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Karyotype analysis of three species of *Allium* (Amaryllidaceae) from Thailand

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Abstract. *Saensouk S, Saensouk P. 2021. Karyotype analysis of three species of* Allium *(Amaryllidaceae) from Thailand. Biodiversitas 22: 3458-3466.* Comprehensive karyotype analysis of three species of *Allium* from Thailand has not been reported. This work aims to study karyotype analysis of three species of *Allium* from Thailand. It is confusing due to the use of the common name or local name or local Thai name and morphologies. The karyotype analysis of three species with two variations in the genus *Allium* from Thailand were studied from root tips and observed under a microscope then karyotype was obtained from 10 metaphase plates. The chromosome numbers of them were found 2n = 16. The karyotype formulas of them were constructed as 14m + 2st (*Allium ascalonicum* L. or shallot), 12m + 2sm + 2st (*A. cepa* L. or onion), 10m + 6sm (1 sat) (*A. cepa* L. or red onion), 10m + 4sm + 2st (*A. sativum* L. or big garlic or Chinese garlic) and 8m + 6sm + 2st (*A. sativum* L. or small garlic or Thai garlic). Chromosome structure differences among the three species appeared in the number of m, sm and st chromosomes and the satellites at the end of the short arm of *A. cepa* L. (red onion). This is new data on chromosome structure of the genus *Allium*. Moreover, the karyotype formulas, chromosome structures and satellites of this study should be used for classification of *Allium* from Thailand.

Keywords: chromosome number, satellite, karyotype, *Allium*, Amaryllidaceae

INTRODUCTION

The genus *Allium* L. was previously classified in the family Alliaceae, but has been reassigned to the family Amaryllidaceae (Fritsch et al. 2010). *Allium* consists of 1006 accepted species, making it one of the largest monocotyledonous genera (Maragheh et al. 2019, International Plant Names Index (IPNI) 2020, KewScience. 2021). In Thailand, *Allium* recognized ten species (Bangkok Forest Herbarium 2014). *Allium* is commonly used in Thai daily life and it is found in home gardens and produce markets. In Thailand, fresh or processed *Allium* species are economic plants that are used as herbs and for cooking. Various products, such as pickled garlic, fried garlic, shallots, pickled shallots and pickled onions, are processed from several species of this genus (Pholhiamhan et al. 2018).

Allium ascalonicum L. and *A. cepa* L. are commonly used to study cell division (both mitosis and meiosis) in various stages, due to the chromosomes of both species being large in size and clearly visible with a light microscope (Donsakul and Phornphisutthimas 2010, Maragheh et al. 2019). The karyotypes of many *Allium* species, from crops and ornamental plants, have been relatively poorly studied (Maragheh et al. 2019). *Allium* about 11 species, including *A. cepa, A. aobonum, A. wakegi, A. oschanini, A. galantum, A. vavilovii, A. fistulosum, A. altaicum, A. ledebourianum, A. schoenoprasum* and *A. sativam* have a chromosome number 2*n* = 16 (Mensinkai 1939; Konvicka and Levan 1972; Seo and Kim 1975; Roy 1978; Verma and Mittal 1978; Vosa 1976; Chen 1989; Wajahatellah and Vahidy 1990; Puizina et al. 1995; Puizina and Papea 1996; Donsakul and Phornphisutthimas 2010; Mukherjee and Roy 2012; Maragheh et al. 2019). Except, some workers are found such as Kumar and Thonger (2018) reported chromosome numbers of the genus *Allium* from Nagaland, North-Eastern Region of India, namely *A. chinense* (2n = 32), *A. tuberosum* (2n = 32), *A. hookeri* (2n = 22) and with basic chromosome number (x = 8). Moreover, Mastall et al. (2018) studied chromosome numbers of and *A. derderianum* from Iran to be 2*n* = 18. The previous karyology study of *Allium* was found 12m+3sm+1st (*A. ascalonicum* from Kumar and Thonger (2018)), 12m + 4sm (*A. sativum* from Maragheh et al. (2019)) and 14m+2sm (*A. sativum* from Kumar and Thonger (2018)). In Thailand, Donsakul and Phornphisutthimas (2010) reported the chromosomes from root tips stained with Giemsa's stain and the karyotype formulas with NF of *Allium ascalonicum* L., *A. cepa* L. var. *aggregatum* G. Don, *A. cepa* var. *viviparum* (Metzg) Alef., *A. fistulosum* L., *A. ampeloprasum* L., *A. sativum* L. (large cloves), *A. sativum* L. (small cloves), and *A. tuberosum* Roxb. In addition from Donsakul and Phornphisutthimas (2010), the chromosome characteristics of *Allium* can be divided into two groups, i.e.: (i) onion group had satellite chromosomes, and (ii) garlic group had secondary constrictions on their chromosomes.

