REVIEW ARTICLE *Taeckholmia 41 (2021): 70-90*

Chromosomes as Sources of Taxonomic Information for Plant Systematics and Evolution

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Abstract

Chromosomes are the units of inheritance within the nuclei of all eukaryote cells and carry the genes as DNA packaged in the chromosomes forming the chromatin fibril with histone proteins. The DNA content of each chromosome is a single linear DNA double helix, which corresponds genetically to a linkage group. The chromosomes are only seen during cell division because of coiling up as chromatin in the non-dividing nucleus. In somatic cells, chromosomes occur as pairs of homologous chromosomes or homologs. During cell division, the chromosomes, condense into individual chromosomes during the stages of division (prophase, metaphase, anaphase, and telophase). The chromosomes are best studied at metaphase in somatic cells as each chromosome appears as two longitudinal chromatids. Each chromosome is composed of two arms separated by the centromere and terminated at the ends by telomeres. One member of each homologous chromosome is inherited from either the male or female plants, these homologs pair as bivalents in the first meiotic prophase. In about 45% of plants, more than two sets of chromosomes have been reported, these are known as polyploid numbers. The chromosome features that have been used as sources of taxonomic information include various attributes of chromosomes; the most common features are: 1) chromosome number, 2) chromosome size and gross morphology, 3) chromosomal variations in number and shape, 4) chromosome's behavior at meiosis, 5) chromosome banding as revealed by differential staining of some chromosome parts and 6) in situ hybridization and chromosome painting to locate genes on chromosomes. A special role is claimed for chromosomal data in taxonomy since chromosomes are the seats of genetic information that guides plant species evolution. The recent molecular cytogenetics approaches are producing a wealth of new data of enormous taxonomic and evolutionary importance, that must be fully integrated with traditional taxonomic data. In this article, the taxonomic attributes of chromosomes are described and examples of their role of chromosomes in flowering plant systematics and evolution are outlined.

Keywords: Chromosomes. Karyotype, Plants, Phylogeny, Systematics, Taxonomy

Introduction

The number of flowering plants (angiosperms) as reported by the Angiosperm Phylogeny Group in 2009 (APG III, 2009) comprised an estimated number of 352,000 species subdivided in 14,559 genera and 405 families (Bremer et al., 2009). In the angiosperm orders and family's classification in the APG IV report in 2016, the member of orders was 64 and the number of families reached 416 (Chase et al., 2016). From an evolutionary perspective, the high level of variability of angiosperms indicates that they have undergone extensive and progressive diversification and adaptive radiation, generating new species adapted to diverse environments in different ecosystems (Soltis and Soltis, 2004; Agrawal et al., 2009). Heslop-Harrison and Schwarzacher (2011) reviewed the evolutionary structure and function of the plant genome and its organization in chromosomes and concluded that plant genomes are remarkably diverse. Plant genomes consist of mostly coding and regulatory DNA sequences present in low copy number, along with highly repeated rDNA (rRNA genes and intergenic spacers), centromeric and telomeric repetitive DNA and transposable elements which is widespread mobile genes (Heslop-Harrison and Schwarzacher, 2011).

Plant speciation and diversification have relied strongly on changes in the nuclear genome, both at the whole ploidy level and at individual chromosome level. Despite this huge range, structural features of chromosomes like centromeres, telomeres and chromatin packaging are well-conserved (Heslop-Harrison and Schwarzacher, 2011). Chromosomes have evolved by fission, fusion, duplication, and insertion events,

Received on 14/05/2022 and accepted on 02/06/2022

allowing evolution of chromosome size and chromosome number. These include major genome alterations, such as whole genome duplication and adaptations that comprised the concomitant or independent occurrence of chromosome numerical and structural changes, which had induced instability and variation that act as driver for genome evolution and adaptation (Guerra; 2008; De Storme and Mason, 2014).

1. Chromosomes Division and Transmission

The word chromosome is derived from the two Greek words "chromos" meaning color and "soma" which means body, i.e., the chromosome is a colored body. Chromosomes can be seen after fixing the cells followed by staining with dyes specific to the DNA like the Feulgen reagent or other stains that adsorb to the chromatin. For methods and information on handling of the chromosomes, see Darlington and LaCour (1976), Fukui and Nakayama (1996). The chromosomes are only seen during cell division because of coiling up the chromatic material in the nucleus. During cell division, the chromosomes, seen as chromatin in the nondividing nucleus, condenses into individual chromosomes during the stages of mitosis.

2.1. Mitosis

Mitosis is the process of cell division in somatic cells in eukaryotic organisms. It is an asexual process by which the daughter cells receive equal and identical number of chromosomes. Since the chromosomes are the carriers of genes, the daughter cells are virtually assured of receiving equal and identical copies of all the genes of the parent cell. In sexually reproducing organisms' mitosis is responsible for the delivery of the genes of parents in the zygote to every somatic cell of the body of the offspring thus making all cells of the organism identical in genetic content. In asexually reproducing organisms, mitosis is responsible for the delivery of the genes to all somatic cells and ensure the transfer of genetic information to successive generations.

Overview of mitosis

- Mitosis is characteristic cell division of all growing tissues in all eukaryotes
- By this process a cell nucleus divide precisely into two equivalent daughter nuclei in karyokinesis followed by the division of the cytoplasm (cytokinesis).
- The process of mitosis serves the duplication of genes present on the chromosomes
- Duplication of genes takes place before the mitosis i.e., in the interphase stage
- The duplication of the chromosomes during the interphase stage is the result of duplication of the DNA the main chemical constituent of the chromosomes. DNA is the substance of the genes and ensures their transmission from parents to offspring.

During mitosis, the duplicated chromosome number of the parent cell is halved in each daughter cell that contains the same number of chromosomes so that the original diploid number is preserved. The stages of mitosis are shown in Fig. 1.

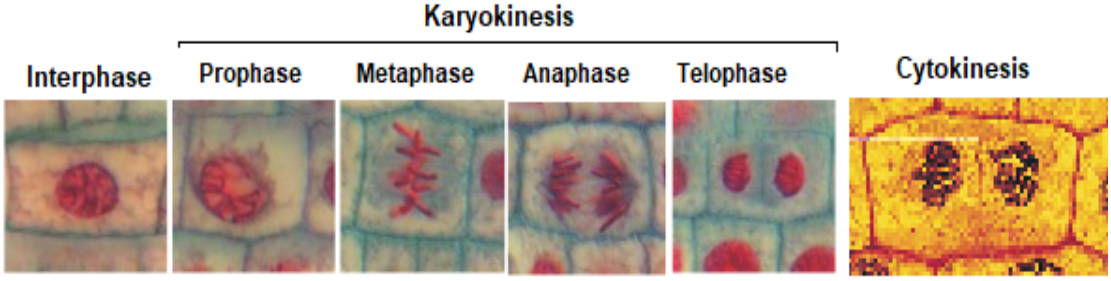

Fig. 1: Photographs illustrating the stages of mitotic stages of mitosis preceded by interphase and followed by cytokinesis

In the diploid cells of eukaryotes, chromosomes occur as pairs of homologous chromosomes or homologs. One member of each homologous pair is inherited from either the male or female parent, these homologs pair as bivalents in the first meiotic prophase (Fig. 2).

2.2. DNA packaging in the chromosome

The chromatin is a viscous, gelatinous substance that contains DNA, RNA, basic proteins called histones, and non-histone (more acidic) proteins. The acidic proteins, which are found in a rather loose association with the DNA and are involved in the maintenance of a higher-order folding of the chromatin and in the forming of the scaffold of the chromosome that hold sister chromatids together. Histones are small proteins that are basic because they have a high content (10-20%) of the basic amino acids, arginine, and lysine. Being basic, histones bind tightly to DNA, which is acid. The DNA strands and the histone proteins exist as a fibril of repeating units called nucleosomes (Kornberg 1974). Duplicates of four main histone proteins, H2A, H2B, H3 and H4, are associated in octamers that contain two of each of them to form the nucleosome core. These histones are very similar in all living eukaryotes and are among the most

conserved proteins of the cell. A piece of DNA that is about 140 bp is coiled on the outside of the octamer histones. Another histone called H1 is not conserved between species and has tissue-specific forms, links adjacent nucleosomes to each other and is associated with about 80 bp of DNA. Thus, every nucleosome involves about 200 bp of DNA. The nucleosome fibril is folded in a specific manner to form a solenoid that is about 30 nm wide, this solenoid is folded further to form a 300 nm filament that is coiled again to form the 600 nm, which is about the width of the chromatid (Fig. 2).

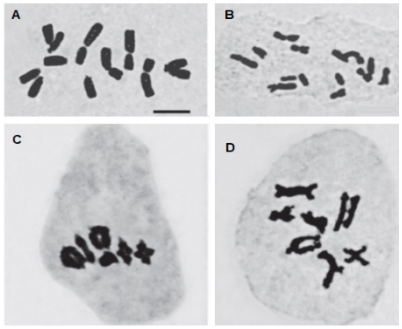

Fig. 2. Photographs of somatic chromosomes of (A), *Callisia repens* (2n = 2x = 12) and (B), *Callisia monandra* (2n = 2x = 14). And of (C), the pollen mother cells with meiotic chromosomes of *Callisia repens*, metaphase I with 6 bivalents and (D) *Callisia monandra*, diakinesis with 7 bivalents. (Scale bar = 5 µm). Grabiele et al. (2015).

