RESEARCH ARTICLE

CYTOMORPHOLOGICAL CHARACTERIZATION OF *OCIMUM BASILICUM* AND *O. TENUIFLORUM* GERMPLASM

Aditi Saha*

Department of Botany, NarasinhaDutt College, Howrah 711101
Email: sahaaditi2007@rediffmail.com

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Abstract: *Ocimumbasilicum* (sweet basil)and *O. tenuiflorum*(holy basil) of the genus *Ocimum*(basil; Family: Labiatae) obtained from Medicinal Plant Garden, Narendrapur are grown in the experimental field plots of Kalyani University (West Bengal plains) and cytotaxonomically characterized. Quantitative estimation of essential oil content from leaves and flower tops are also performed. *O. basilicum* and *O. tenuiflorum* show 2n= 72 and 2n= 36 respectively in meiocytes. The objective of the work is to catalogue the germplasms of the species under study for proper maintenance of genetic stock(s) for future exploration.

Keywords: Basil, Meiosis, Taxonomic characterization, Essential oil, Genetic stock

INTRODUCTION

Ocimumbasilicum (sweet basil)and *O. tenuiflorum*(holy basil) of the genus *Ocimum*(basil; Family: Labiatae) are important source of essential oil (methyl chavicol, eugenol, linalool, camphor and cinnamate – Simon *et al.* 1990; extracted from leaves and flowering tops) apart from possessing immense therapeutic uses (Prakash and Gupta 2005, Datta*et al.* 2010). The essential oil of basil possesses anti-cancerous property (Aruna and Sivaramakrishnan 1996) apart from having insecticidal (Chogo and Crank 1981, Chavan and Nikam 1982) and nematocidal (Chatterjee *et al.* 1982) activities. *O. tenuiflorum* is designated as 'elixir of life' in Ayurveda as it is believed to promote longevity (Puri 2002). Considering the significance, the species should be under sustainable cultivation with proper characterization of the germplasm under study. Such study can be helpful for maintenance of genetic stock(s). With a view to it, present study characterized *Ocimumbasilicum*and *O. tenuiflorum* germplasmscytomorphologically that are grown in Kalyani University campus (Nadia, West Bengal plains). Quantitative assessment of essential oil content (from leaves and flowering tops) of the germplasms is also performed.

MATERIAL AND METHOD

Germplasm

Mother seed stock of*Ocimumbasilicum*L.(moisture content - 8.62%)and *O. tenuiflorum*L. purple type (moisture content – 6.50%) were procured from Medicinal Plant Garden, Narendrapur, Ramkrishna Mission (Voucher specimens deposited in the Herbarium, Botany Department, Kalyani University), West Bengal, India. Seeds of both species were sown in late Novemberin the experimental field plots of Kalyani University(West Bengal plains, latitude: 22˚50' to 24 ˚11' N and longitude: 88˚09' to 88˚48' E, altitude: 9.75 m; sandy loamy soil, pH: 6.85)to raise plants.

Taxonomical studies

The mature plants of both species were examined and the study includes detail description of every part of the species with Olympus dissecting microscope under 10X. Measurements of the leaves, floral parts and seeds were made. On an average 15 seeds were measured in either of the species in a Stereo dissecting microscope. 100 seed weight and moisture content were also determined.

Meiosis

Flower buds of suitable sizes from 5 randomly selected plants (for each species) plants of *O. basilicum* and *O. tenuiflorum* were fixed in Carnoy's fluid (4 to 5 p.m.) and two changes were given in the fixative at an interval of 24h. Anthers were squashed in 2% aceto-carmine solution and well scattered PMCs were scored at metaphase I (MI) and anaphase I (AI). Meiotic analysis was performed from 3 sites (5 plants assessed in each site for both species; data pooled over the plants in each site) in the field to assess variation, if any. Photomicrographs were taken from suitable preparation. Pollen grains from mature buds were also stained in 2% aceto-carmine and uniformly stained pollen grains were considered fertile (Marks 1954).

Acetolysis studies

Acetolysis technique was adopted as per Erdtman (1952) to study the shape, size and ornamentation of the pollen grains in both species.