Currently, shallot, onion and garlic are found in several markets in the world especially in supermarkets, local

markets and home gardens in villages (Pholhiamhan et al. 2018). However, their morphology is similar but differs in size, which is confusing due to the use of the common name or local name in each area in the world such as shallot and red onion (Donsakul and Phornphisutthimas 2010, Pholhiamhan et al. 2018). The karyotype analysis of three *Allium* species are associated mainly with comparisons among different species and varieties, and variations in chromosome number and karyotypes. Finally, chromosome structures and satellites of all species and all variations in this study should be not the same as previous report. It might be new chromosome data report of this genus. Therefore, the aim of this study is to study karyotype analysis of three species of *Allium* from Thailand.

MATERIALS AND METHODS

Plant materials

Three species and two variations of *Allium*, namely *A. ascalonicum* L. (shallot), *A. cepa* L. (onion), *A. cepa* L. (red onion), *A. sativum* L. (big garlic or Chinese garlic) and *A. sativum* L. (small garlic or Thai garlic), were collected from produce markets and home gardens in Maha Sarakham Province, Thailand. The cut roots were obtained from bulbs grown in pots in a nursery at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Thailand. In particular, shallots and red onions are both called shallots by most young Thai people (Table 1). All specimens were deposited at Mahasarakham University Herbarium. The comparative morphologies of all plant materials are presented in Table 1 and Figure 1.

Karyotype observation and analysis

Root tips (1.5–2 cm long) of all *Allium* specimens were pretreated with paradichlorobenzene (PDB) for 6 hours at 4°C, fixed in ethanol-acetic acid (3:1, v:v) for 30 min at room temperature and stored at 4°C or immediately used. Samples were washed in distilled water, then hydrolyzed in 1M HCl for 5 min at 60°C and washed again in distilled water. Root tips were stained and crushed in 2% aceto-orcein (Saensouk and Saensouk 2021 a, b), and observed with taken photographs under a light microscope (Zeiss: Axiostar plus) at 100x magnification. Karyotype formulas were derived from measurements of the metaphase chromosomes in photomicrographs, obtained from 10 metaphase plates. The nomenclature of the chromosome shape for karyotype description followed Levan et al. (1964), Senavongse et al. (2018), Saensouk and Saensouk (2021 a, b). The classification of the karyotype symmetry followed Stebbins (1971), Senavongse et al. (2018), Saensouk and Saensouk (2021a, b).

RESULTS AND DISCUSSION

Botanical nomenclature of *Allium* studied

The botanical nomenclature of three species and two variations of the genus *Allium*, namely *Allium ascalonicum* L. (shallot), *A. cepa* L. (red onion), *A. cepa* L. (onion), *A. sativum* L. (big garlic) and *A. sativum* L. (small garlic), were sourced from plant list database (Maragheh et al. 2019, International Plant Names Index (IPNI) 2020, KewScience. 2021). *A. ascalonicum* L. (shallot), *A. sativum* L. (big garlic) and *A. sativum* L. (small garlic) are accepted names, while *A. cepa* L. (red onion) is an accepted name, but it has a synonym which is *A. cepa* L. var. *viviparum* (Metzg) Alef.; *A. cepa* L. (onion) is an accepted name, while *A. cepa* L. var. *aggregatum* G. Don is a synonym of this species.

Table 1. Comparative analysis of morphological characteristics in three species of *Allium* with two variations of *A. cepa* and *A. sativum* from Thailand

Species of *Allium* (common name/ local Thai name)	Pseudostem height (cm)	Diameter of bulb (cm)	Color of leaf sheaths (Figure 1.A-E)	Leaf size (cm)	Leaf shape	Cloves	Collector no.
A. ascalonicum L. (shallot/Hom-Daeng)	10-15	2	Reddish when young Red when mature (Figure 1.A)	0.3-0.6 x 7-10 cm	Linear with tubular	-	Saensouk 3000
A. cepa L. (onion/Hom-Hua-Yai)	35-40	15-20	Greenish-white or white when young and mature (Figure 1.B)	1-1.3 x 27-35 cm	Linear with tubular	-	Saensouk 3002
A. cepa L. (red onion/Hom-Khaek)	25-30	8	Dark red when young and mature (Figure 1.C)	0.8-0.9 x 20-25 cm	Linear with tubular	-	Saensouk 3001
A. sativum L. (small garlic or Thai garlic/Kra-Tiam-Kleeb-Lek)	20-25	5-7	Grey when young and mature (Figure 1.D)	0.5-0.8 x 15-20 cm	Broadly linear to linear-lanceolate	Small cloves	Saensouk 3003
A. sativum L. (big garlic or Chinese garlic/Kra-Tiam-Kleeb-Yai)	35-40	10-15	Grey when young and mature (Figure 1.E)	1-1.2 x 28-35 cm	Broadly linear to linear-lanceolate	Large leaves	Saensouk 3004