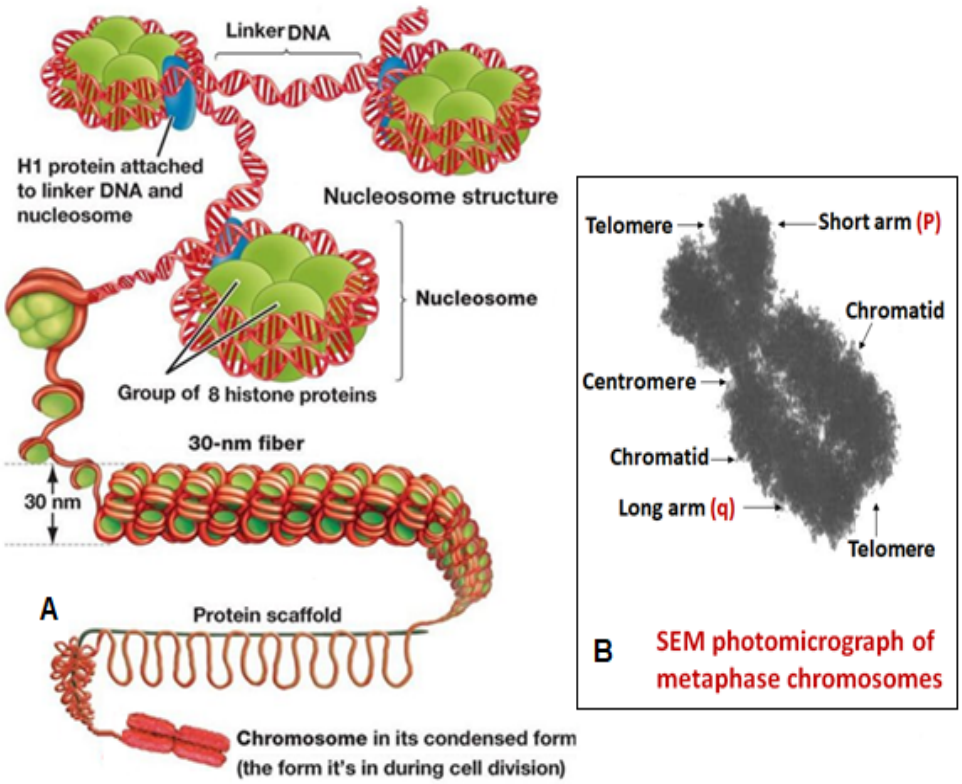

Fig. 3: Diagram of the nucleosome model for packaging of DNA in the chromosome (A) and scanning electron microscope of the metaphase chromosome (B).

1.3. Meiosis

Meiosis is the process of cell division which occur in sexually reproducing organism to produce specialized male and female gametes. In meiosis a diploid cell undergoes two nuclear divisions but only one division of chromosomes, thus because of meiosis the chromosome number is halved. From each diploid cell, meiosis results in four haploid cells which are the gametes in most eukaryotic cells. In plants this process occurs in the anthers and embryo sacs. The first division of the nucleus is called meiosis I (M1) and the second division is called meiosis II (MII). For convenience each of these divisions has been divided into prophase,

metaphase, anaphase, and telophase. The prophase of the first meiotic division is the most complex,

protracted and genetically significant period and therefore has been subdivided into substages (Fig. 4).

Overview of meiosis

- Meiosis keeps the chromosome number constant across generations.
- The two phases of meiosis are designated by roman numerals.
- Ensure that each gamete contains only one member of each homologous pair.
- Produce new combinations of genes through crossing over.
- Meiosis involves two nuclear divisions; it produces four haploid daughter cells, each containing half the number of diploid chromosomes of the parent nucleus.

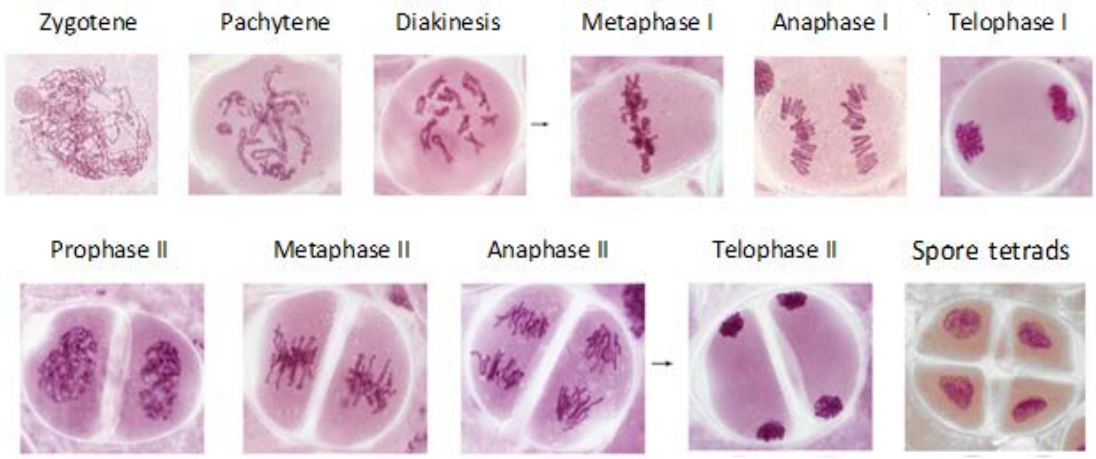

Fig. 4: Photomicrographs of the stages of meiosis in pollen mother cells that leads to the formation of four haploid gametes

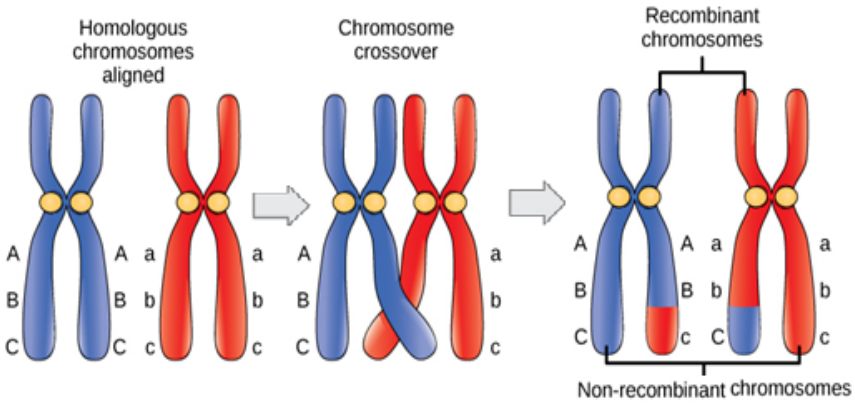

Fig. 5. Diagram illustrating the synapsis of the homologous chromosomes and the crossing over of genes in two non-sister chromatids resulting in the production of recombinant chromosomes. https://in.pinterest.com/pin/506725395544972757/.

The genetic significance of meiosis

1. Since the genetic material was replicated only once but there have been two divisions, each of the four resulting cells contains only half the original number of chromosomes. The full diploid number is restored by fertilization.
2. The chromosomes derived from each parent are randomly divided between the daughter nuclei. This ensures that no offspring is identical to its parents.
3. During prophase I, crossing over also mixes up the genetic material, ensuring that no offspring will be identical to its parents (Fig. 5).
4. Independent assortment and crossing over constitute genetic recombination, which provides the natural variation necessary for the process of evolution.

3. Chromosomes as Sources of Taxonomic Information

For use as taxonomic source of information, chromosomes at mitotic metaphase following treatment of dividing cells in roots with colchicine or another spindle inhibiting chemical (Darlington and LaCour, 1976; Fukui and Nakayama (1996). These chemicals arrest mitosis at metaphase, shorten the chromosomes by over spinalization and make them scattered in the cell to make possible their counting and description. The application of chromosome features as sources of taxonomic information includes the study of various attributes of chromosomes, the most common features are the following (Moore, 1978; Stace, 2000).

1) Chromosome number
2) Chromosome size and gross morphology
3) Chromosomal variations in number and structure
4) Chromosome's behavior of chromosome at meiosis,
5) Chromosome banding as revealed by differential staining of repetitive sequence of DNA known as heterochromatin.
6) In situ hybridization and chromosome painting to locate genes on chromosomes.

A special role is claimed for chromosomal data in taxonomy since chromosomes are the seats of genetic information that guides plant species evolution. It is well known that a taxonomic character should be genetically controlled to be inherited and maintained across generations (Moore, 1978; Stace, 2000).

3.1. Chromosome number

Chromosome number, as a taxonomic character, is probably one of the most stable features of the species, all individual plants of the same species usually have the same number of chromosomes. Chromosome number can be a plesiomorphic characteristic of a large clade or a recurrent trait which arose independently in two or more clades. Therefore, the same number may be found in taxonomically distant taxa e.g 2*n*=16, has been recorded in most *Allium* species (Alliaceae) and *Trifolium* species (Fabaceae). This number is also found in several other genera from both monocots and dicots. This situation is due to the limited numbers of chromosome numbers found in the vast number of species of flowering plants. For the estimated number of about 352,000 species of flowering plants chromosome counts have been made in about 20% The numbers recorded range between 2*n* = 4 in *Haplopappus gracilis* (Jackson, 1959) to 2n = 260 in *Poa litorosa* (Hair and Beuzenberg, 1961) The plant shape and chromosomes of the two species are shown in Fig. 6.

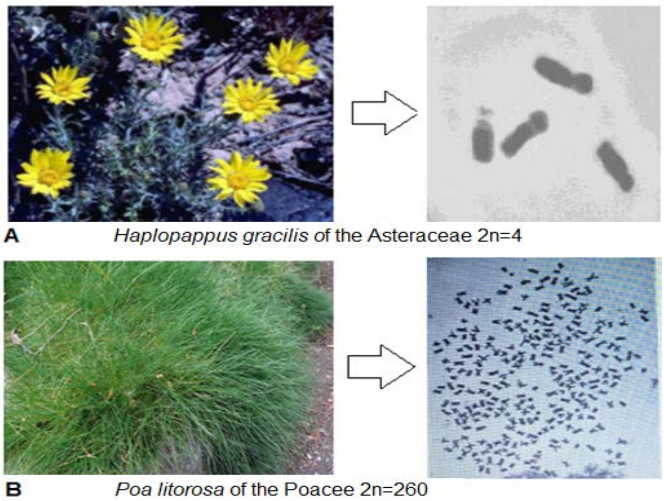

Fig. 6: Photomicrographs of *Haplopappus gracilis* (Asteraceae) and its chromosome number of 2n = 4 (A) and of the *Poa litorosa* (Poaceae) and its 2n = 260 (B).

In cytotaxonomic practice, it is important that voucher specimens should be kept for all plants whose chromosomes have been counted. The persons who identified the material and who counted the chromosomes should also be identified. Apart from their value in allowing the identity of material counted to be checked, voucher specimens may be valuable in allowing taxonomists to find out if any morphological differences are associated with cytological features. However, problems often arise because morphological differences between taxa with different chromosome numbers are often not absolute. In general, infraspecific taxa or populations of the same species differing in chromosome features are often called cytotypes.

