International Journal of Agricultural Technology 2025 Vol. 21(5):2097-2108
DOI: https://doi.org/10.63369/ijat.2025.21.5.2097-2108
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Cytogenetic insights into Katokkon pepper: an indigenous *Capsicum* from Tana Toraja, Indonesia

Yusuf, A. F. [1], Latifah, V. R.[2], Fajriati, I. T.[2] and Daryono, B. S.[1*]

[1]Laboratory of Genetic and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [2]Tropical Biology Department, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

Yusuf, A. F., Latifah, V. R., Fajriati, I. T. and Daryono, B. S. (2025). Cytogenetic insights into Katokkon pepper: an indigenous *Capsicum* from Tana Toraja, Indonesia. International Journal of Agricultural Technology 21(5):2097-2108.

Abstract The Katokkon pepper, an indigenous variety from Tana Toraja, Indonesia is found to be hold a significant local value and is recognized for its antioxidant potential. This research presented the first cytogenetic analysis of the Katokkon pepper which revealed a diploid chromosome number of 2n = 24, with predominantly metacentric chromosomes. The karyotype formula was determined to be 2n = 2x = 24 = 12m. The highest mitotic index (34.24%) was recorded at 07:00 a.m., indicating the peak of mitotic activity. These findings provided essential insights into the chromosomal structure and evolution of the Katokkon pepper, laying the groundwork for future breeding programs which enhances this unique variety.

Keywords: Chromosome number, Karyotype analysis, Mitotic index, Solanaceae

Introduction

The Solanaceae family, encompassing 3,000 to 4,000 species across approximately 90 genera, exhibits extensive morphological and chemical diversity. These species are globally distributed and hold significant economic, ecological, and agricultural importance. Prominent genera within the family include *Solanum, Capsicum, Nicotiana*, and *Physalis* (Gebhardt, 2016). Members of the Solanaceae range from essential crops such as potatoes, tomatoes, eggplants, and peppers to valuable medicinal and ornamental plants, underscoring their versatile applications across various sectors (Padmanabhan *et al*., 2016).

Among these, Capsicum spp., commonly known as peppers, are of considerable global economic interest. However, their nomenclature can be confusing due to regional variations in terminology, with terms such as paprika, pepper, chili, and chile being used interchangeably (Dzoyem *et al*., 2017). This

[*] **Corresponding Author:** Daryono, B. S.; **Email:** bs_daryono@mail.ugm.ac.id

often leads to confusion in the identification and classification of varieties, particularly across different cultures and languages.

The genus *Capsicum* is native to the tropical regions of Central and South America and comprises 27 species, five of which have been domesticated: *C. annuum, C. chinense, C. pubescens, C. frutescens,* and *C. baccatum* (Morris and Taylor, 2017; Ibiza *et al*., 2012). These domesticated species are extensively cultivated, primarily for their fruits, which are utilized in food production (Reddy *et al*., 2024), pharmaceuticals (Sanati *et al*., 2018), and ornamental horticulture (Stommel and Kozlov, 2018). In general, members of the *Capsicum* genus, such as *C. annuum*, *C. chinense*, *C. pubescens*, *C. frutescens*, and *C. baccatum*, along with several wild species, possess a diploid chromosome number of 2n=24. However, some wild species have been confirmed to have a diploid chromosome number of 2n=26, including *C. campylobuccium*, *C. ciliatum*, *C. cornutum*, *C. lancelatum*, *C. mirabile*, *C. schottianum*, *C. buforum*, *C. capylopodium*, and *C. villosum* (Bosland and Vovata, 2012; Pozzobon and Schifino-Wittmann, 2006).

Figure 1. Morphological appearances of Katokkon pepper

The Katokkon pepper, a unique chili variety native to Tana Toraja, South Sulawesi, Indonesia, derives its name from the local term "Katokkon," meaning "large fruit" (Yamamoto *et al*., 2014). This variety is distinguished by its small, bell-shaped pods (Figure 1), thick pericarp, and intense spiciness. When fully ripe, the fruit turns bright red and is commonly used in traditional Torajan cuisine (Daryono and Tammu, 2022). Furthermore, the Katokkon pepper has demonstrated potential as a rich source of antioxidants, including carotenoids, ascorbic acid, and capsaicin (Wijaya *et al*., 2020; Daryono and Tammu, 2022).