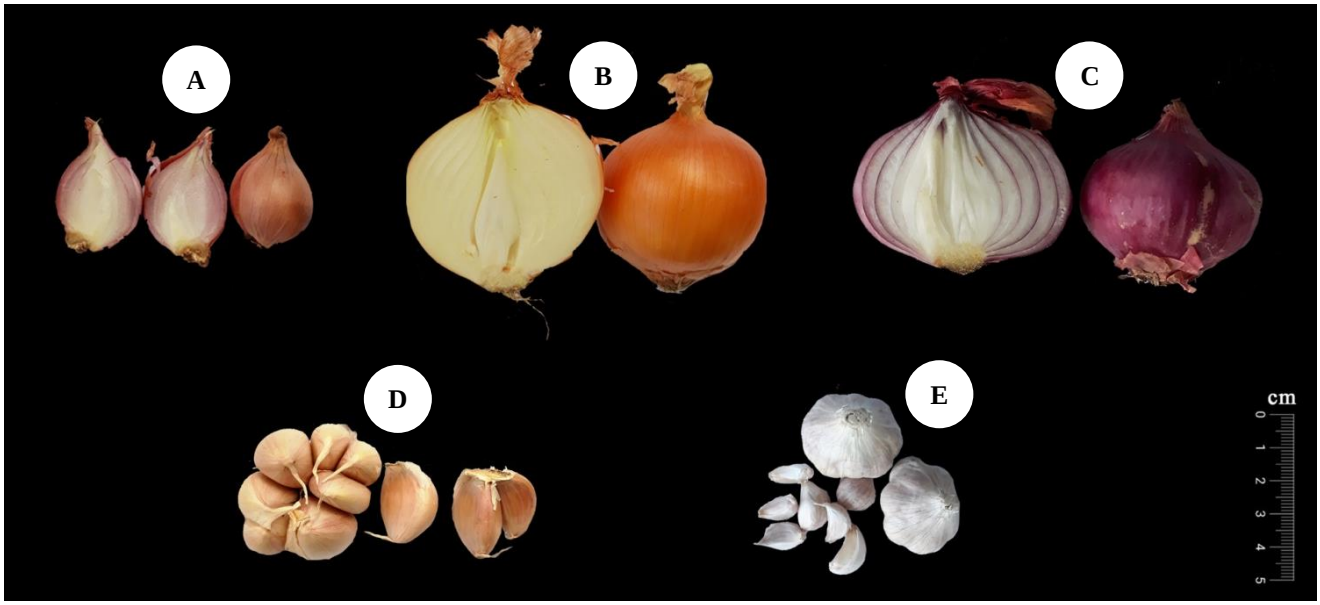

Figure 1. Morphology of bulbs from three species and two variations of *Allium* in Thailand: A. *A. ascalonicum* L. (shallot), B. *A. cepa* L. (onion), C. *A. cepa* L. (red onion), D. *A. sativum* L. (big garlic), E. *A. sativum* L. (small garlic). Scale bar = 5 cm

Karyotype analysis

The studied chromosome numbers of three species and two variations, namely *Allium ascalonicum* L. (shallot), *A. cepa* L. (red onion), *A. cepa* L. (onion), *A. sativum* L. (big garlic) and *A. sativum* L. (small garlic), were found to be 2*n* = 16 and this chromosome number agrees with a previous report by Donsakul and Phornphisutthimas (2010) who reported 2*n* = 16. From result in this study (Table 2) found that the karyology study of *Allium ascalonicum* not consistent with the previous karyotype study by Donsakul and Phornphisutthimas (2010) and Kumar and Thonger (2018). Whereas, the karyotype of *A. cepa* L. (Red Onion) differs from the previous karyotype study by Donsakul and Phornphisutthimas (2010). Moreover, the karyotype of *A. cepa* L. (Onion) disagrees with the previous karyotype study by Donsakul and Phornphisutthimas (2010). The karyotype of *A. sativum* L. differs from the previous karyotype study by Donsakul and Phornphisutthimas (2010), Kumar and Thonger (2018) and Maragheh et al. (2019).

Allium ascalonicum has chromosome number of 2*n* = 16 and its karyotype formula of 14m + 2st (Table 2) consists of seven pairs of metacentric (m) and one pair of subtelocentric (st) chromosomes (Table 3). The chromosomes have length short ranging from 4.37±0.58 to 11.58±0.67 μm, length long ranging from 8.51±0.30 to 14.21±0.58 μm, length total ranging from 14.35±0.88 to 25.79±1.24 μm, relative length ranging from 8.91 to 16.02 % and centromeric index ranging from 0.50 to 0.76 (Table 3, Figures 2A and 3A). The number of chromosome arms (arm number, NF or FN) was found to being 32 (Table 2). This is consistent with the chromosome number 2*n* = 16 reported by Aryavand (1975); Seo and Kim (1975); Vosa (1977); Pandita (1979); Cortes et al. (1983); Bartolo et al. (1984); Talukder and Sen (2000); and Donsakul and Phornphisutthimas (2010). Donsakul and Phornphisutthimas (2010) reported that karyotype formula of *A. ascalonicum* to be 12m + 2sm + 2st (1 sat or one visible satellite chromosome) with NF = 30, which is not consistent with the karyotype formula with without visible satellite chromosome and NF of the present study results due to the effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a, b).

