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. vatium 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 2n = 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 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 A. derderianum from Iran to be 2n = 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. ameloprasum 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 Thongphitsuthimas 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).

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                   | 0.3-0.6 x 7-10 cm | Linear with tubular | -      | Saensouk 3000 |
|                                               |                        |                       | Red when mature                      |                |           |        |              |
| A. cepa L. (onion/Hom-Hua-Yai)                 | 35-40                  | 15-20                 | Greenish-white or white              | 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       | 0.8-0.9 x 20-25 cm | Linear with tubular | -      | Saensouk 3001 |
|                                               |                        |                       | (Figure 1.C)                        |                |           |        |              |
| A. sativum L. (small garlic or Thai garlic/Kra-Tiam-Kleeb-Lek) | 20-25                  | 5-7                   | Grey when young and mature           | 0.5-0.8 x 15-20 cm | Broadly linear to linear-lanceolate | Small cloves | Saensouk 3003 |
|                                               |                        |                       | (Figure 1.D)                        |                |           |        |              |
| A. sativum L. (big garlic or Chinese garlic/Kra-Tiam-Kleeb-Yai) | 35-40                  | 10-15                 | Grey when young and mature           | 1-1.2 x 28-35 cm | Broadly linear to linear-lanceolate | Large leaves | Saensouk 3004 |

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.
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 $2n = 16$ and this chromosome number agrees with a previous report by Donsakul and Phornphisuthimas (2010) who reported $2n = 16$. 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 Phornphisuthimas (2010) and Kumar and Thonger (2018). Whereas, the karyotype of A. cepa L. (Red Onion) differs from the previous karyotype study by Donsakul and Phornphisuthimas (2010). Moreover, the karyotype of A. cepa L. (Onion) disagrees with the previous karyotype study by Donsakul and Phornphisuthimas (2010). The karyotype of A. sativum L. differs from the previous karyotype study by Donsakul and Phornphisuthimas (2010), Kumar and Thonger (2018) and Maragheh et al. (2019).

Allium ascalonicum has chromosome number of $2n = 16$ 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 $4.37\pm0.58$ to $11.58\pm0.67 \mu m$, length long ranging from $8.51\pm0.30$ to $14.21\pm0.58 \mu m$, length total ranging from $14.35\pm0.88$ to $25.79\pm1.24 \mu m$, 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).

