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Quantitative Karyotype Analysis of *Lycopersicon esculentum* cv. Oxheart

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Abstract: The chromosomes were identified individually and standard karyotype of *Lycopersicon esculentum* cv. Oxheart was formulated. Roots were collected from germinating seeds and were pre-treated in a saturated aqueous solution of monobromonaphthalene. After iron-alum mordanting, standard hematoxylin method was followed. 2n = 24 chromosomes were found. Karyotype analysis was carried out following a quantitative method using scatter diagram technique. Only two chromosomes were identified individually and the rest were morphologically characterized. One of the two identified chromosomes had mean total length 3.1µ and mean arm ratio 1.3 whereas, the other one had mean total length 2.5µ and mean arm ratio 2.0. The length of the prometaphase chromosomes ranged from 1.3 to 3.1µ. The karyotype of the studied tomato genotype consists of 8 metacentric and 4 sub-metacentric chromosomes. One pair of satellited chromosomes was also observed.

Key words: *Lycopersicon esculentum* cv. Oxheart, karyotype, quantitative approach

Introduction

Chromosome study is required for genetic improvement of any material. Chromosome analysis involves the characterization of individual chromosomes by their morphological characteristics such as length, centromere location, presence or absence of satellites and number of chromosomes. A detailed study of a set of chromosomes of any material revealing the various chromosomal characteristics indicates its' karyotype. The karyotypic information i.e. the morphology of chromosomes of a species is necessary for a full understanding in plant genetic studies as well as the plant improvement. The size and shape of the chromosomes are important in determining the gross genetic homology between related species of plants or animals. Endo and Gill (1984) postulated that chromosome size and arm ratio data from meiosis cannot be reliably used for identification of somatic chromosomes. Most of the previous works towards determination of karyotypic composition were made from single cell observation. Hence, it was felt important that quantitative approach of karyotype formulation may prove more reliable than single cell observation method (Shahid and Kabir, 1999). Ahmad *et al.* (1983) employed this method to analyze the soybean karyotype.

Tomato has small chromosomes which possess technical difficulties. The chromosome number in tomato is moderately high and their size is considerably small. Therefore, various kinds of technical difficulties are encountered in cytological preparations in the present material. The present research work was carried out to formulate standard karyotype of *L. esculentum* cv.Oxheart by a quantitative approach.

Materials and Methods

The experiments pertaining to the present investigation were carried out in the laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh during 1998-1999.

The tomato genotype, *Lycopersicon esculentum* cv. Oxheart was used for karyotype analysis of root-tip cells at prometaphase. When the young and healthy first-roots (radicles) were about 1 cm long, then the radicles along with the seeds were treated with saturated aqueous solution of monobromonaphthalene for 35 minutes. Occasional shaking was done for uniform treatment of the roots. Then roots were washed thoroughly under running water and fixed in acetic-alcohol (1:3) for at least 15 minutes. Slides were prepared by standard hematoxylin method (Shahid and Kabir, 1999).

Photomicrographs from five cells were made by an Olympus Photomicrography system using Kodak Photomicrographic film and a high contrast developer. Photographic prints were made at a magnification of 2000X.

Five cells of a satisfactory quality were included for the analysis. Photographs were used in determining the position of the centromere. Measurements in millimeters of the chromosome prints were converted to actual lengths in microns (µ). Arm ratios were calculated by dividing the length of the long arm by that of short arm and the conventions proposed by Levan *et al.* (1964) were used to refer different chromosomes. The standard karyotype of the tomato cultivar Oxheart was proposed according to the allocation of the identified and unidentified chromosomes following the schedule proposed by Ahmad *et al.* (1983).

Scatter diagram was prepared for each cell to determine the homologous pairs of chromosomes and a combined scatter diagram was prepared for chromosome identification. Karyotype analysis was performed following the steps listed below.

Preparation of scatter diagram: Scatter diagram was prepared incorporating the total lengths and arm ratios of all chromosomes in each cell. Two nearly proximiting points were considered as a pair of homologous chromosomes and they were circled.

Derivation of haploid values: Averages of the lengths and arm ratios of each pair of chromosomes constituted the haploid complements of that cell.

Combined scatter diagram was prepared incorporating haploid lengths and haploid arm ratios of the 12 haploid chromosome values from each of five cells of *L. esculentum* cv. Oxheart.

