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Comparative Karyotype Analysis and Chromosome Evolution in the Genus *Ocimum* L. from Thailand

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ABSTRACT.– *Ocimum* L. (Lamiaceae) is one of the best-known genera of aromatic herbs in the world for its economic and medicinal importance. Most species are polyploids, harboring a large variation in genome and chromosome compositions. This study reports the comparative karyotype analysis among *O. americanum* L. and four accessions each of *O. basilicum* L., *O. africanum* Lour., *O. gratissimum* L. and *O. tenuiflorum* L. from Thailand. We found chromosome numbers of *O. americanum*, *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. tenuiflorum* to be 2*n*=26, 52, 78, 40 and 36, respectively. All these species have asymmetric karyotypes, but *O. tenuiflorum* exhibited higher level of asymmetry than the other species. Although the chromosome numbers of all the studied species were stable among accessions, differences were found in the karyotypic constitutions. Intra-specific variation in karyotype formulae and satellite chromosomes of *O. basilicum* and *O. africanum* suggest that chromosomal rearrangements are involved in the chromosome evolution of these karyotypically unstable species, indicating that they may be newly formed polyploids. On the other hand, the disproportionate rise between ploidy level and the total karyotype length in the derived and stable polyploids, *O. gratissimum* and *O. tenuiflorum*, points towards a process of diploidization via genome downsizing.

KEY WORDS: basil, chromosomal rearrangement, diploidization, genome downsizing, karyotype asymmetry, polyploidy

INTRODUCTION

The basil genus *Ocimum* L. (Lamiaceae) comprises 65 species (Paton et al., 1999) and has a worldwide distribution. Most species might have originated in tropical Africa and were later introduced to tropical Asia and America (Paton et al., 2004). In Thailand, *Ocimum* is represented by five species, namely *O. americanum* L., *O. basilicum* L., *O. africanum* Lour., *O. gratissimum* L. and *O. tenuiflorum* L. (Smitinand, 2014; Suddee et al., 2005). *Ocimum* has been extensively cultivated and used for culinary purposes and as a source of essential oil. Furthermore, *Ocimum* species are important in the indigenous systems of medicine because of their chemical composition, which not only have antimicrobial properties but also antioxidant activities (Avetisyan et al., 2017; Berić et al., 2008; Hussain et al., 2008; Lawrence, 1992; Suddee et al., 2005).

Due to its economic and medicinal importance, the genus *Ocimum* has received much research attention in recent years, especially in research aiming to resolve taxonomic discrepancies within the genus and to clarify phylogenetic relationships for breeding and cultivation purposes. A

number of molecular genetic markers, including genome-wide markers, such as AFLP, EST-SSR, RAPD and ISSR, and chloroplast DNA sequences, have indicated high level of intra- and inter-specific genetic variation, supporting the observed phenotypic diversity, morphology and chemical compositions among *Ocimum* accessions (Carović-Stanko et al., 2010; Christina and Annamalai, 2014; Khosla, 1995; Kumar et al., 2016; Paton and Putievsky, 1996; Paton et al., 2004; Vieira and Simon, 2000). This diversity is likely derived through hybridization, allopolyploidy and backcross breeding (Pyne et al., 2018).

Cytogenetic studies of *Ocimum* have revealed variation in chromosome numbers, both in terms of intra- and inter-specific variation. Such information can be related to polyploidization and aneuploidization in the genus. The chromosome numbers 2*n*=24, 26 and 64 were observed in *O. americanum* and the chromosome numbers 2*n*=64 and 72 were recorded for *O. africanum*, while *O. basilicum* exhibited the most variable numbers of chromosomes, ranging from 2*n*=48 to 50, 52, 53, 56, 60, 72 and 74. Moreover, individuals with 2*n*=40 and 48 for *O. gratissimum*, and 2*n*=32, 34, 36 and 76 have been reported for *O. tenuiflorum* (Bir and Saggoo, 1985; Carović-Stanko et al., 2010; Dash et al., 2017; Dhasmana, 2013; Edet and Aikpokpodion, 2014; Idowu and Oziegbe, 2017; Khosla, 1988, 1989; Khosla, 1995; Mehra and Gill, 1972; Morton, 1962; Mukherjee and Datta, 2006; Mukherjee et al., 2005; Paton and Putievsky, 1996; Pushpangadan and Sobti, 1982; Ryding, 1994; Thoppil and Jose, 1994; Vij and Kashyap, 1976). Our previous report on chromosome numbers of Thai *Ocimum* and its meiotic chromosome behavior (Lekhapan et al., 2019) revealed a polyploid series based on *x*=13, which involved diploid *O. americanum* (2*n*=26), tetraploid *O. basilicum* (2*n*=52) and hexaploid *O. africanum* (2*n*=78), whereas *O. gratissimum* and *O. tenuiflorum* were proposed to be tetraploids with base numbers *x*=10 (2*n*=40) and 9 (2*n*=36), respectively. This previous work also reported for the first-time interspecific variation in chiasma frequencies in the genus *Ocimum* from Thailand. Such variation in chiasma frequencies possibly reflects a reduction in meiotic recombination mediated by chromosomal rearrangements, which is part of the process transforming the polyploid genome to diploid-like one or diploidization (Lysák and Schubert, 2013). Moreover, the diploidization appear to be species-specific, and may even be variable among populations in response to environmental condition (Alix et al., 2017; Soltis et al., 2016). Therefore, to examine whether chromosomal rearrangements exist in the genome of *Ocimum* species and have independently occurred in specialized environments, we conducted comparative analysis of karyotypes of Thai *Ocimum* species from diverse geographical locations with different climatic and ecological characteristics.