Allium cepa (onion) has chromosome number $2n = 16$, and its karyotype formula of $12m + 2sm + 2st$ (Table 2) consists of six pairs of metacentric (m), and one pair of submetacentric (sm) and one pair of subtelocentric chromosomes (Table 4). The chromosomes have length short ranging from $3.87\pm0.31$ to $8.78\pm0.33 \mu m$, length long ranging from $7.25\pm0.33$ to $11.34\pm0.20 \mu m$, length total ranging from $12.82 \pm 0.91$ to $18.42 \pm 0.68 \mu m$, 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 Phornphisuthimas (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                  | 2n | NF  | Karyotype formula | Visible satellites | Location     | Reference                                      |
|--------------------------|----|-----|-------------------|-------------------|--------------|-----------------------------------------------|
| A. ascalonicum L. (shallot) | 16 | 32  | -                 |                   | South        | Seo and Kim (1975)                            |
|                          | 16 | -   | -                 | -                 | Korean       | Aryavand (1975)                               |
|                          | 16 | -   | -                 | -                 | Iran         | Vosa (1977)                                   |
|                          | 16 | -   | -                 | -                 | India        | Pandita (1979)                                |
|                          | 16 | -   | -                 | -                 | Libya        | Cortes et al. (1983)                          |
|                          | 16 | -   | -                 | -                 | Bartolo et al. (1984) |
|                          | 16 | -   | 12m+3sm+1st       | 1(STR)            | Thailand     | Donsakul and Phornphisuthimas (2010)          |
|                          | 16 | 30  | 12m + 2sm + 2st   | 1(STR)            | Thai         | Present study                                 |
| A. cepa L. (red onion)   | 16 | 30  | 10m + 4sm + 2st   | 1(STR)            | Thailand     | Donsakul and Phornphisuthimas (2010)          |
| (synonym = A. cepa)      | 24 | 48  | -                 | -                 | South Korea  | Seo and Kim (1975)                            |
| L. var. viviparum        | 16 | 24  | 32, 48            | -                 | India        | Kumar and Thonger (2018)                      |
|                          | 16 | -   | -                 | -                 | India        | Mukherjee and Roy (2012)                      |
|                          | 16 | -   | 12m + 3sm + 1st   | -                 | India        | Puizina et al. (1995)                         |
|                          | 16 | -   | -                 | -                 | Brasil       | Vosa (1977)                                   |
|                          | 16 | -   | -                 | -                 | India        | Langer and Koul (1983)                        |
|                          | 16 | 24  | 32, 48            | -                 | Croatia      | Puizina and Papea (1996)                      |
|                          | 24 | -   | -                 | -                 | Croatia      | Gohil and Kaul (1981)                         |
| A. cepa L. (onion) (syn. A. cepa) | 16 | 30  | 10m + 6sm         | 1(STR)            | Thailand     | Present study                                 |
| L. var. aggregatum       | 16 | 24  | 32, 48            | -                 | India        | Vijayavalli and Mathew (1990)                 |
|                          | 16 | -   | -                 | -                 | Japan        | Chen (1989)                                   |
| A. sativum L. (big garlic) | 16 | -   | -                 | -                 | India        | Katayama (1928)                               |
|                          | 16 | -   | -                 | -                 | Sweden       | Levan (1931)                                  |
|                          | 16 | -   | -                 | -                 | Sweden       | Levan (1935)                                  |
|                          | 16 | -   | 1(STR)            | -                 | Sweden       | Mensinkui (1939)                              |
|                          | 16 | -   | -                 | -                 | India        | Khoshoo and Sharma (1959)                     |
|                          | 16 | -   | 2(STR)            | -                 | India        | Khoshoo et al. (1960)                         |
|                          | 16 | 32  | 12m + 2sm + 2st   | 1(STR)            | Thailand     | Donsakul and Phornphisuthimas (2010)          |
|                          | 16 | -   | -                 | -                 | Thailand     | Present study                                 |
| A. sativum L. (big garlic) | 16 | 32  | 12m + 2sm + 2st   | 1(STR)            | Thailand     | Donsakul and Phornphisuthimas (2010)          |
|                          | 16 | 32  | 10m + 2sm + 2t    | -                 | Thailand     | Present study                                 |
|                          | 16 | 32  | 6m + 2sm + 2t     | -                 | Thailand     | Donsakul and Phornphisuthimas (2010)          |

Notes: Visible satellites: 1(STR) = satellite chromosome, 2(STR) = two satellite chromosomes.
16 32 - - - - - - - India Mukherjee and Roy (2012)
16 32 14m+2sm - - - - - - - India Manzum et al. (2014)
16 32 12m + 4sm - - - - - - - India Kumar and Thonger (2018)
16 32 10m + 4sm + 2st - - - - - - - Poland Maragheh et al. (2019)

A. sativum L. (small garlic)

16 32 - - - - - - - Thailand Present study
(big garlic)

16 32 6m + 2sm + 2t (large cloves and small cloves) - - - - - - - Thailand Donsakul and Phornphisutthimas (2010)

16 32 - - - - - - - India Mukherjee and Roy (2012)
16 32 - - - - - - - India Manzum et al. (2014)
16 32 - - - - - - - India Kumar and Thonger (2018)

