonoids show antioxidant activity and their effects on human health are considerable (Pourmorad *et. al.*, 2006). This component was found significantly in the present study.

III. Protein banding pattern

A total of ten varieties/lines of *Trichosanthes dioica* were used for making a comparative analysis of protein band by polyacrylamide gel electrophoresis. All the lines were not found to be available during this study and this is why only ten varieties/lines were used. Moreover, their seeds also could not be stored and that is why their leaves were used for this experiment. Vishal *et. al*. (2012) made seed and leaf protein analysis of *Cleome* species and stated that highest numbers of peptide bands were found to vary in number among five species. In analysis of proteins from both leaves and seeds of *Cleome* species, different polypeptides were obtained and they were compared with those of standard proteins with respect to their Rf values and molecular weight. However, in the present study leaf protein of *Trichosanthes dioica* showed the presence of distinct bands. Some of the bands were found to be somewhat similar in all

the varieties/lines. It was found that there were some bands which were unique to a particular line.

By definition, each variety of any cultivated crop should differ from each other in one or more specific characteristics and they may be chemical, physiological or morphological, and it may be possible to develop one or more test to detect and quantify such differences (McKee, 1973). Electrophoresis is such an excellent means of characterizing varietal variations in terms of protein band. Seed protein profile is apparently typical or diagnostic trait for plant, which reflects the genetic constitution of various species or varieties which might provide us some clues (Ladizinsky and Adler, 1976). However, in the present study leaf protein profile was studied instead of seed protein.

The electrophoresis test, as applied to pointed gourd identification, sperated protein fraction into bands of different mobility and different quality. Three types of bands were found in the present study and those were thin and distinct, diffuse, and thick and dense. These are being supported by different works on different species of plant, since no such study on pointed gourd has been reported till today. Whatever it is, varieties/cultivars/lines of any plant species give bands due to electrophoresis and that are characteristic of genotypes (Wrigley, 1970). The pattern of protein bands obtained from seed extract of any plant is analogous to a set of fingerprints for that particular plant material.

In the present study each variety/line of *T. dioica* had its own pattern and also a different number of bands. Generally protein banding pattern reveals variation in number and intensity of bands and that has been observed distinccly in all the ten varieties and lines studied. Kabir and Singh (1988) found similar type of seed protein in case of six species and two F_1S of *Cicer*. Ladizinsky and Adler (1976) reported similar findings in 88 cultivars of *Cicer arietinum*. So it may be said here that number and types of bands may be species or genotype specific but pattern on the contrary always remains same.

Cucurbitaceae, the gourd family, is one of the largest families of flowering plants, comprising over 940 species and 122 genera distributed in tropical and sub-tropical regions of the world. (Schaefer and Renner, 2011). Several studies have reported the diversity among some of the numbers using different DNA markers like RAPD, ISSR, AFLP etc. at intra-specific level (Schaefer *et. al.*, 2009; Zhang *et. al.*, 2006). Neither of these studies has provided a classification that takes into account proteomics for classifying these members of Cucurbitaceae at intergenus level. Thus, Dudwadkar *et. al.* (2015) conducted a study which provided an insight into variations using seed proteins among members of Cucurbits obtained from diverse genotypes in India. On

segregation of proteins on one dimensional SDS-PAGE demonstrated highly resolved separated bands with optimized M4, than the other methods.

In present study similarity index of protein bands was calculated in all possible ways which revealed variations among the genotypes of *T. dioica*. Highest similarity index was found to be 80.00% and that was between BARI 1 and BARI 2. On the other hand, lowest similarity index was found between BARI 1 and PG006. The similarity index between MALE 1 and any of the female varieties/lines was found to vary from 50.00 to 60.00%. In this study protein bands almost in all the 10 varieties/lines of *T. dioica* were separated and differences were very much distinct. However, Reddy (2009) stated that species were evident in protein profiling as similar banding pattern was observed between cultivars of the same species belonging to the family Cucurbitaceae.

The findings in the present study reveal that the method used for protein extraction has been proved to be efficient for extraction of soluble seed storage proteins. Dudwadkar *et. al.* (2015) says that protein profiling is an efficient method for studying genetic diversity, coupling biochemical method with molecular approaches would help plant taxonomist to understand the variance among plants in further depth. However, proteins being primary gene products regulate genetic systems in many ways and thus, the knowledge with the help of electrophoretic methods provide a constructive idea on the chemotaxonomic properties of plants at inter- and intra species/genetic level.

CHAPTER FIVE

SUMMARY

SUMMARY

The present investigation deals the cytomorphological and biochemical characters of eighteen varieties/lines *Trichosanthes dioica* (Roxb.). All of the varieties/lines are diploid (2n=22). The plant is dioecious in nature. The qualitative characteristics of morphological parts carrying taxonomic importance of all the varieties/lines of *T. dioica* were scrutinized. In case of stem colour eighteen genotypes of pointed gourd showed green, dark green and light green colour. Leaf characters in all the varieties/lines of pointed gourd were found differentially. Six characters were found to be common in all of them. Leaf margin were mostly entire but type and colour varied. Like stem colour leaf colours were green, dark green and light green. Three types of ovary shape were observed and these were oval, cylindrical and spindle. Male flower was found to be bigger in size compared to that of female. In case of fruit characters their colour were found to be green, dark green and light green with different patterns of stripes. The fruit shapes were three types viz. oval, cylindrical and spindle.

