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DAPI Banding Pattern of Chromosomes in Several Brassicaceae Species

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BRASSICACEAE family includes a number of the most important *Brassica* cultivars such as canola, mustard and cabbage, and *Arabidopsis thaliana* as plant biology model species. Chromosome banding is the most probable protocol for karyotyping and to distinguish numerical chromosome abnormalities, duplications, inversions or deletions of chromosome parts, or exchanging a part from one to another chromosome as well. The aim of this study is to illustrate the DAPI banding patterns in 16 Brassicaceae species with small chromosome size, and they have different origin, ploidy level (chromosome number varies from 2n= 14 to 2n= 32). These species belong to five tribes (Brassiceae, Sisymbrieae, Lepidieae, Arabideae and Alyssea. The aim of this study is to illustrate the DAPI banding patterns in 16 Brassicaceae species with small chromosome size, and they have different origin and ploidy level (chromosome number varies from 2n= 14 to 2n= 32). In the current study we investigated different genera belong to five tribes of the family in regard to the position of regions as by using DAPI staining. It was excluded that most of plants with small chromosomes have mostly a centromeric bands and the more the chromosome size the more tendency to have interstitial bands.

Keywords: Brassicaceae, Chromosome banding, DAPI, Plant.

Introduction

Brassicaceae is famous as the cabbage, mustards or crucifer family contains 3700 species belong approximately to 338 genera (Al-Shehbaz et al., 2006). *Brassica* L. is one of the most economically important genera within family Brassicaceae, it includes approximately 80 species worldwide (Amer et al., 2019). The Brassicaceae includes Arabidopsis thaliana which consideredan important model plant in plant biology. The family includes alsoa number of valuable species as weeds (e.g., *Capsella*, *Thlaspi*, *Sisymbrium*, and *Lepidium*), or as ornamental plants (e.g., *Matthiola*, *Hesperis*, and *Lobularia*). Chromosome number in Brassicaceae is highly variable among the species that ranges from n= 4 to 128 and about 37% of species are polyploidy (Warwick & Al-Shehbaz, 2006). However, the taxonomy and systematic of this family are very complicated because of the necessity to sufficient and reliable information in regard to the interpretation of species and genus names. Olin-Fatih & Heneen (1992) reported that classical cytogenetics in *Arabidopsis* and *Brassica* has also been used to recognize the basic karyotype using C-banding.

The Cytogenetics tool is still a useful method to provide excellent information on chromosome sizes, numbers and polyploidy (Jellen, 2016). Heterochromatic regions were the most intensively studied parts of the chromosome and used as chromosome markers in both plants and animals. Heterochromatic regions (HC) could be differentiated from the euchromatic regions by tradition C-banding method or by base-specific

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staining with fluorescent dye, e.g. Hoechst 33258, chromomycin A3 (CMA), 4-6-diamidino-2-phenylindole (DAPI), quinacrin, mythramycin, and others. Caspersson el al. (1968) designed and tested fluorescent dyes that could differentially bind to specific nucleotide pairs in DNA, and they wished to be detectable spectrophotometrically. The first tested molecule was quinacrine mustard dihydrochloride on *Trillium erectum* and *Vicia faba,* revealed brightly fluorescent bands that differntiate the HC regions of these plant chromosomes.

The main HC region in most organisms is the centromere which is considered the most important structure in the cell for chromosomes segregation during meiotic and mitotic divisions. Centromeric region is highly rich in transposable elements and/or satellite repeats and is embedded into the HC pericentromere (Wang et al., 2009). Modern molecular cytogenetics provides a valuable tool to study the HC DNA regions which are informative in molecular chromosome biology, as the investigation of *Arabidopsis* centromere sequences sequence, knob and chromosome painting in *Arabidopsis thaliana* (Murata et al., 1994; Heslop-Harrison et al., 1999; Lysak et al., 2001; Fransz et al., 2016), and several studies utilizing genomic in situ hybridization (GISH) helped for further declaration of chromosome poloidy, genome composition, and pairing in the meiosis of the Brassicaceae (Ali et al., 2004; Xiong & Pires, 2011). The 5S and 25S rDNA markers which indicate the nucleolar organizing regions are localized in Brassicaceae by fluorescent in situ hybridisation (FISH) techniques (Ali et al., 2005). The chromosome number reduction mechanisms and the genome size evolution in *thaliana* compairing with Brassicaceae species have been studied by Lysak et al. (2006, 2009). Many studies have been done to clarify the evolution and systematics of the tribes in this family (Al-Shehbaz et al., 2006; Koch & Mummenhoff, 2006). Kiefer et al. (2014) introduce a preface to informative database on the evolution of Brassicaceae.