Note: STR: subtelomeric region; NF: Fundamental Number

Table 3. Chromosome characteristic of Allium ascalonicum (shallot)

| Chromosome pair | L±SD (µm) | L±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 | 13.09±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 2n = 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 2n = 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
**Table 4.** Comparison of chromosome characteristics of *Allium cepa* (onion) and *A. cepa* (red onion)

| Species                   | Chromosome pair | L±SD (μm) | L±SD (μm) | LT±SD (μm) | AR     | RL   | CI   | Chromosome shape | Structure |
|---------------------------|-----------------|-----------|-----------|------------|--------|------|------|-----------------|-----------|
| *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±0.30 | 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               | 6.33±0.33 | 7.29±0.33 | 11.92±0.67 | 1.58   | 9.72 | 0.61 | Subtelocentric  |           |

Notes: *: satellite chromosome, 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 Phornphisuthimas (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 Phornphisuthimas (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, 2017, 2020). This observed difference could mainly be related to variation in the chromosome condensation levels of measured cells (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, 2015b, 2017, 2020). This observed difference could mainly be related to variation in the chromosome condensation levels of measured cells (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, 2015b, 2017, 2020).

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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### REFERENCES

Aryavand A. 1975. Contribution all’etude cytotaxonomique de quelques angiospermes de l’Iran. Bot Not 128: 299-311.

Bangkok Forest Herbarium. 2014. Thai Plant Names Tem Smitinand Revised Edition 2014. Bangkok, Thailand.

Bartolo G, Brullo S, Pavone P, Terrasi MC. 1984. Cytotaxonomical notes on some Liliaceae of N Cyrenaica. Webbia 38: 601-622. DOI: 10.1080/00087792.1984.10670329.

Battaglia E. 1963. Mutazione chromosomica e cariotipio fondamentale in *Allium sativum* L. Caryologia 16: 1-46. DOI: 10.1080/00087114.1963.10796082.

Chen Ry. 1989. A further research of plant chromosome G-banding. In: D. Hong (ed.). Plant Chromosome Research. Nishiki Print Co, Hiroshima.

Corbet F, Gonzalez-Gil G, Hazen MJ. 1983. C-Banding and sister chromatid exchanges in three species of the genus *Allium* (A. cepa, A. ascalonicum and A. sativum). Caryologia 36: 203-210. DOI: 10.1080/00087114.1983.10797661.

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**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 | L±SD (µm) | LT±SD (µm) | AR (%) | RL (%) | CI (Structure) | Chromosome shape |
|------------------|-----------------|-----------|------------|--------|--------|---------------|-----------------|
| *A. sativum* L.  | 1  | 11.09±0.44 | 12.35±0.44 | 1.44±0.88 | 1.11  | 15.02 | 0.53 | Metacentric   |
| (big garlic)     | 2  | 8.45±0.28  | 14.79±0.58 | 2.34±0.85 | 1.75  | 14.89 | 0.64 | Submetacentric|
| (small garlic)   | 3  | 8.57±0.44  | 12.88±0.42 | 2.15±0.86 | 1.50  | 13.74 | 0.60 | Submetacentric|
| (Thai garlic)    | 4  | 9.21±0.58  | 11.12±0.44 | 2.03±1.02 | 1.21  | 13.02 | 0.55 | Metacentric   |
|                  | 5  | 8.80±0.56  | 10.32±0.39 | 1.92±0.96 | 1.17  | 12.25 | 0.54 | Metacentric   |
|                  | 6  | 4.23±0.44  | 12.79±0.44 | 1.70±0.88 | 3.03  | 10.90 | 0.75 | Subtelocentric|
|                  | 7  | 7.35±0.58  | 9.01±0.58  | 1.63±1.15 | 1.22  | 10.48 | 0.55 | Metacentric   |
|                  | 8  | 7.06±0.41  | 8.06±0.50  | 1.52±1.09 | 1.14  | 9.69  | 0.53 | Metacentric   |

Note: LA: length of the arm; p: long arm; q: short arm; TL: total arm length; CS: chromosome shape; AR: Arm Ratio.
Donsukul T, Phornphusuthim S. 2010. Karyotypes of six species in *Allium* (Alliaceae) in Thailand. Naresuan Univ J 18: 34-39.