Allium cepa (onion) has chromosome number 2*n* = 16, and its karyotype formula of 12m + 2sm + 2st (Table 2) consists of six pairs of metacentric, one pair of submetacentric (sm), and one pair of subtelocentric chromosomes (Table 4). the chromosomes have length short ranging from 3.87±0.31 to 8.78±0.33 μm, length long ranging from 7.25±0.33 to 11.34±0.20 μm, length total ranging from 12.82 ± 0.91 to 18.42 ± 0.68 μm, relative length ranging from 10.13 to 14.55 % and centromeric index ranging from 0.51 to 0.75 (Table 4, Figures 2B and 3B). The number of chromosome arms (arm number, NF or FN) is found to being 32 (Table 2). Donsakul and Phornphisutthimas (2010) reported that karyotype formula of *A. cepa* (onion) to be 12m + 2sm + 2st (1 sat) with NF = 30, which is the same as the karyotype formula in the present study results but differs in without visible satellites and NF due to the effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a,b).

Table 2. Chromosome number and karyotype analysis of three species and two variations of *Allium* from previous reports and the present study

Species	2*n*	NF	Karyotype formula	Visible satellites	Location	Reference
A. ascalonicum L. (shallot)	16	32	-		South Korean	Seo and Kim (1975)
	16	-	-	-	Iran	Aryavand (1975)
	16	-			Brasil	Vosa (1977)
	16	-			India	Pandita (1979)
	16	-			-	Cortes et al. (1983)
	16	-			Libya	Cortes et al. (1983)
	16	-			-	Bartolo et al. (1984)
	16	-			India	Talukder and Sen (2000)
	16	-	12m+3sm+1st		India	Kumar and Thonger (2018)
	16	30	12m + 2sm + 2st	1(STR)	Thailand	Donsakul and Phornphisutthimas (2010)
	16	**32**	**14m + 2st**		**Thailand**	***Present study***
A. ascalonicum L. (shallot)	16	32	-		South Korea	Seo and Kim (1975)
			12m+3sm+1st		India	Kumar and Thonger (2018)
	16	30	12m + 2sm + 2st	1(STR)	Thailand	Donsakul and Phornphisutthimas (2010)
	16	**32**	**14m + 2st**		**Thailand**	***Present study***
A. cepa L. (red onion) (synonym = *A. cepa* L. var. *viviparum*)	16	30	10m + 4sm + 2st	1(STR)	Thailand	Donsakul and Phornphisutthimas (2010)
	24	48	-	-	India	Mukherjee and Roy (2012)
	16, 24	32, 48	-	-	Croatian	Puizina et al. (1995)
	16	-	-	-	Brasil	Vosa (1977)
	16	-	-	-	India	Langer and Koul (1983)
	16, 24	32, 48	-	-	Croatian	Puizina and Papea (1996)
	24	-	-	-	India	Gohil and Kaul (1981)
	16	**32**	**10m + 6sm**	**1(STR)**	**Thailand**	***Present study***
A. cepa L. (red onion) (synonym = *A. cepa* L. var. *viviparum*)	16	30	10m + 4sm + 2st	1(STR)	Thailand	Donsakul and Phornphisutthimas (2010)
	24	48	-	-	India	Mukherjee and Roy (2012)
	16, 24	32, 48	-	-	Croatian	Puizina et al. (1995)
	16, 24	32, 48	-	-	Croatian	Puizina and Papea (1996)
	24	-	-	-	India	Gohil and Kaul (1981)
	16	**32**	**10m + 6sm**	**1(STR)**	**Thailand**	***Present study***
A. cepa L. (onion) (syn. *A. cepa* L. var. *aggregatum*)	16	-	-	-	India	Vijayavalli and Mathew (1990)
	16	32	-	-	Japan	Chen (1989)
	16	30	12m + 2sm + 2st	1(STR)	Thailand	Donsakul and Phornphisutthimas (2010)
	16	32	-	-	India	Mukherjee and Roy (2012)
	16	**32**	**12m + 2sm + 2st**	**-**	**Thailand**	***Present study***
A. sativum L. (big garlic)	16	-	-	-	-	Katayama (1928)
	16	-	-	-	Sweden	Levan (1931)
	16	-	-	-	Sweden	Levan (1935)
	16	-	-	1(STR)	-	Mensinkai (1939)
	16	-	-	-	India	Khoshoo and Sharma (1959)
	16	-	-	2(STR)	India	Khoshoo et al. (1960)
	16			2(STR)	-	Battaglia (1963)
	16	32	-	-	Sweden	Konvicka and Levan (1972)
	32	-	-	-	-	Novak (1974)
	16	32	-	-	India	Verma and Mittal (1978)
	32	-	-	-	India	Roy (1978)
				-	India	Verma and Mittal (1978)
	16	-	-	-	India	Gohil and Kaul (1981)
	16	-	-	-	India	Kumar and Subramaniam (1986)
	16	-	-	-	India	Vijayavalli and Mathew (1990)
	16	32	-	-	Pakistan	Wajahalatullah and Vahidy (1990)
	16	32	-	-	India	Jacobkutty and Bhavanandan (1997)
	16	32	6m + 2sm + 2t (large cloves and small cloves)	-	Thailand	Donsakul and Phornphisutthimas (2010)

