Journal of Tropical Biodiversity and Biotechnology
Volume 11, Issue 01 (2026): jtbb20339
DOI: 10.22146/jtbb.20339

Short Communication

Karyomorphological Study on 21 Shallot Cultivars (*Allium cepa* L. var. *ascalonicum*) Released by BALITSA Indonesia in 1984-2018

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Keywords:
Allium cepa L. var. *ascalonicum*
Chromosome Variation
Cytogenetic
Karyotype
Shallot
Submitted:
12 March 2025
Accepted:
23 July 2025
Published:
05 January 2026
Editors:
Miftahul Ilmi
Annisaa Widyasari

ABSTRACT
Shallot (*Allium cepa* L. var. *ascalonicum*) is a vegetable commodity with consistently increasing demand each year in Indonesia. BALITSA Research Centre released 21 superior cultivars between 1984 and 2018 to support production, but there were no research on their karyotype characteristics. The 21 cultivars were studied during prometaphase mitosis using the squash method to identify chromosomal variations. All cultivars shared the same chromosome number, 2n = 16, but exhibited differences in chromosome size and shape, most of which were metacentric, submetacentric, and subtelocentric. The findings will inform breeding programs in Indonesia to improve shallot quality.

How to cite:
Masithoh, D. et al., 2026. Understanding Karyomorphological Study on 21 Shallot Cultivars (*Allium cepa* L. var. *ascalonicum*) Released by BALITSA Indonesia in 1984-2018. *Journal of Tropical Biodiversity and Biotechnology*, 11(1), jtbb20339. doi: 10.22146/jtbb.20339

The genus *Allium* (shallot) is one of the most species-rich genera of monocots worldwide (Kobrlová et al. 2024), with 1,200 species identified mostly in the northern hemisphere (Govaerts et al. 2021; G. Kim et al. 2023; Ahmad et al. 2024). There are more than 100 *Allium* species, of which 50 % are endemic to the Greek flora (Dimopoulos et al. 2016). One among the many diverse *Allium* plants, shallots. They have been cultivated since 3,200–2,700 BC, evidenced by the discovery of Egyptians and Ancient Greeks artefacts such as shallot paintings on statues, monuments, and stones. This evidence is further supported by Ancient Greek relics about shallot cultivation from 4,000 years ago.

Shallots (*Allium cepa* L. var. *ascalonicum*) are widely known and cultivated in many countries, including throughout Indonesia. In Indonesia, shallots are among the most popular and economically important horticultural crops (Mallor et al. 2011). Their high value stems are coming from the diverse applications in daily life, spanning culinary, medicinal, and agricultural uses. Culinarily, shallots are widely used as a key seasoning to enhance the flavour of various traditional and modern dishes (Ricciardi et al. 2020; Liao et al. 2022). Medicinally, they have long been utilised in traditional practices as a natural antibiotic to reduce fever, treat ulcers, manage diabetes, lower blood sugar and cholesterol levels, and prevent the hardening of blood vessels (Alfiyyah et al. 2020; Putra et al. 2021; Karavelioğlu & Hoca 2022; Mardani et al. 2023). These health benefits are largely attributed to bioactive compounds such as alliin and allicin, which exhibit bactericidal, antimicrobial, anticancer, and anti-inflammatory properties (Marefati et al. 2021; Alam et al. 2023; Habibah et al. 2023; Savitri et al. 2023). In agriculture, shallots are also valued for their by-products, which can be used as organic fertilizer (Banu 2020; Rinzani et al. 2020) and as natural pesticides to help manage pests in an environmentally friendly manner (Nilan et al. 2019; Alivianingsih et al. 2020; Mulyati 2020). This wide range of benefits highlights the strategic importance of shallots not only for food and health but also for sustainable agricultural practices.

Due to high demand, increasing shallot production is important to support Indonesia's food security. The great need and an increase in population results in an increasing demand for shallots every year (Wospakrik 2017). Efforts to overcome these problems require shallot breeding initiatives. Seeds have the potential to become a high-quality genetic resource material through the application of breeding programs (Singh et al. 2020). The initial step is to obtain primary genetic information of these plants through karyotype analysis. It is needed to support genetic studies as well as enhance the efficiency of breeding activities. It is also utilised to distinguish individuals from each species. From 2004 to 2018, the Vegetable Crops Research Institute (BALITSA Research Centre) has released 21 superior shallot cultivars to increase shallot production in response to the growing demand in Indonesia. However, the karyotype diversity of the 21 shallot cultivars remains understudied. Therefore, this study aims to identify the size of arm lengths and chromosome shapes of the 21 shallots cultivars based on cytological characteristics. It is expected to add insights into knowledge in supporting the shallot plant breeding program to improve the quality of shallots produced in Indonesia, as well as information on genetic resources and material for the preparation of shallot libraries.