Corresponding chromosomes in five haploid complements of present material were determined through a grouping technique applied to a combined scatter diagram based on chromatin length and arm ratio. Thus, appearance of the group of two, three or five scatters indicated the distinct and individually identifiable chromosomes. Then the unidentified chromosomes were characterized on a probabilistic inference.

Results and Discussion

General considerations: Cells in which the chromosomes were in a satisfactory state for analysis, occurred rarely. Five plates for prometaphase chromosomes of *L. esculentum* cv. Oxheart were included for karyotype analysis; one of these five plates is shown in Fig. 1. Chromosome number was always found to be 2n = 24.

Conceptual basis of the method and of "standard morphology": Cells with well spread metaphase chromosomes having distinct morphology were selected for study. Information about morphological properties of the chromosomes of the complement are required for establishing the standard karyotype of a species. Since the morphology of a chromosome is altered by contraction, measurements of that chromosome in different cells may not be directly comparable. Thus the mean length and mean arm ratio of that chromosome over a series of cells subjected to similar cytological treatment may represent the best estimate of its "standard morphology". On this basis, two propositions were considered for present method of karyotype analysis. First, in a

Table 1: Mean lengths and mean arm ratios of the two identified chromosomes of *L. esculentum* cv. Oxheart

Chromosome name	Length		Arm ratio	
	x ± S.E. μ	Coefficient of variation of the data sample (%)	x ± S.E.	Coefficient of variation of the data sample (%)
m_1	3.1 ± 0.15	10.6	1.3 ± 0.07	12.6
sm_1	2.5 ± 0.15	13.7	2.0 ± 0.19	21.7

Table 2: Proportion of the complement total length occupied by the two identified chromosomes in present material

Cell	Haploid total length (μ)	Total lengths of the two identified chromosomes (μ)	Proportion of the haploid total length occupied by the 2 chromosomes (%)	Mean (%)
A	25.4	5.9	23.2	
B	25.3	5.7	22.4	
C	22.5	5.1	22.3	22.7
D	25.5	5.8	22.7	
E	24.2	5.5	22.9	

Table 3: The allocation of unidentified chromosomes in *L. esculentum* cv. Oxheart karyotype to different morphological categories

Length (x) classes (μ)	Arm ratio (y) classes	Total No. of chromosomes in five haploid sets	Mean No. of chromosomes per haploid set	No. of identified chromosomes with names	Proposed No. of unidentified chromosomes	Total No. of chromosomes	Assigned chromosomes number
2.5 ≤ x	y< 1.7	7	1.4	1 (m_1)	nil	1	1
,,	1.7≤y< 3.0	2	0.4				
,,	3.0≤y						
2.3≤x < 2.5	y< 1.7	1	0.2				
,,	1.7≤y< 3.0			1(sm_1)	nil	1	2
,,	3.0≤y						
2.1≤x < 2.3	y< 1.7	4	0.8		1		
,,	1.7≤y< 3.0	5	1		1	1	3
,,	3.0≤y					1	4
1.9≤x < 2.1	y< 1.7	11	2.2		2	2	5,6
,,	1.7 ≤ y< 3.0	9	1.8		1	1	7
,,	3.0≤y						
1.7≤x < 1.9	y < 1.7	13	2.6		3	3	8, 9, 10
,,	1.7≤ y < 3.0	5	1		1	1	11
,,	3.0≤y						
x < 1.7	y < 1.7	2	0.4		1	1	12
,,	1.7≤y< 3.0	1	0.2				
,,	3.0≤y						
Column totals		60	12		10		

Table 4: Morphological features of the proposed standard karyotype in *L. esculentum* cv. Oxheart.

Chromosome No.	Name of identified chromosome	Total length (x) μ	Arm ratio (y)	Chromosome type
1	m_1	3.1	1.3	m
2	sm_1	2.5	1.5	sm
3		2.1≤ x < 2.3	y< 1.7	m
4		2.1≤x< 2.3	1.7≤ y< 3.0	sm
5		1.9≤x< 2.1	y< 1.7	m
6		1.9≤x< 2.1	y< 1.7	m
7		1.9≤x< 2.1	1.7≤ y< 3.0	sm
8		1.7≤x< 1.9	y< 1.7	m
9		1.7≤x< 1.9	y< 1.7	m
10		1.7≤x< 1.9	y< 1.7	m
11		1.7≤x< 1.9	1.7≤ y< 3.0	sm
12		x< 1.7	y< 1.7	m
				8m + 4 sm

scatter diagram of total length and arm ratios of all chromosomes of five cells, the points produced by the same chromosome were clustered in a specific region. The mean of these points were considered to constitute the standard morphology of that chromosome.