MATERIALS AND METHODS

Live plant materials of *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. tenuiflorum* were collected from four locations in Thailand, including Chiang Mai (Northern), Nakhon Ratchasima (Eastern), Phra Nakhon Si Ayutthaya (Central) and Trang (Peninsula) provinces, except for *O. americanum*, which has limited distribution and therefore sample of this species were collected from Prachuab Khiri Khan (South-western) province (Table 1

TABLE 1. Karyotypic characteristics of *Ocimum americanum*, *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. tenuiflorum*: Karyotype formula, chromosome length (CL), total karyotype length (TKL), intrachromosomal (A1) and interchromosomal (A2) asymmetry indices and Stebbins's classification of karyotype symmetry.

Species	Location	Karyotype formula	CL (μm)	TKL (μm)	A1 index	A2 index	Stebbins's type
O. americanum	PKN[1]	2n=26=18m+2sm+6a(1 sat)	0.97-2.82	49.85	0.33	0.30	2B
O. basilicum	CMI[2]	2n=52=28m+8sm+12a(1 sat)+4t	0.78-2.55	90.75	0.39	0.28	2B
	NMA[3]	2n=52=32m+6sm+12a(1 sat)+2t	0.85-2.72	87.96	0.37	0.31	2B
	AYA[4]	2n=52=32m+8sm+10a(1 sat)+2t	0.88-3.06	99.39	0.38	0.29	2B
	TRG[5]	2n=52=32m+8sm+10a(1sat)+2t	0.77-2.76	95.20	0.38	0.30	2B
O. africanum	CMI[2]	2n=78=48m(1sat)+20sm+2a+8t	0.90-3.24	153.93	0.36	0.30	2B
	NMA[3]	2n=78=46m+22sm(1sat)+6a+4t	1.03-3.40	165.75	0.35	0.26	2B
	AYA[4]	2n=78=48m+14sm(1sat)+2a+14t	1.00-3.16	147.82	0.37	0.28	2B
	TRG[5]	2n=78=48m+14sm+4a+12t	0.92-2.97	143.70	0.40	0.28	2B
O. gratissimum	CMI[2]	2n=40=24m+16t	0.63-1.92	49.92	0.46	0.28	2B
	NMA[3]	2n=40=24m+16t	0.75-1.58	45.33	0.47	0.25	2B
	AYA[4]	2n=40=24m+16t	0.63-1.63	44.25	0.45	0.28	2B
	TRG[5]	2n=40=24m+16t	0.58-1.79	46.75	0.46	0.27	2B
O. tenuiflorum	CMI[2]	2n=36=10m+4sm+22t	0.65-1.62	36.39	0.69	0.31	3B
	NMA[3]	2n=36=10m+4sm+22t	0.84-2.13	49.11	0.67	0.31	3B
	AYA[4]	2n=36=10m+4sm+22t	0.60-1.85	39.63	0.67	0.33	3B
	TRG[5]	2n=36=10m+4sm+22t	0.51-1.94	41.76	0.69	0.36	3B

[1]Prachuab Khiri Khan, [2]Chiang Mai, [3]Nokhon Ratchasima, [4]Phra Nakhon Si Ayutthaya, [5]Trang

and Fig. 1). All plant materials were grown in pots at Chulalongkorn University, Bangkok, Thailand. Voucher specimens were deposited at Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Sciences, Chulalongkorn University (BCU).

From each species and each location, one individual plant was used in this chromosome study. Chromosomes were prepared from actively growing root tips pretreated with saturated *p*-dichlorobenzene solution for 1–3 hours before being fixed in Carnoy's solution (1 part of glacial acetic acid: 3 parts of 95% ethanol, v/v) for a minimum of one hour. The root tips were then hydrolyzed in 1 N HCl for 15 minutes, after which they were stained and squashed in 1% aceto-orcein for 15 minutes. The chromosome slides were examined using an Olympus BX51 microscope and the images were captured under x1000 magnification with an Olympus DP71 camera.