The research objects used were 21 shallot cultivars from Indonesia, released by the Vegetable Crops Research Institute (BALITSA Research Centre) from 1984 to 2018. Of these, 20 cultivars are collections from Balitsa, including BM1 Bima Brebes, BM1 Maja Cipanas, BM3 Kuning, BM4 Kramat 1, BM5 Kramat 2, BM6 Sembrani, BM7 Katumi, BM8 Pikatan, BM9 Trisula, BM10 Pancasona, BM11 Mentes, BM12 TSS Agrihorti 1, BM13 TSS Agri-

horti 2, BM14 Violetta 1 Agrihorti, BM15 Violetta 2 Agrihorti, BM16 Violetta 3 Agrihorti, BM18 Ambassador 1 Agrihorti, BM19 Ambassador 2 Agrihorti, BM20 Ambassador 3 Agrihorti and BM21 Ambassador 4 Agrihorti and one cultivar BM17 Super Philip, was a collection from the Bima Agriculture Office of West Nusa Tenggara. These 21 cultivars sampled were selected because they have been certified by BALITSA Research Centre and are cultivated in various shallot centers in Indonesia. All of the shallot cultivars were then brought to Laboratory Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia for further chromosome investigation.

The chromosome preparation of the 21 shallot cultivars analysed in this study was carried out based on the protocol described by (Hillis & Moritz 1996). Shallot bulbs from each cultivar were germinated in petri dishes at the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Root tips were collected and pre-treated in 0.03 % colchicine solution at 4 °C for 24 hours. Following this, the samples were fixed in 45 % acetic acid at 4 °C for 15 minutes, hydrolysed in 1 N hydrochloric acid at 55 °C for 3 to 5 minutes, and then squashed using 1 % aceto -orcein. Prepared slides were examined under an Olympus BX-41 microscope.

Chromosome measurements were carried out using Ideokar v.1.3 software (Mirzaghaderi & Marzangi 2015). Classification of mitotic prometaphase chromosomes was based on centromere position according to the criteria proposed by (Levan et al. 2009): metacentric chromosomes had a centromeric index ranging from 37.50 to 50.00, submetacentric from 25.00 to 37.49, subtelocentric from 12.50 to 24.99, and telocentric from 0 to 12.49. The resulting data on chromosome size and centromere position were used to assemble karyograms using Ideokar v.1.3, while the use of CorelDRAW Graphics Suite 2018 v.20.1.0.708 program (Arisuryanti & Wibowo 2016) to edit the karyotype.

Mitotic analysis in this study focused on specific morphological characteristics of prometaphase chromosomes, including chromosome size and centromeric index. Data were analyzed through chromosome observations made during the metaphase (Mercado et al. 2020; Pinky et al. 2022). The mitotic activity in the 21 shallot cultivars examined predominantly occurred between 09:00 and 10:00 a.m. (WIB – Western Indonesian Time). The somatic prometaphase chromosomes displayed a diploid number of 2n = 16 (Figure 1), consistent with a previous finding conducted by (Arisuryanti et al. 2009). Although the 21 shallot cultivars exhibited the same diploid chromosome number, differences were observed in chromosome size and centromeric index, resulting in variation in the karyotype formula (Figure 1 and Table 1).

As shown in Figure 1 and Table 1, five distinct karyotype formulas were identified among the shallot cultivars: 16M, 14M+2SM, 12M+4SM, 10M+6SM, and 10M+4SM+2ST. The majority of cultivars (a total of eight) exhibited the 16M karyotype, including BM2 Bima Brebes, BM10 Pancasona, BM13 TSS Agrihorti 2, BM14 Violetta 1 Agrihorti, BM15 Violetta 2 Agrihorti, BM19 Ambassador 2 Agrihorti, BM20 Ambassador 3 Agrihorti, and BM21 Ambassador 4 Agrihorti. The presence of exclusively metacentric chromosomes in these eight cultivars indicated the absence of chromosomal mutations. In general, plant species that have not undergone extensive cultivation tend to retain a fully metacentric karyotype, reflecting chromosomal stability.

