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RESEARCH ARTICLE

Chromosome Number and Mitotic Time of Two Species of the Apiaceae Family

Ganies Riza Aristya[a]*, Afifah Nur Aini Putri[a], Nurina Tahta Afwi Maulina[a], Franciscus Rico Kusuma[a], Muhammad Fauzi Arif[b]

[a]Laboratory of Genetics and Breeding, Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia; [b]Laboratory of Biology, Department of Biology, Faculty of Mathematics and Life Sciences, Mulawarman University, Samarinda, 75242, Indonesia

Abstract In Indonesia, the production yield and demand in vegetable cultivation must be balanced with improved production quality. Research is needed to enhance the quality of plant production, particularly in the genetic breeding of carrots and celery. This study aimed to characterize the shape, number, and size of chromosomes in two species of the Apiaceae family, specifically carrot (*Daucus carota* L.) and celery (*Apium graveolens* L.). The study applied a modified squash method, incorporating procedures for chromosome preparation such as root fixation, cell maceration, and chromosome staining. Mitotic phases were observed under the microscope, and the analysis was conducted using Image Raster 3 for length measurement, Photoscape v3.6 and Corel Draw X6 for generating karyotypes, and Microsoft Excel 2007 for creating the idiograms. The results indicate differences in the mitotic timing and chromosome characteristics between the two Apiaceae species. Carrot species (*D. carota* L. 'Kuroda' and 'Nantes') exhibited mitotic division from 9:30 to 11:00 am with a chromosome number 2n = 18. Celery species (*A. graveolens* L. 'Summer' and 'Amigo') showed mitotic division occurring from 11:30 am to 12:45 pm with a chromosome number of 2n = 22. This study can help enhance the quality of plant production, particularly in the genetic breeding of carrots and celery.

Keywords: Apiaceae, chromosome, karyotype, squash method.

Introduction

Indonesia is recognized as an agrarian country that allows the cultivation of various beneficial vegetable crops for human life. Most vegetables play a crucial role in fulfilling dietary needs and improving nutrition as they contain essential vitamins, fiber, and minerals. One family often classified as beneficial vegetables is the Apiaceae family [1–3], especially carrots and celery. Carrots (*Daucus carota* L.) are known for their high vitamin A content, which helps maintain eye health [4, 5]. Additionally, carrots contain other vitamins, such as C, K, B1, B2, B3, B5, B6, B9, and E [6, 7]. Another main component found in carrots is *α*-carotene and *β*-carotene, which is converted into vitamin A [8, 9]

On the other hand, celery (*Apium graveolens* L.) belongs to the celery vegetable type and is known for its strong taste. Celery is used as a seasoning ingredient and also serves as a medicinal plant [10, 11]. Celery is known to contain saponins, flavonoids, polyphenols, proteins, sulfur, calcium, iron, phosphorus, and vitamins A, B1, and C [11, 12]. The content of celery, such as flavonoids, saponins, and tannins, has been proven to prevent cardiovascular diseases, improve fertility, reduce cholesterol and fat levels, as well as lower cholesterol or hypertension, and skeletal disease [12–16].

The increasing usefulness of carrots and celery has triggered a growing demand. Therefore, it is essential to enhance the quality of carrots and celery. One way to improve plant quality is through plant breeding, such as polyploidization, which involves chromosomal duplication in an organism [17, 18]. Polyploidization, or chromosomal duplication in an organism, is one form of plant breeding that can be performed [19]. Before conducting polyploidization, it is crucial to determine an organism's chromosome number and mitotic division time, especially for plants of the Apiaceae family [20, 21].

***For correspondence:**
ganies_riza@ugm.ac.id

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In Indonesia, carrots (*D. carota*) commonly used in the market are the Kuroda and Nantes cultivars, while celery (*A. graveolens*) widely used in the market includes the celery cultivars Summer and Amigo. Both of these carrot and celery species are considered to proliferate, be productive, strong, and resistant to pests. As genetic breeding research has yet to be conducted on carrots and celery in Indonesia, it is necessary to conduct genetic breeding research to enhance the production of these two plants in Indonesia. aimed to characterize the shape, number, and size of chromosomes of the Apiaceae family (*D. carota* 'Kuroda' and 'Nantes') and celery (*A. graveolens* 'Summer' and 'Amigo').