Despite its local significance, the taxonomic classification of Katokkon remains ambiguous, Some studies have identified it as *C. annuum* (Tammu *et al*., 2021), *C. chinense* (Sjahril *et al*., 2023), or as a variety under *C. annuum var. chinense* (Al-Amanah *et al*., 2022), Additionally, one registered variety is classified as *C. frutescens* under registration number 96/BR/PVL/08/2017 (Daryono and Tammu, 2022). This taxonomic uncertainty arises from the morphological similarities shared by the five species most commonly cultivated in Indonesia (Djarwaningsih, 2005).

Morphological characteristics provide limitations for species identification, especially in plants with high phenotypic variability, such as the Katokkon pepper. To overcome these limitations, molecular marker-based identification, such as DNA barcoding, has emerged as a powerful validation tool (Hebert and Gregory, 2005; Kress, 2017). In addition to molecular techniques, chromosome analysis plays a vital role in species identification and taxonomic classification by providing insights into genetic relationships. This field of study, known as cytogenetics, examines the behavior of chromosomes during plant growth, development, and reproduction, with a focus on hereditary traits (Kannan and Zilfalil, 2009).

Cytogenetic studies typically involve the analysis of chromosome numbers, chromosomal rearrangements, and nuclear-cytoplasmic genome interactions (Harun *et al*., 2024). Common techniques employed in cytogenetic research include karyotyping, Fluorescence in situ hybridization (FISH), and comparative genomic hybridization (GISH) (Shakoori, 2017; Silva *et al*., 2018). These methods enable researchers to detect chromosomal abnormalities and variations that may affect species classification and genetic diversity.

Chromosome mapping and banding techniques further enhance our understanding of genetic relationships and evolutionary linkages between species (Kumar *et al*., 2021). By comparing karyotypes—complete sets of chromosomes within a cell—scientists can infer phylogenetic relationships, shedding light on how different species are related at the genetic level. These comparative studies are crucial for accurately classifying plants within the correct genus or family,

and for unraveling the broader evolutionary history of plant lineages (Cao *et al*., 2024).

In the context of the Katokkon pepper, cytogenetic and molecular approaches hold significant potential in resolving its ambiguous taxonomic classification and clarifying its relationship to other *Capsicum* species. To date, no cytogenetic studies have been conducted on the Katokkon pepper, leaving a critical gap in understanding its chromosomal features. The research aimed to address this gap by conducting a karyological analysis of somatic cells from the Katokkon pepper to determine its diploid chromosome number, karyotype structure, and other key quantitative characteristics.

Materials and methods

Research area

This study was conducted in the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, from August to September 2024. The research involved germination and cytogenetic analysis of Katokkon pepper. Plant materials were obtained from a local farmer in Tana Toraja, North Sulawesi, Indonesia.

Chromosome slide preparation

Root apical meristems (RAM) from germinated seeds were used to identify optimal meristematic tissue for cytogenetic analysis. Chromosome slides were prepared using an improved acetic orcein squash technique, with several modifications. Root tips were collected at 30-minute intervals between 07:00 and 10:00 WIB (Western Indonesian Time). Samples were fixed in 45% glacial acetic acid at 4°C for 15 minutes, followed by hydrolysis in 1 N HCl at 55°C for 7 minutes. After hydrolysis, root tips were stained with 1% aceto-orcein for 24 hours. Squashes were prepared to spread the cells, then observed under a light microscope at 400x magnification. Photomicrographs were taken using the *OptiLab Viewer software*.

Mitotic index determination

The mitotic index (MI) was calculated to determine the proportion of cells undergoing mitosis. Cells in different mitotic stages and the total number of cells were counted at each time interval. The MI was calculated using the formula MI

= (Nm / N) × 100, where Nm represents the number of mitotic cells, and N is the total number of observed cells.

Karyotype and idiogram construction

Karyotype construction was performed using three well-spread prometaphase plates using *Corel Draw 2020*. The karyotype formula was established following the method described by (Levan *et al*., 1964). Ideograms were constructed based on the mean arm length of each chromosome.