The 14M+2SM karyotype was identified in three cultivars: BM3 Kuning, BM11 Mentes, and BM18 Ambassador 1 Agrihorti. Another eight cultivars exhibited the 12M+4SM karyotype, namely BM1 Maja Cipanas, BM4 Kramat 1, BM5 Kramat 2, BM6 Sembrani, BM7 Katumi, BM9 Trisula, BM16 Violetta 3 Agrihorti, and BM17 Super Phillip. Additionally, the 10M+6SM and 10M+4SM+2ST karyotypes were each observed in a single

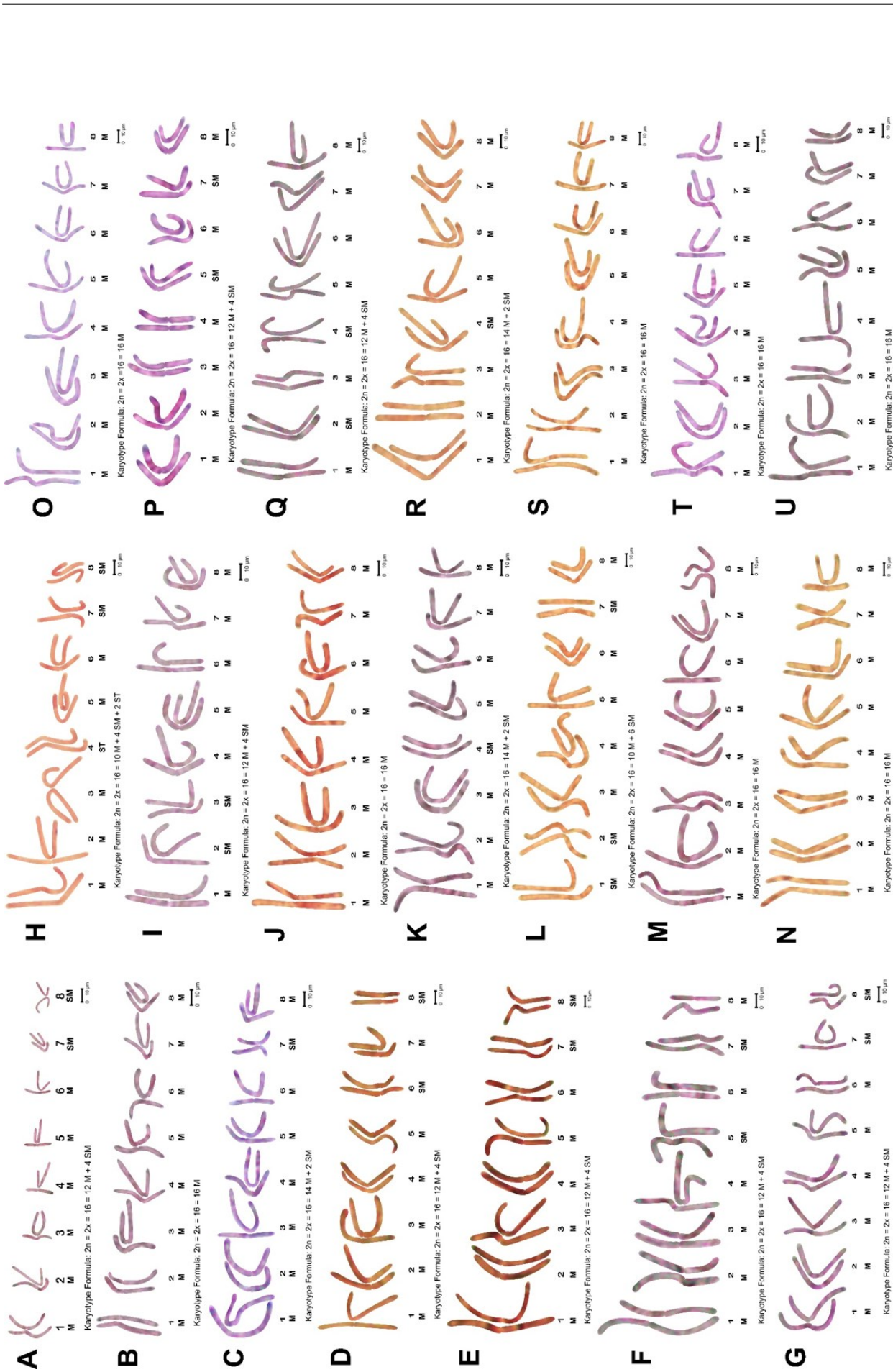

Figure 1. Photomicrophotographs of karyotypes of the 21 shallot cultivars from Indonesia: A. BM1 Maja Cipanas; B. BM2 Bima Brebes; C. BM3 Kuning; D. BM4 Kramat 1; E. BM5 Kramat 2; F. BM6 Sembrani; G. BM7 Katumi; H. BM8 Pikatan; I. BM9 Trisula; J. BM10 Pancasona; K. BM11 Mentes; L. BM12 TSS Agrihorti 1; M. BM13 TSS Agrihorti 2; N. BM14 Violetta 1 Agrihorti; O. BM15 Violetta 2 Agrihorti; P. BM16 Violetta 3 Agrihorti; Q. BM17 Super Philip; R. BM18 Ambassador 1 Agrihorti; S. BM19 Ambassador 2 Agrihorti; T. BM20 Ambassador 3 Agrihorti; and U. BM21 Ambassador 4 Agrihorti. The scale bar represents 10 µm.

Table 1. Chromosome size and karyotype formula of the 21 Indonesian shallot cultivars.