Secondly, two nonhomologous chromosomes were identifiable individually on morphological basis in a combined scatter diagram, if the mean location of one chromosome occurred no less than one standard deviation away from that of the adjacent one, with respect to either total length or arm ratio. If such a difference did not exist, these two chromosomes would not be identifiable individually, unless any particular marker features such as a satellite exist on one of them. The chromosomes which were not identifiable individually could be assigned to different morphological categories on a probability basis.

Derivation of haploid values: A scatter diagram was prepared for each cell incorporating lengths and arm ratios of 24 chromosomes. Each chromosomes and its corresponding point on the diagram was numbered. The chromosomes were paired by circling the corresponding points on the scatter diagram on the basis of proximity of two points. The 12 pairs of points were considered to represent homologous chromosome pairs. Averages of the lengths and arm ratios of each pair of chromosomes constituted the haploid complement of that cell. The process was repeated for each for the five cells under study. Chromosome pairs were then numbered from 1 to 12 within each cell approximately, but not strictly, in the increasing order of length and arm ratio.

The comparability of measurements of chromosomes in the five cells under study was determined by examining the variation in haploid total lengths of the cells analyzed. The values ranged from

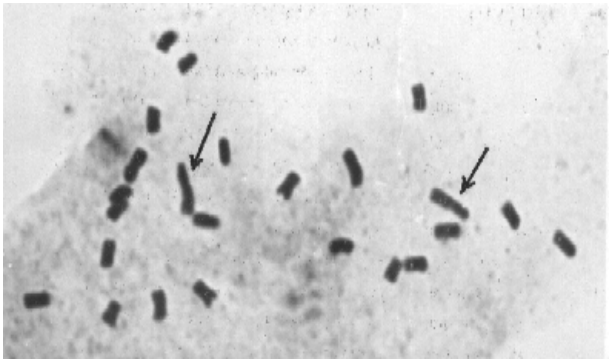

Fig. 1: Representative root tip complement of *L. esculentum* included for karyotype analysis. Arrow indicates satellite chromosomes. This particular cell was considered as Plate A

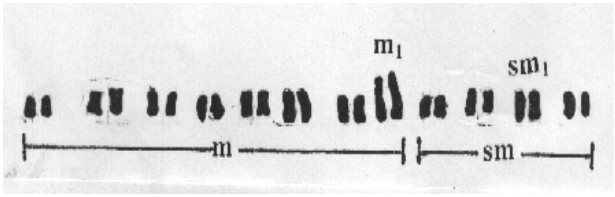

Fig. 2: Karyogram of *L. esculentum* cv. Oxheart constructed from plate A, Fig. 1. Identified chromosome pairs are indicated by specific names (m1, sm1). The m1 chromosome pair is the satellite one. [Abbreviations: metacentric (m), submetacentric (sm)].

22.5 to 25.5μ with a mean of 24.6 ± 1.3 and a coefficient of variation of 5.1% (Table 2) which indicated the proper selection of cells for photomicrography and precision in taking measurement. Thus there was, on average, a similar degree of contraction of the chromosomes in five cells.

Individual chromosome identification: Corresponding chromosomes in different haploid complements were then determined through a grouping technique applied to the combined scatter diagram of the five haploid complements involving 60 chromosomes. The data used were the original haploid values for arm ratio and chromosome length values.

Ideally, if the morphology of all chromosome pairs was distinct and reproducible across the cells, the five points representing the haploid homologues of each chromosome should cluster closely, and 12 such clusters should be recognizable. Where the morphology of non-homologous was not distinct, their clusters would be expected to overlap and lack of reproducibility of morphology for a chromosome in different cells would result in diffused clusters. Regardless, each cluster must contain one point (chromosome) from each cell studied, in haploid complement.