	16	32	-	-	India	Mukherjee and Roy (2012)
	32	-	-	-	India	Manzum et al. (2014)
			14m+2sm		India	Kumar and Thonger (2018)
	16	32	12m + 4sm	-	Poland	Maragheh et al. (2019)
	16	**32**	**10m + 4sm + 2st**	**-**	**Thailand**	***Present study*** **(big garlic)**
A. sativum L. (small garlic)	16	-	-	-	-	Katayama (1928)
	16	-	-	-	Sweden	Levan (1931)
	16	-	-	-	Sweden	Levan (1935)
	16	-	-	1(STR)	-	Mensinkai (1939)
	16	-	-	-	India	Khoshoo and Sharma (1959)
	16	-	-	2(STR)	India	Khoshoo et al. (1960)
	16			2(STR)	-	Battaglia (1963)
	16	32	-	-	Sweden	Konvicka and Levan (1972)
	32	-	-	-	-	Novak (1974)
	16	32	-	-	India	Verma and Mittal (1978)
	32	-	-	-	India	Roy (1978)
				-	India	Verma and Mittal (1978)
	16	-	-	-	India	Gohil and Kaul (1981)
	16	-	-	-	India	Kumar and Subramaniam (1986)
	16	-	-	-	India	Vijayavalli and Mathew (1990)
	16	32	-	-	Pakistan	Wajahalatullah and Vahidy (1990)
	16	32	-	-	India	Jacobkutty and Bhavanandan (1997)
	16	32	6m + 2sm + 2t (large cloves and small cloves)	-	Thailand	Donsakul and Phornphisutthimas (2010)
	16	32	-	-	India	Mukherjee and Roy (2012)
	32	-	-	-	India	Manzum et al. (2014)
			14m+2sm		India	Kumar and Thonger (2018)
	16	32	12m + 4sm	-	Poland	Maragheh et al. (2019)
	16	**32**	**8m + 6sm + 2st**	**-**	**Thailand**	***Present study*** **(small garlic)**

Note: STR: subtelomeric region; NF: Fundamental Number

Table 3. Chromosome characteristic of *Allium ascalonicum* (shallot)

Chromosome pair	Ls±SD (μm)	Ll±SD (μm)	LT±SD (μm)	AR	RL (%)	CI (Structure)	Chromosome shape
1	11.58±0.67	14.21±0.58	25.79±1.24	1.23	16.02	0.55	Metacentric
2	11.56±0.88	11.56±0.58	23.11±1.46	1.00	14.36	0.50	Metacentric
3	9.14±0.74	12.72±0.88	21.86±1.62	1.39	13.58	0.58	Metacentric
4	8.73±0.42	12.76±0.67	21.49±1.08	1.46	13.35	0.59	Metacentric
5	8.56±0.88	11.34±0.67	19.90±1.55	1.32	12.36	0.57	Metacentric
6	4.37±0.58	13.69±0.58	18.05±1.15	3.13	11.21	0.76	Subtelocentric
7	7.87±0.58	8.56±0.58	16.43±1.15	1.09	10.20	0.52	Metacentric
8	5.84±0.58	8.51±0.30	14.35±0.88	1.46	8.91	0.59	Metacentric

Note: LA: length of the arm; p: long arm; q: short arm; TL: total arm length; CS: chromosome shape; AR: Arm Ratio

Allium cepa (red onion) has chromosome number of 2*n* = 16, and its karyotype formula of 10m + 6sm (1 sat) consists of five pairs of metacentric, three pairs of submetacentric chromosomes and one visible satellite at the end of the short arm, pair 6, bar 11 of the submetacentric chromosome (Table 4). The chromosomes have length short ranging from 4.63±0.33 to 9.13±0.33 μm, length long ranging from 7.10±0.33 to 10.49±0.33 μm, length total ranging from 11.92 ± 0.67 to 19.62 ± 0.67 μm, relative length ranging from 9.72 to 15.99% and centromeric index ranging from 0.53 to 0.65 (Table 4, Figures 2C and 3C). The number of chromosome arms (arm number, NF or FN) is found to being 32 (Table 2). The present results agree with the previous studies by Vosa (1977), Langer and Koul (1983) and Puizina and Papea (1996), while Mukherjee and Roy (2012); Puizina et al. (1995); Puizina and Papea (1996). Gohil and Kaul (1981) reported a chromosome number of 2*n* = 3x = 24, which disagrees with this study. In addition, the karyotype formula of this species is 10m + 4sm + 2st with one visible satellite (NF =30), (Donsakul and Phornphisutthimas 2010) which is different from the karyotype formula of the present study due to the effects of environmental factors, and NF is different in this study results (NF = 32) also due

to the effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a, b).