Shallot Cultivars	Chromosome length (µm)			Centromere Index Score (NIS)	Chromosome Length Ratio (RLK)	Karyotype Formula
	Short Arm [p (µm)]	Long Arm [q (µm)]	Total Length [p+q (µm)]			
BM1 Maja Cipanas	2.34 ± 0.03 – 8.65 ± 0.06	4.04 ± 0.04 – 8.79 ± 0.02	6.74 ± 0.08 – 17.45 ± 0.07	30.73 ± 2.47 – 49.60 ± 0.12	1.02 ± 0.01 – 2.27 ± 0.26	2n = 2x = 16 = 12M+4SM
BM2 Bima Brebes	4.25 ± 0.12 – 7.89 ± 0.15	5.41 ± 0.03 – 10.35 ± 0.08	10.16 ± 0.11 – 17.23 ± 0.07	38.79 ± 0.43 – 48.50 ± 0.86	1.06 ± 0.04 – 1.58 ± 0.03	2n = 2x = 16 = 16M
BM3 Kuning	3.00 ± 0.02 – 8.77 ± 0.13	3.90 ± 0.07 – 10.75 ± 0.10	7.65 ± 0.25 – 19.52 ± 0.23	32.15 ± 0.21 – 49.21 ± 0.23	1.03 ± 0.01 – 2.11 ± 0.02	2n = 2x = 16 = 14M+2SM
BM4 Kramat 1	2.17 ± 0.09 – 6.23 ± 0.10	4.26 ± 0.20 – 7.29 ± 0.10	6.70 ± 0.19 – 13.52 ± 0.00	29.55 ± 1.16 – 48.41 ± 0.00	1.07 ± 0.00 – 2.39 ± 0.13	2n = 2x = 16 = 12M+4SM
BM5 Kramat 2	2.71 ± 0.05 – 8.38 ± 0.10	5.91 ± 0.13 – 10.61 ± 0.16	8.93 ± 0.14 – 18.99 ± 0.26	30.35 ± 0.08 – 49.75 ± 0.21	1.01 ± 0.01 – 2.30 ± 0.01	2n = 2x = 16 = 12M+4SM
BM6 Sembrani	3.13 ± 0.10 – 9.48 ± 0.10	5.59 ± 0.10 – 14.47 ± 0.07	9.68 ± 0.20 – 23.95 ± 0.17	30.48 ± 0.91 – 49.96 ± 0.00	1.00 ± 0.00 – 2.28 ± 0.10	2n = 2x = 16 = 12M+4SM
BM7 Katumi	2.43 ± 0.06 – 8.06 ± 0.10	4.60 ± 0.07 – 8.19 ± 0.06	8.69 ± 0.02 – 16.25 ± 0.16	27.55 ± 0.62 – 49.60 ± 0.13	1.02 ± 0.01 – 2.63 ± 0.08	2n = 2x = 16 = 12M+4SM
BM8 Pikatan	3.15 ± 0.07 – 9.75 ± 0.04	6.61 ± 0.07 – 14.49 ± 0.10	10.16 ± 0.02 – 24.24 ± 0.07	20.94 ± 0.23 – 48.53 ± 0.52	1.06 ± 0.02 – 3.78 ± 0.05	2n = 2x = 16 = 10M+4SM+2ST
BM9 Trisula	4.44 ± 0.08 – 7.99 ± 0.10	5.98 ± 0.07 – 11.91 ± 0.07	11.11 ± 0.17 – 19.35 ± 0.00	32.36 ± 0.05 – 47.91 ± 0.03	1.09 ± 0.00 – 2.09 ± 0.00	2n = 2x = 16 = 12M+4SM