Chromosome measurements

Basic chromosome data were analyzed using *Image Raster 3.0 software*. Five well-spread prometaphase plates, previously selected, were used to measure the short arm (p), long arm (q), absolute length, relative length, arm ratio, centromere index, and chromosome type.

Results

Mitotic Index of Tana Toraja's Katokkon pepper

Approximately one thousand cells (mean of 1217.17 cells) were identified during each sampling time to determine the mitotic division timing in Katokkon pepper (Table 1). The highest mitotic index, which indicates the peak of cell division, was recorded at 07:00 a.m., with a percentage of 34.24%. The mitotic index then gradually decreased to 18.28% at 09:30 a.m. Optimal karyotype identification was achieved during prometaphase, where condensed chromosomes had formed and were dispersed in the cytoplasm, allowing for clearer chromosome counting compared to other phases. This study confirmed that prometaphase in Katokkon pepper was most frequently observed at 07:30 a.m., with a percentage of 2.15%.

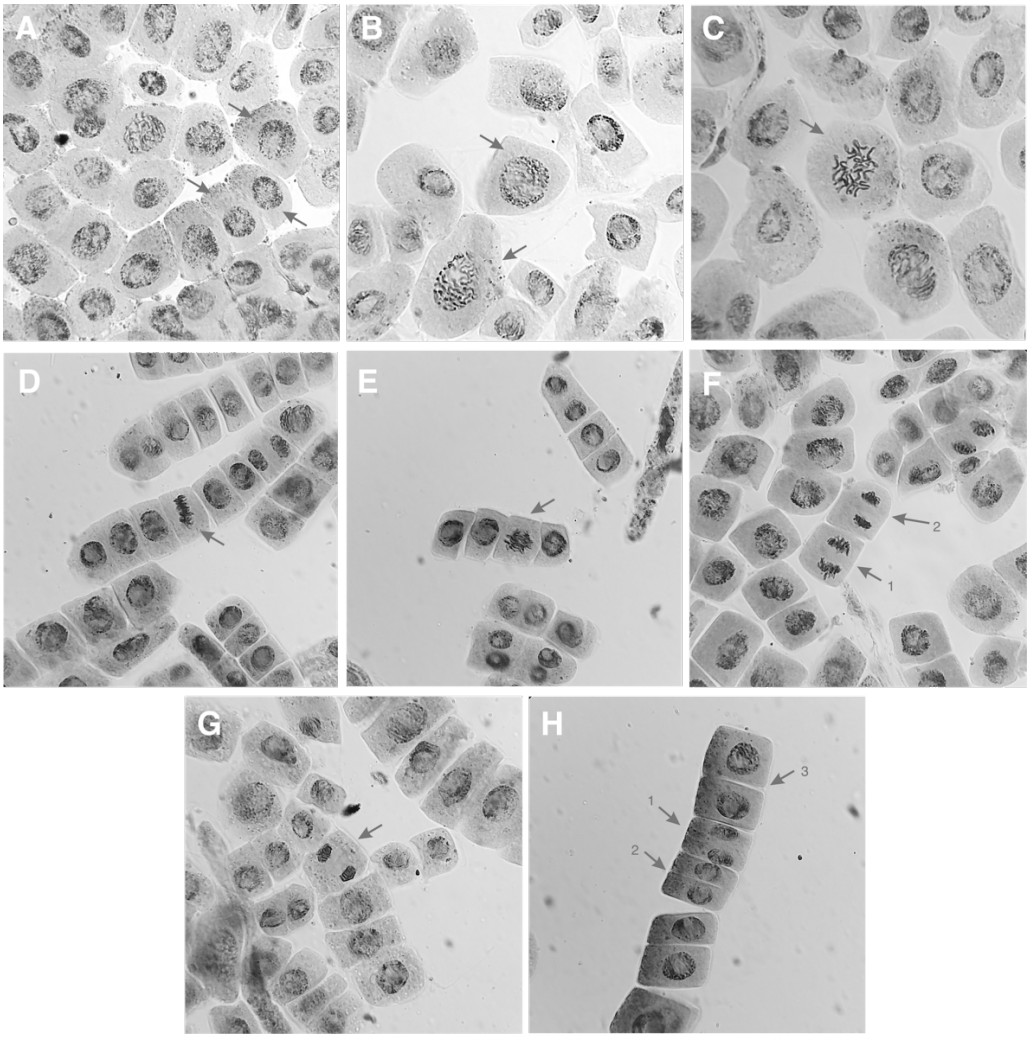

Figure 2. Photomicrographs showing the stages of mitotic cell division in Katokkon pepper: (A) Interphase, (B) Prophase, (C) Prometaphase, (D) Metaphase, (E, F) Anaphase, and (G, H) Telophase and Cytokinesis, The numbers in certain images indicate sequential stages of the process

