Abrahamia
An International Journal for Plant Sciences
Abrahamia
Vol. 3 (2)
141-145
2017
ISSN: 2395-4108

Karyotype analysis in cultivars of 'Sweet basil'- *Ocimum basilicum* Linn. (Lamiaceae)

Mariamma Cherian* & Radhamany P. M.
Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India
*Corresponding author: cherian.mariamma@gmail.com

Abstract

Karyomorpholgy in three *cultivars* of *Ocimum basilicum* (Lamiaceae) is analyzed for the first time. Cytological studies in the different cultivars showed 2n= 48: cultivar-1-*O.basilicum* var. *citriodorum*, 2n= 48= 6M+14m+4sm; cultivar2- *O.basilicum* var. *pilosum*, 2n= 48= 6M+ 15m+3sm and cultivar3- *O.basilicum* var.*purpurascens*, 2n= 48= 8M+12m+3sm+1st chromosomes. The chromosomes are small in size and ranged in length from 0.86 to 3.86µm. The karyotype parameters used differentiated the cultivars under study intraspecifically. They revealed 1B and 2B karyotypes. The cultivar with 2B karyotype, three sm chromosomes and one st has more asymmetrical karyotype compared to the others. Slight variations were also seen in mean arm ratio, TF%, and A1 and A2 values.

Keywords: Cytology, karyotype, *Ocimum basilicum*

Introduction

The genus *Ocimum* (Lamiaceae), which includes sweet basil, offers a wide diversity among its more than 50 species (Darrah,1980), particularly regarding plant growth, morphology, physical appearance, essential oil content and seed oil composition (Morales *et al*., 1993). Extracts of the plant are used in traditional medicines. Members have been shown to contain biologically active constituents that are insecticidal, nematicidal or fungistatic (Deshpande and Tipnis, 1977; Chatterjee *et al*., 1982; Oxenham *et al*., 2005). *Ocimum* taxonomy is confused due to interspecific hybridization, polyploidization, and the existence of chemo types or chemical races with similar morphology. Most commercial basil cultivars available in the market belong to the species *O. basilicum* (Simon and Reiss-Bubenheim, 1987). However, a major difficulty in the use of Lamiaceae species for pharmaceutical purposes is the individual variability due to genetic and biochemical heterogeneity (Vieira and Simon, 2000).

As karyomorphology provide comparative data useful in the analysis of genetic relationships and variations within or among the species (Abou-El-Enain, 2006) the present study reports the karyotypes of 3 cultivars of *O. basilicum* (Darrah, 1974) namely *citriodorum* type (cultivar 1), dwarf type namely *pilosum* (cultivar 2) and purple type namely *purpurascens* (cultivar 3). A perusal of literature revealed that cytological studies in *O. basilicum* have mainly been limited to chromosome counts (Vaarama, 1947; Morton, 1962; Sanjappa, 1979; Cherian and Kuriachan, 1981; Bir and Saggoo, 1985; Paton and Putievsky, 1996; Murin, 1997; Idowu and Oziegbe, 2017). Detailed karyomorphological data have been reported only for a few recent cases such as Archana *et al*. (2013) and Edet and Aikpokpodion (2014) despite its economic importance and wide distribution.

Materials and methods

Sample collection

Ocimum basilicum commonly known as sweet basil is introduced into gardens as a medicinal herb. Representative samples of the three different cultivars namely cultivar 1-*O.basilicum* var. *citriodorum* (v.no.7002*)*, cultivar 2- *O.basilicum* var. *pilosum* (v.no. 7005*)*, and cultivar 3- *O.basilicum* var. *purpurascens (*v.no. 7004*)* were collected from different localities in Kerala, Tamil Nadu and Karnataka. Voucher specimens are kept in the Herbarium of Department of Botany (KUBH), University of Kerala.