Twelve characters were used for determining the genotypic diversity among eighteen varieties/lines of *T. dioica*. In the metroglyph analysis two most variable characters i.e., stem length and leaf area were used for determining X and Y axis, respectively and thus, for construction of metroglyph pattern. There were three clusters comprising of one or more varieties/lines out of eighteen which were studied. Cluster I was represented by only two varieties/lines namely PG008 and PG023. Cluster II was formed by three varieties/lines while cluster III was found to be formed with the maximum number (thirteen) of varieties/lines. The ray's pattern on the glyph among the clusters revealed a marked variation for the presence or absence of rays and mostly inclined rays.

Interphase nuclear phenotype of all the varieties/lines of *T. dioica* were studied in present investigation. Interphase chromosome volume (ICV) was measured. The range of ICV was from 0.111 to 0.356μ^3 in KALI BOMBAY and PG023, respectively. Interphase nuclear structure was found to be chromocentric. Number of chromocentre ranged from 13.23 to 18.04 and they were found in PG012 and PG005, respectively. Heterochromatin% was also measured and they ranged from 12.12 to 19.98 in BARI 1 and PG019, respectively.

To identify the chromosomes through somatic karyotype, shoot tips of all the varieties/lines of *T. dioica* were collected. Shoot tips of 1.5cm length were appropriate for obtaining maximum number of metaphase plates. At least three well spread metaphase plates were observed for this investigation. Somatic chromosomes were measured from photomicrographs. For making quantitative karyotypic analysis the

method proposed by Ahmed *et. al*. (1983) was adopted considering the bases of scatter diagram of total chromatin lengths (TCL) and arm ratios (AR) of all chromosomes in a number of cells. The cells with well spread metaphase chromosomes having more or less distinct morphology are presented. Lengths and ratios of representative complement of shoot tip chromosomes in eighteen varieties/lines of *T. dioica* were measured. Data were taken at the desirable stage for each of the three cells and were plotted in separate scatter diagrams. The average values of total length and arm ratio were calculated constituting the haploid complement of that cell. Then the chromosomes of haploid complements were numbered in decreasing order of total chromatin length and increasing order of arm ratio within the same length. The chromosomes were distributed to the various morphological categories using also probabilistic inferences, specially on the chromosome frequency in a given class per haploid set (A=Large, B=Medium, C=relatively short and D=short). The morphological features of the haploid complement in the varieties/lines of *T. dioica* have been presented in the result chapter. The standard karyotypes were proposed for *T. dioica* (eighteen varieties/lines) on the basis of centromeric formula along with range and average of chromatin length per chromosome. Data on chromosome morphology, i.e., length, arm ratio, TCL%, TF% and chromosome type were also determined.

The present cytological investigation revealed the presence of a heteromorphic pair of sex chromosome (assumed) in male plants. Diploid male was, therefore, supposed to be heterogametic with 20+XY and the females were homogametic with 20+XX. The arm ratio was 0.93 in X and 1.00 was in Y chromosome. The total length of X chromosome in MALE 1 was recorded as 2.46µm and Y chromosome was 2.54µm. The total chromatin length (TCL) ranged from 25.03µm to 41.80µm and they were found in PG020 and BARI 2, respectively. The X chromosomes (sex regulatory) were found as different pairs in the different varieties/lines separately. In all the female varieties/lines of pointed gourd all the chromosome pairs were found to be very much homomorphic and so called sex chromosomes were assumed to be carrier of sex controlled genes in functional state.

Preliminary phytochemical analysis of metabolic extracts of leaves of pointed gourd (*Trichosanthes dioica*) revealed the presence of alkaloids, flavonoids, glycosides, steroids and tannins as constituents in extracts. The intensity in terms of concentration of the phytochemicals were marked as low, medium and high.

A collection of eighteen varieties/lines of *T. dioica* were studied for their chlorophyll-a, b and sugar content in leaves and also studied for quantitative estimation of antioxidant capacity (%), phenol, proline and protein in fruits of all the varieties. The maximum value of chlorophyll-a was recorded in PG003 and the minimum value was recorded in

PG011, having 0.366 and 0.056. On the other hand, the value of chlorophyll-b ranged from 0.021 to 0.234 and they were found in PG020 and MALE 1, respectively. Total chlorophyll (a + b) was found in PG003 with highest value and the lowest was found in PG011. The highest value of sugar content was found in PG023 and the lowest value was recorded in PG028. The highest value of antioxidant capacity (%) was measured in PG019 while the lowest value was found in PG006. It was observed that the highest value of phenol was recorded in PG012 and the lowest value was obtained by PG003. Estimation of proline in fruits of the varieties/lines of *T. dioica* were determined. The highest value of proline was found in PG020 and the lowest value was recorded in PG003. The highest value of protein was measured in PG020 while the lowest value was found in PG018.

A total of ten varieties/lines lines of *T. dioica* were studied for their protein banding pattern. Polyacrylamide gel electrophoresis was used for determining their protein profiles. Photograph of stained gels showed bands at different Rf values. Protein banding pattern showed variations in number and intensity of bands in different varieties/lines of *T. dioica*. A total of 47 bands were observed. The types of bands were decided on the basis of their colour and intensity, like thin and distinct, diffused, and thick and dense. Similarity was considered as the number of pairs of similar bands on the basis of their Rf values in the varieties/lines and dissimilarity was also indicated by a number of different kinds of bands. Similarity index was calculated in all possible ways which varied among the varieties/lines. Highest similarity index between BARI 1 and BARI 2 was found to be 80.00% and the lowest similarity index (14.28%) was found between BARI 1 and PG006.

CHAPTER SIX

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