The present study shows the chromosome numbers of *A. cepa* (onion) and *A. cepa* (red onion) are the same, but have differences in the karyotype formula 12m + 2sm + 2st of *A. cepa*. (onion) and 10m + 6sm (1 sat) of *A. cepa* (red onion) and one visible satellite due to differences in the morphology of both variations of onions (Table 1), i.e. the pseudostem, including the bulb, and leaves of red onion are shorter and smaller than the pseudostem of onion; the color of the leaf sheaths of red onion has a dark red color when young and mature, while the color of the leaf sheaths of onion has been found to be greenish-white or white when young and mature. While, the scientific name of onion and red onion from many literature studies and international databases, such as http://www.ipni.org (International Plant Names Index (IPNI) 2020) and http://apps.kew.org/wcsp/ (KewScience 2021), were found to be the same for both variations, indicating both are the same species. Therefore, differences in the karyotype formula, chromosome structure of both variations (onion and red onion) due to the variation in morphology and effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a,b).

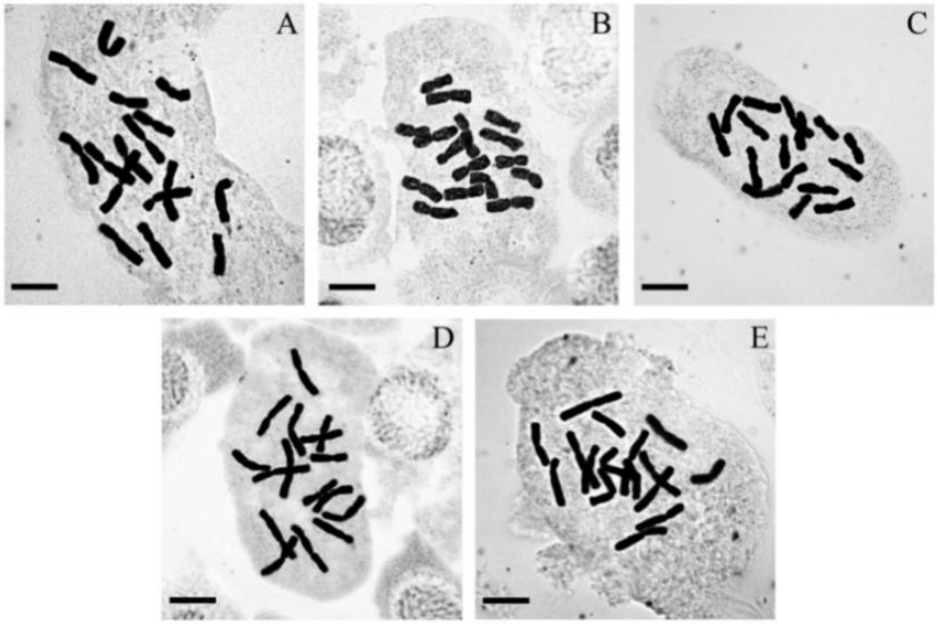

Figure 2. Somatic chromosome at metaphase of: A. *A. ascalonicum* (shallot), B. *A. cepa* (onion), C. *A. cepa* (red onion), D. *A. sativum* (big garlic or Chinese garlic), and E. *A. sativum* (small garlic or Thai garlic). Scale bars = 10 μm

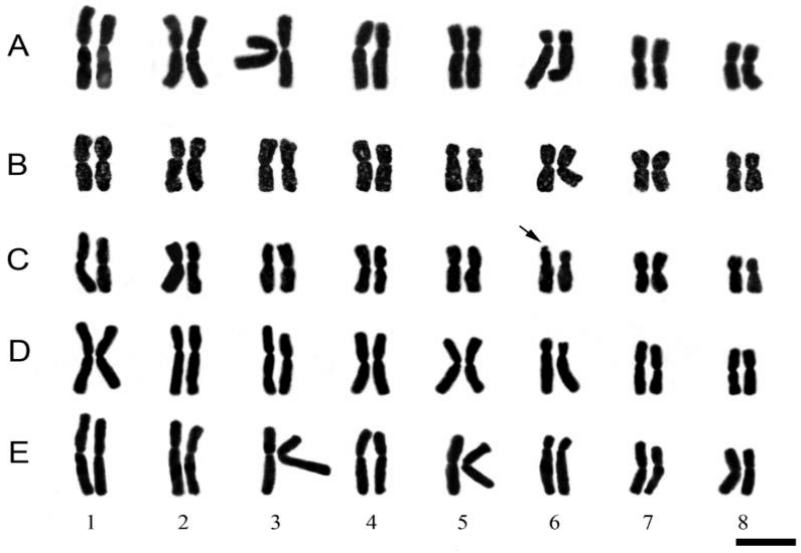

Figure 3. Karyotypes of: A. *A. ascalonicum* (shallot), B. *A. cepa* (onion), C. *A. cepa* (red onion), D. *A. sativum* (big garlic or Chinese garlic), and E. *A. sativum* (small garlic or Thai garlic). Arrows indicate satellite. Scale bar = 10 μm