3.2. Chromosome size and gross morphology

Chromosomes vary in size, both absolute and relative, shape and volume. In cytotaxonomic studies, it is customary to use chromosome length to describe its size. The chromosome length in plants ranges from

0.5-30µ. The chromosomes within a karyotype may be of similar or different length. The most used aspect of chromosome is the position of centromere. The centromere may be median in metacentric chromosomes, submedian in submetacentric chromosomes, subterminal in acrocentric chromosome or terminal in telocentric chromosomes (Fig. 7). The terminology of centromere position and of chromosome types is based on the arm ratio value according to a procedure proposed by Levan *et al.* (1964) and summarized in Table 1.

Table 1: Symbols used for chromosome types and the corresponding centromere position, arm ration and chromosome type as proposed by Levan *et al.* (1964).

The symbol used	Centromere position	Arm ratio	Chromosome type
M	Median point	1.0	Metacentric
m	Median region	1.0 – 1.7	Metacentric
sm	Sub median region	1.7 – 3.0	Submetacentric
st	Subterminal region	3.0 – 7.0	Sub telocentric
t	Terminal region	> 7.0	Acrocentric
T	Terminal point		Telocentric

Another useful aspect of chromosome morphology is the presence and distribution of secondary constrictions. These constrictions are also called nucleolar organizers (NO) because they contain the DNA coding for proteins involved with the structure of nucleoli and because nucleoli have been observed to be associated with this part of chromosomes especially during the early stages of prophase I of meiosis. Nucleolar organizers may be located near the end of the chromosome separating a small part known as satellite. The recording of satellites is sometimes problematic because they are often variable in appearance and because it is not always possible to obtain consistent results. Nucleolar organizers may also be located at the middle of one chromosome arm next to the centromere. When located adjacent to the centromere, an extended constriction is observed and is called *compound constriction*, which is composed of the centromere and nucleolar organizer (Fig. 7).

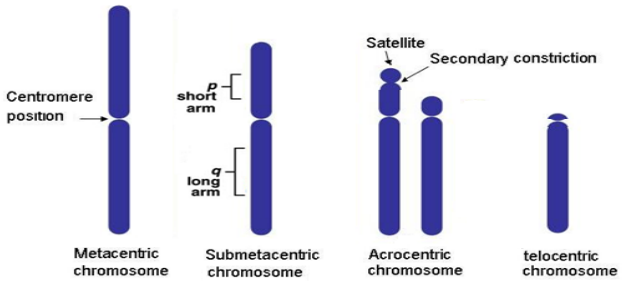

Fig. 7: Diagrammatic illustration of the chromosome types, based on the chromosome arm ratio by dividing the length of the long arm (p) by the length of the short arm (q), centromere position and the presence of secondary constriction and satellite.

3.3. The karyotype

In the classical cytotaxonomic studies the term karyotype (Fig. 8) is applied to describe the appearance of somatic chromosomes at mitotic metaphase. To construct a karyotype, the chromosomes of several C-mitotic metaphase cells is photographed, enlarged to a known magnification and the total length of each chromosome and that of its two arms are measured. Based on these measurements, the chromosomes are arranged in homologous pairs in order of decreasing length and arm ratio values.

3.4. Karyotype symmetry

The process of karyotyping involves the identification, characterization and interpretation of the complete chromosome set of a species or a subspecific rank that may sometimes be termed cytotype. Some workers have devised a few formulas to summarize karyological data. The most widespread form may illustrate the chromosome number and the number of each chromosome type as determined by the arm ratio value. The formula for *Allium cepa*, which has a symmetric karyotype, is 2n = 16 = 14 m + 2sm and for *Allium erdeii* which has an asymmetric karyotype is 2*n* = 16 = 12 m + 2 t + 2T as reported by Badr (1977) and illustrated in Fig. 9. The karyotype is also represented diagrammatically by the basic chromosome set and is then called as idiogram or karyogram (Fig. 8C).

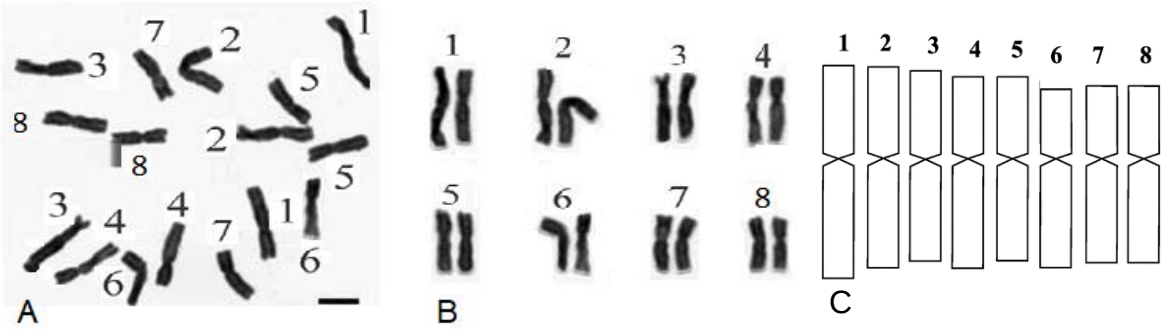

Fig. 8: Photomicrograph of *Allium cepa* chromosomes (A) and the karyotype of the same chromosomes arranged in order of length and arm ratio (B) and the ideogram representing the basic chromosome number (C). Scale bar = 4 μm.

Fig. 9: The karyotype of *Allium subhirsutum* with 2n = 14 and x = 7 (A) and of *Allium erdelii* with 2n = 16 and x = 8 including two telocentric chromosomes (B).

The study of gross morphology of chromosomes over a wide range of plant species showed that within angiosperms, the karyotype may be symmetrical i.e., with little variation between chromosomes in length and arm ratio or asymmetrical with substantial differences among the chromosomes in these two aspects. The value of the mean arm ratio and the values of the standard error of mean chromosome length and mean arm ratio indicate the degree of asymmetry. However, the karyotype symmetry is more frequently determined by calculating the total form percent (TF%) as

$$TF\% = \frac{\text{Total length of short arms} \times 100}{\text{Total length of the chromosome complement}}$$

Perfect symmetry is obtained when TF% is 50 (Sarbhoy, 1980).

The asymmetry of the karyotype may also be based on the relations between the number of chromosome and their length and expressed as the intrachromosomal asymmetry index (A_1) and the inter-chromosomal asymmetry index (A_2) as estimated by Zarco (1986) as follows:

$$A_1 = 1 - \sum pi/qi \div n \quad \text{and} \quad A_2 = S / X$$

The intrachromosomal asymmetry index (A_1) ranges from zero to one. The equation is formulated to obtain lower values when chromosomes tend to metacentric. n_i is the number of homologous chromosome pairs. pi is the average length for short arms in every homologous chromosome pair and qi is the average length for their long arms. On the other hand, for calculating the inter-chromosomal asymmetry index (A2) X = mean chromosome length (MCL) and S is its standard deviation. Two more measures, coefficients of variation (CV) are centromeric index (CV_{CI}) and chromosome length (CV_{CI}) which represent the degree of heterogeneity within a chromosome complement in terms of chromosome length (inter-chromosomal variation) and centromere position (intrachromosomal variation), respectively are often used (Paszko, 2006).

In general, symmetrical karyotype is comprised of metacentric chromosomes of similar length and arm ratio and is considered relatively primitive, while asymmetrical one composed of different types of chromosomes of variable length and arm ration and is more advanced. However, a symmetrical karyotype may arise from asymmetrical one through changes in chromosome structure throughout their evolution and

are responsible for changes karyotype asymmetry and even in aneuploid changes in chromosomes number (Stebbins, 1971; Stace 1980). The karyotype asymmetry index is a good expression of the general morphology of plant chromosomes. It would therefore be advantageous to have a uniform system whereby the karyotypes of related genotypes and species could be compared (Stace, 2000).

3.5. Chromosome variations

Natural chromosomal changes are important for the induction of variation in living organisms and essential for the evolution to proceed. Chromosomal variations are of two types: changes in number and changes in structure; Changes in number involve the addition or loss of complete sets of chromosomes and termed polyploidy or be the addition or deletion of one or few chromosomes and termed aneuploidy Stebbins (1971). Polyploidy is the occurrence of exact multiples of the gametic number of a diploid cytotype or species which is known as the base or basic number (*x*). For example, in the genus *Festuca* (Poaceae) there are species with 2n = 14, 28, 42, 56 and 70. Such numbers are called diploid, tetraploid, hexaploid, octaploid and decaploid respectively. In addition to the *Festuca* example mentioned above, well known polyploidy series is also found in *Allium (Alliaceae)*, *Triticum* (Poaceae) and *Artemisia* (Asteraceae). Polyploidy in angiosperms was described with reference to the genetic insight to the phenomenon by Datta et al. (2016) and its impact on the taxonomy of plants has been addressed by several authors, the most comprehensive may be those written by Leitch and Bennett (1997) and by Guerra (2008).

The genome in the diploid plants may be represented as AA, then the tetraploid derived from them will be designated as AAAA. These tetraploids, and other polyploids which are derived by chromosome doubling and comprised of multiples of the same genome are called autopolyploid or autoploids. Another type of polyploidy is known to arise by chromosome doubling in a diploid plant that is a hybrid between two plants with two unlike genomes (AB), the derived tetraploids are designated as AABB and are called allopolyploids (Fig. 10).

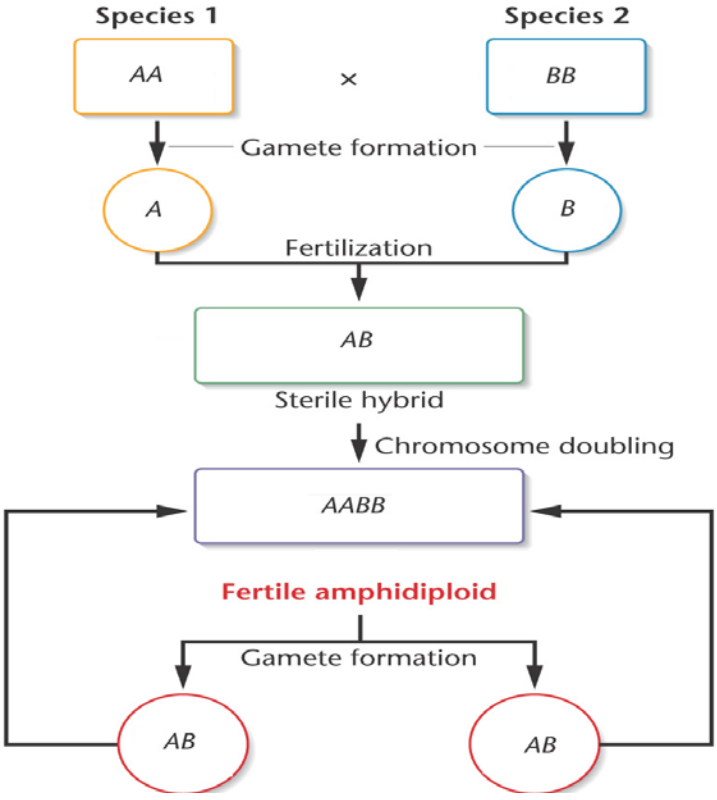

Fig. 10. Diagram illustrating that allopolyploids arise by the doubling of the chromosome number of a F1 hybrid between two different species.

