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CARROT (*DAUCUS CAROTA* L.) CHROMOSOME ANALYSIS AND THEIR IMPACT ON GENETIC DIVERSITY

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SUMMARY

Carrot (*Daucus carota* L.) is a widely cultivated root crop due to its substantial nutritional values, including elevated levels of essential vitamins and antioxidants. The apposite study aimed to determine the chromosomes' mitotic time numbers. The research used a modified squash method, including chromosomes preparation steps, such as fixation, maceration, and staining. The comparison of two carrot cultivars, i.e., Berastagi and Ta-Fung, highlighted similarities in chromosome morphology, size, and karyotype formulas, with some disparity in chromosome formula unveiling their unique genetic attributes and distinctions. The established mitotic times and chromosomal formulas emerged as 09:00 AM–10:00 AM ($2n = 2x = 18 m$) and 09:00 AM–10:10 AM ($2n = 2x = 14 m + 4 sm$) for cultivars Berastagi and Ta-Fung, respectively, with their respective karyotypes and ideograms. In chromosome count, the congruence between the two cultivars highlights their shared genetic foundation and, albeit with structural variations. Such primary data of the presented research lays the foundation for future breeding research for improvement in both carrot cultivars.

Keywords: Carrot (*D. carota* L.), chromosome numbers, chromosome morphology and size, mitotic times, genetic diversity, ideograms, karyotypes, squash method

Key findings: In carrot (*D. carota* L.), the chromosome's exact number, formulas, and karyotype have yet to be determined for two carrot cultivars grown in Indonesia. However, through this research, the mitotic times and chromosomal formulas' establishment occurred as 09:00 AM–10:00 AM ($2n = 2x = 18 m$) and 09:00 AM–10:10 AM ($2n = 2x = 14 m + 4 sm$) for cultivars Berastagi and Ta-Fung, respectively, with their karyotypes and ideograms.

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INTRODUCTION

Carrot (*Daucus carota* L.) represents as one of promising agricultural commodities in Indonesia (Sari *et al.*, 2021). Carrot is a popular staple crop known for its nutritional values, containing vitamins A, C, K, and antioxidants, such as, carotenoids and anthocyanin (Marcelino *et al.*, 2020; Boadi *et al.*, 2021; Ibrahim *et al.*, 2023). Carrots cultivated for generations hold the highest economic values, sought after by communities since their cultivation began in Central Asia (Que *et al.*, 2019; Elechi *et al.*, 2022).

Generally, the carrots grow in tropical climates in cool areas, such as, mountainous and highland areas, with temperatures around 21 °C (Hochmuth *et al.*, 2021). Carrot cultivation involves planting in moderately cool regions, providing moderate watering and good soil aeration (Kjellenberg, 2007; Sari *et al.*, 2021). Carrots thrive best with total sunlight exposure in soil, with a pH of 6–7 (Heininonen, 1990). After planting in the soil, the germination period ranges from 10 to 21 days, and need approximately 2.5–3 months before ready for harvest (Lenaerts *et al.*, 2019).

As a widely utilized plant, the availability of past data regarding carrot karyotypes and ideograms is essential to serve as a foundation for further studies (Kadluczka and Grzebelus, 2021; Ou *et al.*, 2022; Singh *et al.*, 2022). The chromosomal data gathered could serve to produce superior variants and explore the plant variations undergoing evolution, and determine their relationships with other species (Iovene *et al.*, 2008). The two carrot cultivars Berastagi and Ta-Fung used in this study were particularly interesting for differences in their origins, reflected by the structural variations in their tubers.

Previous different studies have highlighted the degree of similarity between cultivated carrot genotypes. Understanding the chromosomes could eventually lead to heighten breeding efforts, resulting in cultivars with more expressed desirable traits (Li *et al.*, 2017; Puchta and Houben, 2023). Based on the above discussion, the presented study sought to analyze and characterize the mitotic

times and chromosomes (number, shape, and size) of the two carrot (*D. carota* L.) cultivars, Berastagi and Ta-Fung. Focusing on the chromosomal data of these cultivars could contribute to further research on its genetic make-up with different breeding aspects.