*Corresponding Author

Table 1. Metaphase I configurations of two species of *Ocimum*

District	*O. basilicum* (2n=72)			*O. tenuiflorum* (2n=36)		
	PMCs scored	II	I	PMCs scored	II	I
Site I	172	35.40	1.20	192	17.20	1.60
Site II	198	35.12	1.76	186	17.72	0.56
Site III	166	35.64	0.72	174	17.92	0.16
χ^2 test of heterogeneity probability		>0.05	<0.05		>0.05	<0.05

Table 2. Quantitative estimation (in per cent) of essential oil in two species of *Ocimum*

District	Genotypes			
	O. basilicum		*O. tenuiflorum*	
	Leaf	Inflorescence	Leaf	Inflorescence
Site I	0.380-0.412	0.180-0.182	0.312-0.315	0.241-0.246
Site II	0.391-0.402	0.160-0.168	0.290-0.298	0.224-0.229
Site III	0.355-0.371	0.160-0.169	0.301-0.306	0.191-0.199
χ^2 test of heterogeneity probability	>0.05	>0.05	>0.05	>0.05

Figure plate 1 (a-b) Plant types of *Ocimum* spp. **(a)** *Ocimum basilicum* and **(b)** *Ocimum tenuiflorum*.

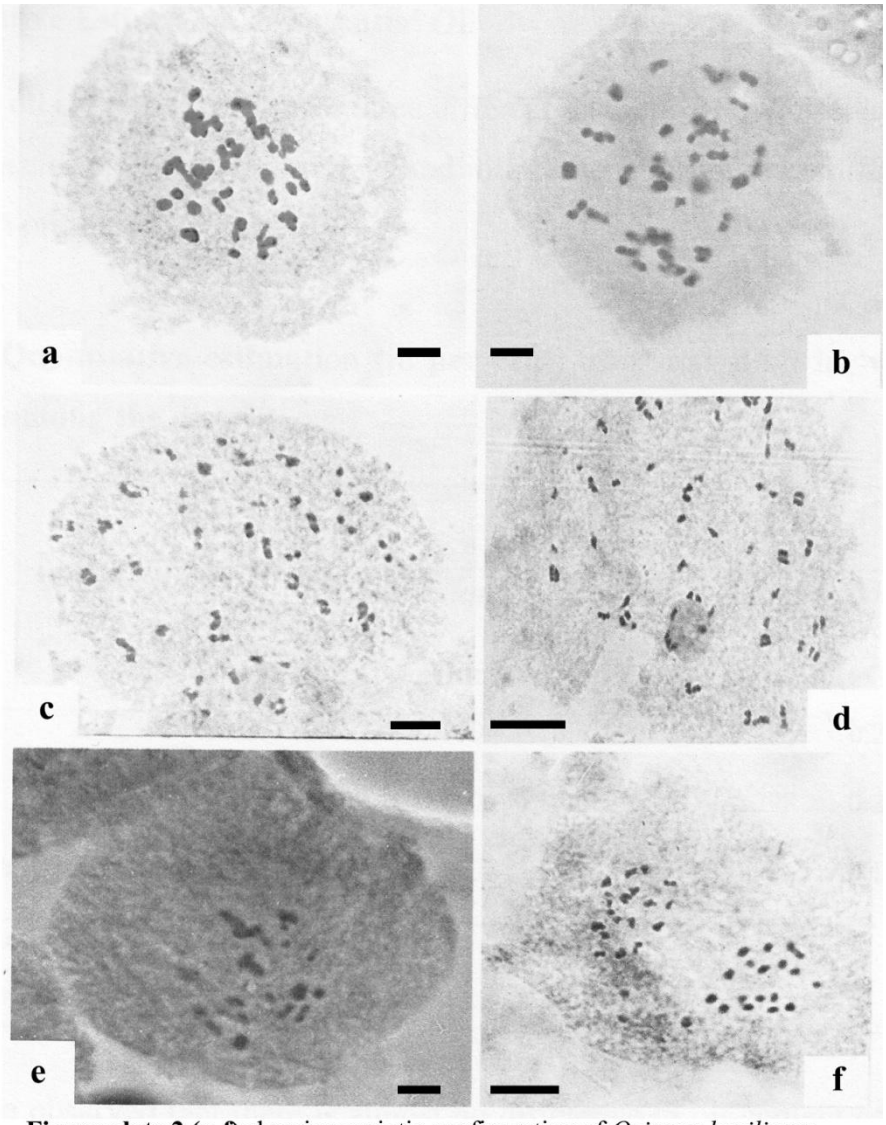

Figure plate 2 (a-f) showing meiotic configuration of *Ocimum basilicum* **(a-d)** and *O. tenuiflorum* **(e-f). (Fig a-d)** MI showing **a -**36II**, b-**33II+6I**, c-**30II +12I, **d-**29II+14I**; e-** MI showing 18II**, f-** AI with 18:18 separation
scale bar = 2 μm