Chromosome parameters, chromosome length (CL) and total karyotype length (TKL), were determined and karyotype formulae were established based on measurements of three to five well-spread metaphase cells. Karyotypes were arranged in decreasing lengths according to the metaphase chromosome sizes. Chromosome classification followed that of Levan et al. (1964): m = metacentric chromosome with arm ratio of 1.0–1.7; sm = submetacentric

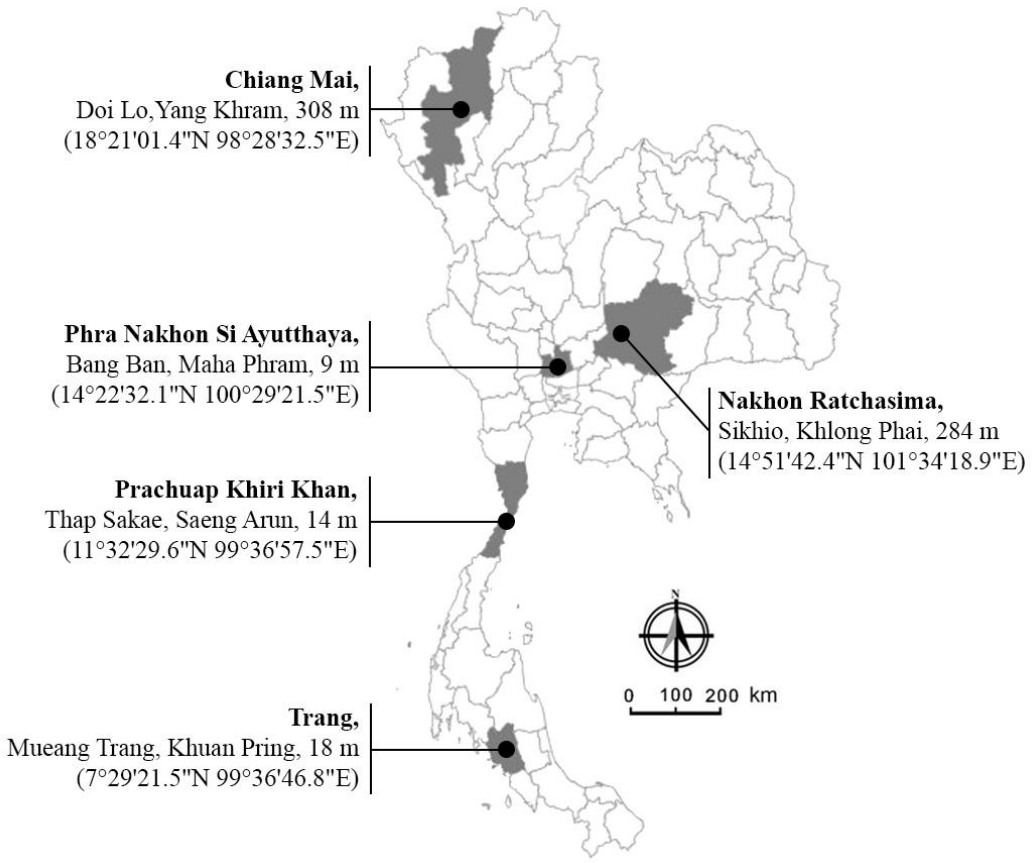

FIGURE 1. Map locations and geographic coordinates of the studied accessions of five *Ocimum* species from Thailand.

chromosome with arm ratio of 1.7–3.0; a = acrocentric chromosome with arm ratio of 3.0–7.0; and t = telocentric chromosome with arm ratio higher than 7.0. Satellite chromosomes were abbreviated in the present study as 'sat' and characterized by the position of the secondary constriction, which is also known as the nucleolar organizer region (NOR), the active site of ribosomal RNA gene transcription into a nucleolus. NORs have become part of the karyotype as their position on chromosomes is conserved to the point that it can reflect phylogenetic relationship among closely related species (Pederson, 2011). Satellite chromosomes were classified into three types according to Battaglia (1955): microsatellite = a spheroidal satellite of small size, *i.e.* having a diameter equal or less than one half the chromosomal diameter; macrosatellite = a spheroidal satellite of large size, *i.e.* having diameter greater than one half the chromosomal diameter; linear satellite = a satellite having the shape of a long chromosomal segment. Karyotype asymmetry were classified according to Stebbins (1971), while the intrachromosomal asymmetry index (A1)

and interchromosomal asymmetry index (A2) were calculated following Zarco (1986). A scatter diagram was constructed to evaluate the relationship between asymmetry indices A1 and A2.

RESULTS

Chromosome numbers, karyotype formulae and karyotypic characters were obtained from *O. americanum* and the four accessions each of the other four *Ocimum* species from Thailand (Table 1). Somatic metaphase chromosomes are illustrated (Fig. 2) to show chromosome morphology and relative size. The chromosome numbers were found to be variable among different *Ocimum* species, but not within species. Each species, represented by different locations, showed stable somatic (2*n*) chromosome number. The chromosome numbers of *O. americanum*, *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. tenuiflorum* were 2*n*=26, 52, 78, 40 and 36, respectively. Despite the constant chromosome number within species, intra-specific variation in karyotype formulae was discovered in *O. basilicum* and *O. africanum*. Other karyotype characteristics, *i.e.* chromosome length, total karyotype length, A1 and A2 indices and Stebbins's degrees of asymmetry appeared to be variable among *Ocimum* species and accessions.