The most known allopolyploid plant is the bread wheat (*Triticum aestivum*), which is a hexa-allopolyploidal with 2n = 42. comprised of three genomes AABBDD. The mitotic chromosomes and the karyotype of the bread wheat are illustrated in Fig. 11.

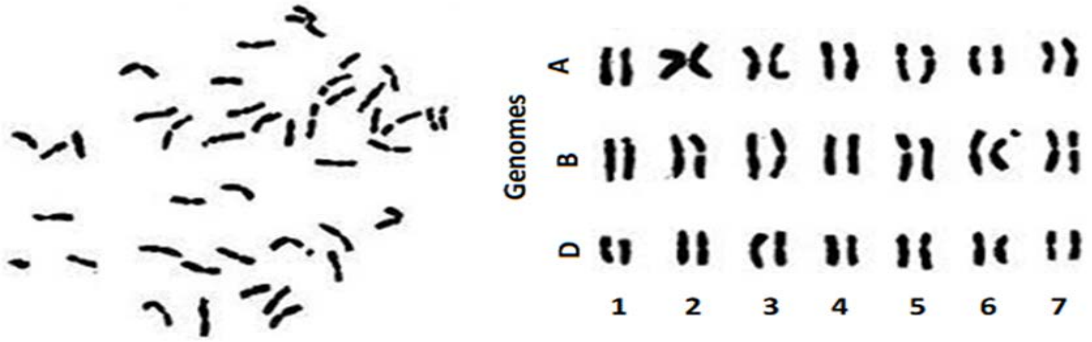

Fig. 11: Photographs of the wheat *Triticum ae*stivum (Poaceae); 42 chromosomes composed of three genomes A, B and D each comprised of 2n = 14.

Changes in the chromosome number may be attained by means other than polyploidy, for example by stepwise increases or decreases by one or few chromosomes. These changes are known as aneuploidy. In aneuploid plants chromosome number varies from the normal diploid or a polyploid level not in multiples of the basic number but by one or few chromosomes. Examples of aneuploidy are found in the genus *Vicia* (Fabaceae) where numbers of 2n = 10, 12, 14, 24 and 28 have been reported and in the genus *Plantago* (Plantaginaceae) with 2n = 8, 10, 12, 16, 20, 25, 30 and 36. The gain of a chromosome is feasible at all ploidy levels with no harmful effect on the plant, however loss of a chromosome in a diploid genome may be lethal, although it can be tolerated more readily at higher ploidy levels because of the buffering effect of multiple genomes. A diploid with one extra chromosome is known as a trisomy (i.e., that chromosome is represented three times) and a diploid with one chromosome missing is known as monosomy. Normal diploids are called disomic where each chromosome in the karyotype is represented twice. Cases where the interrelationships between the different ploidy levels and different taxa at each ploidy level are complicated and are often referred to as ploidy complexes. In these cases, species delimitation is extremely problematic, since ploidy levels in this species are not represented by completely distinctive morphological characters, some taxonomists regard this complex as a single polymorphic species (Badr, 1977).

The types of chromosomal structural changes result from spontaneous chromosome breakage. The type of structural changes, in the chromosomes, depend on the behavior of the broken ends. They may remain broken or rejoin with other ends in the same or different chromosomes. These changes are often recognized as four different types, these are deficiencies (deletions), duplications (additions), inversions (paracentric or pericentric) and translocations (interchanges) (Stace, 2000). Structural chromosomal changes initially occur in a heterozygous condition since chromosomes breaks occur with a rather low frequency (Fig. 12).

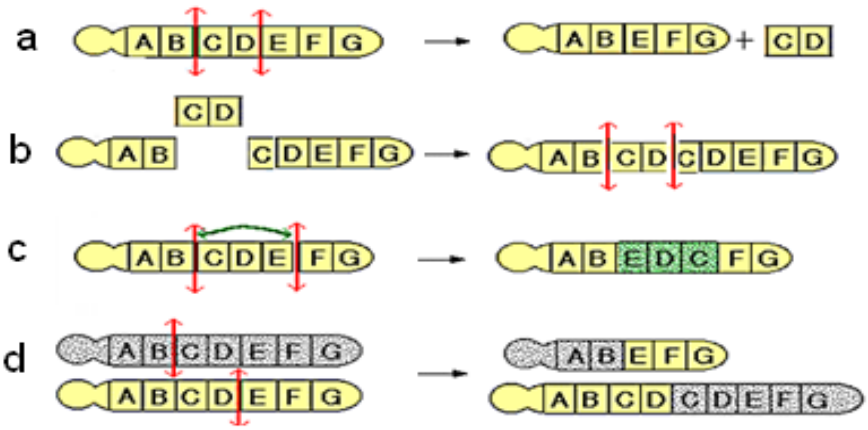

Fig. 12: Diagrammatic illustrations of the four major types of chromosomal structural changes; a = Deletion, b = Duplication, c = Inversion, d = Translocation

3.6. Chromosome behavior at meiosis

The appearance of chromosomes and the way they behave at meiosis are frequently used as means for indicating the relationships of species and below the species level, i.e. subspecies, varieties, ecotypes and populations. Meiosis is studied in the gamete forming sex organs without pretreatment using squash techniques and staining in orcein or carmine. In normal diploid species, two homologous sets of chromosomes are present and pair regularly in bivalents at the pachytene of prophase I but become clearly visible at metaphase I. Chiasma frequency can be used to estimate the homology between the two sets of chromosomes occurring in a hybrid. In polyploid species, where three or more sets of chromosomes are present, pairing analysis could indicate the type of polyploidy involved. In autopolyploids, chromosomes pair in trivalents or quadrivalents depending on the number of homologous chromosomes in the karyotype. In allopolyploid species, chromosomes pair in bivalents because chromosome sets from different parents may fail to pair. The study of meiosis proved the derivation of *Allium trifoliatum* subsp. *trifoliatum* from *A. hirsutum* and *A. trifoliatum* subsp. *hirsutum* and the allopolyploid nature of *A. neapolitanum* in which chromosomes pair in quadrivalents, trivalents, bivalents and some failed to pair at all as illustrated in Fig. 13.

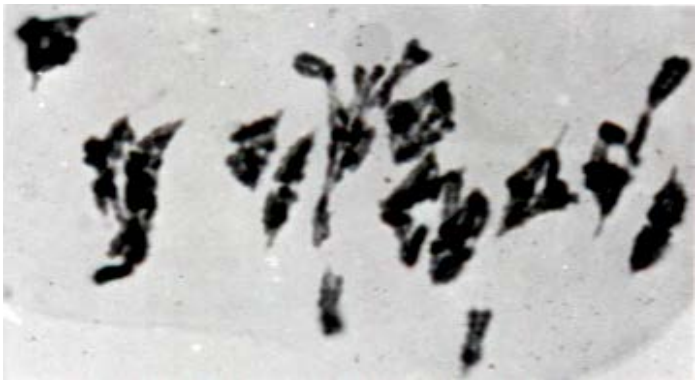

Fig. 13: Chromosome pairing in the pentaploid *Allium neapolitanum* with 2n = 35 and *x* = 7. The configurations indicate that this species is segmental allopolyploid

The study of chromosome behavior at meiosis is important in taxonomic, phylogenetic, and gene-ecological investigations for the following reasons:

1) It may show whether hybridization has occurred through the evolution of a species
2) Indicate structural differences between sets of chromosomes in the karyotype
3) Explain the causes of sterility
4) Suggest the derivation of a species from another

3.7. Chromosome banding

The use of chromosome information in plant taxonomy remained unchanged over some decades until the 1970s, only in a few isolated plants such as *Trillium* species was further differential chromosome segments differentiation possible by cold treatments (Fig. 14) as reported by Bailey (1958). That finding however, enabled the differential staining of heterochromatic regions in chromosomes; a step that had encouraged attempts to reveal structural variation of chromosomes based on identification and distribution of heterochromatin. Chromosome banding, which produce characteristic staining pattern for each chromosome pair made it possible to identify each chromosome in the karyotype and to identify structural rearrangements with much greater accuracy. A band is defined as a part of a chromosome that is clearly distinguished from adjacent parts by virtue of a lighter or darker staining intensity. Bands that stain light by one method may stain dark by another. Chromosome banding can be defined as a lengthwise variation in staining properties along a chromosome, induced by application of a variety of chromosome treatments by specific reagents, dyes, singly or in combination. It refers both to the process of producing banding patterns and to the patterns themselves. The chromosomes are visualized as consisting of a continuous series of light and dark bands (Badr and Elkington 1977).