Materials and Methods

The 16 Brassicaceae species which were used in the current study have different origin and different ploidy level. The chromosome number varies from 2n= 14 to 2n= 32. These species belong to five tribes (Brassiceae, 5 species, Sisymbrieae, 6 species, Lepidieae, 3 species, Alysseae, one species and Arabideae, one species), the tribal assignments are given according to Janchen (1942) as shown in Table 1. Chromosome preparations from young flower buds were done according to Mandáková & Lysak (2008). The whole inflorescences of each species were fixed in ethanol: chloroform: acetic acid (6:3:1) overnight and stored in 70% ethanol at -20°C until use. The flower buds were rinsed in distilled water then in citrate buffer (10mM sodium citrate, pH 4.8; 2-5min), afterword incubated in an enzyme mixture (0.3% cellulase, 0.3% cytohelicase, and 0.3% pectolyase; Sigma-Aldrich) in the citrate buffer at 37°C for 3hrs., then washed in the citrate buffer. The smallest (very young) anthers were put on a microscope slide and disintegrated by a needle in a drop of citrate buffer. Then was softened by adding 30µL of 60% acetic acid and spread by stirring with a needle on a hot plate at 50°C for maximum 2min.Chromosomes were fixed by adding100µL of ethanol: acetic acid (3:1). The slide was dried with a hair dryer. The suitable slides which have good separated mitotic chromosomes were postfixed in 4% formaldehyde in water for 10min. and air-dried. For denaturing the chromosomes, the slides were immersed in a mixture contained 70% formamide in 2×SSC (v/v) in water bath at 70°C for 5min. then washed in 2×SSC and dried in ethanol series 50, 70 and 100% and left at room temperature overnight. The chromosomal DNA were counterstained with 2µg mL DAPI mounted in Vectashield (Vector) and chromosome images were captured with a Zeiss Axioplan 2 epifluorescence microscope equipped witha Spot2e CCD camera. The images were processed for contrast and sharpness using Adobe Photoshop C.S. Middle east version software.

Results

The chromosome number of the studied species varies from 2n= 14 to 2n= 32 (Table 1), and they belong to five tribes (Brassiceae, 5 species, Sisymbrieae, 6 species, Lepidieae, 3 species, Alysseae, one species and Arabideae, one species). All species in this investigation are diploid except five species are polyploid, three belong to tribe Sisymbriea, *A. griffithiana* (2n=4x=32), *A. suecica* (2n=4x=26), and *Arabidopsis arenosa* (2n=4x=32)], one species, *Camelina microcarpa* (2n=?x=48) belongs to tribe Lepidieae, and *Rorippa palustris* (2n=4x=32) belongs to tribe Arabideae.

DAPI-bands were detected in all species,

indicating the presence of AT-rich HC. In general, in most of the studied species, the common pattern of DAPI-banding was centromeric especially that all of the studied species with small chromosomes (Fig. 1, 2).

Figure 1 shows five species belong to tribe Brassiceae, *Cordylocarpus muricatus* (2n=2x=16), *Eruca sativa* (2n=2x=22),) *Sinapis arvensis* L. (2n=2x=18), *Diplotaxis siifolia* (2n=2x=20), and *Sinapidendron frutescens* (2n=2x=20), in addition to three species belong to tribe Sisymbrieae, *Arabidopsis griffithiana* (2n=2x=32), *Arabidopsis Carpatica* (2n=2x=16) and *Arabidopsis suecica* (2n=2x=26).