All chromosome counts from *O. basilicum* produced 2*n*=52, but karyotype formulae and other characters varied among locations. The karyotype formulae of *O. basilicum* were 2*n*=28m+8sm+12a(1 sat)+4t for Chiang Mai and 2*n*=32m+6sm+12a(1 sat)+2t for Nakhon Ratchasima. Although these two accessions had different karyotype formulae, they showed the same microsatellite location, on the short arm of chromosome 16. On the other hand, the two other *O. basilicum* accessions, from Phra Nakhon Si Ayutthaya and Trang, shared the same karyotype formula that consisted of 2*n*=32m+8sm+10a(1 sat)+2t, however, their satellite sizes and locations were different. A linear satellite was found on the long arm of chromosome 16 in the accession from Phra Nakhon Si Ayutthaya, while the microsatellite was found on the short arm of the same chromosome pair in the accession from Trang. A measurable difference in the length of chromosomes and the total length of karyotype was found among all four accessions of *O. basilicum*. The chromosome length varied from 0.77 to 0.88 μm for the smallest chromosome pair and from 2.55 to 3.06 μm for the largest pair, whereas the total karyotype length was from 87.96 to 99.39 μm (Table 1, Fig. 2B-E, Fig. 3B-E).

Taken together, chromosome counts of *O. africanum* obtained from different locations resulted in a stable number 2*n*=78. Karyotype of the accession from Chiang Mai was formulated as 2*n*=48m(1 sat) +20sm+2a+8t, in which the macrosatellite was localized on the long arm of chromosome 25, whereas the chromosome complement of Nakhon Ratchasima accession was 2*n*=46m+22sm(1 sat)+6a+4t, in which the macrosatellite was found on the short arm of chromosome 25. The other two accessions of *O. africanum*, from Phra Nakhon Si Ayutthaya and Trang, had karyotype formulae of 2*n*=48m+14sm(1 sat)+2a+14t and 2*n*=48m+14sm+4a+12t, respectively. The single macrosatellite on the long arm of chromosome 25 was found only in the accession from Phra Nakhon Si Ayutthaya. The chromosomes of all studied accessions of *O. africanum* were on average longer than those of *O. basilicum* and other species examined. They ranged from 0.90 to 1.03 μm in chromosome length for the