Karyotype analysis

Root tips for mitotic analysis were collected between 12.30 pm to 1.00 pm. The meristems were pretreated with 0.002M 8- hydroxyquinoline over a period of 3h at 8^0C and fixed in Semen's fluid (Sharma and Sharma, 1965). Chromosomes were stained in 2% acetocarmine. Five clear preparations of chromosome complements for each individual were measured for the characterization of the karyotypes of each cultivar. The values taken for chromosome morphology were length of chromosome arms and total length of each haploid chromosome. Levan *et al*'s (1964) classification of median point (M), median region (m), submedian (sm), subterminal (st), terminal region (t) and terminal point (T) based on the arm ratio of 1.0, 1.0 to 1.7, 1.7-3.0, 3.0 -7.0, 7.0 - ∞ and ∞ respectively was used for comparison. These values were used to calculate the mean chromosome length (MCL), mean arm ratio (MAR) and total form percentage (TF %). Karyotype asymmetry was estimated based on the relation between the chromosome arms A1 (intra chromosomal asymmetry index) and length A_2 (inter chromosomal asymmetry index) using the equations of Zarco (1986) and the categories of Stebbins (1971a) as A_1 =1-[∑ (b/B/n] and A_2 = s / x, where b and B are the mean length of short and long arms of each pair of homologues respectively, n is the number of homologous chromosome pairs, s is the standard deviation and x equals to the mean chromosome length.

Results and Discussion

Karyotype analysis

Cytological studies in the three cultivars of O. basilicum showed 2n=48 chromosomes (Fig. 1-3). The chromosomes are small in size and ranged in length from 0.86 to 3.86µm. The karyomorphological data (Table 1- 4; Fig. 4 - 6) showed that the chromosome complement in the different populations has a more or less similar pattern with m (median region) chromosomes in overwhelming proportion, with few metacentric (M) and sub metacentric (sm) chromosomes. One population has a sub terminal chromosome also. Terminal chromosomes are absent in all populations. The karyotypes of the three populations are more or less symmetrical as indicated by the total form percentages (TF %) of 39.65, 41.78 and 43.83, which are resolved into 1B and 2B karyotype categories.

Investigation of the current and previous

chromosome counts (Vaarama, 1947; Morton, 1962; Sanjappa, 1979; Cherian and Kuriachan, 1981; Bir and Saggoo, 1985; Paton and Putievsky, 1996; Murin, 1997; Archana *et al*., 2013) of *O.basilicum* revealed the same chromosome number (2n=48), though Edet and Aikpokpodion (2014) and Idowu and Oziegbe (2017) reported 2n = 60 and 2n= 52 respectively for the species. These results showed that the number 2n=2x=48 is conservative in the species. Thus it appears that the chromosome evolution occurred in a conservative manner in the species. The chromosomes in general are small sized (Table 4) as indicated by the mean chromosome length (MCL). The highest MCL value is recorded in cultivar 3 (1.94µm) and the lowest in cultivar 1 (1.32µm). All the three studied cultivars have karyotypes comprising mostly of metacentrics as indicated by the mean arm ratio (MAR). The highest value (MAR =1. 51) is found in cultivar 3 whereas the lowest value 1.34 is found in cultivar 1. Though the different samples under the present study shared the same basic chromosome number of x=24, a predominance of metacentric (m) chromosomes, and relatively symmetrical karyotypes (1B and 2B) the karyomorphology shows significant differences in the finer details. Cultivar1 without any st chromosome, lowest mean arm ratio (1.34 ±0.03) and intra chromosomal asymmetry index (0.22) and the highest TF %(43.83 ±0.02) has the most symmetric karyotype and is the most primitive member. Cultivar 3 with three sub median (sm) chromosomes and a sub terminal (st) chromosome and the highest mean arm ratio (1.51 ±0.02) and intra chromosomal asymmetry index (0.25) has the most asymmetrical karyotype and represent the most progressive evolution level. According to Stebbins (1971b) the change of centromere from median to sub terminal or terminal position and increasing intra karyotypic size differences of chromosome are basic factors, which bring about evolution of chromosome morphology. He also proposed that increasing asymmetry without any change in the number

of centromeres or of independent chromosomes results from pericentric inversions and unequal translocations of chromosome arms.

The differences in chromosome morphology between the three populations indicate genetic diversity in general. In order to elucidate the infra phylogeny and evolution of the species in its broad sense genetic variability studies involving Random polymorphic DNA analysis has also been carried out. The data yielded positive results, which revealed a mean genetic variability of 0.13 among the cultivars based on Nei and Li's similarity coefficient in accordance with the karyotypic data (Cherian and Radhamany, 2018 in press). On the basis of our data and data from previous literature in the species it appears that chromosome evolution in the different cultivars of *O. basilicum* must have occurred by gene rearrangements than by chromosome alterations. The different cultivars under the present investigation show seed set, though they are propagated naturally through vegetative means. Hence the chromosomal structural variability observed in the species might be an adaptation gained during evolution to acclimatize at the various geographical conditions. Such species are very successful in evolution, as they have retained the capacity of generating variability through sexual reproduction (Sharma and Sen, 2002). The chromosomal variability observed during the present study as correlated with the diversity in basil based on appearance, flavours, fragrances, industrial, edible, and drying oils and natural pigments (Phippen and Simon, 1998) may help in the analysis of genetic relationships and variations within the species.