BM10 Pancasona	4.03 ± 0.10 – 8.76 ± 0.07	5.60 ± 0.09 – 10.29 ± 0.13	9.63 ± 0.19 – 18.52 ± 0.14	38.67 ± 0.58 – 49.56 ± 0.12	1.02 ± 0.00 – 1.59 ± 0.04	2n = 2x = 16 = 16M
BM11 Mentes	4.83 ± 0.05 – 8.53 ± 0.05	6.01 ± 0.07 – 10.48 ± 0.10	10.89 ± 0.01 – 19.01 ± 0.05	34.91 ± 0.65 – 49.82 ± 0.15	1.01 ± 0.00 – 1.86 ± 0.05	2n = 2x = 16 = 14M+2SM
BM12 TSS Agri-horti 1	4.07 ± 1.10 – 7.17 ± 0.06	6.61 ± 0.07 – 12.56 ± 0.06	11.28 ± 0.00 – 19.37 ± 0.01	33.44 ± 0.38 – 48.90 ± 0.62	1.04 ± 0.03 – 1.99 ± 0.03	2n = 2x = 16 = 10M+6SM
BM13 TSS Agri-horti 2	3.61 ± 0.06 – 8.53 ± 0.06	4.45 ± 0.03 – 8.58 ± 0.05	8.06 ± 0.09 – 17.11 ± 0.11	40.44 ± 0.79 – 49.85 ± 0.03	1.01 ± 0.00 – 1.47 ± 0.05	2n = 2x = 16 = 16M
BM14 Violetta 1 Agrihorti	4.37 ± 0.05 – 7.29 ± 0.14	4.90 ± 0.07 – 8.63 ± 0.11	9.27 ± 0.12 – 15.32 ± 0.09	41.14 ± 0.17 – 49.34 ± 0.61	1.03 ± 0.03 – 1.43 ± 0.01	2n = 2x = 16 = 16M
BM15 Violetta 2 Agrihorti	4.57 ± 0.06 – 11.01 ± 0.13	5.70 ± 0.09 – 11.19 ± 0.06	10.55 ± 0.05 – 22.20 ± 0.19	39.42 ± 0.05 – 49.58 ± 0.15	1.02 ± 0.01 – 1.54 ± 0.00	2n = 2x = 16 = 16M
BM16 Violetta 3 Agrihorti	1.99 ± 0.03 – 5.75 ± 0.06	3.29 ± 0.03 – 6.74 ± 0.08	5.29 ± 0.05 – 11.96 ± 0.13	33.72 ± 0.20 – 49.95 ± 0.05	1.00 ± 0.00 – 1.97 ± 0.02	2n = 2x = 16 = 12M+4SM
BM17 Super Phillip	2.95 ± 0.02 – 5.79 ± 0.10	4.58 ± 0.07 – 7.73 ± 0.05	7.53 ± 0.09 – 12.85 ± 0.00	34.26 ± 0.06 – 49.58 ± 0.07	1.02 ± 0.00 – 1.92 ± 0.00	2n = 2x = 16 = 12M+4SM
BM18 Ambassador 1 Agrihorti	4.06 ± 0.10 – 11.35 ± 0.04	5.33 ± 0.05 – 11.89 ± 0.10	9.39 ± 0.15 – 22.84 ± 0.14	35.90 ± 0.15 – 49.69 ± 0.13	1.01 ± 0.01 – 1.79 ± 0.01	2n = 2x = 16 = 14M+2SM

Description: M = metacentric; SM = submetacentric; ST = subtelocentric; T = telocentric.

Table 1. Contd.