Materials and Methods

Materials

Glacial acetic acid 45% was used in the root fixation process. HCl 1 N was used for maceration of the fixed root tips. Sterile distilled water was used to rinse the treated material. Aceto-orcein dye was utilized to stain chromosomes during mitosis. Glycerin served to enhance the refractive index. Acetone was applied to seal the slides for observation. Observations were conducted under a light microscope.

Methods

The seeds of carrot (*D. carota* 'Kuroda' and 'Nantes') and celery (*A. graveolens* 'Summer' and 'Amigo') were germinated in a petri dish moistened with water by placing the seeds on a tissue paper. The seeds were left for seven days until the seedlings grew, while the tissue paper was kept wet. The method used in this research was a modified version of the Squash method by Nathewet *et al* (2009) [22] . Root tips were taken from germinated carrot and celery seeds in a petri dish containing wet tissue, with the cutting performed approximately every hour and later continued every half an hour. The root tips were cut approximately 0.5 cm. After that, fixation was performed. The root tip was placed in a flacon bottle using a brush and immediately fixed with 45% glacial acetic acid for 15 minutes. During fixation, the flacon bottle had to be kept at a temperature of 4˚C. After 15 minutes, the 45% glacial acetic acid was removed, and the root tip sections were washed thrice with distilled water. Hydrolysis involved macerating the root sections in the flacon bottle that had been washed by dripping them into a 1 N HCl solution until submerged.

The sample was incubated at a temperature of 55 ˚C for 10 minutes. After that, it was rewashed with distilled water three times. The cleaned root tips were stained with 1% aceto-orcein for approximately 4 hours. The root tips were taken from the flacon and positioned on a glass slide for squashing. Any residual aceto-orcein was eliminated, and the root sections were cut approximately 2 mm at the tip with the deepest coloration. The sections were then dripped with glycerin and covered with a cover glass, then pressed with the tip of the brush until the root was crushed. To prevent the preparation from drying quickly, the edges of the cover glass were sealed with acetone and labeled.

Data Analysis

The chromosome numbers for each cultivar were directly determined by observing images under the microscope at the prometaphase stage using the Image Raster v3. To calculate the mitotic time, the presence of each mitotic phase was assessed visually in three fields of view, with three repetitions each. The cutting time with the highest percentage of prometaphase presence was used as a reference for the initial stage of the mitotic time range.

Chromosome analysis was conducted by karyotyping, involving measuring the short arm (p) and long arm (q), determining chromosome size, calculating the centromere index, identifying chromosome shape, and constructing an idiogram. This procedure was executed manually with Image Raster v3, Photoscape v3.6, Corel Draw X6, and Microsoft Excel 2007. The measurements of chromosome length data were input into Microsoft Excel 2007 to compute absolute chromosome length, relative chromosome length, and standard deviation. Subsequently, the data comprising chromosome length measurements were employed to determine the centromere index and the ratio of chromosome arm length to short arm length [23, 24]. The standard of centromere index and the ratio of chromosome arm length to short arm length followed from [24].

The size, shape, and configurations of chromosomes in each cultivar were examined descriptively. The karyotype was constructed by arranging chromosomes based on their absolute length from longest to shortest using the Corel Draw X6. Subsequently, the generated karyotype was used as the foundation for building the idiogram, which was created with Microsoft Excel 2007.

Results and Discussion

Some vegetables classified in the Apiaceae family include carrots and celery. Carrots and celery belong to the order Apiales (Umbelliflorae) and the family Apiaceae (Umbelliferae) due to their flowers being arranged in umbels, their hermaphroditic nature, actinomorphic structure, and pentamerous arrangement [25, 26]. Their sepals are small, their petals are large, and their stamens are arranged in a circle [27]. Polyacetylene compounds in some Apiaceae vegetables exhibit cytotoxic activity [28, 29]. Compounds composing polyacetylenes include falcarinol and falcarindiol, with falcarindiol being the main compound responsible for the bitter taste in carrots [30].