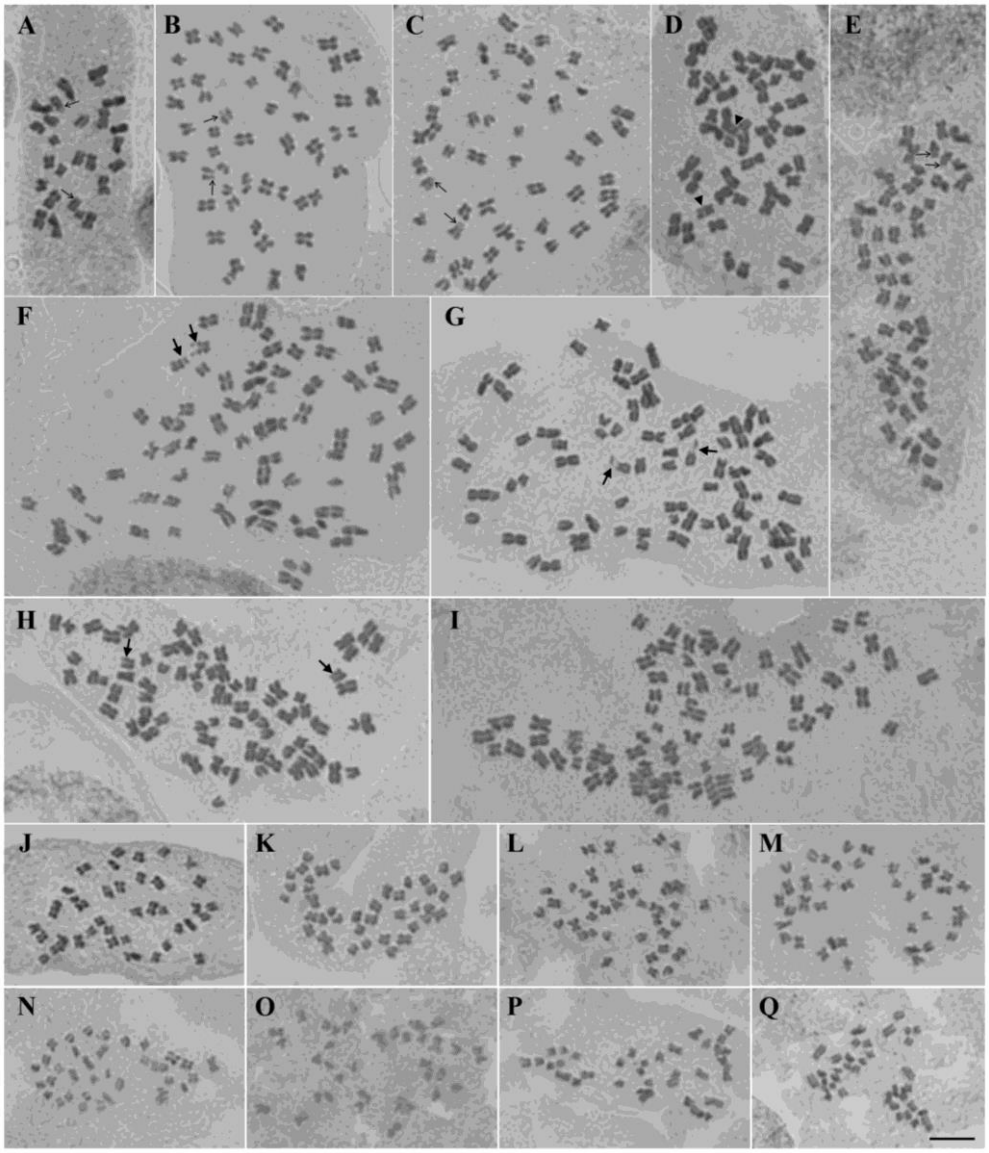

FIGURE 2. Somatic metaphase chromosomes of 17 *Ocimum* accessions. A: *O. americanum* from Prachuab Khiri Khan; B-E: *O. basilicum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang; F-I: *O. africanum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang; J-M: *O. gratissimum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang; N-Q: *O. tenuiflorum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang. The thin arrows, the thick arrows and the arrowheads point to secondary constriction of microsatellite, macrosatellite and linear satellite chromosomes, respectively. Scale bar = 5 μm

smallest pair and from 2.97 to 3.40 μm for the largest pair, and the total karyotype length was 143.70–165.75 μm (Table 1, Fig. 2F-I, Fig. 3F-I).

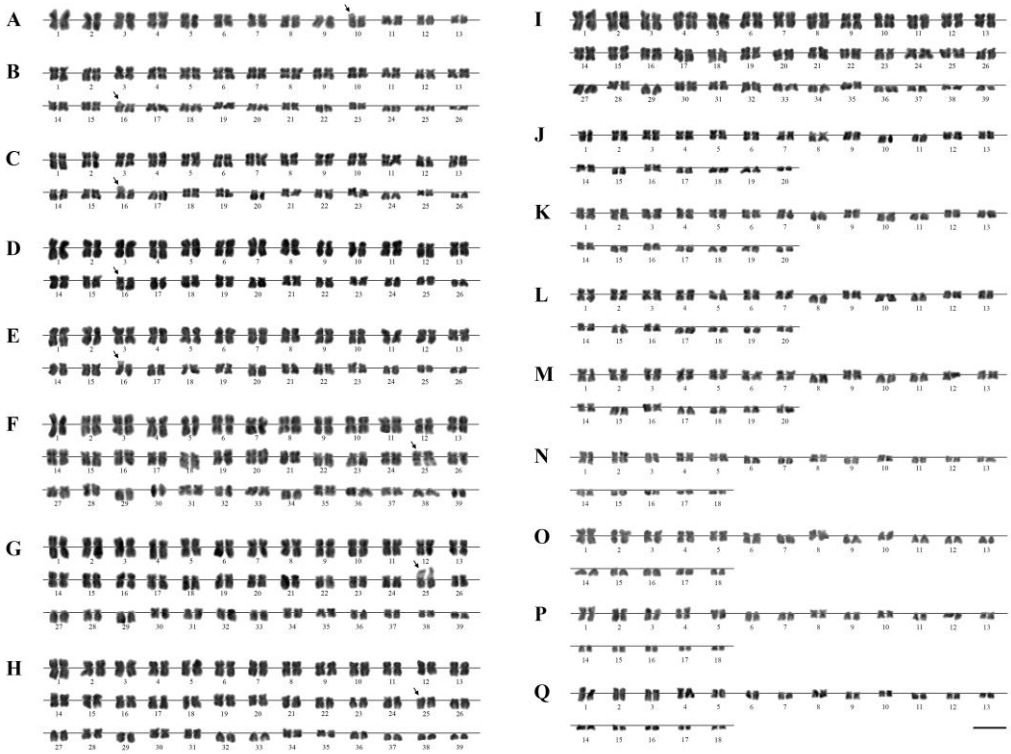

FIGURE 3. Karyotypes of 17 *Ocimum* accessions. A: *O. americanum* from Prachuab Khiri Khan; B-E: *O. basilicum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang; F-I: *O. africanum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang; J-M: *O. gratissimum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang; N-Q: *O. tenuiflorum* from Chiang Mai, Nakhon Ratchasima, Phra Nakhon Si Ayutthaya and Trang. The arrows point to satellite chromosomes. Scale bar = 5 μm

On the other hand, the four accessions of *O. gratissimum* demonstrated a uniform karyotype formula of 2*n*=40=24m+16t. No satellite could be detected in this species. The chromosome length ranged from 0.58 to 0.75 μm for the smallest pair and from 1.58 to 1.92 μm for the largest pair, whereas the total karyotype length was 44.25–49.92 μm (Table 1, Fig. 2J-M, Fig. 3 J-M). Likewise, all examined accessions of *O. tenuiflorum* showed uniform karyotype of 2*n*=36=10m+ 4sm+22t, without a satellite. The chromosome length ranged from 0.51 to 0.84 μm for the smallest pair and from 1.62 to 2.13 μm for the largest pair, and the total karyotype length was 36.39–49.11 μm (Table 1, Fig. 2N-Q, Fig. 3N-Q).

The species *O. americanum*, which was represented by a single location, showed no karyotypic variation. Its karyotype formula was 2*n*=26=18m+2sm+6a(1 sat), in which the microsatellite was located on the short arm of chromosome 10, and individual chromosomes ranged in length between 0.97 and 2.82 μm, with the total karyotype length of 49.85 μm. (Table 1, Fig. 2A, Fig. 3A).