RESULT AND DISCUSSION

Taxonomical characterization

Taxonomical description of the plant species of *Ocimum* are given below

O. basilicum (Fig. 1a)

Annual branched herb, 66.6 cm high, 4 angular, hairy throughout, prominent along the 4-angles or ridges of the stem, solid, green to dull green, leaves opposite-decussate, simple, lanceolate to ovate lanceolate, or broadly elliptic, 2 to 3 cm long, and 1.5 cm wide, acute, margin serrate-dentate, base cuneate, venation unicostate, 3-4 pairs of alternate secondary, hairy on both surfaces, gland dotted, dull green petiolate, petioles 1 cm long, hairy; inflorescence with about verticillasters, mostly 6 flowers in a whorl, 3 to 5 in each axis of the bract, finally forming spikes of 30 cm long, inflorescence bract elliptic, 7 mm long and 2.5 mm wide, purple-copper coloured, long hairy, hairs silky, midvein distinct; flowers complete, bisexual, hypogynous, 2 lipped, white, pedicillate, pedicels slender 4-angular, 1.2 to 2 cm long, purple, short hairy; calyx gamosepalous, 1.6 to 1.8 mm long, united part of 6 to 8 mm, free lobes 8 to 10 mm long, posterior sepal broadly ovate, 5.5 to 8 mm long and 10 mm wide, hairy on the outer surface, more acute, long hairy, greenish purple, smaller than the posterior sepal and the anterior sepals, anterior sepals triangular-dentate 8 to 14 mm long and 6 mm wide, acute to acuminate, long hairy, purple-green, longer than the lateral calyx lobes, base of the calyx tube on the posterior end just above the pedicel tumid; corolla 2-lipped, tubular part 1.73 to 2.22 mm long, glabrous both within and without, light pinkish white, posterior lobe oblong, shallowly 2-lobed at apex, 2.4 to 3.2 mm long and 1.4 mm wide, glabrous,

all white; stamens 4, didynamous, filaments of posterior-lateral pairs long slender, 3.4 to 3.9 mm long, attached above the base, not bifurcate not dentate, white, glabrous, anthers 2-celled, ovoid, dorsifixed, dehiscence longitudinal, latrorse, orange, glabrous; carpels 2, syncarpous, ovary ellipsoid, inconspicuously 4-lobed, 0.4 to 0.6 mm across glabrous, style 6.4 to 7.6 mm long, pinkish to deep pinkish on the upper end, glabrous, stigma bifid, pinkish, glabrous.

O. tenuiflorum (Fig. 1b)

Annual to perennial herb, branched, hairy; leaves opposite decussate, ovate to oblong, 4.3 to 5 cm long, and 3.1 cm wide, apex rounded, margin serrate with setae on serrate end, base rounded venation unicostate reticulate with 4(5) pairs of pinnate secondaries, basal 2 pairs opposite, 3rd pair sub-opposite 4(5th) pair alternate, arched and united with the surface adjacent secondary in the intramarginal region (brochidodromous), distinct on the lower surface, veins purple to deep purple above, major veins depressed above, plumose hairy on both surfaces, gland-dotted below, greenishpurple above, dull green beneath, petiolate, petioles slender 8 to 10 mm long, slightly ridged, ciliate, hairs unicellular, woolly white, greenish with slight purple shade; inflorescence with primary rachis, 7.2 cm long with 11 whorls (verticels) of 6 flowers each, secondary rachis opposite decussate, 3 to 4.5 cm, 5 to 7 verticels, 6 flowered or rarely less in number, inflorescence bract 2, ovate, 2.5 mm long and 2 mm wide, apex acute margin ciliate, entire, base cordate, rounded or rarely sub-truncated, surface pubescent more on the lower surface, purple green, later brownish, venation 3-veined, 2 basal inconspicuous; flowers complete, bisexual, hypogynous, zygomorphic, pentamerous white with slightly pink, ebracteate, pedicillate, pedicels long slender, 2.6 to 3 cm long, arched and curved, purple, hairy; calyx gamosepalous, 2.4 to 3 mm long, bilobed; sepals 5, trimorphic, posterior one broadly ovate or broadly orbicular, 1.9 mm wide, apex apiculate, entire,hairy on the outer surface and margin,accrescent in fruit,lateral 2 sepalsdentate, 2 mm long, purple, hairy, anterior 2 sepals dentate to acuminate,2.5 mm long, purple, hairy, tubular part 1.5 mm long; corollagamopetalous,bilabiate, 5 mm long, tubular part 2.2 mm, petals oblong, 1.7 to 2.5 mm long, rounded, white with slightly pinkish, , posterior to larger than the lateral and anterior; stamens 4, didynamous (2+2), filaments long slender to filiform, 3.8 to 4.5 mm long, epipetalous, anthers 2-celled, oblong ovoid, dorsifixed, dehiscence longitudinal, dull yellowish, glabrous; carpels 2, syncarpous, ovary superior, 4-lobed, 0.3 to 0.5 mm long, style slender, about 5.5 mm long pinkish white, glabrous.