The banding techniques fall into two fundamental groups, those resulting in bands distribution along the length of whole chromosome, such as Quinacrine and Giemsa bands, which demonstrate patterns of DNA synthesis, and those that stain a restricted number of specific bands or structures, and include methods, which reveal centromeric (constitutive heterochromatin) bands (C-bands), telomeric bands (T- bands), and

nucleolus organizing regions (NOR-bands). Various causes have been reported for occurrence of chromosome bands; these include the presence of repetitive DNA, differences in the composition of DNA, the protein components, and the degree of DNA packing in the chromosomes

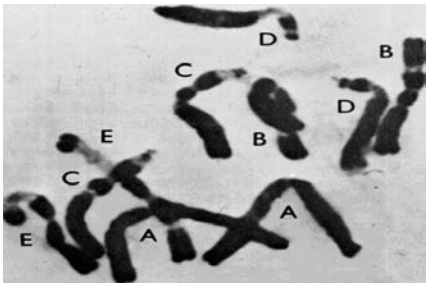

Fig. 14: The chromosomes of *Trillium grandiflorum* as revealed by the Feulgen staining method following cold treatment

Giemsa banding can be induced by several staining protocols, but only one type (C-banding) is of important significance for plant taxonomy. The C-bands are revealed after treating fixed and squashed cytological preparations with an alkali (most often barium hydroxide) for the denaturation of chromosomal DNA followed by incubation in a hot salt solution (2X-SSC) and staining in Giemsa at neutral pH. C-band are generally assumed to represent regions of constitutive heterochromatin and are constant, as implied by the word "constitutive", as they are visible through division and interphase. There is some evidence that C-bands reveal satellite DNA, which is a highly repeated DNA sequences and that alteration in the DNA-protein complex in the chromosomes may be important in producing these bands. There is a consensus that all banding techniques reveals repetitive sequences of DNA, but the type of DNA sequences made visible by these methods vary from one protocol to another. Figure 15 illustrates different banding styles in a *Vicia faba* chromosome one and a *Scilla sibirica* chromosome one after staining with different types of basic dyes, fluorescence dyes and Giemsa protocols. The repetitive sequences of DNA that have been revealed as bands are considered of three classes (Guerra et al. 2000).

a. Tandem arrays which correspond to the densely stained heterochromatin revealed by the banding techniques
b. Dispersed repeat families corresponding to introns and transposons
c. Multigene families where several genes are clustered in complex loci

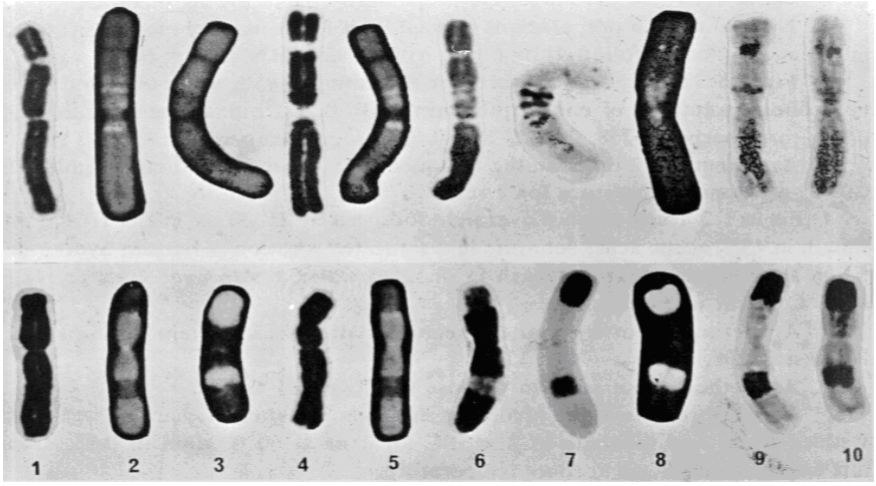

Fig. 15: Above is chromosome one of *Vicia faba* and below is chromosome one of *Scilla sibirica* stained with four types of basic dyes (1&4), and four fluorescence dyes (2, 3, 5 & 6) and two Giemsa protocols (7-10).

Vosa and Marchi (1972) published the first report on fluorescent and Giemsa C-bands in plants. Although both fluorescence and C-bands are assumed to represent heterochromatic chromosomal segments composed of repetitive DNA sequences, C-bands may or may not be coincident with enhanced or reduced fluorescent bands (Fig. 16). Fluorescence banding patterns are revealed by staining of chromosomes with DNA base-specific fluorochromes e.g. quinacrine and its

derivatives, ethidium bromide and commercial dyes e.g. Hoechst 33258, and DAPI and examination using UV light as a source of illumination. Fluorescent regions (bands) revealed are assumed to correspond to A=T rich DNA sequences, but not all A=T rich sequences react strongly to all fluorochromes and some fluorochromes reveal G=C rich DNA sequences (Babu and Verma, 1987). Figure 16 illustrates that the two types of bands coincide to each other in the chromosomes of *Allium flavum* (Alliaceae). However, the C-bands and fluorescent bands may represent several different chromosomal structural situations (Badr and Elkington, 1977).

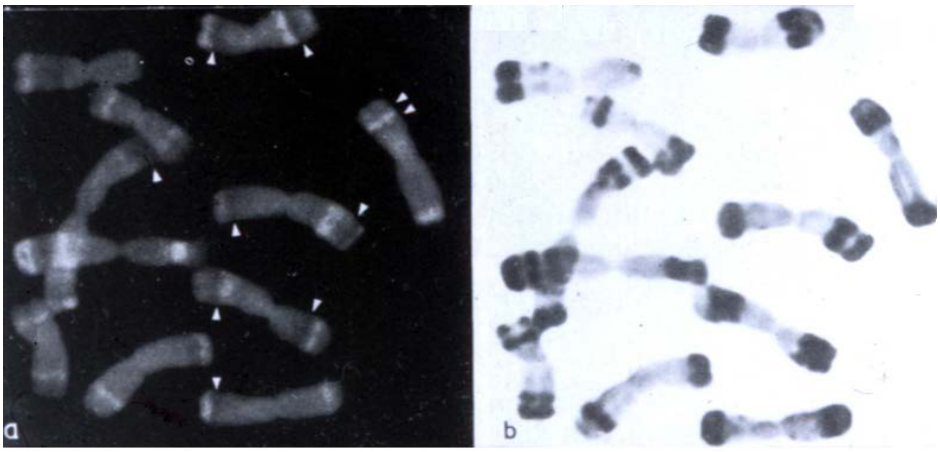

Fig. 16: Photomicrographs of 11 chromosomes of *Allium flavum* showing fluorescence Q- bands (a) and Giemsa C-bands (b)

Constitutive heterochromatin is chromosome-specific and species-specific and can be used for chromosome identification; it is cold-sensitive, late-replicating, and genetically inert, and usually contains highly repetitive DNA sequences. If we are using chromosome banding as evidence in taxonomic studies; biochemical explanation for their existence is not essential. However, one important point is that if we carry out a comparative study, which is always the case in taxonomy, it is important to use the same technique for each type of bands on every taxon. In systematic studies chromosome banding may be applied for the following objectives.

- Identification of individual chromosomes in the karyotype of the same species.
- Differentiation of otherwise similar chromosomes and karyotypes in different species
- Identification of parental genomes in a hybrid genome.
- Elucidating the origin of polyploids and aneuploids.
- Demonstration of chromosomal rearrangement due to structural changes

3.8. In situ hybridization

This is a technique usually used to locate genes in the genomes. It relies on the fact that a length of a single-stranded DNA fragment will bind to a homologous length of DNA in the nucleus or chromosomes. In practice a DNA probe is labeled, either radioactively or with a high fluorescent chemical group, and hybridized with chromosomal DNA in cytological preparations. In this way genes of known DNA sequence in one organism can be in the genome of the same or other organism. This technique can also be used for genome painting, which is a method for elucidation of the origin of taxa that have resulted from interspecific hybridization (Bennett *et al.* 1992). In this case a DNA extracted from a suspected parent is cleaved into fragments, using one or more of the restriction endonucleases, these fragments are labeled and used for hybridization with chromosome preparations (Schweizer et al., 1990).

Fluorescence in-situ hybridization (FISH)

Fluorescence in-situ hybridization is a method used to identify specific parts of the chromosome and have widely accepted tool in gene mapping on chromosomes. For example, if the sequence of a certain gene is known, the chromosome in which the gene is located is unknown, FISH can be used to identify the chromosome in question and the exact location of the gene. Braz et al. (2020) developed two oligonucleotide-based probes that hybridize to 24 chromosomal regions in maize. The maize individual chromosomes show distinct FISH signal patterns, which allow their universal identification from three *Zea* species. and two additional wild *Zea* species based on individually identified chromosomes. The basic procedures of the FISH are as illustrated in Fig. 17 and the steps of FISH may be summarized as follows: -

1) Make a probe complementary to the known sequence. When making the probe, label it with a fluorescent marker, e.g., fluorescein
2) Spread the root tip chromosomes on a microscope slide and denature them, add the probe and hybridize to its complementary site

3) Wash off the excess probe and look at the chromosomes in a fluorescence microscope using a UV illumination. The probe will show fluorescent signals illustrating the location of the gene on the chromosome (Fig. 18).

In addition to the FISH techniques, several methods called chromosome painting are particularly useful in differentiating the chromosomes from each other in the karyotype and in mapping genes on the chromosomes. In the most common of these methods, the chromosomes are separated using cytometric methods and DNA of one chromosome is labeled and used as probe for hybridization with the chromosomes of many chromosome spreads to distinguish the chromosome pair complementary to the probe.