MATERIALS AND METHODS

The latest research commenced from March to July 2023 at the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. The two carrot cultivar (Berastagi and Ta-Fung) used in this study were procurements through different means. Cultivar Berastagi seeds came from the seed store, while cultivar Ta-Fung seeds from local farmers in Magelang, Central Java, Indonesia.

Before planting, soaking carrot seeds in water for one hour transpired, preceding transfer to moist growth media for germination. After 7–10 days, the emerged root tips (1–2 cm) obtained fixing with 45% glacial acetic acid. Following three washes with distilled water, maceration used 1 N HCl. The samples' staining employed 1% aceto-orcein for 4 h. Once left to stain on a glass slide continued with the samples carefully cut at the ends, covered with a coverslip, squashed, and observed under a microscope (Olympus BX41, objective magnification 40–100×), coupled with a reflected light illuminator, providing additional magnification at 1.25×, 1.6×, and 2×.

Capturing photos used the OptiLab Viewer v3.0. Chromosome images processing engaged Adobe Illustrator to sharpen, enhance, and trace the full chromosomes. Taking measurements used Image Raster, with karyotypes and ideograms developed by arranging the chromosomes. The mitotic index calculation depended on the percentage of cells that undergo mitosis in each phase. The ideograms construction proceeded in Microsoft Excel, with chromosomes sorted by length and centromere regions aligned on a horizontal baseline.

RESULTS AND DISCUSSION

Mitotic time

Observing the cells fixed at different times determined the mitotic time ranges for the carrot cultivars Berastagi and Ta-Fung. All fixation treatments happened in the GMT+7 time zones (Western Indonesian Time). The analysis of the root cells of the cultivar Berastagi with varying fixation times revealed the mitotic time range occurred between 09:00 and 10:00 AM. In the cell, the observed prophase fixed from 09:00 to 09:25 AM, characterized by the apparent condensation of chromosomes within the cell nucleus (Figure 1). Prometaphase was most distinctly notable in cells fixed between 09:35 and 09:45 AM, displaying entirely discernible chromosomes that are countable individually without clumping. Subsequently, the metaphase observation was also around 09:45 to 09:50 AM, with chromosomes aligning due to the

movements they underwent. From 09:50 to 09:55 AM, cells entered into anaphase, evident from the movement of chromosomes toward new poles of the cells. Finally, cells underwent telophase between 09:55 and 10:00 AM, seen from the faintly emerging nuclei.

For carrot cultivar Ta-Fung, the cells' observation indicates differences in the mitotic time range. Prophase occurs around 09:00 to 09:30 AM, while the prometaphase took place approximately from 09:30 to 09:50 AM, which was longer than the prometaphase duration in the cultivar Berastagi. The time ranges for metaphase, anaphase, and telophase were approximately 09:50–09:55 AM, 09:55–10:00 AM, and 10:00–10:10 AM, respectively (Figure 2). According to past studies, the differences in the mitotic time range between the cultivars Berastagi and Ta-Fung referred to cultivar variations, along with the influence of circadian rhythms, temperature, and hormonal balance in each genotype (Farshadi *et al.*, 2020; Cain *et al.*, 2021).