Figure 2 shows the other three species which belong to tribe Sisymbrieae, *Arabidopsis arenosa* (2n=2x=32), *Sisymbrium officinale* (2n=2x=14) and (2g) *A. halleri* (2n=2x=16), in addition to three species belong to tribe Lepidieae, C*amelina microcarpa* (2n=?x=48), *Thlaspi arvense* (2n=2x=14), and *Iberis sempervirens* (2n=2x=22), moreover, one species belongs to tribe Arabideae, *Rorippa palustris* (2n=4x=32), and the last one *Ptilorichum spinosum* (2n=2x=32) belongs to tribe Alysseae. The DAPA banding patterns in the different studied species were as fellowes. *Eruca sativa* chromosomes (2n=22, tribe Brassiceae) bear the DAPI bands at the metacentric, submetacentric or subtelomeric positions (Fig. 1b).

A thick block of centromeric bands were observed almost on all chromosomes of seven species, five species out of them belong to tribe Sisymbrieae, *A. halleri* (2n=16, Fig. 2g), *A. griffithiana* (2n=4x=32, Fig. 1f), *A. suecica* (2n=4x=26, Fig. 1h), *Arabidopsis arenosa* (2n=4x=32, Fig. 2a) and *Sisymbrium officinale* (2n=14, Fig. 2c)], in addtion to one species belongs to tribe Arabideae (*Rorippa palustris* 2n=4x=32, Fig. 2b), and one species belong to tribe tribe Brassiceae (*Sinapis arvensis* L. 2n=18, Fig. 1c) .

Two species belong to tribe Brassiceae [*Sinapidendron frutescens* (2n=20, Fig. 1e) and *Cordylocarpus muricatus* (2n=16, Fig.1a)], and one species belongs to tribe Lepidieae (*Iberis sempervirens* 2n=22, Fig. 2f), exhibited strong medium-sized centromeric sharp-bands, whereas *A. Carpatica* (2n=16, tribe Sisymbrieae) showed normal medium-sized centromeric bands (Fig. 1g).

TABLE 1. Chromosome number and the origin for 16 Brassicaceae species

Species Ploidy, 2n Origin	Tribe
Cordylocarpus muricatus 2x =16 Botanical Garten Berlin-Dahlem	Brassiceae
Eruca sativa2x = 22shop	
Sinapis arvensis L.*2x* =18Botanical Garten Frankfurt a.M. 462	
Diplotaxis siifolia2x=20Jardin Bot de Bordeaux, 212, Tarifa (Spain)	
Sinapidendron frutescens 2x=20Madeira	
A.griffithiana4x = 32S hurob, Uzbekistan	Sisymbrieae
Cardaminopsis carpatica2x=16Besenova, Slovakia	
A.suecica4x=26Koornneef M., Wageningen University, Netherlands	
A.arenosa4x=32 Dolni Benesov, Czech Republic	
Sisymbrium officinale2x=14BG,IPK, Gatersleben, Germany	
A.halleri2x=16Zuberec, Slovakia	
Camelina microcarpa?x=48BG University of Copenhagen, No. 519, Denmark	Lepidieae
Thlaspi arvense2x=14 BG, IPK, Gatersleben, Germany	
Iberis sempervirens2x= 22 BG, IPK, Gatersleben, Germany	
Ptilorichum spinosum2x=32 BG, IPK, Gatersleben, Germany Alysseae	
Rorippa palustris *4x*=32 BG, IPK, Gatersleben, GermanyArabideae	

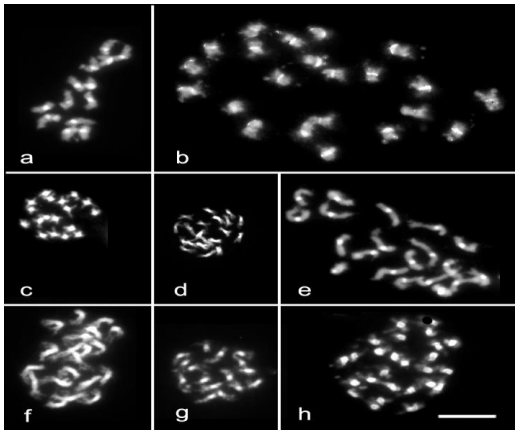

Fig. 1. The DAPI- banding patterin of chromosomes in species a) *Cordylocarpus muricatus*, b) *Eureca sativa*, c) *Sinapis arvensis*, d) *Diplotaxis siifolia*, e) *Sinapidendron frutescens*, f) *Arabidopsis griffithiana*, g) *Arabidopsi carpatica*, h) *Arabidopsis suecica* [Scale Bar = 5.0 µm]