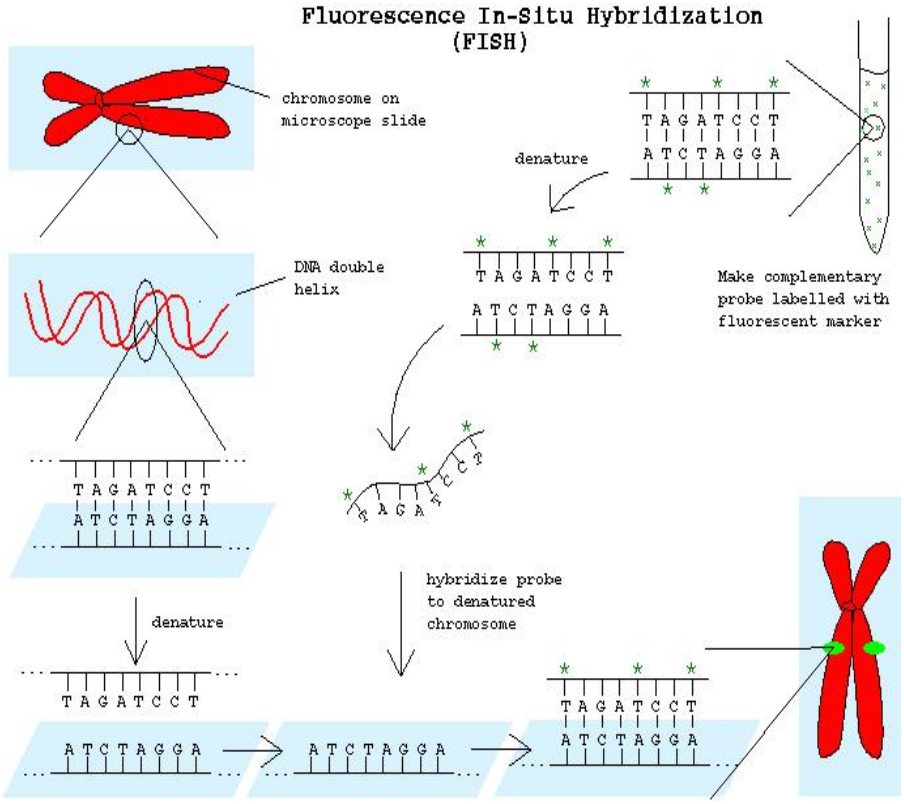

Fig. 17: Diagrammatic representation of the steps of FISH technique

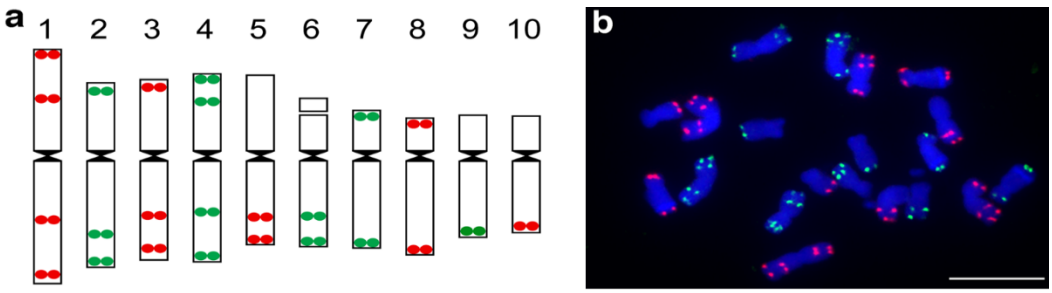

Fig. 18: Barcode oligo-FISH probes for maize chromosomes identification. a). Oligos were selected from a total of 24 chromosomal regions, 12 red regions and 12 green regions to show predicted locations of the oligo-FISH signals, b) FISH mapping of the two oligo-FISH probes on metaphase chromosomes prepared from maize inbred B73. Bar = 10 µm. (Braz et al., 2020).

4. Examples of the Role of Chromosome Information in Plant Taxonomy

4.1. Basic guidelines

There has been much discussion about the value of chromosomal features, relative to other characters, in plant classification and phylogeny reconstruction of plant taxa. Because chromosomes are the seats of genes and relate to the mechanism of heredity than morphological, anatomical, and chemical characters, they have been regarded as of no predominant importance. However, a distinction

must be made between the value of cytological information as providing comparative data and its value for suggesting evolutionary relationships and deducing phylogenetic interpretations.

To find rules or tendencies correlated with the systematic position of a species or a higher taxon, a comprehensive survey of the karyotypes of as large number of species as possible is required. Considerable progress in clarifying the phylogenetic relations between species came about because of the analysis of the karyotypes, and the simultaneous study of the geographic distribution and the ecological preferences of closely related species (Stebbins, 1971).

An increase in the number of chromosomes is usually associated with a reduction in chromosome size. The comparison of the karyotypes of closely related species revealed some conspicuous, though not universally applicable patterns of distribution. In summary, the distribution of chromosome sizes shows several trends.

1) In spore-producing pteridophytes, smaller chromosomes are associated with heterosporous genera such as *Selaginella* and *Isoetis* or families such as Marsiliaceae and Salviniaceae, while larger chromosomes are typical for homosporous groups.

2) Fern species exhibit high chromosome numbers, and these are a result of polyploidy. In the so-called Adder's tongue fern (*Ophiglossum*) the base number of chromosomes is 120. The diploid species of this genus have $2n=2x=240$ chromosomes. This demonstrates the high end of the number of chromosomes that are found in eukaryotic species.

3) Gymnosperms such as Cycads and Conifers have on average larger chromosomes than most other plants. They are surpassed by a few angiosperm genera such as *Paeonia*, *Lilium*, *Trillium* and *Tradescantia*, and *Allium.*

4) Wooden angiosperms have without exception small and usually hardly distinguishable chromosomes. Between related species and genera exist often no detectable differences.

5) Related species and genera of herbaceous angiosperms display considerable differences in the sizes of their chromosomes. The shapes of the chromosomes do provide no clue for the phylogenetic position of the family, nor do they allow predictions of the chromosome size of any species.

6) Chromosome sizes of significant difference have two, partly complementary causes:
   a) Species with larger chromosomes have more active genes than species with smaller chromosomes.
   b) Species with large chromosomes contain large amounts of non-coding DNA, also called repetitive DNA or heterochromatin.

7) Apart from analyzing the chromosomal numbers of distinct species, interpretations of their variability in larger phylogenic groups of plants are feasible. As a result, the chromosomal numbers of herbaceous angiosperms have been shown to vary by the factor of 100, while this factor is 14 in woody angiosperms and just 2 in conifers. The chromosomal numbers of cycads do not vary at all.

8) An increase in the morphological variability correlates usually with an increase of the chromosomal variability. Herbaceous angiosperms use a wide range of reproductive strategies. The sizes of their populations are far smaller than that of woody species. Optimized genotypes can easily be accumulated and thus the speed of evolution is increased.

For the use of chromosomal information in suggesting evolutionary relationships and phylogenetic speculations, experience has given the following guidelines:

1) Polyploid plant species or cytotypes are derived from diploid ones.

2) Symmetric karyotypes are more often found in less evolved species in most genera

3) Primary basic numbers give rise to secondary basic numbers by duplication or stepwise increase or decrease.

4) Molecular cytogenetic approaches such as chromosome banding, and in situ hybridization and chromosome painting are increasingly applied

5) Chromosomal data can determine many processes involved in evolutionary change or can act as a marker of them, but they do not do so equally in all groups of plants.

4.2. Examples of chromosomes contribution in plant taxonomy

In numerous groups of plants chromosomal information has provided evidence for classifications which better express natural grouping of many plant taxa compared to their current grouping and pointed out to events of evolutionary change. The following are general examples at different taxonomic levels:

(1) Chromosomal variations in the Ranunculaceae

In Ranunculaceae, the chromosome set falls into two distinct types, the large R-type found in *Ranunculus* and the small T-type found in *Thalictrum*. When taken in conjunction with chromosome number, chromosomal information provided a more satisfactory basis for subdivision of

the family than that produced by the fruit type. In this family the two genera *Nigella* and *Adonis*, both with x = 6, are delimited together. In the genus *Ranunculus* chromosomal variation contributed to the reticulate evolution of the species and validated taxonomic concepts in the insights when combined with the analysis of morphological, karyological and molecular data (Hörandl et al., 2009).

Also, in the Ranunculaceae, the genus *Anemone*, two small groups belong to the closely related clades: the montane/alpine Baldensis clade and the more temperate Multifida clade. The evolution of allohexaploid *A. baldensis* (AABBDD, 2n = 6x = 48) from Europe and allotetraploid *A. multifida* (BBDD, 2n = 4x = 32) from America was analyzed by Mlinarec et al. (2012). The results indicated that *A. multifida* originated from the crosses of diploid members of the *A. multifida* (donor of the A and B subgenomes) and *A. baldensis* groups (donor of the D subgenome). The A and B subgenomes are closely related to the genomes of *A. sylvestris, A. virginiana* and *A. cylindrica*, indicating that these species or their progeny might be the ancestral donors of the B subgenome of *A. multifida* and A and B subgenomes of *A. baldensis*. The results further indicated that both polyploids have undergone genomic changes such as interchromosomal translocation affecting B and D subgenomes and changes at the rDNA sites. GISH staining of the chromosomes showed that *A. multifida* has lost the 35S rDNA loci characteristic of the maternal donor (B subgenome) and maintained only the rDNA loci of the paternal donor (D subgenome).

Also, in the Ranunculaceae, molecular phylogenetic studies on the specie relationships in the genus *Clematis.* which grow in the Mediterranean basin, indicated that interspecific hybridization is rather common in the genus, and artificial hybridization has been widely applied to produce new *Clematis* cultivars for nearly two centuries (Lyu et al., 2021). These findings demonstrated that plants of *Clematis pinnata* is not a self-evolved clade and should not be treated as a species. The study also suggested that interspecific hybridization is a common mechanism in *Clematis* to generate diversity and variation and may play an important role in the evolution and diversification of species. It was concluded that morphological diversity caused by natural hybridization may overstate the real species diversity in the genus *Clematis.*

The genus *Paeonia* with *x*=5 and very large chromosomes was separated from the family Ranunculaceae in the distantly related family Paeoniaceae. The floral vasculature and leaf architecture of Ranunculaceae and Paeoniaceae differ from those of the Dilleniaceae by characters that determined their divergent evolutionary pathways early in angiosperm phylogeny. The basic chromosome number n = 5 is shared by Paeonia and Glaucidium and is discordant to both Ranunculaceae and Dilleniaceae. The correlation of other characters supports the inclusion of *Glaucidium* in the Paeoniaceae and its separation from *Paeonia* at generic level only (Melville, 1983).

(2) Chromosome changes and the phylogeny of Solanaceae

Solanaceae is a cosmopolitan family with several economically important species. There have been different views regarding the taxonomic treatment of the family. Current treatments of the Solanaceae (D'Arcy 1991) recognized two main subfamilies. The Solanoideae with curved embryos contained in flattened discoid seeds and the Cestroideae with straight or slightly bent embryos in subglobose seeds. The Solanoideae have been viewed traditionally as the primitive subfamily, with the divergence of the Cestroideae occurring early during its diversification (D'Arcy 1979, 1991, Armstrong 1986). On the other hand, Olmstead and Palmer (1992), based on molecular results, argued that the Cestroideae is more primitive than the Solanoideae. Badr et al. (1997) investigated the contribution of chromosomal variations to the taxonomic relationships of 45 taxa in the two main subfamilies of the Solanaceae; the Solanoideae and the Cestroideae and examined the relationships within and between species are discussed and their phylogeny based on chromosomal variation is proposed (Fig. 19).

In contrast to the traditional sub-familial delimitation of the Solanaceae, (D'Arcy 1979, 1991; Armstrong 1986), chromosomal variations indicated that subfamily Cestroideae is less evolved than subfamily Solanoideae. These data support the isolation of *Cestrum* in a separate tribe. The isolation of tribe Salpiglossideae in a separate family as proposed by Hutchinson (1973) and the delimitation of both *Datura* and *Hyoscayamus* as two separate tribes as done by D'Arcy (1991) was strongly supported by cytological data. In agreement with D'Arcy (1991) the delimitation of *Nicandra* in a separate tribe was further recommended.