Meiotic analysis

O. basilicum (Fig. 2a-d)

PMC squashes showed 2n=72 chromosomes in all cases. The plants formed 36II in metaphase I cells (Table 1). PMCs with 35II+2I, 34II+4I, 33II+6I, 32II+8I and 31II+10I were also found. Bivalent frequency among the plants varied from 35.12 to 35.64 per cell and those bivalents (random distribution evidenced from χ^2 test of heterogeneity χ^2= 0.265 at 9 DF, p>0.95) tended to form variable groups at MI; although univalent range:from 0.72 to 1.76/cell(non-randomly distributed, χ^2= 21.27 at 9 DF, p<0.01) were often associated in groups with bivalent. AI segregation of chromosome is mostly balanced and rarely cell from laggards varied from 1-4/PMC. Pollen fertility among the samples ranges between 46.85% and 53.12%.

O. tenuiflorum (Fig. 2e-f)

The species had 2n=36 chromosomes in the meiocytes always (Table 1)and range from 17.20 to 17.92(random distribution, χ2= 0.504 at 9 DF, p>0.95).Univalent per cell is found to vary0.16 to 1.60(non-randomly distribution, χ2= 104.96 at 9 DF, p<0.01) at MI. while the rest had17II+2I, 16II+4I, 33II+6I and 13II+10I. AI is cytologically (18:18) balanced with occasional formationof laggards; bridges and cells with unequal separation of chromosomes. Pollen fertility was also noted.

Present investigation reports 2n=72 chromosomes in *O. basilicum* which is rather contrary to 2n=16 (SZ Borsos 1970) and 2n=48 (Morton 1962, Mehera and Gill 1972, Puspangadan*et al.* 1975, Sanjappa 1979, Puspangadan and Sobti 1982); however the number 2n=72 has earlier been reported in *Ocimum*for the species *O. americanum*(Puspangadan and Sobti 1982). Critical analysis of taxonomic data of the genus *Ocimum* is provided that *O. americanum*is a synonym of *O. basilicum*L. (Morton 1962, Banerjee and Maity 2000). *O. tenuiflorum*has 2n=36 chromosomes always in meiocytes. Bir and Sagoo (1980) and Singh (1980) also reported 2n=36 chromosomes for the species but it also stated to be as n=16 (Mehera and Gill 1972, Khosla and Sobti 1985), n=16+0-3B (Vij and Kashyap 1976), n=17 (Singh 1980) and 32 (Tischler 1938).

Pollen morphology

O. basilicum

Average pollen size 75.68µ × 75.32µ (ranges: 68.00µ × 68.00µ to 80.00µ × 80.00µ); spheroidal in shape; zonocolpate, colpi 6, deep; surface with small reticulation, uniform, pentagonal rarely hexagonal, super reticulate; wall structure-tectate columellate, collumellae numerous, distinct.

O. tenuiflorum

Average pollen size 32.49µ × 29.58µ (ranges: 29.58µ × 26.10µ to 34.80µ × 33.10µ); spheroidal to oval; zonocolpate, colpi 6, deep; surface reticulate, reticulum small with often irregular size and shape; uniform, wall structure-tectate columellate,collumellae numerous, distinct.

Bio-chemical analysis

Quantitative estimation of essential oil

Essential oil content analysed for three different districts following steam distillation in two species of

*Ocimum*from leaf and inflorescence have been presented in table 2.

It has been observed that there is almost no difference in oil content of two species of *Ocimum*among these three districts.

CONCLUSION

The study is significant for proper indexing of the germplasms under study that can act as an important genetic resource in sustainable cultivation.

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