Acknowledgements

The first author is thankful to Department of Science and Technology, Govt. of India and University Grants Commission, New Delhi for the award of Women Scientist Fellowship for carrying out this study. We thank Dr. S. Suhara Beevy, Head, and Dr. G. M Nair, Former Head, Department of Botany, University of Kerala for providing facility and rendering help to carry out this work.

Table 1
Karyotype details of *O. basilicum* cultivar 1

no. of chromo-some	LA	SA	TCL	arm ratio LA/SA	centromere position	RCL	F%	TF%	karyo type sym metry	karyotype formula
1	1.21	0.86	2.07	1.41	sm	6.53	41.55			
2	1.03	0.86	1.89	1.20	m	5.96	45.50			
3	0.86	0.86	1.72	1.00	M	5.43	50.00			
4	0.86	0.86	1.72	1.00	M	5.43	50.00			
5	0.94	0.68	1.62	1.38	m	5.11	41.98			
6	0.86	0.68	1.54	1.26	m	4.86	44.16			
7	0.86	0.68	1.54	1.26	m	4.86	44.16	43.83	1B	6M+14m +4sm
8	0.86	0.60	1.46	1.26	m	4.61	41.10			
9	0.68	0.68	1.36	1.00	M	4.30	50.00			
10	0.68	0.68	1.36	1.00	m	4.30	50.00			
11	0.68	0.68	1.36	1.00	M	4.30	50.00			
12	0.68	0.52	1.20	1.31	m	3.79	43.33			
13	0.68	0.52	1.20	1.31	m	3.79	43.33			
14	0.68	0.52	1.20	1.31	m	3.79	43.33			
15	0.60	0.52	1.12	1.15	m	3.53	46.43			
16	0.68	0.43	1.11	1.58	m	3.50	38.74			
17	0.68	0.43	1.11	1.58	m	3.50	38.74			
18	0.68	0.43	1.11	1.58	m	3.50	38.74			
19	0.52	0.52	1.04	1.00	M	3.28	50.00			
20	0.52	0.52	1.04	1.00	M	3.28	50.00			
21	0.68	0.34	1.02	2.00	sm	3.22	33.33			
22	0.68	0.34	1.02	2.00	sm	3.22	33.33			
23	0.68	0.34	1.02	2.00	sm	3.22	33.33			
24	0.52	0.34	0.86	1.53	m	2.71	39.53			
Total	17.8	13.89	31.69	32.12						
Mean			1.32	1.34						

LA=Long arm; SA=Short arm; TCL=Total chromosome length; F%=;TF%=Total form percentage

Table 2
Karyotype details of *O. basilicum* cultivar 2

No.of chromo-some	LA	SA	TCL	Arm ratio LA/SA	Cen-tromere position	RCL	F%	TF%	Karyo ty pe sym metry	Karyotype formula
1	2.30	1.15	3.45	2.00	sm	8.09	33.33			
2	1.61	1.15	2.76	1.40	m	6.48	41.67			
3	1.26	1.15	2.41	1.10	m	5.65	47.72			
4	1.38	0.92	2.30	1.50	m	5.40	40.00			
5	1.15	0.92	2.07	1.25	m	4.86	44.44			
6	1.15	0.92	2.07	1.25	m	4.86	44.44			
7	1.15	0.69	1.84	1.67	m	4.32	37.5			
8	1.15	0.69	1.84	1.67	m	4.32	37.5			6M+15m
9	1.15	0.69	1.84	1.67	m	4.32	37.5			+3sm
10	0.92	0.92	1.84	1.00	M	4.32	50.00	39.65	2B	
11	0.92	0.92	1.84	1.00	m	4.32	50.00			
12	0.92	0.92	1.84	1.00	M	4.32	50.00			
13	1.15.	0.46	1.61	2.50	sm	3.78	28.57			
14	0.92	0.69	1.61	1.34	m	3.78	42.85			
15	0.92	0.69	1.61	1.33	m	3.78	42.85			
16	0.92	0.69	1.61	1.33	m	3.78	42.85			
17	0.92	0.46	1.38	2.00	sm	3.24	33.33			
18	0.69	0.69	1.38	1.00	M	3.24	50.00			
19	0.69	0.69	1.38	1.00	M	3.24	50.00			
20	0.69	0.69	1.38	1.00	M	3.24	50.00			
21	0.77	0.57	1.34	1.35	m	3.14	43.53			
22	0.69	0.46	1.15	1.50	m	26.98	40.00			
23	0.69	0.46	1.15	1.50	m	26.98	40.00			
24	0.46	0.46	0.92	1.00	M	2.16	50.00			
Total	24.57	16.90	42.62	33.36						
Mean			1.77	1.39						