(3) Chromosomes and the taxonomy of *Astragalus*

In the genus *Astragalus* (Fabaceae), the basic division between the Old World and the American species is consistent with cytological information. The Old-World species have *x*=8 with common polyploidy whereas, the American species form a lineage with *x*=11, 12, 13, 14 and 15 with a rare occurrence of polyploidy. The karyotype of 35 taxa representing 24 species of the Egyptian *Astragalus* were described by Badr and Sharawy (2007). A diploid number of 2n =16 and x = 8 was recorded in 28 taxa representing 17 species, including six polyploid counts. Meanwhile, numbers based on x =7 were recorded in four taxa of which three counts

are tetraploid with 2n = 28 representing the three species *Astragalus annularis*, *A. mareoticus* and *A. vogelii*. In addition, numbers based on x=6 were encountered in *A. trimestris* (2n = 12 and 2n = 24) and *A. boeticus* (2n = 30). The chromosomes in Egyptian *Astragalus* are generally small with a mean size ranging between 0.82 µm and 1.59 µm. Short chromosomes were particularly found in *A. vogelii* (MCL=0.82 µm) and *A. boeticus* (MCL= 0.87 µm), whereas longer chromosomes were scored in *A. sinaicus* (MCL=1.59 µm).

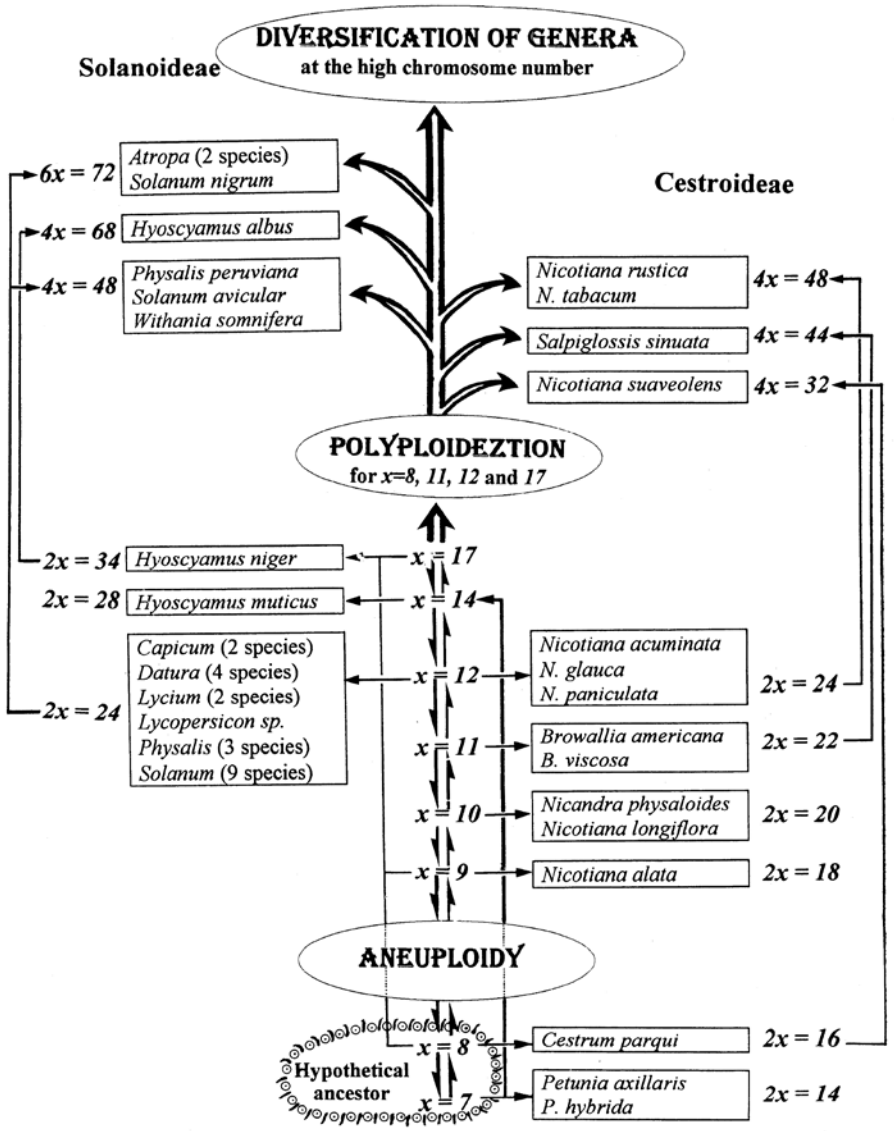

Fig. 19. A proposed phylogeny of 45 taxa of Solanaceae based on variations in chromosome number through aneuploidy and polyploidy (Badr et al., 1997).

The grouping of the examined species, based on chromosomal criteria and analysis of karyotype data, also support the grouping of the species in section Sesamei as proposed by Podlech (1986). However, karyotype features and the analyses of chromosomal data do not support the grouping together of four species in section Harpilopus as proposed by Podlech (1986), these are *A. corrugatus* (2n = 32) and *A. hauarensis* (2n = 16), A. trimestris (2n = 12; 24) and A. mareoticus (2n = 28). On the other hand, karyological similarities are evident among the examined four species of section Chronopus i.e. *A. dactylocarpous*, *A. fruticosus*, *A. sieberi* and *A. trigonus*. All four species have 2n=16 with closely similar chromosome length, arm ratio TF% and A1 values. The karyotype features may not justify delimitation of *A. hispidulus* in a separate section and indicated its association with *A. carpinus* and *A. dactylocarpous, A. fruticosus, A. sieberi, and A. trigonus*. All these species have 2n =16 and similar TCL and mean arm ratio but slightly different A1 value (Badr and Sharawy, 2007).

(4) Wheat speciation and domestication

Wheat has played a major role in the development of the world civilization. The domestication of wheat was a major event in world civilization because it allowed humans to change from nomadic hunter gathers to permanent residents in communities and societies. The development of the modern allohexaploid bread wheat from diploid and tetraploid ancestors could have taken place by hybrids of *Triticum urartu* (AA) with *Aegilops speltoides* also known as *Triticum searsii* (BB) to give *Triticum turgidum* (AABB) and the hybridization of this tetraploid species with *Triticum tauschii* (DD) to produce the hexaploid bread wheat *Triticum aestivum* (AABBDD)

Archaeological evidence has shown that *Triticum turgidum* (AABB), known as durum wheat, was being grown in both Mesopatamia (Tigris and Euphrates River Valley) and in the Nile River Valley 8,000 years ago. Because wild *T. tauschii* is found only in the mountain region of western Iran and northern Irak, it is thought that the hybridization that produced *T. aestivum* occurred in these regions. It has been suggested that this occurred as recently as 8,000 years ago, which coincides with the development of collective settlements by man. The genome increases in allopolyploid species increased spike size and grains number compared to the diploid einkorn wheat (Fig. 24).

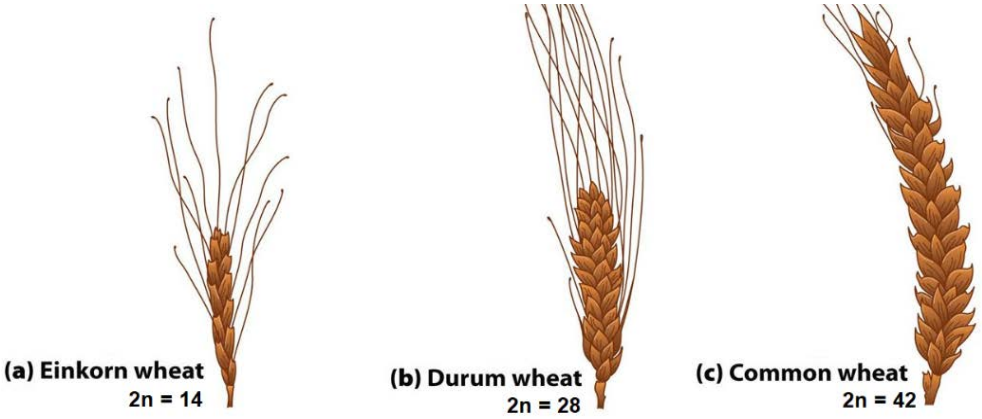

Fig. 24: Diagrams illustrating the increased spike size and grains number with the increase in chromosome number during wheat species domestication

(5) Chromosomal information and speciation of Roses

In the genus *Rosa* (Rosaceae) Hurst (1925) reviewed the chromosomal information and reported that the base number of chromosomes (x) is 7, for diploid rose 2*n* = 2*x* = 14, the tetraploid rose species have 2n = 4x = 28 chromosomes, the pentaploid roses have 2*n* = 5*x* = 35 chromosomes and the hexaploid rose has 2n = 6*x* =4 2 chromosomes. The spontaneous polyploid species of *Rosa* are clearly not duplicated diploids but differential polyploids, with differential sets of chromosomes since they show in their taxonomic characters various combinations of differential characters of the five differential diploid species. For example, the tetraploid sub-species *Rosa gallica* has the genome formula AACC, the pentaploid *Rosa canina* AABDE, the hexaploid *Rosa moyesii* AABBEE and the octoploid *Rosa. acicularis* BBCCDDEE.

The current classification of the present-day *Rosa* is based on cytological, genetical, and morphological characters combined. The genus may be divided into nine sections according to the numbers of somatic genomes of chromosomes present in the species and may be sub-divided into 15 sub-sections according to the numbers of gametic genomes present in the species. The 211 species in the genus may be classified, according to the possible combinations of the differential genomes of chromosomes and other characters. Hurst (1941) concluded that the major groups of the garden roses are descended from seven main species, namely, *R. rubra, R. phoenicea, R. moschata, R. canina, R. chinensis, R. gigantea, and R. lutea*. The specific characters of these species can still be traced in the current garden Roses. In fact, Roses have undergone extensive reticulate evolution involving interspecific hybridization, introgression and polyploidization. De Vries and Dubois (1996) sated that only 8 to 20 rose species are thought to have contributed to the present complex hybrid rose cultivars, namely Rosa × hybrida. The diploid Chinese rose. (*Rosa* chinensis) was introduced to Europe in the 18th century. This species is considered one of the main species that participated in the subsequent extensive process of hybridization with roses from the European, Mediterranean, and Middle Eastern (mostly tetraploid) sections (Raymond et al. (2018). Among the breeding traits originating from Chinese roses, the capacity of recurrent flowering as well as color and scent signatures are key traits for Ros quality.