Overall, karyotypes of all five *Ocimum* species in this study were generally asymmetrical. The ratios of m/sm to a/t chromosomes, calculated from karyotype formulae, were 3.3:1, 2.3-3.3:1, 3.9-6.8:1 and 1.5:1 for *O. americanum*, *O. basilicum*, *O. africanum* and *O. gratissimum*, respectively. In contrast, the chromosome compositions of *O. tenuiflorum* exhibited a decreased proportion of m/sm to a/t chromosomes, with the ratio of 0.6:1, reflecting higher karyotype asymmetry.

The asymmetry indices A1 and A2 according to Zarco (1986) are also presented in Table 1. The results showed that *O. tenuiflorum* accession from Trang had the highest A1 and A2 values, representing the largest difference in the length of chromosome arms and the highest variation in length among chromosomes, respectively. In contrast, karyotypes of *O. americanum* and *O. gratissimum* accessions from Nakhon Ratchasima had the lowest values of A1 and A2, respectively, and thus being the least asymmetrical. The scatter diagram of A1 and A2 asymmetry indices (Fig.4) separated the *Ocimum* species into three groups. The first group with low values of A1 and low to median values of A2 included accessions of *O. americanum*, *O. basilicum* and *O. africanum*. The other two groups were species-specific. The group of *O. gratissimum* accessions had median values of A1 and low values of A2, in the opposite direction compared to the first group, whereas the group of *O. tenuiflorum* accessions had high values of both A1 and A2. This species, *O. tenuiflorum*, exhibited the Stebbins's 3B type of karyotype asymmetry, while all other species had 2B asymmetrical karyotypes (Table 1).

DISCUSSION

Karyotypic variation and chromosomal rearrangements

The present study provides full description of karyotypes of five Thai *Ocimum* species. The chromosome numbers 2*n*=26, 52, 78, 40 and 36 belong to *O. americanum*, *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. tenuiflorum*, respectively. These results are in complete agreement with our previous study of meiotic chromosome numbers in the genus *Ocimum* from Thailand (Lekhapan et al., 2019). Our results also match other reports to a certain extent (Bir and Saggoo, 1985; Carović-Stanko et al., 2010; Dash et al., 2017; Idowu and Oziegbe, 2017; Khosla, 1988, 1989; Khosla, 1995; Morton, 1962; Mukherjee and Datta, 2006; Mukherjee et al., 2005; Paton and Putievsky, 1996; Pushpangadan and Sobti, 1982; Ryding, 1994; Thoppil and Jose, 1994; Vij and Kashyap, 1976). Although intraspecific variation in chromosome number was not detected in the present study, the karyotype formulae of the most known species in cultivation, *O. basilicum* and *O. africanum*, are polymorphic across geographically diverse populations. Our karyotype formulae are also different from those described in other reports (Dash et al., 2017; Dhasmana, 2013; Edet and Aikpokpodion, 2014; Idowu and Oziegbe, 2017). The incongruence in karyotype formulae could probably be attributed to chromosomal rearrangements, such as deletion, duplication, inversion and translocation, in the polyploid genomes of *O. basilicum* and *O. africanum*.

The present study found variation in the size and position of satellites (sat) among species and accessions. Three types of satellite chromosomes were identified among the accessions of *O. americanum*, *O.*