LA=Long arm; SA=Short arm; TCL=Total chromosome length; F%=;TF%=Total form percentage

Table 3
Karyotype details of *O. basilicum* cultivar 3

no.of chro-mosome	LA	SA	TCL	arm ratio LA/SA	cen-tromere position	RCL	F%	TF%	karyo type sym metry	karyotype formula
1	2.76	1.10	3.86	2.51	sm	8.29	28.50			
2	1.66	1.10	2.76	1.51	m	5.93	39.86			
3	1.38	1.10	2.48	1.25	m	5.32	44.35			
4	1.38	0.83	2.21	1.66	m	4.74	37.56			
5	1.24	0.96	2.20	1.29	m	4.72	43.64			
6	1.10	1.10	2.20	1.00	M	4.72	50.00			
7	1.10	1.10	2.20	1.00	M	4.72	50.00			
8	1.34	0.83	2.17	1.61	m	4.66	38.25	41.78	2B	8M+12m+
9	1.10	0.83	1.93	1.33	m	4.14	43.01			3sm+1st
10	1.10	0.83	1.93	1.33	m	4.14	43.01			
11	1.10	0.83	1.93	1.33	m	4.14	43.01			
12	1.10	0.83	1.93	1.33	m	4.14	43.01			
13	1.10	0.83	1.93	1.33	m	4.14	43.01			
14	1.10	0.83	1.93	1.33	m	4.14	43.01			
15	0.83	0.83	1.66	1.00	M	3.56	50.00			
16	0.83	0.83	1.66	1.00	M	3.56	50.00			
17	0.83	0.83	1.66	1.00	M	3.56	50.00			
18	0.83	0.83	1.66	1.00	M	3.56	50.00			
19	0.83	0.83	1.66	1.00	M	3.56	50.00			
20	1.10	0.55	1.65	2.00	sm	3.54	33.33			
21	1.10	0.28	1.38	3.93	st	2.96	20.29			
22	0.83	0.55	1.38	1.51	m	2.98	39.86			
23	0.83	0.28	1.11	2.96	sm	2.38	25.23			
24	0.55	0.55	1.10	1.00	M	2.36	50.00			
Total	27.12		46.58	36.21						
Mean		19.46	1.94	1.51						

LA=Long arm; SA=Short arm; TCL=Total chromosome length; F%=;TF%=Total form percentage.The degree of symmetry was estimated as per the scheme proposed by Huziwara (1962) and Stebbins (1971b).

Table 4.
Karyotype details of *O. basilicum* cultivars

Parameters	Cultivar 1 (μm)	Cultivar 2 (μm)	Cultivar 3 (μm)
			1.12
M LA	17.8 ± 0.02	24.57 ± 0.02	1.13±0.03
M SA	13.89 ±0.03	1.90 ±0.02	19.46 ±0.03
MCL ±SE1	1.32 ±0.03	1.77 ±0.03	1.94 ±0.03
MAR ±SE2	1.34 ±0.03	1.39 ±0.03	1.51 ±0.02
TF%	43.83 ±0.02	40.75 ±0.02	41.77 ±0.02
A1	0.22	0.23	0.25
A2	0.02	0.01	0.02
KC	1B	2B	2B
KF	6M+14m+4sm	6M+15m+3sm	8M+12m+3sm+1st

MLA=mean length of long arm; MSA=mean length of short arm; MCL=mean chromosome length; MAR=mean arm ratio; TF% total form percentage; A1=intra chromosomal asymmetry index; A2= inter chromosomal asymmetry index; KC= karyotype asymmetry; KF= karyotype formula