The cell division stages of Katokkon pepper from Tana Toraja are illustrated in Figure 2. Interphase, which involves cell growth and DNA synthesis, is characterized by non-condensed chromatin with smooth granules and nucleolus white spot indices, as shown in Figure 2A. Mitosis begins with prophase, where chromatin starts to condense, followed by the breakdown of the nuclear envelope (Figure 2B). Prometaphase displays condensed chromatins, as

shown in Figure 2C. Sister chromatids then align at the center of the cell, forming a metaphase plate structure at the equator between opposite poles (Figure 2D).

Table 1. Mitotic index of Katokkon Pepper from Tana Toraja

Time	n	Cells in mitotic phase and interphase, %						MI,
		p	*pm*	*m*	*a*	*t*	*i*	%
7.00	1177	29.9	1.8	1.4	0.4	0.8	65.8	34.24
7.30	1025	24.2	2.2	1.2	0.9	1.7	70	30.05
8.00	1252	23.6	1.8	1.4	0.4	1.1	71.7	28.27
8.30	1391	18.2	1.9	2.4	0.8	0.7	76	24.01
9.00	1249	15.9	1.4	0.6	0.6	0.6	80.8	19.22
9.30	1209	14.9	0.8	1.3	0.3	1	81.7	18.28

p: prometaphase; pm: prometaphase; m: metaphase; a: anaphase; t: telophase; i: interphase; MI; mitotic index.

In early anaphase (Figure 2E), the two sets of chromosomes, attached to the mitotic spindle (microtubules), begin migrating toward opposite poles. By late anaphase (Figure 2F), identical sets of chromosomes have moved to each pole. Early telophase (Figure 2G) is marked by the formation of a cell plate to facilitate cytokinesis, eventually producing two identical daughter cells, as shown in Figure 2H.

Karyotype and chromosome profile of Tana Toraja's Katokkon pepper

Photographs of the karyotypes of Katokkon pepper from Tana Toraja provide a clear depiction of the chromosomal structure, which predominantly exhibits a metacentric type (Figure 3). The idiogram presented in Figure 4 illustrates a basic chromosome number of x = 12. Katokkon pepper has a chromosome number of 2n = 24, which aligns with the chromosomal characteristics of many other species within the *Capsicum* genus. Chromosome sizes range from 1.21 to 3.05 μm, with a centromeric index of 40% (mean of 42.29 ± 1.90) and a mean arm ratio of 1.37 ± 0.10. Basic chromosome set information for Katokkon pepper from Tana Toraja is provided in Table 2. Based on these data, the karyotype formula for Katokkon pepper is 2n = 2x = 24 = 12m.

Figure 3. Karyogram representative of Katokkon pepper chromosome. M, metacentric

Table 2. Basic chromosome set information for Katokkon pepper

Chr.	Mean ± SD, µm			RL	AR	CI
	LA	SA	AL			
1	1.82 ± 0.43	1.23 ± 0.17	3.05 ± 0.38	12.57	1.47	40.42
2	1.41 ± 0.12	1.22 ± 0.10	2.63 ± 0.15	10.82	1.15	46.41
3	1.43 ± 0.18	0.96 ± 0.18	2.40 ± 0.15	9.86	1.49	40.13
4	1.31 ± 0.16	1.03 ± 0.07	2.33 ± 0.17	9.61	1.27	43.99
5	1.33 ± 0.18	0.92 ± 0.10	2.25 ± 0.15	9.25	1.45	40.82
6	1.21 ± 0.15	0.93 ± 0.06	2.14 ± 0.11	8.80	1.31	43.31
7	1.10 ± 0.08	0.85 ± 0.10	1.95 ± 0.05	8.03	1.29	43.71
8	1.04 ± 0.09	0.79 ± 0.13	1.83 ± 0.08	7.51	1.32	43.18
9	0.98 ± 0.07	0.72 ± 0.07	1.71 ± 0.10	7.02	1.36	42.40
10	0.90 ± 0.07	0.61 ± 0.09	1.51 ± 0.04	6.20	1.48	40.27
11	0.76 ± 0.11	0.54 ± 0.09	1.30 ± 0.09	5.36	1.40	41.63
12	0.71 ± 0.14	0.50 ± 0.11	1.21 ± 0.15	4.97	1.43	41.19