Fritsch RM, Blattner FR, Gurushidze M. 2010. New classification of *Allium* L. **subg. melanosorum** (Webb & Berthel.) Rouy (Alliaceae) based on molecular and morphological characters. Phytom 49: 145-220.

Gohil RN, Kaul R. 1981. In Chromosome number reports LXXII. Taxon 30: 707.

International Plant Names Index (IPNI). 2020. http://www.ipni.org/; Accessed 30 May 2021.

Jacobkutty ML, Bhavanandan KV. 1997. Karyotype studies in *Allium sativum*. J Cytol Genet 32: 125-128.

Katayama Y. 1928. The chromosome number in *Phaseolus* and *Allium*, and an observation on the size of stomata in different species of *Triticum*. J Sci Agric Soc Tokyo 303: 52-54.

KewScience. 2021. World Checklist of Selected Plant Families (WCSP). http://apps.kew.org/wcsp/ Accessed 30 May 2021.

Khosloo TN, Atal CK, Sharma VB. 1960. Cytotaxonomical and chemical investigations on the northwest Indian garlic. Res Bull Panj Univ 28: 37-47.

Khosloo TN, Sharma VB. 1959. Chromosome number of north Indian garlic. Curr Sci 26: 62.

Konvicka O, Levan A. 1972. Chromosome studies in *Allium sativum*. Hereditas 72: 129-148. DOI: 10.1111/j.1601-5223.1972.tb01355.x.

Kumar S, Thonger T. 2018. Karyomorphology of five *Allium* species from Nagaland, North-Eastern Region of India. Jordan J Biol Sci 11: 9-15.

Kumar V, Subramaniam, B. 1986. Chromosome Atlas of Flowering Plants of the Indian Subcontinent, Monocotyledons. Kew Scientific Publishing Co., CA.

Langer A, Koul AK. 1983. Studies on nucleolus and nuclear chromosomes in angiosperms VII. Nature of nucleolar chromosome polymorphism in *Allium cepa var. viviparum* (Metzg) Alef. Cytologia 48: 323-332. DOI: 10.1508/cytologia.48.323.

Levan A, Fredya K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosome. Hereditas 52: 201-220. DOI: 10.1111/j.1601-5223.1964.tb01953.x.

Levan A. 1931. Cytological studies in *Allium*. A preliminary note. Hereditas 15: 347-356. DOI: 10.1111/j.1601-5223.1931.tb02557.x.

Levan A. 1935. Cytological studies in *Allium* VI. The chromosome morphology of some diploid species of *Allium*. Hereditas 20: 289-330. DOI: 10.1111/j.1601-5223.1935.tb0192.x.

Manzum AA, Sultana SS, Warasy AA, Begum R, Alam SS. 2021. Molecular cytogenetic characterization and interpretation of their karyotypes. *Allium* species from North Eastern India. Molecular Cytogenetics 14 (2): 243-264. DOI: 10.3897/MolCytogenetics.v14i2.11680.

Mehrjerdi, M, Norouzim, Rezaei J. 2018. Karyomorphology of *Allium cepa* L. Caryologia 27: 720-725.

Mensinkai SW. 1939. Cytogetic studies in the genus *Allium*. J Genet 39: 1-45. DOI: 10.1007/BF02982816.

Mukherjee A. Roy SC. 2012. Karyotype analysis of five species of *Allium*. Indian J Fund Appl Life Sci 2: 374-383.

Novak FJ. 1974. The changes of karyotype in callus cultures of *Allium sativum* L. Caryologia 27: 45-54. DOI: 10.1080/00087114.1974.10796560.

Pandita TK. 1979. Cytological investigations of some monocots of Kashmir. [Dissertation], Chandigarh University, Chandigarh State.

Pholthiamoorn R, Saensouk S, Saensouk P. 2018. Ethnobotany of Phu Thai ethnic group in Nakhon Phanom Province, Thailand. Walailak J Sci Technol 15 (10): 679-699. DOI: 10.48048/wjst.2018.3737.