References

Abou-El-Enain, M. M. (2006) Interspecific and phylogenetic relationships in the genus *Companula L. s. str.* Companu-laceae): Karyological approach. Cytologia 139: 93-100

Archana, D., Shivnaz, Tand, N., Anand, Dash, D.K. (2013). Somatic chromo-somal studies in *Ocimum basilicum* L and *Ocimum sanctum* L. Internat J Phyto med 5 (3): 330- 340

Bir, S. S., Saggoo M. I. S. (1985) Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India). Proc Indian Acad Sci (Plant Sci) 94: 619- 626

Chatterjee, A., Sukul, N.C., Laskal, S., Ghoshmajumdar, S. (1982) Nematicidal principles from two species of Lamiaceae. J Nematol 14:118-120

Cherian, M., Kuriachan, P. I. (1981) In: IOPB chromosome number reports LXXII. Taxon 30: 707- 708

Cherian, M., Radhamany, P. M. (2017) Genetic diversity In *Ocimum basilicum* L. and *Ocimum tenuiflorum* (L.) Keng (Lamiaceae) varieties as Revealed by RAPD analysis. Abrahamia 2 in press

Darrah, H., H. (1980) *The cultivated basils*. Buckeye Printing Co., MO.

Darrah, H., H. (1974) Investigations of the cultivars of basils (*Ocimum*). Econ Bot 28: 63– 67

Deshpande, R.S., Tipnis, H. P. (1977) Insecticidal *activity of Ocimum basilicum* L. Pesticides 11: 11-12

Edet, O. U., Aikpokpodion, P. O. (2014) Karyotype Analysis of *Ocimum basilicum* in South Eastern Nigeria. Amer J Pl Sci 5: 126-131

Huziwara, Y. (1962) Karyotype analysis in some genera of Compositae VIII. Further studies on the chromosome of *Aster*. Amer J Bot 49:116–119

Levan A., Fredga K. and Sandberg A. A., 1964 *-Nomenclature for centromeric position on chromosomes*. Hereditas, 52: 201-220.

Morales, M. R., Charles, D. J., Simon, J. E. (1993) New aromatic lemon basil germplasm. In Janick,& J. E. Simon (Eds), *New crops* (pp 632–635). Wiley: NewYork

Morton, J. K. (1962) Cytotaxonomic studies on the West African Labiatae. J Linn Soc London (Bot) 58: 231-283

Murin, A. (1997) Karyotaxonomy of some medicinal and aromatic plants. Thaiszia 7: 75-88

Oxenham, S. K., Svoboda, K. P., Walters, D. R. (2005) Antifungal activity of the essential oil of basil (*Ocimum basilicum*) J Phytopath 153 (3):174 – 180

Paton, A., Putievsky, E. (1996) Taxonomic problems and cytotaxonomic relationships between varieties of *Ocimum basilicum* and related species (Labiatae). Kew Bul 5:1–16

Phippen, W. B., Simon J. E. (1998) *Anthocyanins in basil* (*Ocimum basilicum* L.). *J. Agr. Food Chem*., 46: 1734-1738.

Sanjappa, M. (1979) In IOPB chromosome number reports LXIII. Taxon 28: 275

Sharma, A., Sen, S. (2002) *Chromosome Botany.* Science Publishers, Inc, Enfield: USA

Sharma, A. K. Sharma A. (1965) *Chromosome techniques: Theory and Practice* Butterworth and Co., Ltd

Stebbins, G. L. (1971a) Adaptive radiation of reproductive characteristics in angiosperm seeds and seedlings. Ann Rev Ecol Syst 2: 237- 260

Stebbins, G. L. (1971b) *Chromosomal Evolution in Higher Plants*, Edward Arnold: London

Vaarama, A. (1947) Some chromosome numbers in the genera *Anglica*, *Ocimum*, *Satureja*, *Thymus* and *Cnicus*. Arch Soc Zool Bot Fennicae Vanamo 2: 55-59

Vieira, R. F., Simon J. E. (2000) Chemical characterization of basil found in the markets and used in traditional medicine in Brazil. Econ Bot 54:207-216 Zarco, C. R. (1986) A new method for estimating karyotype asymmetry Taxon 35: 526- 530

Received: 25 October 2017
Revised and Accepted: 12 December 2017