LA: long arm, SA: short arm, AL: absolute length, RL: relative length, AR: arm ratio, CI: centromeric index.

Discussion

The chromosome number of several species within the *Capsicum* genus, including *C. annuum, C. frutescens, C. pubescens, C. chinense,* and *C. baccatum*, has been consistently observed as 2n = 24. Some wild species of *Capsicum*, such as *C. buforum, C. capylopodiume*, and *C. cornutum*, have been found to possess a chromosome number of 2n = 26 (Pozzobon and Schifino-Wittmann, 2006). Karyotype analysis of three prometaphase plates confirmed that Katokkon pepper has 2n = 24 chromosomes, which is consistent with chromosome numbers reported in previous studies for various members of the genus *Capsicum* (Cheema and Pant, 2013; Lanteri and Pickersgill, 1993; Limaye and Patil, 1989;

Souza *et al*., 2015). Karyotype formula of Katokkon pepper is slightly different from the other previous karyotype studies in *Capsicum*. Most of them reported that *Capsicum* sp. has a relatively symmetrical karyotype, but with at least one pair of chromosomes identified as sub-metacentric or sub-telocentric (Alcorcés, 2001; Rohami *et al*., 2010; Souza *et al*., 2015; Moscone *et al*., 2007). Stebbins (1971) has argued that the evolution of the karyotype in higher plants is generally from symmetry to asymmetry. Primitive plants have symmetrical karyotypes, whereas asymmetrical karyotypes are a secondary and more recent trait that has emerged in more evolutionarily advanced taxa. Differences in morphology, size, and number of chromosomes are common in intraspecific populations. These are known as 'cytotypes' (Stebbins, 1971).

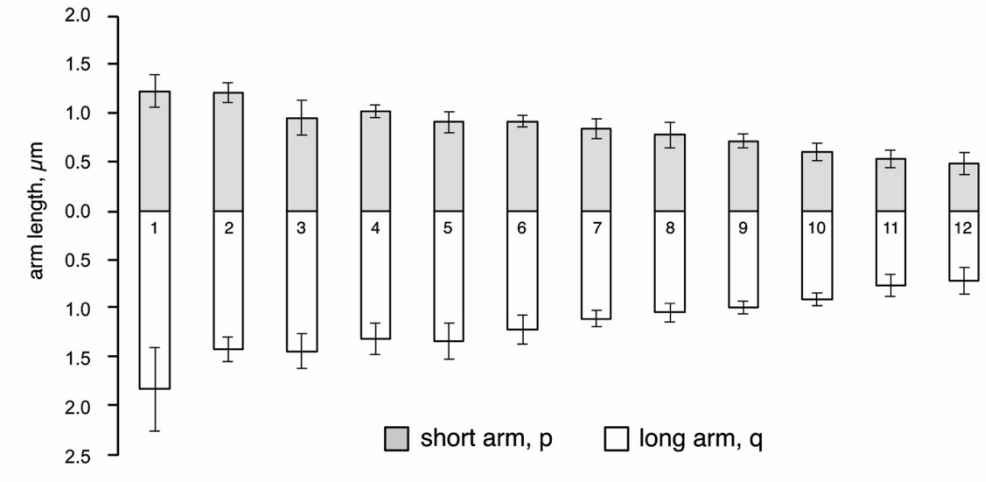

Figure 4. Idiogram of Katokkon pepper from Tana Toraja

This study provides the first cytogenetic analysis of Katokkon pepper, a local variety from Tana Toraja, Indonesia. The results revealed that Katokkon pepper has a diploid chromosome number of 2n = 24, with predominantly metacentric chromosomes. The highest mitotic index of 34.24% was recorded at 07:00 a.m., marking the peak of cell division activity. These findings offer critical insights into the chromosomal structure and evolution of Katokkon pepper, laying a strong foundation for future breeding programs aimed at enhancing this unique variety.