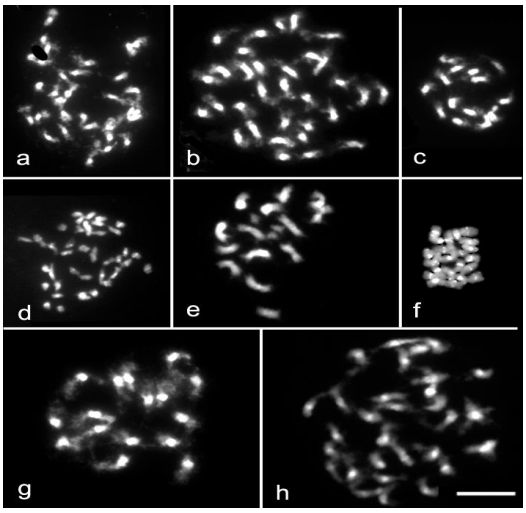

Fig. 2. DAPI- banding patterin in a) *Cardaminopsis arenosa*, b) *Rorippa palustris*, c) *Sisymbrium officinale*, f) *Iberis sempervirens*, e) *Thlaspi arvense*, d) *Camelina microcarpa*, g) *Arabidopsis halleri*, h) *Ptilorichu spinosum* [Bar= 5.0µm]

Ptilorichum spinosum (2n=32) belongs to tribe Alysseae, revealed differentiation in the size and strangeness of centromeric bands (Fig. 2h). The banding pattern was not equally distibuted in three species, in two species of tribe Lepidieae [*Thlaspi arvense* (2n=14, Fig. 2e) and *Camelina microcarpa* (2n=?x=48, Fig. 2d)], in additon to one species belongs to tribe Brassiceae (*Diplotaxis siifolia* 2n=20, Fig. 1d), some chromosomes of these species were completely stained with DAPI and some were partially stained, whereas the rest of chromosomes exhibeted interstitial faint bands.

Discussion

The patterns of chromosome banding can verify speciation and the relationship among the different plant species by revealing the changes in the karyotype of chromosomes, that could be important in evolution (Bickmore, 2001). The range of normal metaphase chromosome size is from less than 1 to more than 10mm, but lower and upper stable ranges for chromosome size are apparently determined by the size of genome, number and karyotype structure of chromosome in given species (Schubert, 2007).

One of the most attracted chromosomal components, which had been exclusively studied is mostly HC, because of it is lacking the unique genes, its function still unknown and it stains differently from the rest of the chromosome (Guerra, 2000). HC is varying qualitatively and quantitatively between species and varieties, and it was observed that the distribution of HC is concentrated in proximal, interstitial and telomeric regions (Heitz, 1933). The physical distribution of repetitive sequences and genes, which are crucial components for understanding the evolution of plants via the genome organization, has been extremely investigated by sequencing whole genome or large stretches of DNA sequences in some plants (ex. *Arabidopsis thaliana*). Caspersson et al. (1968) were the first who discovered the chromosome banding in plants by using quinacrin dihydrochloride which was known as Q-banding. Pardue & Gall (1970) noted that the centromeres of chromosomes darkly stained than other chromosomal regions during in situ hybridization investigations of labeled satellite DNA to mouse chromosomes. Arrighi & Hsu (1971) develop a modified technique by staining the chromosome preparations by Giemsa after denaturing with 0.07M NaOH followed by incubated in 2× SSC for several hours. They postulated that the repetitive DNA in HC reannealed faster than the euchromatin of the chromosomes which leads to stain differentiation, darkness and thickness of these regions depend on the amount of repetitive DNA in the HC regions. Then a variety of banding patterns has been developed by using alternative treatments before Giemsa staining which could discriminate the chromosomes of many species. In addition, the chromosome banding patterns by

many base-specific binding fluorochromes, AT rich chromatic regions (DAPI and Hoechst 33258) or GC rich regions (7-aminoactinomycin D and Chromomycin A3) depending on absorption and emission spectra (Schweizer, 1981; Verm & Babu, 1995).