(6) Chromosome banding and *Allium* species evolution

The genus *Allium* L. comprises more than 800 species (Fritsch et al. 2010) that were divided in 15 monophyletic subgenera, based on nuclear ribosomal DNA ITS sequences. (Friesen et al. 2006). In the genus *Allium*, the New World species, which all have *x*=7 and symmetric karyotype, are grouped with close relatives in the Old World with x = 7 and species with *x* = 8 and x = 9 in subgenus *Molium*. The karyotype of the species with *x* = 8 and 9 in this subgenus is asymmetric and includes telocentric chromosomes, as in the karyotype of *Allium erdelii* and are derived from *x* = 7 by centromere division of one or two chromosomes. The other subgenera in *Allium* have *x* = 8 but the karyotype is symmetric and comprised of metacentric and submetacentric chromosomes (Badr, 1977). The contribution of chromosome banding in species systematics and evolution in *Allium* are outlined here.

In subgenus *Rhizirideum*, the basic chromosome number is *x* = 8 and the karyotype is highly symmetric and telomeric bands are on almost all chromosomes of most species. This subgenus is large and morphologically diverse and banding style does not appear to be useful tool in sorting out species and sectional delimitation. Vosa, (1973) and El-Gadi and Elkington (1977) examined the karyotype of several taxa in the cepa group of *Allium* and two allied species using fluorochromes and Giemsa staining techniques. The chromosomes of all species have terminally located heterochromatic segments which show reduced Quinacrine-fluorescence and deeply stained band by the Giemsa technique as demonstrated in the chromosomes of (Fig. 20). Together with morphological similarities these characteristics indicate a close relationship between the alliums of the cepa group and the two allied species *A. schoenoprasum* and *A. ledebourianum*. Based on chromosome morphology and C-band endowment a hybrid origin of the viviparous forms of the onion: *Allium cepa* var. viviparum, *Allium aobanum* and *Allium wakegi* was confirmed.

In section *Codonoprasum* of subgenus *Allium*, where karyotype is like that of subgenus Rhiziridium with x = 8 and the karyotype is symmetric, the banding style appears excessive. The banding style in this section is characterized by telomeric and intercalary bands leaving sizeable pericentric region devoid of bands as in *A. flavum*, *A. pulchellum* and *A. stamineum*, all with 2n = 16. In this group banding polymorphism among and within populations is common. In general, enhanced, or reduced fluorescence bands often reveal Giemsa bands in this group as demonstrated in the chromosomes of *A. flavum* (Vosa, 1973). See Fig 18.

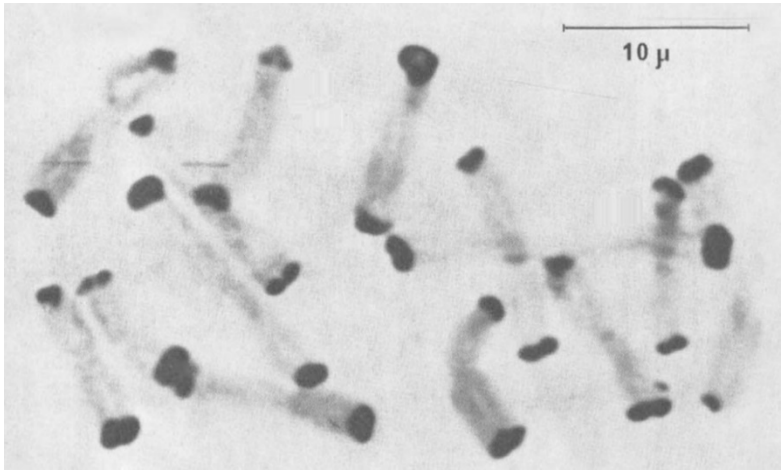

Fig. 20: Photomicrographs of *Allium ataicum* chromosomes showing terminal and nucleolar Giemsa C-bands

In subgenus *Molium*, Plants grow in the Mediterranean Basin and North America. Plants from North America are characterized by a basic number of *x*=7 with symmetric karyotypes. Most of these species possess narrow bands on the nucleolar chromosomes as in *Allium fibrillum* and *A. unifolium*. In addition, *A. acuminatum* chromosomes showed narrow centromeric bands, whereas *A. cernuum* chromosomes showed thin telomeric bands. In this regard this species resembles the members of subgenus Rhiziridium. Interestingly *A. cernum* is the only species with a bulb borne on a rhizome in the *Molium* group. Its banding style suggests its removal from subgenus *Molium to* subgenus Rhiziridium (Fig. 21). One *Molium* species from Europe *A. moly* resembles those of North America in having narrow bands on the nucleolar chromosomes (Badr and Elkington, 1977; 1984; Cai and Chinnappa, 1987).

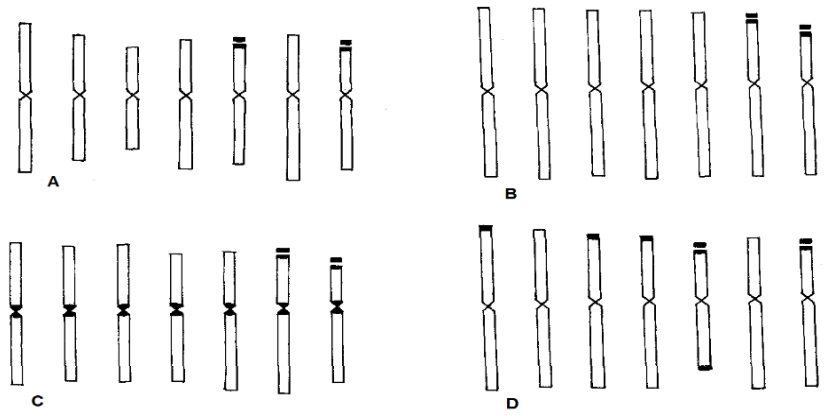

Fig. 21: Karyograms of C-banded chromosomes of the North American species, *Allium fibrillum* (A), *A. unifolium* (B) with bands on the nucleolar chromosomes, *A. acuminatum* with centromeric bands (C) and *A. cernuum* with temeric bands (D)

On the other hand, the Mediterranean species of subgenus *Molium* are interesting in illustrating the chromosomal variation in speciation. From this group of species, *A. subhirsutum*, *A. neopolitanum* and *A. trifoliatum* are morphologically similar. *A. subhirsutum* is essentially diploid with 2n = 14 and like the North American species and exhibits C-bands on one pair of nucleolar chromosomes in the karyotype (Fig. 22). Nucleolar organizing region in this species is adjacent to the centromere and together with the centromere compose a compound constriction. On the other hand, *A. neopolitanum* is usually polyploid with 2n = 28, but 2n = 35 and 40 were also recorded. C-banding of this species indicates that the two genomes of the karyotype resemble each other in karyotype characteristics and banding style.

Another polyploid situation in *Allium* involves *A. oleraceum*, which is known to have different polyploid cytotypes. Chromosome banding for material with 2n = 32 and 2n = 40 were studied. The banding pattern of the 2*n* = 32 cytotype resembles that of *A. paniculatum* (2*n* = 24) and *A. pulchellum* regarding the fluorescence banding but differ to some extent in C-banding style. The tetraploid cytotype of *A. oleraceum* appear to be of an allopolyploid origin, which may involve *A. paniculatum*. It was possible to hybridize *A. paniculatum* and *A. oleraceum* and thus again chromosome banding similarity confirms genetic affinity between closely related species, as judged by their ability to cross, as in the case of *A. subhirsutum* and *A. trifoliatum*.

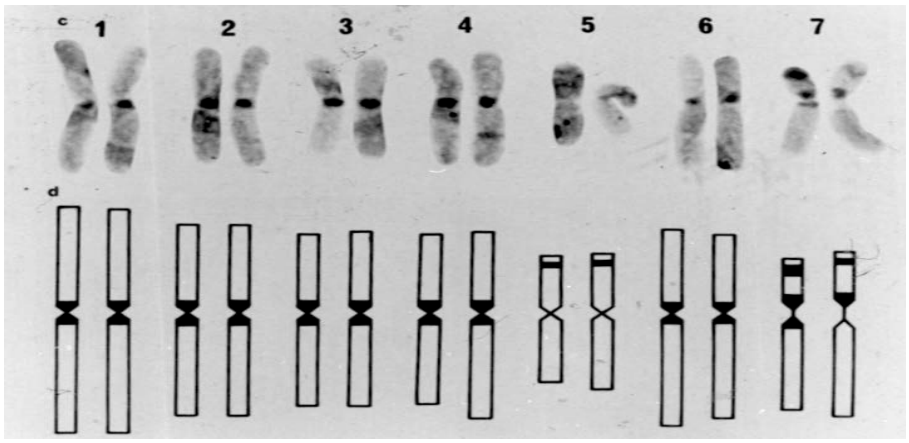

Fig. 22: Karyotype and ideogram of C-banded chromosomes of *Allium trifliatum* subsp. *hirsutum* (2n=14), six chromosomes having centromeric C- bands

Conclusions

Chromosomal criteria have provided strong evidence for the classification, biosystematics, and evolution of plants. These criteria are often types of chromosomal numerical variations which include major genome alterations, such as whole genome duplication of the same chromosome sets (auto-polyploidy) or of hybrids chromosome sets (allo-polyploidy) and/or stepwise increase or decrease in

chromosome number (aneuploidy). Chromosomal variations also comprise the concomitant or independent occurrence of chromosome structural changes, which had induced instability and variation that act as driver for genome evolution and adaptation. These may have been occurred as deletions, addition, inversion, or translocation of chromosomal segments or transposition of DNA segments in the same chromosome or to other chromosomes. In practical plant systematics, the chromosomal information may be regarded as variations in chromosome number, karyotype symmetry, pairing at meiosis, chromosome banding and/or in-situ hybridization to locate genes on chromosomes. The given example of chromosomal changes associated with the phylogeny of taxa in the Ranunculaceae and Solanaceae and on the species evolution in some such as genera of *Astragalus*, *Triticum*, *Rosa* and *Allium*, are only a few of countless situations that clearly indicated the crucial role of chromosomal changes in the elucidating phylogeny and evolution of plant taxa. Chromosomal variations are particularly useful in addressing speciation, domestication, and breeding where hybridization had played a crucial role in species evolution as in *Rosa*, *Triticum* and *Allium*.

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