Two well-known carrot cultivars planted in Indonesia are the Kuroda and Nantes [31, 32]. Kuroda, a seed variety from the Known-You Seed company, has a somewhat firm texture but a sweet taste, with harvested roots measuring around 19 × 5.5 cm and weighing 400 grams. This cultivar can be harvested after 100-110 days. On the other hand, the Nantes cultivar is a seed from Saribenih Unggul. The fruit's color turns red after harvesting, and it has a higher water content than other cultivars, with fruit length ranging from 18-20 cm. This cultivar has high production quality and can be harvested after 85 days.

Two well-known celery cultivars in Indonesia are the Amigo and Summer cultivars [33, 34]. Amigo, a seed from Panah Merah, is characterized by young green leaves, long stalks, numerous productive shoots, erect plant growth, and short stature. It is suitable for cultivation at medium to high altitudes. Meanwhile, the Summer celery cultivar, a seed from the Known-You Seed company, is suitable for cultivation in lowland areas. The leaves of the 'Summer' celery are dark green. This cultivar has the advantage of having firm and not easily broken leaf stalks, making it resistant to damage during transportation. The sturdy stalks do not have fibers and do not age quickly.

Cytogenetic research on carrots has not progressed as much as in many other species. There have been several studies on the *D. carota* plant, including *D. broteri* (2n = 20), *D. capilifolius* (2n = 18) with a chromosome formula of 10m + 6sm + 2st, *D. carota* var. Sativus (2n = 18) with a chromosome formula of 8m + 6sm + 4st, *D. crinitus* (2n = 22) with a chromosome formula of 4m + 14m + 4st, *D. hispidifolius* (2n = 22), *D. pusillus* (2n = 22) with a chromosome formula of 6m + 14sm + 2st, and *D. glochidiatus* (2n = 44) [35]. According to [36], carrots are diploid organisms possessing nine pairs of relatively short chromosomes (2n = 18). The estimated genome size is 473 mega base pairs, which is four times larger than that of *Arabidopsis thaliana*, one-fifth the size of the corn genome, and roughly equivalent to the size of the rice genome. It was demonstrated that the chromosome number of *A. graveolens* is 2n = 22 [37]. The cell cycle of *D. carota* 'Kuroda' and 'Nantes', as well as *A. graveolens* 'Summer' and 'Amigo', was observed using Nathewet *et al*'s (2009) [22] modified squash method. According to the findings, most plants in the Apiaceae family undergo mitotic division primarily between 09:00 and 12:45 pm. Specifically, *D. carota* exhibits mitotic division from 09:00 to 11:00 am, while *A. graveolens* predominantly undergoes it between 11:00 am and 12:45 pm.

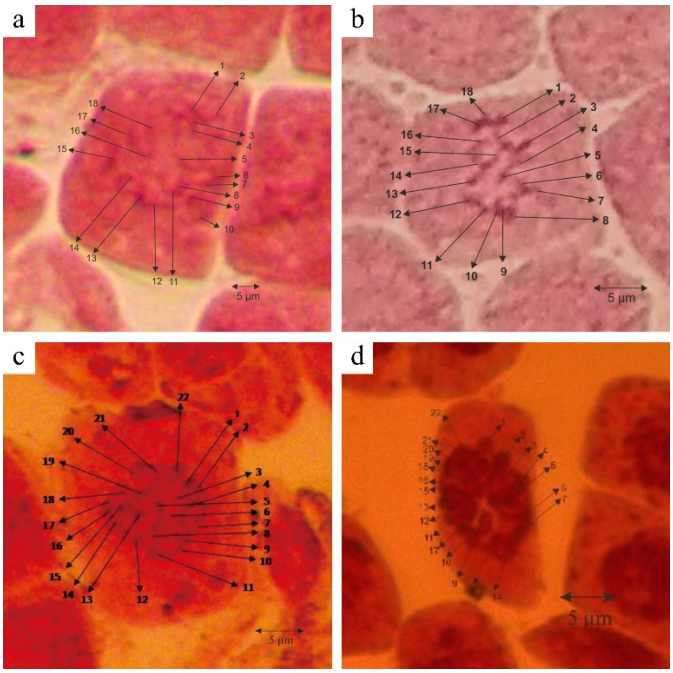

Figure 1. The number of chromosomes of carrot (*D. carota*) and celery (*A. graveolens*) cultivars (a) 'Kuroda', (b) 'Nantes', (c) 'Summer' and (d) 'Amigo'. Line bar = 5 µm