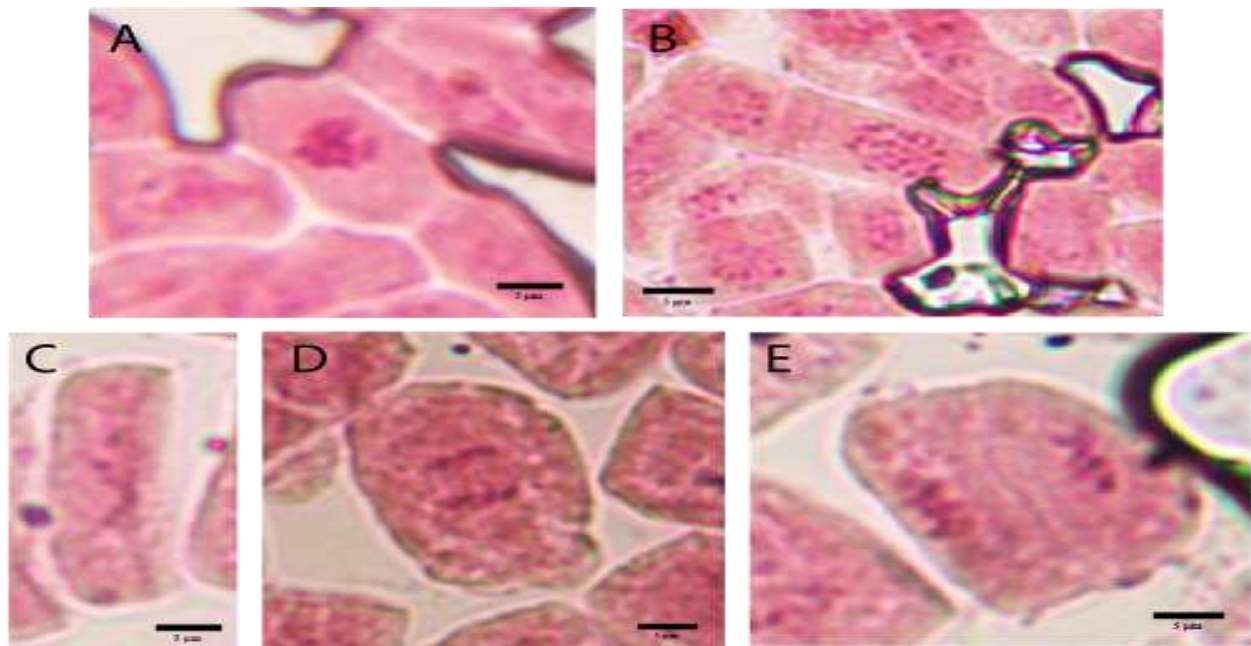


Figure 1. Mitotic time of carrot cultivar Berastagi at various fixation times: A. Prophase (time range: 09:00–09:35), B. Prometaphase (time range: 09:35–09:45), C. Metaphase (time range: 09:45–09:50), D. Anaphase (time range: 09:50–09:55), E. Telophase (time range: 09:55–10:00). Line bar = 5 µm.

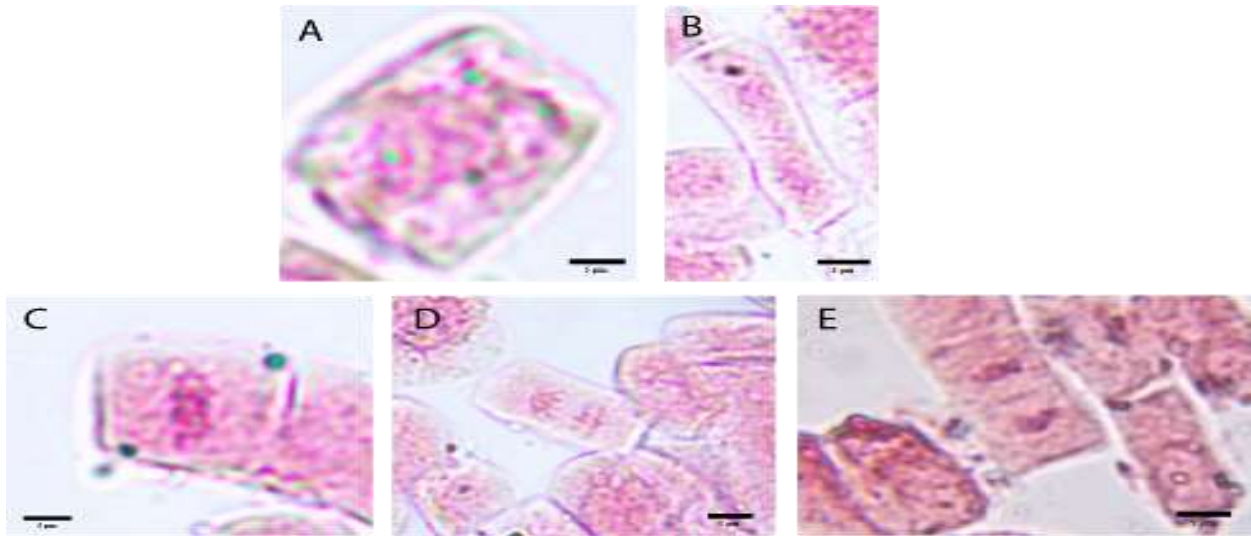


Figure 2. Mitotic time of carrot cultivar Ta-Fung at various fixation times: A. Prophase (time range: 09:00–09:30), B. Prometaphase (time range: 09:30–09:50), C. Metaphase (time range: 09:50–09:55), D. Anaphase (time range: 09:55–10:00), E. Telophase (time range: 10:00–10:10). Line bar = 5 µm.