Acknowledgements

This work was financially supported by the Riset Asistensi program under the grant number 10534/UN1.P4/PT.01.03/2024. The author gratefully acknowledges the financial support

from the Directorate of Research, Universitas Gadjah Mada, and extends appreciation to Rachmawati Dwi Hasanah for her valuable help in data collection at the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Al-Amanah, H., Sjahril, R., Haring, F., Riadi, M. and Larekeng, S. H. (2022). Mapping Distribution of *Capsicum annuum var. chinense* in Tana Toraja and Surrounding Districts (Indonesia) Based on Fruit Morphology. Biodiversitas, 23.

Bosland, P. W. and Vovata, E. J. (2012). Peppers: Vegetables and Spices *Capsicums*`. 2nd ed. CAB International, United Kingdom.

Cao, R. B., Chen, R., Liao, K. X., Li, H., Xu, G. B. and Jiang, X. L. (2024). Karyotype and LTR-RTs Analysis Provide Insights into Oak Genomic Evolution. BMC Genomics, 25:328.

Cheema, S. K. and Pant, M. R. (2013). Karyotype Analysis of Seven Cultivated Varieties of *Capsicum annuum* L. Caryologia, 66:70-75.

Daryono, B. S. and Tammu, R. M. (2022). Genetic Potential Characteristics and Utilization of Katokkon Pepper from Toraja, Indonesia. 1st ed. UGM Press, Yogyakarta, Indonesia.

Djarwaningsih, T. (2005). *Capsicum* spp. (Chilli): Origin, Distribution, and Its Economical Value. Biodiversitas, 6:292-296.

Dzoyem, J. P., McGaw, L. J., Kuete, V. and Bakowsky, U. (2017). Anti-Inflammatory and Anti-Nociceptive Activities of African Medicinal Spices and Vegetables. Medicinal Spices and Vegetables from Africa, Elsevier, pp.239-270.

Gebhardt, C. (2016). The Historical Role of Species from the Solanaceae Plant Family in Genetic Research. Theoretical and Applied Genetics, 129:2281-2294.

Alcorcés, N. (2001). Chromosome Studies of Four Selections of *Capsicum chinense* Jacq. Revista Científica UDO Agrícola, 1:34-41.

Harun, A., Fang, Z. and Chen, C. (2024). The Contributions of Cytogenetics, Genetics, and Epigenetics to the Stability of Plants Polyploidy. Discover Plants, 1:11.

Hebert, P. D. N. and Gregory, T. R. (2005). The Promise of DNA Barcoding for Taxonomy V. Savolainen, ed. Systematic Biology, 54:852-859.

Ibiza, V. P., Blanca, J., Cañizares, J. and Nuez, F. (2012). Taxonomy and Genetic Diversity of Domesticated *Capsicum* Species in the Andean Region. Genetic Resources and Crop Evolution, 59:1077-1088.

Kannan, T. P. and Zilfalil, B. A. (2009). Cytogenetics: Past, Present and Future. Malaysian Journal of Medical Sciences, 16.

Kress, W. J. (2017). Plant DNA Barcodes: Applications Today and in the Future. The Journal of Systematics and Evolution, 55:291-307.

Kumar, S., Kiso, A. and Abenthung Kithan, N. (2021). Chromosome Banding and Mechanism of Chromosome Aberrations. in: Cytogenetics - Classical and Molecular Strategies for Analysing Heredity Material, M. L. Larramendy and S. Soloneski, eds. IntechOpen.

Lanteri, S. and Pickersgill, B. (1993). Chromosomal Structural Changes in *Capsicum annuum* L. and *C. chinense* Jacq. Euphytica, 67:155-160.

Levan, A., Fredga, K. and Sandberg, A. A. (1964). Nomenclature for Centromeric Position on Chromosomes. Hereditas, 52:201-220.