Shallot Cultivars	Chromosome length (µm)			Centromere Index Score (NIS)	Chromosome Length Ratio (RLK)	Karyotype Formula
	Short Arm [p (µm)]	Long Arm [q (µm)]	Total Length [p+q (µm)]			
BM19 Ambassador 2 Agrihorti	2.48 ± 0.08 – 10.45 ± 0.03	2.93 ± 0.06 – 12.32 ± 0.06	5.41 ± 0.14 – 21.33 ± 0.08	38.61 ± 0.02 – 48.99 ± 0.04	1.04 ± 0.00 – 1.59 ± 0.00	2n = 2x = 16 = 16M
BM20 Ambassador 3 Agrihorti	4.47 ± 0.10 – 8.70 ± 0.08	5.63 ± 0.05 – 11.88 ± 0.10	10.10 ± 0.15 – 19.15 ± 0.07	37.96 ± 0.30 – 48.55 ± 0.03	1.06 ± 0.00 – 1.63 ± 0.02	2n = 2x = 16 = 16M
BM21 Ambassador 4 Agrihorti	2.89 ± 0.06 – 6.28 ± 0.05	4.43 ± 0.05 – 7.85 ± 0.04	7.32 ± 0.11 – 13.72 ± 0.12	39.48 ± 0.23 – 49.42 ± 0.01	1.02 ± 0.00 – 1.53 ± 0.01	2n = 2x = 16 = 16M

Description: M = metacentric; SM = submetacentric; ST = subtelocentric; T = telocentric.

cultivar: BM12 TSS Agrihorti 1 and BM8 Pikatan, respectively. The presence of submetacentric and subtelocentric chromosomes among these cultivars suggests the occurrence of chromosomal mutations, specifically pericentric inversions that involve the centromere. This type of mutation results in a reversed chromosome segment that spans both the short (p) and long (q) arms, as well as the centromere itself. A similar phenomenon was reported by (Arisuryanti et al. 2018) in two garlic (*Allium sativum* L.) cultivars, which exhibited submetacentric chromosomes due to frequent selection practices.

Cytological analysis also revealed diversity in chromosome size (Table 1). The results showed that the shortest lengths of chromosome short arms, long arms, and total chromosome length were observed in the BM16 Violetta 3 Agrihorti cultivar. In contrast, other cultivars exhibited varying lengths of short arms, long arms, and total chromosome size. The twenty-one shallot cultivars released by the Vegetable Crops Research Institute (BALITSA Research Centre) possess a diploid chromosome number of 2n = 16, which are classified into five distinct karyotype types: 16M, 14M+2SM, 12M+4SM, 10M+6SM, and 10M+4SM+2ST. The chromosome size and chromosome shape among these twenty-one cultivars exhibited considerable variation. This variation in chromosome size indicated the presence of genetic diversity among the 21 shallot cultivars examined. Genetic variation is a critical factor in breeding programs, as it enables the development of high-quality shallot varieties.

The use of cytological characteristics is considered critical for species identification (Y. Kim et al. 2023), in understanding plant evolution (Hosseini & Yaghoobi 2024), and hybrid populations (Sayadi et al. 2021). Therefore, the chromosome or karyotype structures presented in this study may be utilised for the classification of *Allium* species (Maragheh et al. 2019; Saensouk & Saensouk 2021). However, some members of the genus *Allium*, such as *Allium sativum*, *Allium fistulosum*, and *Allium cepa*, possess the same diploid chromosome number (2n = 16) and also share similar karyotype structures despite belonging to different species (Guetat et al. 2015; Arisuryanti et al. 2009; Arisuryanti et al. 2018; Abdali & Miri 2020). Therefore, molecular approaches are necessary to accurately determine cultivar identity, which in turn facilitates more effective breeding programs (Peruzzi et al. 2017; Hao et al. 2023). With the availability of new cytogenetic baseline data from the twenty-one shallot cultivars released by the Vegetable Crops Research Institute (BALITSA Research Centre), further research is needed. This includes analyses of the relationships between karyotype and phenotypic traits such as growth, disease resistance, and bulb quality; studies on the influence of karyotype on breeding outcomes and genetic selection; and the development of kar-

yotype-based genetic markers for shallot breeding and selection programs. Therefore, this study did not only provides novel cytogenetic baseline data but also opened avenues for integrated and applied follow-up research.

AUTHORS CONTRIBUTION

DM designed the research, collected plant samples, performed laboratory work, analysed the data, and wrote the manuscript. P, TA, BSD designed the research, supervised all the processes from the field work to laboratory analysis, and wrote the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank the Genetics and Breeding Laboratory Faculty of Biology Universitas Gadjah Mada for valuable helps during conducting research, and the Vegetable Crops Research Institute (BALITSA Research Centre), which has sent 21 shallot cultivars.

CONFLICT OF INTEREST

The authors declared we have no conflicts of interest to disclose.

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