The circadian rhythm synchronizes the biological processes in an organism with the light-dark cycle that occurs within a day (Farshadi *et al.*, 2020). In plants, the circadian rhythm influence comprised two primary factors, i.e., light and air temperature (Srivastava *et al.*, 2019). This rhythm intersects with the TTFL (transcriptional-translational feedback loop), which balances cellular cycles and other metabolic functions based on the environmental conditions and plant requirements (Srivastava *et al.*, 2019). One cycle associated with TTFL is the expression of TOC-TIC genes, linked with various biological processes, such as, mitosis, photosynthesis, flowering, and plant senescence (Richardson and Schnell, 2020). The imported cultivar Ta-Fung adapts by adjusting its cell cycle through alterations in TOC-TIC expression, responding to the variations in day's duration and temperature at the experimental location.

In this study, the second mitotic time of both carrot cultivars lasted approximately one hour and fell within a similar time range, around 09:00 to 10:00 AM. These findings align with previous studies on cell cycles in plants, revealing consistent mitotic times

ranging from 1 to 1.5 hours across various plants, although with varying interphase times in various crop plants (Scofield *et al.*, 2014; Qi and Zhang, 2020). Factors, such as, plant stress and the growth environment influenced the mitosis and interphase varied durations in the cell cycle of different plants (Farshadi *et al.*, 2020). Based on these facts, the imported carrot cultivar Ta-Fung might adapt its mitotic timing according to the climate at the research location.

The results revealed a high similarity in the timings of the mitotic phase between the two carrot cultivars, occurring around 09:00–10:00 AM. In the mitotic durations, the resemblance is referring to the close genetic relatedness between the two individuals. Taxonomically, both cultivars stem from the same carrot plant. These two carrot cultivars are also products of selective breeding and maintain the capacity to interact due to their significant genetic proximity, escorted by the inheritance of robust traits within their genes (Domblides and Domblides, 2020).

Based on this study, the presented research organized the observed chromosomes into karyotypes and ideograms, which can serve as elementary data for further breeding

investigations in these two carrot cultivars. Prior studies involving mitotic timings among various cultivars have yielded similar findings and determined mitotic times and chromosome numbers through tissue fixation, squashing, and microscopic observation (Aristya *et al.*, 2015). The findings demonstrated a resemblance in mitotic timings and chromosome formulas among the strawberry cultivars, differing only in their ploidy levels (Aristya *et al.*, 2015; Aristya and Alya, 2019).

Chromosome measurement

Chromosome pairing from both carrot cultivars is a diploid (2n), resulting in the chromosome formula, i.e., $2n = 18$. The matching chromosome formula and configuration indicated a close relationship between the two cultivars. The chromosome formulas of these two cultivars align with past studies conducted by Iovene *et al.* (2008), who performed karyotyping and FISH on various cultivated carrot cultivars, and reported similar findings. The recent results also got support from the research work of Iorizzo *et al.* (2016), who determined the chromosome formula ($2n = 18$) for most carrots cultivated worldwide. The Japanese carrot cultivar 'Kuroda (*D. carota*)' possessed similar genomic composition and features of large, cylindrical root, and shares a mitotic period similar to the presented two cultivars (Xu *et al.*, 2014). The relationship between the studied cultivars suggested a

parallel evolution and adaptation grown in similar environmental conditions (Iovene *et al.*, 2008; Iorizzo *et al.*, 2016).

Karyotype and ideogram

The karyotype and ideogram for both carrot cultivars Berastagi and Ta-Fung preceded construction by measuring and re-arranging the acquired chromosomes. The main purpose was to examine the chromosomal characteristics that differentiate the two cultivars in this study, focusing on the quantity, size, and structural variations of the chromosomes. The constructed karyotype from the chromosomes of the cultivar Berastagi has a formula of $2n = 2x = 18$ (Figure 3). Nine pairs of diploid chromosomes were evident in each somatic cell of this cultivar. The chromosomes, with an average length of $1.39 \mu\text{m}$, exhibited a metacentric shape across all the chromosomes through their centromere positions.