The research results are consistent with Hore's study (1977) [38], which stated that the number of chromosomes in the Umbelliferae family, especially *A. graveolens*, is 2n = 22. These results show no differences between the two cultivars, both Summer (Figure 1c) and Amigo (Figure 1d). Therefore, the number of chromosomes in *A. graveolens* 'Summer' and 'Amigo' is 22n = 22x = 22. Meanwhile, in Figure 1, it can be observed that both carrot cultivars have the same number of chromosomes, which is 2n = 18. This chromosome number is consistent with [36] research, where carrots (*D. carota*) are diploid species, having nine pairs of relatively short chromosomes (2n = 18). Based on the prometaphase results, chromosomes were arranged according to the length of the short arm, the length of the long arm, and the absolute length to determine the homologous chromosomes of each pair. The centromere index of each chromosome and its type can be determined by comparing the length of the short arm and the absolute length of the chromosomes [39, 40].

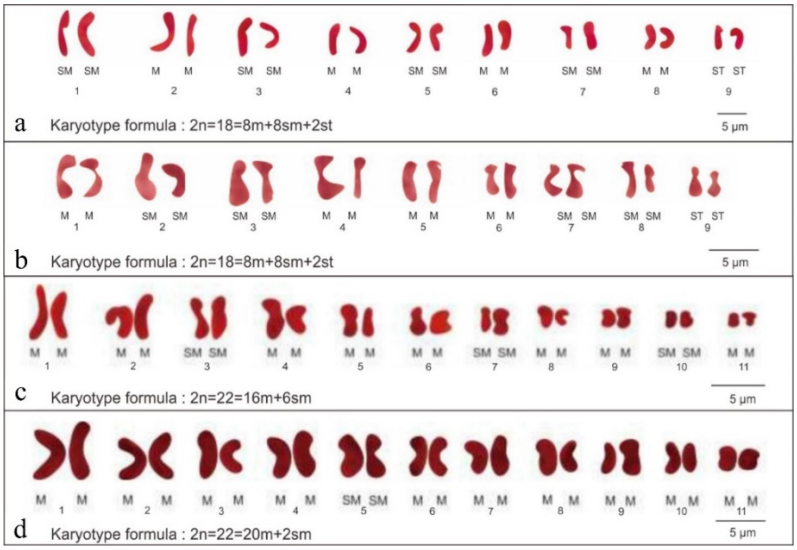

Figure 2. Chromosomes karyotype carrot (*D. carota*) and celery (*A. graveolens*) cultivars (a) 'Kuroda', (b) 'Nantes', (c) 'Summer', (d) 'Amigo'. Line bar = 5 µm

The karyotype of the carrot plant cultivar Kuroda (Figure 2) is illustrated in the karyotype formula for this cultivar, which is 2n = 18 = 8m + 8sm + 2st, with an absolute chromosome length ranging between 1.2 and 7.6 µm. The Kuroda cultivar has the same karyotype formula as the Nantes cultivar, which is 2n = 18 = 8m + 8sm + 2st, with an absolute chromosome length ranging between 0.58 to 2.87 µm. Karyotypes are categorized into two types (symmetric and asymmetric) [41]. Plants with an asymmetric karyotype are considered more advanced than those with a symmetric karyotype. Asymmetric karyotypes consist of chromosomes with diverse forms and result from new cultivation [41, 42]. According to the results of this study, the karyotype of the carrot plant cultivars Kuroda and Nantes falls under the asymmetric karyotype type, with a diverse formula comprising metacentric, submetacentric, and subtelocentric-type chromosomes.