Allium sativum (big garlic) was determined to have the chromosome number 2*n* = 16, and its karyotype formula of 10m + 4sm + 2st, consists of five pairs of metacentric, two pairs of submetacentric and one pair of subtelocentric chromosomes (Table 5). the chromosomes have length short ranging from 4.23±0.44 to 11.09±0.44 μm, length long ranging from 8.06±0.50 to 14.79±0.58 μm, length total ranging from 15.12 ± 0.91 to 23.44 ± 0.88 μm, relative length ranging from 9.69 to 15.02% and centromeric index ranging from 0.53 to 0.75 (Table 5, Figures 2D and 3D). The number of chromosome arms (arm number, NF or FN) is found to being 32 (Table 2). The chromosome number in this study is 2*n* = 16, which agrees with Katayama (1928), Levan (1931 and 1935), Mensinkai (1939), Khoshoo and Sharma (1959), Khoshoo et al. (1960), Roy (1978), Verma and Mittal (1978), Kumar and Subramaniam (1986), Wajahalatullah and Vahidy (1990), Jacobkutty and Bhavanandan (1997), and Mukherjee and Roy (2012). while, Sharma and Bal (1959) reported the chromosome number 2*n* = 18 and Novak (1974) and Manzum et al. (2014) reported the chromosome number 2n = 32, which are different from this study results. Moreover, Khoshoo et al. (1960); and Battaglia (1963) found 2*n* = 16 with two satellite pairs, except Mensinkai (1939) which found only one satellite pair and differs from the current study. Donsakul and Phornphisutthimas (2010) recognized the karyotype formula 6m + 2sm + 2t with NF = 32 of large cloves (big garlic in this study) which disagrees with this study results due to the effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a, b), while NF is the same in this study (NF = 32).

Allium sativum (small garlic) was determined to have the chromosome number 2*n* = 16 and its karyotype formula of 8m + 6sm + 2st consists of four pairs of metacentric, three pairs of submetacentric and one pair of subtelocentric chromosomes (Table 5). the chromosomes have length short ranging from 4.50±0.71 to 12.50±0.58 μm, length long ranging from 8.30±0.44 to 15.58±0.82 μm, length total ranging from 15.42 ± 1.02 to 26.90 ± 1.34 μm, relative length ranging from 9.33 to 16.26 % and centromeric index ranging from 0.54 to 0.77 (Table 5, Figures 2E and 3E). The number of chromosome arms (arm number, NF or FN) is found to being 32 (Table 2). The chromosome number of this study is consistent with the studies of several workers (Katayama 1928; Levan 1931 and 1935; Mensinkai 1939; Khoshoo and Sharma 1959; Khoshoo et al. 1960; Roy 1978; Verma and Mittal 1978; Kumar and Subramaniam 1986; Wajahalatullah and Vahidy 1990; Jacobkutty and Bhavanandan 1997; and Mukherjee and Roy 2012;). While, Novak (1974) and Manzum et al. (2014) reported the chromosome number 2n = 32, which is not consistent with the present study. The chromosome number 2n = 16 with two satellite pairs was earlier reported (Khoshoo et al. 1960; Battaglia 1963; and Mensinkai 1939, while Mensinkai (1939) found only one satellite pair, which differs from current study. Donsakul and Phornphisutthimas (2010) recognized the karyotype formula 6m + 2sm + 2t with NF = 32 of small cloves (small garlic in this study). The karyotype formula differs from this study due to the effects of environmental factors. NF is different in this study results due to effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a, b).

Table 4. Comparison of chromosome characteristics of *Allium cepa* (onion) and *A. cepa* (red onion)

Species	Chromosome pair	Ls±SD (μm)	Ll±SD (μm)	LT±SD (μm)	AR	RL (%)	CI (Structure)	Chromosome shape
A. cepa L. (onion)	1	8.49±0.35	9.94±0.33	18.42±0.68	1.17	14.55	0.54	Metacentric
	2	6.72±0.33	11.34±0.20	18.06±0.53	1.69	14.26	0.63	Submetacentric
	3	8.78±0.33	8.98±0.30	17.76±0.63	1.02	14.03	0.51	Metacentric
	4	6.47±0.30	9.63±0.33	16.10±0.63	1.49	12.72	0.59	Metacentric
	5	3.87±0.31	11.44±0.44	15.32±0.75	2.96	12.10	0.75	Subtelocentric
	6	6.77±0.33	8.08±0.33	14.85±0.67	1.19	11.73	0.54	Metacentric
	7	5.96±0.33	7.33±0.33	13.29±0.67	1.23	10.50	0.55	Metacentric
	8	5.57±0.58	7.25±0.33	12.82±0.91	1.30	10.13	0.57	Metacentric
A. cepa L. (red onion)	1	9.13±0.33	10.49±0.33	19.62±0.67	1.15	15.99	0.53	Metacentric
	2	6.66±0.13	10.38±0.33	17.04±0.47	1.56	13.89	0.61	Submetacentric
	3	7.01±0.33	8.71±0.30	15.71±0.63	1.24	12.81	0.55	Metacentric
	4	7.38±0.33	8.32±0.33	15.70±0.67	1.13	12.80	0.53	Metacentric
	5	6.61±0.33	8.38±0.33	14.99±0.67	1.27	12.22	0.56	Metacentric
	6*	4.96±0.43	9.37±0.33	14.34±0.77	1.89	11.69	0.65	Submetacentric
	7	6.24±0.33	7.10±0.33	13.34±0.67	1.14	10.87	0.53	Metacentric
	8	4.63±0.33	7.29±0.33	11.92±0.67	1.58	9.72	0.61	Submetacentric

Notes: *: satellite chromosome, LA: length of the arm; p: long arm; q: short arm; TL: total arm length; CS: chromosome shape; AR: Arm Ratio.