The constructed karyotype from the chromosomes of the cultivar Ta-Fung has a formula of $2n = 2x = 18$ (Figure 4). Similar to the cultivar Berastagi, nine pairs of diploid chromosomes were visible in each somatic cell. The chromosomes in the cultivar Ta-Fung appeared with an average length of $1.56 \mu\text{m}$, comprising seven pairs of metacentric chromosomes and two pairs of submetacentric chromosomes, based on their centromere positions.

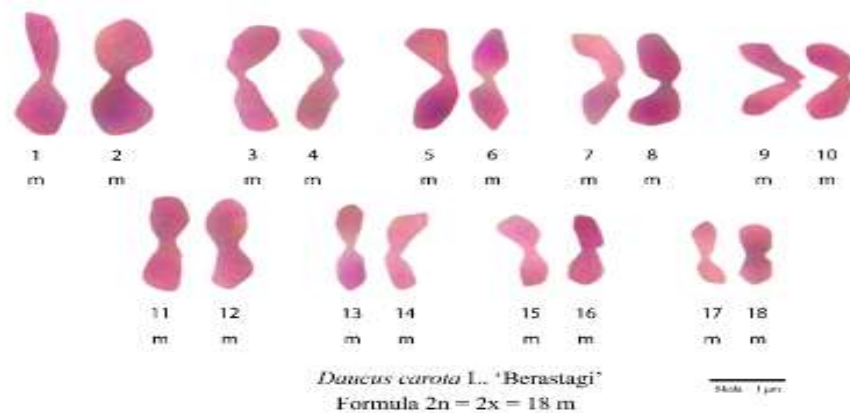


Figure 3. Karyotype of the carrot cultivar Berastagi.

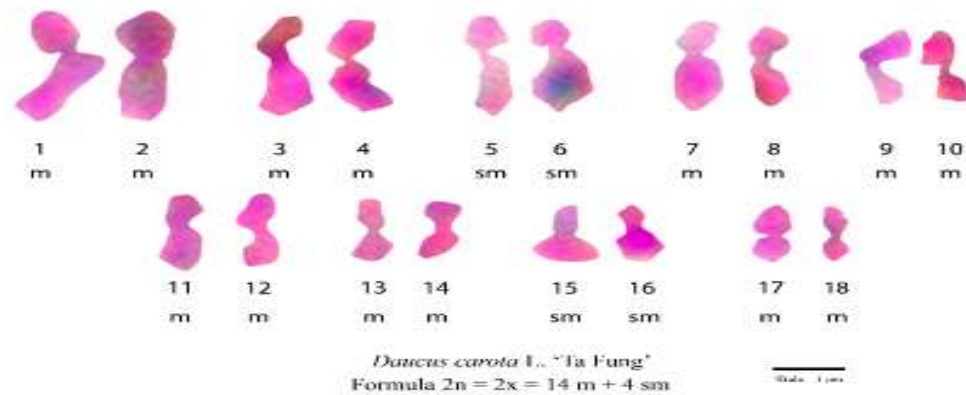


Figure 4. Karyotype of the carrot cultivar Ta-Fung.