Based on Figure 2c, *A. graveolens* chromosomes are dominated by metacentric types, with eight pairs of chromosomes in pairs 1, 2, 4, 5, 6, 8, 9, and 11. In contrast, chromosomes with submetacentric types are found in pairs 3, 7, and 10. These metacentric chromosomes have centromere indices ranging from 37.50 to 50.00, while submetacentric types have centromere indices between 25.00 and 37.49. Thus, the chromosome formula for *A. graveolens* 'Summer' is determined to be 2n = 22 = 16m + 6sm. The length of these chromosomes ranges from 1.06 to 5.03 µm. Different results are shown in the karyotype of the Amigo cultivar. Almost all chromosomes have metacentric types with centromere indices ranging from 37.50 to 50.00, and only one homologous chromosome has a submetacentric type (Figure 2d). In contrast to the Summer cultivar, which has submetacentric chromosome types, in the Amigo cultivar, chromosome pairs 3, 7, and 10 have metacentric types. Meanwhile, chromosome pair 5, which has a metacentric type in the Summer cultivar, has a submetacentric type in the Amigo cultivar. The chromosome formula for *A. graveolens* 'Amigo' is 2n = 22 = 20m + 2sm, different from the Summer cultivar. The estimated length of chromosomes in the Amigo cultivar ranges from 1.84 to 5.64 µm. There is a correlation between chromosome length and chromosome type because chromosome type is determined from the ratio between the short arm and the total length of the chromosome.

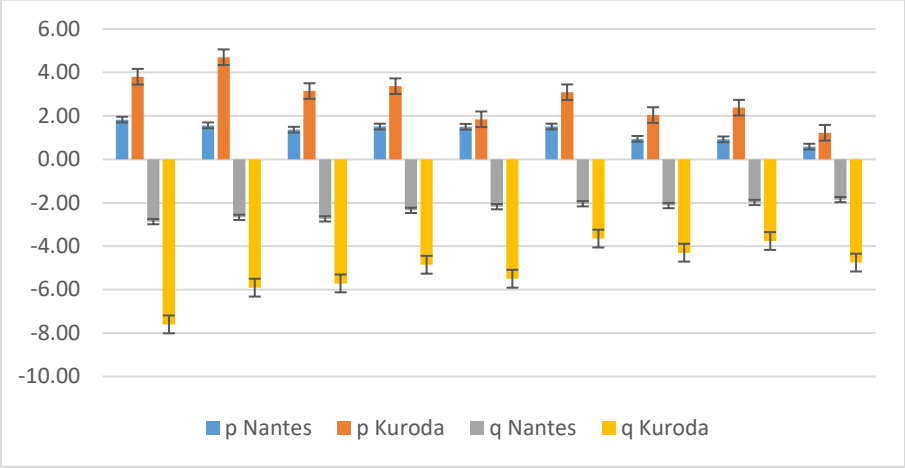

Figure 3. Comparison idiogram between carrot (*D. carota*) cultivars

Examining the short and long arms of chromosome pairs in the two carrot cultivars reveals varying outcomes (Figure 3a). This suggests that the Kuroda cultivar's longer chromosome arms influence distinct phenotypic characteristics. In the Kuroda cultivar, this results in a shorter phenotype with thick fruit flesh, while the Nantes cultivar has a more extended phenotype with thinner fruit flesh.

According to Figure 4, the Summer cultivar's short arm (p) length varies from 0.45 to 2.51 µm, and the long arm (q) length ranges from 0.61 to 2.52 µm. In the Amigo cultivar, the short arm (p) exhibits a range of 0.83 to 2.84 µm, while the long arm (q) varies from 1.01 to 3.00 µm. These results indicate that the comparison of the length of the short arm (p) to the long arm (q) in the Summer cultivar is shorter than in the Amigo cultivar. This comparison of the long and short arms can also affect the phenotype outcomes of both cultivars. Differences in chromosome arm length can impact gene density, expression patterns, and the arrangement of regulatory elements [41]. This may lead to variations in phenotypic traits such as fruit size, leaf shape, flowering time, and stress tolerance as observed in the Summer and Amigo cultivars. Variations in chromosome arm length might play a significant role in the growth, development, and adaptability differences between these two cultivars.