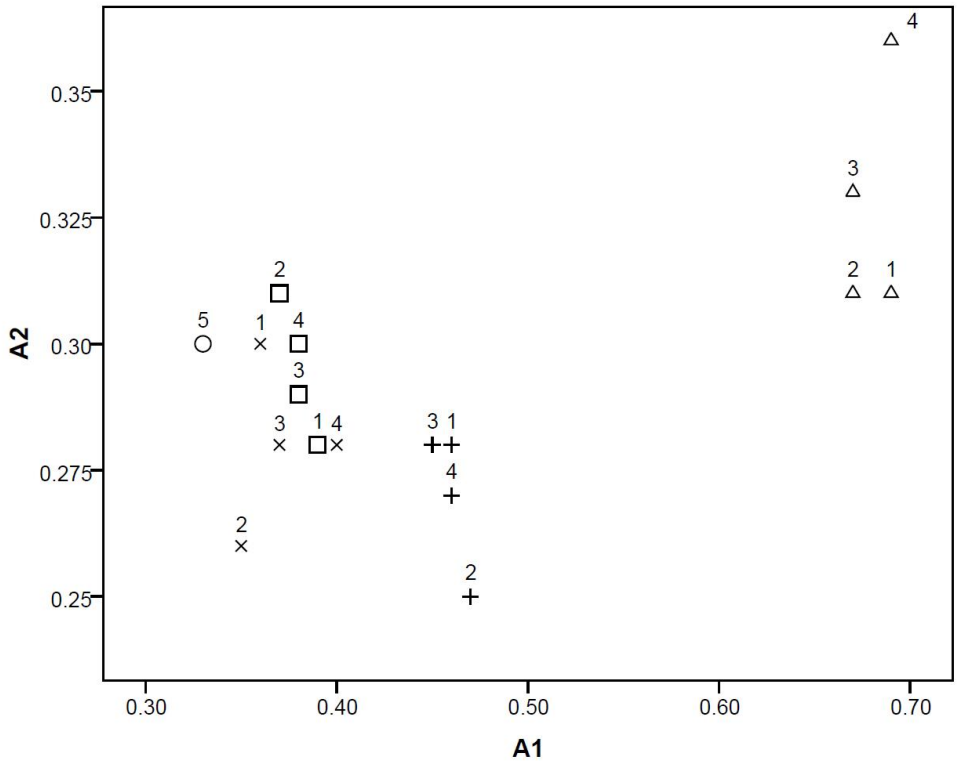

FIGURE 4. Scatter diagram representing the intrachromosomal (A1) and interchromosomal (A2) asymmetry indices. *Ocimum* species are indicated by symbols: ○ = *O. americanum*, □ = *O. basilicum*, × = *O. africanum*, ＋ = *O. gratissimum* and △ = *O. tenuiflorum*. *Ocimum* accessions were indicated by numbers: 1 = Chiang Mai, 2 = Nakhon Rachasima, 3 = Phra Nakhon Si Ayutthaya, 4 = Trang and 5 = Prachuab Khiri Khan

basilicum and *O. africanum*. This implies some kinds of chromosomal rearrangements. For instance, the difference in the position and the size of satellites on chromosome 16 among the four locations of *O. basilicum*, i.e. a "microsatellite" on the short arm of chromosome 16 in the accessions from Chiang Mai, Nakhon Ratchasima and Trang, but a "linear satellite" on the long arm of the same chromosome in the accession from Phra Nakhon Si Ayutthaya, could be explained by 'inversion' or a re-positioning of the centromere. In Arabideae, a large and diverse clade within the Brassicaceae, a re-positioning of centromeres was often found to accompany rapid sequence evolution while the genes on the chromosomes remain collinear (Mandáková et al., 2020). Such centromere position shifts are likely to facilitate the post-polyploid diploidization process in plant species (Bomblies et al., 2016). Similarly in *O. africanum*, a "macro-satellite" was observed on the long arm of chromosome 25 in the accessions from Chiang Mai and Phra Nakhon Si Ayutthaya, while it was observed on the short arm of the same chromosome in the accession from Nakhon Ratchasima. This, once again, implies pericentric inversion or centromere shift. This macrosattelite seems to have disappeared from the accession from Trang, implying 'deletion' of a chromosomal

region. In addition, the larger macrosatellite in *O. africanum*, compared with the smaller microsatellite in *O. americanum* and *O. basilicum*, could indicate 'insertion' or "amplification" of the ribosomal repeats, or 'deletion' in the latter. Changes in the size, position and number of the ribosomal genes per genome are thought to be part of the diploidization process returning polyploids to a diploid-like state over time (Garcia et al., 2017). Karyotypic variation among populations has been reported in various plant taxa, such as *Crotalaria incana* (Fabaceae), *Fragaria* species (Rosaceae), *Lolium perenne* (Poaceae), and it is often suggested to be the result of chromosomal rearrangements (Nathewet et al., 2010; Özer et al., 2018; Tapia et al., 2018). Many empirical studies have found large-scale chromosomal rearrangements, e.g. inversion, linked to adaptive phenotypes and reproductive isolation (Hoffmann and Rieseberg, 2008; Lowry and Willis, 2010). This may also apply to the present study. The karyotypic variation found among geographically different locations of *O. basilicum* and *O. africanum* may represent independent rearrangements of chromosomes in response to environmental factors prevalence at different localities. Chromosomal rearrangements are likely to be a key role in the evolution of stable polyploidy, for example they create physical differentiation between homoeologous chromosomes and resulting in a diploid-like behavior at meiosis coupled with a reduction in recombination and chiasma frequency (Leitch and Bennett, 2004). The variation in chiasma frequencies among different populations of *O. basilicum* and *O. africanum*, reported in our previous study (Lekhapan et al., 2019), may be controlled in the same way by chromosomal rearrangements.

Evidence of genome downsizing

Generally, chromosome sizes of *O. americanum*, *O. basilicum* and *O. africanum* were comparable — their chromosomes were in the same size range (0.77–3.40 μm). On the other hand, the chromosomes of *O. gratissimum* and *O. tenuiflorum* were evidently smaller than those three species. The length of their individual chromosomes was in the range of 0.58–1.92 μm and 0.51–2.13 μm, respectively. In addition, the total karyotype length of *O. gratissimum* (44.25–49.92 μm) and *O. tenuiflorum* (36.39–49.11 μm) were close to that of the diploid *O. americanum* (49.85 μm), even though they were proposed to be tetraploids according to their chromosome numbers. In line with the flow cytometry study of the genus *Ocimum* worldwide (Rewers and Jedrzejczyk, 2016), nuclear DNA contents of all *O. gratissimum* accessions were found to be similar to *O. americanum* var. *americanum*, while *O. tenuiflorum* accession from India had lower nuclear DNA content than those *O. americanum*. This disproportionate rise of genome size up the ploidy level and the total karyotype length in *O. gratissimum* and *O. tenuiflorum*, combined with its small-sized chromosomes, appears to indicate genome downsizing, which is a feature of diploidization. The mechanisms that drive genome downsizing were proposed to be chromosomal rearrangements, which frequently result in dysploid changes (Schranz et al., 2006). For example, pericentric inversion and reciprocal translocation events followed by the elimination of a small mini-chromosome can lead to chromosome number reduction and centromeric DNA loss. In addition, the loss of DNA through activation of transposons, homoeologous recombination and elimination of specific DNA sequences, such as 18S-26S and/or 5S