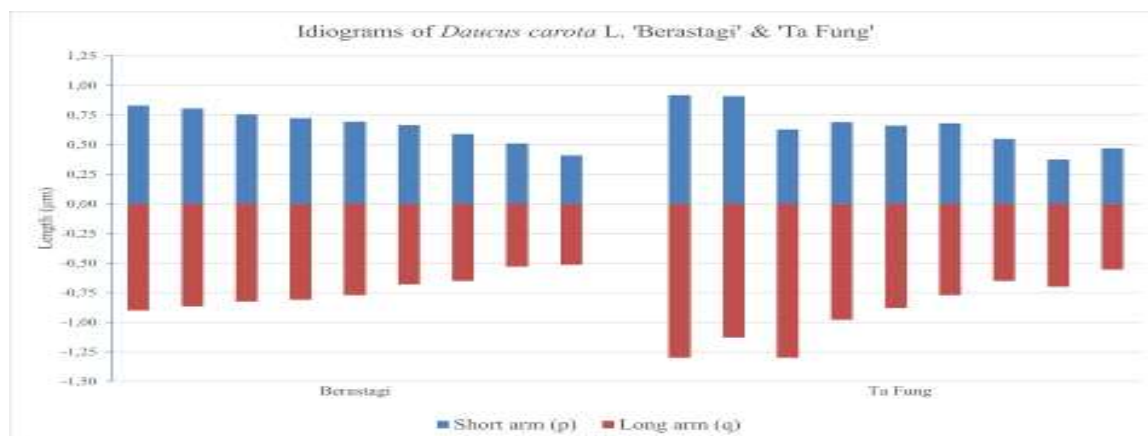


Figure 5. Combined ideogram of the carrot cultivars Berastagi and Ta-Fung. Short arms (p) are colored blue, long arms (q) are colored red.

The pertinent study further demonstrated both carrot cultivars share a similar karyotype, providing evidence for a close relationship between them with 18 sets of chromosomes. The distinction lies in the two pairs of submetacentric chromosomes (numbers 5, 6, 15, and 16) in the cultivar Ta-Fung. In contrast, cultivar Berastagi entirely consisted of metacentric chromosomes. The variation in chromosome shape aligns with the findings of Iovene *et al.* (2008), indicating diverse chromosome forms among the cultivated carrots. The chromosomes of the cultivar Berastagi exhibited a length range of 0.88–1.75 μm , whereas in the cultivar Ta-Fung, the said range was 1.01 to 2.24 μm . In

carrot cultivars, the measured chromosome lengths also agreed with prior research formulating the range of chromosome lengths at 0.89–3.65 μm in carrot genotypes (Iovene *et al.*, 2008; Que *et al.*, 2019). The chromosome shape and length variations directly influence the root phenotype of both cultivars (Brainard *et al.*, 2022; Mathew and Shimelis, 2022). Notably, the cultivar Berastagi possesses a conical-shaped root with a pointed tip, while the cultivar Ta-Fung displays a cylindrical root shape with a blunt tip.

In both carrot cultivars, the ideograms provides an overview of their respective chromosome characteristics (Figure 5). Both cultivars possess a diploid chromosome

formula (2n) with a chromosome number 18. Its depiction is the nine pairs of chromosomes in each cultivar. The chromosome count manifested by the similarity between the two studied cultivars, indicating their equivalence at the species level. The difference between these two cultivars lies in their chromosome length. The chromosomes in the cultivar Ta-Fung were generally larger than in the cultivar Berastagi.

Additionally, cultivar Ta-Fung features two pairs of submetacentric chromosomes based on their centromere positions, contrasting the entirely metacentric chromosomes in cultivar Berastagi. This distinction showed manifestation phenotypically in various aspects, such as, root size, growth duration, and growth characteristics of the two cultivars (Mahmoudi and Mirzaghaderi, 2021). Ta-Fung being an imported cultivar, exhibits a differing chromosome formula compared with the local cultivar Berastagi. The differences might be the cultivar's adaptation to its respective cultivation environmental conditions.

Carrot in comparison to Apiaceae

Apiaceae is a plant family encompassing various flowering plants, some of which include coriander, cumin, celery, and carrots. Apiaceae consists of 434 genera, with approximately 3,780 species that thrive in diverse habitats, ranging from moderate to tropical climates (Wang, 2021). Distinguishing features of the family Apiaceae include its shrub-like growth form, inconspicuous inflorescence enclosed by a cluster of compound flowers, and tiny fruits. The seeds within these fruits grouped into smaller sections called mericarps, which aid in propagation (Simpson, 2010). Plants within the family Apiaceae are common for their high adaptability to their environment, evident in morphological adaptations, such as, compound leaves, hollow stems, and compound inflorescences with wind-dispersed seeds (Ajani and Bockhoff, 2021). These adaptations serve to enhance survival and maximize seed dispersion, aligned with the ancestral habitats of the family Apiaceae in Central Asia to the Middle East, characterized by flat terrains and

strong winds (Ajani and Bockhoff, 2021). Carrots classification under the family Apiaceae depended on characteristics, such as, compound leaves, hollow stems, and umbrella-like inflorescence structures (Kjellenberg, 2007; Que *et al.*, 2019).