Differences in karyotype within the same species with different varieties are likely to occur because, although chromosomes carry inherited traits from parents, changes can still happen [43]. Structural changes in chromosomes, such as fragmentation, deficiency, duplication, and translocation, can alter the arrangement of chromosome karyotypes [44, 45]. Deviations in chromosomes can occur under certain conditions, leading to changes in morphology [46]. However, the chromosome arrangement of the population has evolved [47, 48].

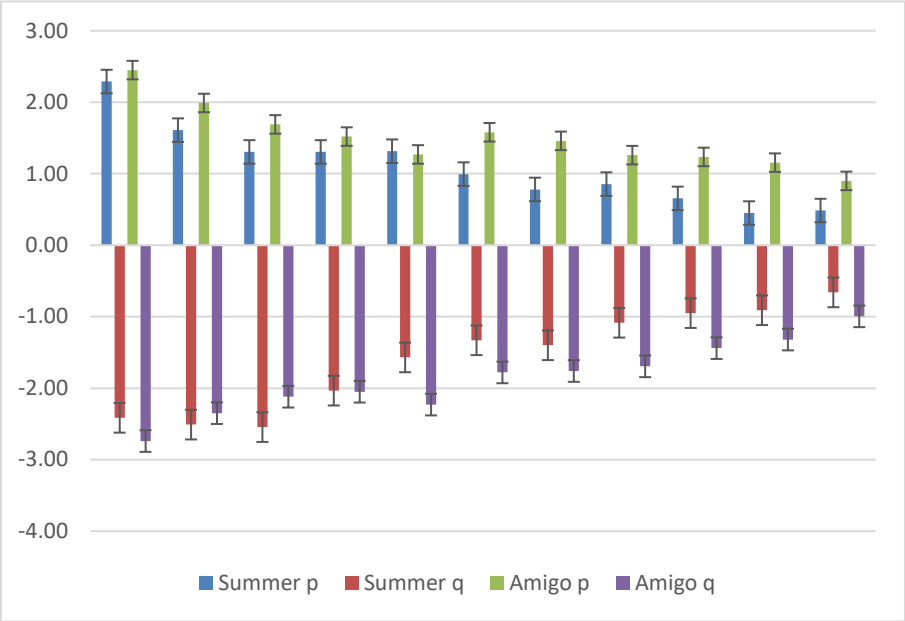

Figure 4. Comparison idiogram between celery (*A.graveolens*) cultivars

The differences in karyotype and the absolute arm length of the two carrot cultivars explain the phenotypic differences that emerge in both cultivars. Morphological differences, such as color, size, and thickness of fruit flesh, result from variations in the chromosomal arrangement of the two cultivars, affecting their phenotypes [49]. Differences in chromosome size may be due to the degree of chromatin condensation or the accumulation of different heterochromatin [50, 51]. In contrast, differences in chromosome shape may occur due to translocation, resulting in different satellite positions for each chromosome [19]. The reduction in chromatin length can be considered one of the symptoms of the evolutionary process [52]. Additionally, variations in both carrot cultivars may also occur due to the processes of domestication and cultivation [53]. The domestication process can lead to changes in the gene arrangement of a species to support its life in different environments compared to the wild type [53, 54]. This is also the cause of differences between the phenotype and genotype of wild-type plants and those resulting from domestication [55].

Conclusions

The mitotic time, chromosome number, karyotype, and idiogram of the two species in the Apiaceae family differ, each displaying its unique characteristics. In the carrot species (*D. carota* L. 'Kuroda' and 'Nantes'), mitotic initiation occurs from 9:30 to 11:00 am, and the chromosome number is 2n=18. The relative length of chromosome arms ranges from 1.22 to 7.6 µm in the Kuroda cultivar and from 0.58 to 2.87 µm in the Nantes cultivar. For celery species (*A. graveolens* L. 'Summer' and 'Amigo'), mitotic begins from 11:30 am to 12:45 pm, and the chromosome number is 2n = 22. The relative length of chromosome arms ranges from 1.06 to 5.03 µm in the Summer cultivar and from 1.84 to 5.64 µm in the Amigo cultivar.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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