Limaye, V. A. and Patil, V. P. (1989). Karyomorphological Studies in the Genus *Capsicum* Sinn. Cytologia, 54:455-463.

Morris, W. L. and Taylor, M. A. (2017). The Solanaceous Vegetable Crops: Potato, Tomato, Pepper, and Eggplant. Encyclopedia of Applied Plant Sciences, Elsevier, pp.55-58.

Moscone, E. A., Scaldaferro, M. A., Grabiele, M., Cecchini, N. M., Sánchez García, Y., Jarret, R., Daviña, J. R., Ducasse, D. A., Barboza, G. E. and Ehrendorfer, F. (2007). The Evolution of Chili Peppers (*Capsicum* - Solanaceae): A Cytogenetic Perspective. Acta Hortic, 137-170.

Padmanabhan, P., Cheema, A. and Paliyath, G. (2016). Solanaceous Fruits Including Tomato, Eggplant, and Peppers. Encyclopedia of Food and Health, Elsevier, pp.24-32.

Pozzobon, M. T. and Schifino-Wittmann, M. T. (2006). A Meiotic Study of the Wild and Semi-Domesticated Brazilian Species of Genus *Capsicum* L. (Solanaceae). Cytologia, 71:275-287.

Reddy, D. N., Mylabathula, M. M. and Al-Rajab, A. J. (2024). Industrial Demand and Applications of Capsaicin. Capsaicinoids, M.K. Swamy and A. Kumar, eds. Springer Nature Singapore, Singapore, pp.249-291.

Rohami, M., Mohammadi, A., Khosroshahli, M., Ahmadi, H. and Darandeh, N. (2010). Karyotype Analysis of Several Ecotypes of *Capsicum annuum* L. in Iran. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38:177-180.

Sanati, S., Razavi, B. M. and Hosseinzadeh, H. (2018). A Review of the Effects of *Capsicum annuum* L. and its Constituent, Capsaicin, In Metabolic Syndrome. Iranian Journal of Basic Medical Sciences, 21.

Shakoori, A. R. (2017). Fluorescence In Situ Hybridization (FISH) and Its Applications. Chromosome Structure and Aberrations, T. A. Bhat and A. A. Wani, eds. Springer India, New Delhi, pp.343-367.

Silva, G. S., Souza, M. M., De Melo, C. A. F., Urdampilleta, J. D. and Forni-Martins, E. R. (2018). Identification and Characterization of Karyotype in *Passiflora* Hybrids Using FISH and GISH. BMC Genetics, 19:26.

Sjahril, R., Riadi, Muh., Saleh, I. R., Novitasari, Galla, E. A., Kasmiati, Trisnawaty, A. R. and Panga, N. J. (2023). Katokkon Pepper (*Capsicum chinense* Jacq.) Ploidy Determination by Morphological Characteristic and Flow Cytometry Analysis. AGRIVITA Journal of Agricultural Science, 45:288-295.

Souza, W. R. D. N., Almeida, A. C. D., Carvalho, R. D., Ferreira, R. L. and Peron, A. P. (2015). Karyotypic Characterization of *Capsicum* sp. Accessions. Acta Scientiarum-agronomy, 37:147.

Stebbins, G. L. (1971). Chromosomal Evolution in Higher Plants (Contemporary Biology). University Park Press, London.

Stommel, J. R. and Kozlov, M. (2018). Ornamental Pepper (*Capsicum annuum* L.) Cultivars Comprising the Christmas Lights Cultivar Series. Horticultural Science, 53:391-394.

Tammu, R. M., Nuringtyas, T. R. and Daryono, B. S. (2021). Colchicine Effects on the Ploidy Level and Morphological Characters of Katokkon Pepper (*Capsicum annuum* L.) from North Toraja, Indonesia. Journal of Genetic Engineering and Biotechnology, 19.

Wijaya, C. H., Harda, M. and Rana, B. (2020). Diversity and Potency of *Capsicum* spp. Grown in Indonesia. in: *Capsicum*, A. Dekebo, ed. IntechOpen.

Yamamoto, S., Djarwaningsih, T. and Wiriadinata, H. (2014). History and Distribution of *Capsicum chinense* in Indonesia.

(Received: 16 October 2024, Revised: 23 June 2025, Accepted: 29 July 2025)