Actually, clear groups existed for only two sets of five points. It was not possible to delineate groups within the remainder of the scatter diagram because of close positioning of many points, presumably indicating superimposition or substantial overlap of the remaining 10 conceptual groups. These two chromosomes fall in the category of individually identifiable ones. One of the two identified chromosomes were of "m" and another one of "sm" type. These two chromosomes were named by "m_1" and "sm_1" respectively. The two identified chromosomes occupied *ca.* 22.7% of the haploid complement total length (Table 2). One of these two identified chromosomes had the mean total length 3.1μ and arm ratio 1.3. The other one had the mean total length 2.5μ and arm ratio 2.0 (Table 1).

A representative karyogram was constructed from the photographs of the chromosomes of plate shown in Fig. 1 (Fig. 2). Following the classification of Levan *et al.* (1964) chromosome pairs in the karyogram were grouped into "m" (metacentric) and "sm"(submetacentric) types, there being no "st"(subtelocentric) and "t" (telocentric) types. The chromosomes are arranged in the decreasing order of length within each type. The identified chromosomes are indicated in karyogram.

Allocation of unidentified chromosomes: All haploid chromosomes were classified according to length and arm ratio (Table 3), using total length of the chromosome. A class interval of 0.2μ for length and the ranges for arm ratio recommended by Levan *et al.* (1964) was used. This classification was superimposed on the combined scatter diagram of the haploid complements as a grid of length and arm ratio classes.

The unidentified chromosomes were distributed to the various morphological classes using probabilistic inferences based on the frequency of chromosomes in a given class per haploid set (Column 4, Table 3), occurrence of points in the combined scatter diagram and the original data on length and arm ratio of the chromosomes. The number of unidentified chromosomes allocated to the various morphological classes is given in column 6, Table 3. Finally, all 12 chromosomes in the haploid complement were numbered from 1 to 12 in decreasing order of length following Rhoades (1955), and increasing order of arm ratio within a length class (column 8, Table 3). Each chromosome was allocated a serial identification number, and each of the two chromosomes identified individually also carried a specific name based on its arm ratio (Table 1). The positions of the chromosomes are determined in the combined scatter diagram in a two dimensional array using grouping technique based on mean value for length and arm ratio for the identified chromosomes and arbitrarily within the relevant class boundaries for the unidentified ones.

Satellited chromosomes: Chromosome with a visible satellite could be seen in this material only in occasional cells. Usually only one, rarely two and never more than two chromosomes with satellites were detected in any cell.

Standard karyotype: The morphological features of the chromosomes are summarized in Table 4. The proposed standard karyotype consists of 8 "m" and 4 "sm" chromosomes.

The identification of chromosomes and their homologues become complicated by the conventional method of karyotypic analysis because of variation in arm ratio and total length between and within the cells. Particularly when more than one pair of chromosomes have similar length and arm ratio. Karyotype analysis by conventional methods becomes not much reliable. To avoid such problems a quantitative method of karyotype analysis (Ahmad *et al.*, 1983) was applied to identify the chromosomes individually and their homologues in *L. esculentum* cv. Oxheart. Although many cells had well spread chromosomes in the present preparations, however only a few of them had a moderate degree of contraction rendering suitable for measurements. Only the best five cells were selected for analysis. Thereby the individual chromosome of a cell was found to be well distributed in the scatter diagram and haploid values of the chromosomes could be determined easily.

The diploid chromosome number in *L. esculentum* cv. Oxheart was 2n = 24. Similar results were obtained by Lesley and Lesley (1935). Large satellite present in the chromosome of this cultivar. Lesley and Lesley (1935) reported that karyotype of the species of tomato differed in the size of the satellite of the nucleolus organizing chromosome.

The identified chromosomes occupied *ca.* 23% of total complement length of this cultivar. Those chromosomes which could not be identified individually were characterized through probabilistic inferences. The karyotypic formula for this cultivar

was found to be 8m + 4 sm. Presence of more than 50% metacentric chromosome in this cultivar indicated a symmetrical karyotype according to the karyotype classification proposed by Stebbins (1958).

The chromosomes were identified individually through applying a grouping technique. Two chromosomes could be identified individually and remaining ten chromosomes were characterized morphologically through probabilistic inferences. One of these two identified chromosomes had the mean total length 3.1µ and mean arm ratio 1.3. The other one had the mean total length 2.5µ and mean arm ratio 2.0.

The karyotypic composition for present material is 8m + 4sm chromosomes. More than 50 percent metacentric chromosome was found in this genotype, which denoted a symmetrical karyotype. A standard karyotype of the tomato cultivar Oxheart has been formulated.

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