Mason (2013) clarified that the features of eukaryotic chromosomes such as shape, size and composition of proteins, DNA and RNA, and in their number and redundancy as well may differ by subjecting to many changes during the evolution, and therefore they vary between and within individual organisms. Classical cytogenetics still informative and appropriate in many poorly-examined clades, but unfortunately it cannot differentiate between most of Brassicaceae species with small chromosomes, particularly in karyotyping of C-banding patterns in chromosomes, but the several greatly informative studies by using only classical cytogenetics, makes it helpful approach to investigate the ploidy level, meiotic chromosome behavior in natural species and interspecific hybrids in Brassicaceae. Cytogenetics approach solved the clue in the relationship among the six agriculturally *Brassica* species, *B. nigra*, *B. rapa*, *B. juncea*, *B. oleracea*, *B. carinata* and *B. napus*. There are very few published studies on Brassicaceae. In rapeseed HC analysis, Olin-Fatih (1994) studied the metaphase chromosomes G-banding pattern of *B. oleracea, B. napus* and *B. campestris,* which were darkly and lightly purple-stained chromatin and not precisely as C-bands. Moreover, the secondary constriction had a faint blue color and discriminated from the rest of the nucleolus chromosomes. Olin-Fatih & Heneen (1992) invistigated the diploid *Brassica* chromosomes by C-banding treatment and noticed that the number of bands along the chromosome was influenced by the de-condensation of the chromosome. Olin-Fatih (1996) stated that C-band polymorphism of intraspecific rapeseed chromosomes is still poorly studied.

Hasrerok & Maluszynska (2000a) indicated the numbers of active and inactive rDNA loci using in situ hybridization and silver staining methods in three allotetraploid species of *Brassica* (*B. napus, B. juncea* and *B. carinata*) and their diploid ancestors (*B. campestris, B. oleracea* and *B. nigra*). Hasrerok & Maluszynska (2000b) could differentiate among undistinguishable chromosome of *B. oleracea* (genome C), *B. nigra* (genome B) and *B. campestris* (genome A) by arm ratio and FISH. Hasrerok & Maluszynska (2000c) measured the *B. napus* chromosomes and observed that their size is small (1.53–3.3µm), and this species has simple and uniform C-banding patterns, that represented mostly by pericentromeric and telomeric HC bands. In another study Amosova et al. (2014) examined chromosomal and genetic polymorphism of 13 *B. napus* by studying C-banding and DAPI banding. A further declaration of relationships at chromosomal level and genetic control of meiosis in family Brassicaceae by utilizing GISH (Ali et al., 2004; Xiong & Pires, 2011). The nucleolar organizing regions (ribosomal 45S rDNA) and 5S rDNA markers are precisely localized in many Brassicaceae tribes by FISH techniques (Ali el al., 2005).

Guerra (2000) summarized the obtained results with the C-banding techniques of 105 species, distributed in 32 monocotyledons and 58 dicotyledons genera, and made a comparison to identify the patterns of HC distribution in plants in relation to chromosome size. He observed that the monocotyledons, *Triticum, Gibasis*, *Allium*, *Scilla*, etc, represented species and genera with large chromosomes are well studied, while dicotyledons species with small chromosomes and are mainly less studied. It was supposed that the highly decreased frequency of intercalary HC regions in small chromosomes could be partially because of difficulties in the technique to locate such bands on the very small chromosomes, in general, the most common in small chromosomes were proximal bands. Therefore, the chromosome size partially determines the distribution of C-bands and the band frequency increase with increasing the chromosome size. Additionally, the karyotypes of 11 out of the 34 species with small chromosomes were found to be only pericentromric (or proximal) C-bands, whereas in only 12 out of the 71 species with large and medium-sized chromosomes, C-bands were more frequently to be interstitially. The reported examples of pericentromric C-bandsin species with small chromosomes in that publication were; *Vellozia pattens* (Melo et al., 1997), *Rollinia pulchrinervis* (Morawetz, 1981), *Schippia concolor* (Röser, 1994), *Callisia* sp. (Jones & Kenton, 1984), *Costus pulverulentus* (Guerra, 1988), *Sesbania tetraptera* (Forni-Martins et al., 1994), *Crepis vesicaria* (Guerra, 1982), *Hypochoeris brasiliensis* (Ruas et al., 1995),

Paeonia tenuifolia (Schwarzacher-Robinson, 1986), and *Helleborus foetidus* (D´Amato & Bianchi, 1989).