rDNA copies, were found to be associated with genome downsizing (Schubert and Vu, 2016). The evolutionary forces that possibly contribute to this phenomenon are natural selection to reduce the biochemical cost of synthesizing and replicating the extra DNA in newly formed polyploids, or natural selection to enhance homologous pairing by augmenting differentiation between homoeologous chromosomes, for stabilization of the newly formed polyploids (Leitch and Bennett, 2004). The chromosomal evidence for genome downsizing in *O. gratissimum* and *O. tenuiflorum* could, therefore, indicate rapid evolutionary changes leading to polyploid stability (Lekhapan et al., 2019). However, these two species are likely to have undergone independent evolutionary changes, as indicated by their contrasting karyotype formulae. For example, the ratios of metacentric and submetacentric chromosomes to telocentric chromosomes were 1.5:1 in *O. gratissimum*, but 0.6:1 in *O. tenuiflorum*. Their chromosome base numbers are also different: x=10 and x=9, respectively. Descending dysploidy, an evolutionarily fixed decrease in base chromosome number (x), is viewed as one of the mechanisms that turn polyploids into functional diploids (Mandáková and Lysak, 2018).

Karyotype asymmetry

Karyotype symmetry is one of the cytological characters used to study plant evolution. A symmetrical karyotype is defined when the chromosomes are mostly metacentric or submetacentric with invariable size. An asymmetry of karyotype can increase through the shift of centromere position from median to subterminal or terminal or through gradual changes in chromosome size, resulting in heterogeneous karyotype. There is a predominant trend in flowering plants toward increasing asymmetry of the karyotype (Stebbins, 1971). In our study, most of the *Ocimum* accessions exhibited the Stebbins's 2B type of karyotype asymmetry, except for the accessions of *O. tenuiflorum* that had the 3B type. Based on the view of Stebbins, the 2B type is more symmetrical than the latter 3B type. Such evidence further supports the derived genome (the more asymmetrical karyotype, 3B) of *O. tenuiflorum* compared with *O. americanum*, *O. basilicum*, *O. africanum* and *O. gratissimum*. The 2B type in *O. basilicum* was also suggested by Idowu and Oziegbe (2017), while in contrast, *O. americanum* had the highly symmetric karyotype 1A. Dash et al. (2017) proposed, based on karyotype formulae, that *O. basilicum*, *O. gratissimum* and the purple form of *O. tenuiflorum* are primitive in nature while the green form of *O. tenuiflorum* relatively advanced. Edet and Aikpokpodion (2014) suggested asymmetrical karyotypes for African cytotypes of *O. basilicum* cytotypes (2n=48 and 2n=60), while, based on the mean centromeric index values (TF%), Dhasmana (2013) suggested that *O. basilicum* and *O. tenuiflorum* are two extremes in the evolutionary pathway.

The asymmetry indices A1 and A2 also revealed close genetic relationship among *O. americanum*, *O. basilicum* and *O. africanum* (see the scatter diagram in Fig. 4). The diagram also revealed some degree of genetic relationship between these three related species and *O. gratissimum* and this could be because of the similarity in shape and size of chromosomes as demonstrated by the indices A1 and A2. In contrast, *O. tenuiflorum* was placed in a separate position of chromosome evolution, owing to the extreme values of its karyotype asymmetry. This assumption is supported by

the infrageneric classification of the genus *Ocimum* based on parsimony analysis of morphological characters (Paton et al., 1999). This morphological analysis placed *O. americanum*, *O. basilicum* and *O. africanum* together in section *Ocimum* of subgenus *Ocimum*, and *O. gratissimum* in section *Gratissima* of the same subgenus, but *O. tenuiflorum* in subgenus *Gymnocymum*, section *Hierocymum*. This is in full agreement with molecular phylogenetic studies, which always grouped *O. americanum*, *O. basilicum* and *O. africanum* in the same cluster, whereas *O. gratisiimum* and *O. tenuiflorum* were placed in separate positions from the rest of the species investigated (Carović-Stanko et al., 2010; Christina and Annamalai, 2014; Kumar et al., 2016).

In conclusion, we have provided comparative karyotype analysis of *O. americanum* and four accessions each of *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. tenuiflorum*. The results indicate the process of post-polyploid diploidization via chromosomal rearrangements and genome downsizing in the genus *Ocimum*. In the future, integrated analyses of genome size, molecular cytogenetic and molecular phylogenetic based on NGS (next-generation sequencing) approaches are required in order to reveal the detailed genome evolution and genetic relationships among *Ocimum* species and accessions.

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