Puizina J, Papea D. 1996. Cytogenetical evidence for hybrid structure and origin of diploid and triploid shallots (*Allium cepa var. viviparum*, Liliaceae) from Dalmatia (Croatia). Pl Syst Evol 199: 203-215. DOI: 10.1007/BF00984905.

Puizina J, Solic ME, Papes D. 1995. Mediterranean chromosome number reports 5 (524-527). Fl Mediit 5: 337-340.

Roy SC. 1978. Polyploidy in *Gnema* banding pattern in *Allium sativum*. Cytologia 43: 97-100. DOI: 10.1508/cytologia.43.97.

Saensouk P, Saensouk S. 2021a. Diversity and cytological studies on the genus *Amomum* Roxb. former *Elettarioïpsis* Baker (Zingiberaceae) in Thailand. Biodiversitas 22 (6): 3209-3218. DOI: 10.15417/biodiv/d220624.

Saensouk S, Saensouk P. 2021b. Comparative chromosomal features for four Apocynaceae species from Northeastern Thailand. Biologia (2021). DOI: 10.1007/s11756-021-00783-0.

Seo BB, Kim JH. 1975. Karyotypic analyses based on heterochromatin distribution in *Allium fistulosum* and *Allium ascalonicum*. Korean J Bot 18: 92-100.

Sharma AK, Bal AK. 1959. A study of spontaneous fragmentation in two varieties of *Allium sativum* and interpretation of their karyotypes. In Proc. 46th Indian Science Congress Association, New Delhi.

She CW, Jiang XH, Ou LJ, Liu J, Long KL, Zhang LH, Duas WT, Zhao W, Hu JC. 2015b. Molecular cytogenetic characterisation and phylogenetic analysis of the seven cultivated *Vigna* species (Fabaceae). Plant Biol 17: 268-280. DOI: 10.1111/plb.12174.

She CW, Jiang XH. 2015a. Karyotype analysis of *Lablab purpureus* (L.) Sweet using fluorochrome banding and fluorescence in situ hybridization with rDNA probes. Czech J Genet Plant Breed 51 (3): 110-116. DOI: 10.17221/32/2015-CJGPB.

She CW, Mao Y, Jiang XH, He CP. 2020. Comparative molecular cytogenetic characterization of five wild *Vigna* species (Fabaceae). Comp Cytogenet 14 (2): 243-264. DOI: 10.3897/CompCytogen.v14i2.11545.

She CW, Wei L, Jiang XH. 2017. Molecular cytogenetic characterization and comparison of the two cultivated *Canavalia* species (Fabaceae). Comp Cytogenet 11 (4): 579-600. DOI: 10.3897/compcytogen.v11i4.13604.

Stebbins GL. 1971. Chromosomal Evolution in Higher Plants. Addison Wesley Publishing Co., CA.

Talukder K, Sen S. 2000. Chromosome characteristics in some *Allium* spp. and assessment of their interrelationship. Nucleus (Calcutta) 43: 46-57.

Verma SC, Mittal RK. 1978. Chromosome variation in the common garlic, *Allium sativum* L. Cytologia 43: 383-396. DOI: 10.1508/cytologia.43.383.

Vijayavalli B, Mathew PM. 1990. Cytotax. Liliaceae Allied Fam. India: Continental Publishers, Kerala.

Vosa CG. 1976. Heterochromatic patterns in *Allium* 1. The relationship between the species of the *Cepa* group and its allies. Heredity 36: 383-392. DOI: 10.1038/hdy.1976.45.

Vosa CG. 1977. Heterochromatic patterns and species relationship. Nucleus 20: 33-41.

Wajahatullah MK, Vahidy A. 1990. Karyotyping and localization of nuclear organizer regions in garlic *Allium sativum* L. Cytologia 53: 501-504. DOI: 10.1508/cytologia.53.501.