Carrots seemed to have originated from the Central Asian region, specifically in areas of Turkey, Tajikistan, and Uzbekistan (Que *et al.*, 2019). Historical carrots had small-sized, bitter-tasting roots, and apparently contrasting to today's cultivated carrots with large, crunchy roots, and a sweet flavor (Kjellenberg, 2007). Carrots' discovery was by ancient humans who led nomadic lives, relying on hunting and gathering for sustenance. Modern-day carrots exhibited varying colors, shapes, and sizes to suit human preferences (Kjellenberg, 2007). The differences between early cultivated carrots and contemporary cultivars manifest in their roots' length and chromosome shapes, leading to diverse carrot characteristics based on growing conditions (Iovene *et al.*, 2008; Stelmach, 2021). The carrots adaptability, high nutritional content, appealing taste profile, and versatile texture for culinary use make carrots a commonly favored vegetable (Varshney and Mishra, 2022).

The carrot's distribution across various regions presents numerous opportunities for the plant to experience gene flow. Mandel and Brunet (2019) elucidates seed dispersal is the most significant trigger for gene flow in carrots. Moreover, the high genetic similarity among the wild carrot populations raises the possibility of high outcrossing, leading to gene flow among populations in different geographical areas. The selective domestication of carrots over thousands of years has given rise to attributes supportive of consumption, resulting in modern carrots with diverse colors, flavors, root shapes, and varying nutritional content (Kjellenberg, 2007).

One well-studied member of the family Apiaceae is celery (*Apium graveolens*). Celery is a supplementary ingredient, additive, and a snack due to its crisp texture and fresh flavor (Lidar and Purnama, 2021). The cultivation of celery originated in Ancient Greece, primarily serving as animal fodder during that period.

With a chromosome formula of $2n = 2x = 22$, celery shares several similarities with the *Daucus* genus. Additionally, similar organic compounds like flavonoids and terpenoids, along with their high adaptability to the environment, reflect the resemblance between these two plants (Song et al., 2020). Over time, celery has evolved with elongated and succulent stems, distinct from carrots that develop colorful, thick, and cylindrical roots (Li et al., 2022). The celery cultivars used in the study also exhibited similar morphology to the foreign cultivars (Song et al., 2020). These past findings of the above studies support the outcomes of this research.

The genus *Daucus* within the family Apiaceae comprises approximately 3,800 species divided into more than 450 genera (Simpson, 2010). The species within this genus exhibit a high degree of similarity, ranging from their lifestyles and distribution to their seed morphology (Kadluczka and Grzebelus, 2021). Wild carrots from earlier periods exhibit a remarkable resemblance to the cultivated carrots today, demonstrated by their shared chromosome count ($2n = 18$) and consistent chromosome components across wild and cultivated species (Iovene et al., 2008; Mandel and Brunet, 2019).

Among all *Daucus* species, only *D. carota* subsp. *sativus* continues cultivation due to its large root, a feature not observed in other *Daucus* plants (Kjellenberg, 2007). The development of the root in this subspecies refers to the crossbreeding of various *Daucus* species, driven by its high propensity for outcrossing and its plant adaptability, thereby, promoting gene flow among the species (Mandel and Brunet, 2019; Abekova et al., 2022; Amangeldiyeva et al., 2023). The substantial size of the root in this subspecies is a result of its extended two-year life cycle, offering significantly more growth time compared to the average one-year life cycle of most other *Daucus* species (Hauser and Shim, 2007). Referring to the study by Mandel and Brunet (2019), an estimation indicates the two attributes of subsp. *sativus* (developed root and extended life cycle) originated from two separate *Daucus* species that experienced outcrossing due to a short geographical

separation between species in the Central Asian plains (Kjellenberg, 2007; Mandel and Brunet, 2019).

CONCLUSIONS

This study explored the mitotic time, chromosome number, and karyotype of the two carrot (*Daucus carota* L.) cultivars, Berastagi and Ta-Fung, extensively grown in Indonesia. The significant variation in chromosome characteristics between the two cultivars highlights their genetic relationship and similarities to a certain extent. Their shared ancestral lineage further complements this within the family Apiaceae. Understanding the mitotic time, chromosome number, and chromosome formula is crucial for optimizing treatment effects to enhance the quality of carrot through various applications.

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