Table 5. Comparison of chromosome characteristic of *A. sativum* (big garlic or Chinese garlic) and *A. sativum* (small garlic or Thai garlic)

Species	Chromosome pair	Ls±SD (*μ*m)	Ll±SD (*μ*m)	LT±SD (*μ*m)	AR	RL (%)	CI (Structure)	Chromosome shape
A. sativum L. (big garlic or Chinese garlic)	1	11.09±0.44	12.35±0.44	23.44±0.88	1.11	15.02	0.53	Metacentric
	2	8.45±0.28	14.79±0.58	23.24±0.85	1.75	14.89	0.64	Submetacentric
	3	8.57±0.44	12.88±0.42	21.45±0.86	1.50	13.74	0.60	Submetacentric
	4	9.21±0.58	11.12±0.44	20.32±1.02	1.21	13.02	0.55	Metacentric
	5	8.80±0.56	10.32±0.39	19.12±0.96	1.17	12.25	0.54	Metacentric
	6	4.23±0.44	12.79±0.44	17.02±0.88	3.03	10.90	0.75	Subtelocentric
	7	7.35±0.58	9.01±0.58	16.36±1.15	1.22	10.48	0.55	Metacentric
	8	7.06±0.41	8.06±0.50	15.12±0.91	1.14	9.69	0.53	Metacentric
A. sativum L. (small garlic or Thai garlic)	1	12.50±0.58	14.39±0.76	26.90±1.34	1.15	16.28	0.54	Metacentric
	2	8.59±0.58	15.58±0.82	24.17±1.26	1.81	14.63	0.64	Submetacentric
	3	7.59±0.82	13.76±0.44	21.95±1.26	1.81	13.29	0.63	Submetacentric
	4	8.91±0.83	12.93±0.62	21.24±1.45	1.45	12.86	0.61	Submetacentric
	5	9.12±0.71	10.92±0.82	20.04±1.53	1.20	12.13	0.55	Metacentric
	6	4.50±0.71	15.38±0.76	19.89±1.47	3.42	12.04	0.77	Subtelocentric
	7	6.82±0.83	8.77±0.58	15.60±1.41	1.29	9.44	0.56	Metacentric
	8	7.12±0.58	8.30±0.44	15.42±1.02	1.17	9.33	0.54	Metacentric

Note: LA: length of the arm; p: long arm; q: short arm; TL: total arm length; CS: chromosome shape; AR: Arm Ratio.

This study showed that the chromosome numbers with NF of *Allium sativum* (big garlic) and *A. sativum* (small garlic) are the same which is the same with study of Donsakul and Phornphisutthimas (2010), but differ in the karyotype formula, chromosome structure due to differences in the size of the morphology in both variations of garlic, i.e. big garlic has large cloves with large leaves, while small garlic has small cloves with small leaves (Table 1) and because of the effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a, b) which disagrees with studied of Donsakul and Phornphisutthimas (2010) because they reported the same in karyotype formula and chromosome structure of variations of garlic.

Such discrepancies in karyotype formula were probably due to differences in analyzed materials and mitotic stages used, and difficulty in identifying chromosomes using the classical staining technique before (She et al. 2015a, 2015b, 2017, 2020). This observed difference could mainly be related to variation in the chromosome condensation levels of measured cells (She et al. 2015a, 2020). Therefore, differences in the chromosome structures due to the variation in morphology of *Allium* species, such as variation in cloves of both variations garlic, in size and color of both variation onion (Table 1) and because of the effects of environmental factors such as water, air, soil and maybe nutrients (Saensouk and Saensouk 2021a, b).

In conclusion, The somatic chromosome number of *Allium ascalonicum* (shallot), *A. cepa* (red onion), *A. cepa* (onion), *A. sativum* (big garlic) and *A. sativum* (small garlic) in this study are found the same number $2n = 16$. The chromosome structure differences among the three species appeared in the number of m, sm and st chromosomes and the visible satellites, which are the visible bands at the end of the short arm of the chromosome structure of *A. cepa* (red onion). chromosome structures and satellites of all species and all variations in this study are not the same. chromosome structures and satellites of this study could be used for classification in *Allium ascalonicum* (shallot), *A. cepa* (onion), *A. cepa* (red onion), *A. sativum* (big garlic or Chinese garlic) and *A. sativum* (small garlic or Thai garlic) from Thailand.

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