Furthermore, Lin et. al. (2005) reported that HC at pericentromeric regions of soybean (Glycine max) comprises mostly of conserved repetitive sequences (tandem repeat STR102 and other retroelements) only in soybean and its closely wild relative species *G. soja*, that indicates a rapid evolution of these repetitive sequences. Afterward, Schmutz et al. (2010) sequenced *G. max* genome and found that about 33% of approximately 46000 *G. max* genes are existed in the HC regions. The repetitive sequences in *Arabidopsis* genome are found to be concentrated in the HC pericentromeric regions too, whereas most of the protein-coding genes are found in the euchromatic arms that are largely devoid of repetitive sequences (She et al., 2007). She & Jiang (2015) analyzed the karyotype of *Lablab purpureus* by using fluorochrome banding, it was observed that only the pericentromeric regions of all long arms of the chromosomes and the NOR regions exhibited DAPI positive bands. Begum & Alam (2016) studied varieties of *Cicer arietinum* L. (chickpea) by differential fluorescent banding and they found that the DAPI-bands were so unique for the chromosomes of each variety, and some terminal and few centromeric bands were observed in the nine chickpea varieties that could easily be used as chromosome markers. The occurrence of genomic alteration within these varieties could be characterized authentically by fluorescent banding analysis. Therefore, cytogenetic techniques are useful and hopeful to expose the general pattern of genome evolution. Cautiously selected whole genome sequences over the entire Brassicaceae family, combined with a deep understanding on genome and chromosome evolution and robust comprehensive phylogenetic hypothesis, will upshot in an essential depth understanding the entire large plant family evolution (Couvreur et al., 2010). All those observed pericentromeric banding pattern in the plants with small chromosomes highly supported our results in the species under study, since there are no previous chromosome banding investigation on them.

Conclusion

Since there are no previous investigations to explain the chromosome banding patterns on the species under study, it could be explained depending on the whole above observations and explanations in different publications on the banding pattern in the species with small chromosome, why the species under study have only pericentromeric DAPI-bands.

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: HA performed the DAPI banding experiment part. HA, SH and AH wrote the manuscript, participated in the data discussion, data analyses, and drafting of the manuscript, all authors have read and approved the manuscript.

Ethical approval: Not applicable.

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أنماط الشرائط الكروموسوميه بالصبغة الفوسفوريه DAPI في عدة انواع من العائلة الكرنبيه

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تضم العائلة الكرنبية عددًا من أهم أصناف الكرنب مثل الكانولا والخردل والكرنب ويعتبر الأرابيدوبسيس ثاليانا من أحسن النبات كنموذج بيولوجيي لصغر حجم الجينوم الخاص به. وتعد الشرائط الكروموسوميه هي البروتوكول الأكثر احتمالًا لدراسة الهيئه الكروموسوميه ولتمييز الأختلالات الكروموسومات العددية numerical aberrations، أو التكرار duplications أو الانقلابات في جزء من الكروموسوم inversions أو حذف أجزاء من الكروموسوم deletions، أو تبادل جزء من كروموسوم إلى آخر أيضًا translocations. الهدف من هذه الدراسة هو توضيح أنماط الشرائط الكروموسوميه بأستخدام صبغة DAPI في 16 نوعًا من العائله الكرنبيه ذات حجم الكروموسوم الصغير، ولها أصول وراثيه مختلفه، ومستوى التعدد الكروموسومي (يختلف عدد الكروموسوم من (2n= 14 إلى 2n= 32). و تنتمي هذه الأنواع إلى خمس عشائر هي Brassiceae، Sisymbrieae ، Lepidieae، Arabideae و Alyssea. ووجد أن الأنوع التي تحت الدراسه وذات حجم الكروموسومات الصغيره تفتقر إلى وجود الشرائط الكروموسوميه البينيه والتي تعكس أماكن الهيتيروكروماتين العالي التكرار ولا يظهر بها سوي السنترومير ويتفق هذا مع الدراسات التي اجريت على أنواع نباتيه أخرى حيث أستنتج منها أن معظم النباتات ذات الكروموسومات الصغيرة لها في الغالب شرائط كروموسوميه مركزية في منطقة السنترومير وكلما زاد حجم الكروموسوم الميل إلى وجود الشرائط الكروموسومى.