Cytomorphological Study of the F_2 Variants of F_1 Hybrids of *Ocimum gratissimum* L. ($2n=40$) and *Ocimum viride* Willd. ($2n=40$)

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Ocimum (family Lamiaceae) is an important aromatic group of herbaceous plants with many of its species serve as natural source of various essential oils useful in perfumery, cosmetics and pharmaceutical industries. Also used as a pot herb and find diverse utility in a variety of purposes in native medicines.

In the present communication, cytomorphological characteristics of F_2 variants of interspecific F_1 hybrids involving *O. gratissimum* and *O. viride* are reported. The study was initiated to evaluate the breeding prospects of F_2 variants and to determine the correlation if any between the phenotypic features and the chromosome numbers present in these variants.

Materials and methods

Morphological observations are made from the living plant species which have been raised and maintained in the experimental medicinal garden of this laboratory. The technique employed for the cytological studies and for raising the hybrids is the same as reported earlier (Khosla and Sobti 1984, 1985).

Observations

The partial fertile experimentally produced F_1 hybrids of *O. gratissimum* $\times$ *O. viride* and the hybrids produced from its reciprocal crosses on selfing gave 1.4% and 1.9% seed set respectively (Khosla and Sobti 1986). The seeds collected from the selfed inflorescences of both the reciprocal hybrids were sown and F_2 populations were raised. Out of 200 healthy looking seeds collected from each of F_1 hybrids, 20 to 35 seeds showed germination and 12 to 20 seeds eventually grew to maturity.

The morphological appearance, chromosome number, herbage and oil yield of all the F_2 plants raised from both the reciprocal crosses were recorded (Tables 1, 2). A perusal of the tables showed that some of the F_2 segregants have a very high herb and oil yield and are higher to that of the parents and even to the F_1 hybrids, especially, the new variants with $2n=80$ chromosomes out yield F_1 hybrids and their parents. On the basis of chromosome numbers alone the F_2 population could be grouped into six categories having chromosome numbers $2n=40, 42, 44, 45, 48$ and 80 . Selections were carried out from these F_2 plants representing at least one plant from each group showing promising combinations of desirable characters for herb and essential oils. The six selected plants were then investigated in greater details for their morphology, cytology, pollen fertility and essential oil characters.

Table 3 gives the comparative morphological account of the six selected plants from the F_2 population which covers a wide spectrum of morphological variants (Fig. 1). The plants are numbered as 1 to 6 on the basis of their chromosome numbers viz., $2n=40, 42, 44, 45, 48$ and 80 respectively. The average herb and oil yield in each of these selectants are appreciably high. Plant nos 1 and 6 gave an average herb yield as high as 950 gm and 4120 gm respec-

tively which is a record yield when compared to the yield of F_1 hybrids or their parents. The oil yield in these variants is also variable and significantly high.

Fig. 1(a-g). Key diagram and polygraphs of six selected F_2 plants of *O. gratissimum* $\times$ *O. viride* respectively.

Mitotic studies of these six selected plants showed the presence of $2n=40, 42, 44, 45, 48$ and 80 chromosomes respectively (Figs. 2-7). Their detailed karyotype analyses are tabulated in Table 4, the chromosome length varied from 0.6μ to 1.75μ and relative length from 1.24μ to 4.17μ in these selectants. The chromosomes belong to five different groups viz. A, B, C, D and E respectively depending upon their absolute length. The total chromatin length as expected showed increase with increase in the chromosome numbers which ranged from 39.15μ to 80.40μ in these F_2 plants.

A detailed chromatin study of the six selectants during different stages of meiosis except plant no. 6 revealed various irregularities (Table 5, Figs. 8-13). The major irregularities encountered are a) relatively large number of unpaired chromosomes at diakinesis—metaphase I, b) partial or loose pairing (pseudobivalents), c) precocious disjunction of many bivalents, d) failure of terminalization, nondisjunction and bridge formation, e) disorientation of ir-

regular bivalents, f) erratic segregation of univalents, g) formation of restitution nuclei, h) formation of microspores of different sizes and numbers, and i) formation of micronuclei.

The course of meiosis after metaphase I is also highly irregular and Table 6 represents the variation in microspore numbers at quartet stage. The pollen grains developed from these

Table 1. Screening of F_2 population (20 plants) of the interspecific hybrid of *O. gratissimum* ($2n=40$) $\times$ *O. viride* ($2n=40$)

Plant number	Morphological appearance like	Chromosome number ($2n$)	Herbage yield per cutting (gm)	Oil yield (MFB)
*1	F_1 hybrid	44	620	4.35
2	New variant	40	350	2.1
*3	<i>O. gratissimum</i>	45	600	5.0
4	<i>O. viride</i>	42	450	0.9
5	New variant	80	3950	2.5
6	<i>O. gratissimum</i>	45	170	2.2
*7	New variant	48	690	2.5
*8	<i>O. viride</i>	42	710	3.5
*9	New variant	80	4120	3.8
10	<i>O. viride</i>	44	500	2.2
*11	F_1 hybrid	40	950	4.4
12	<i>O. gratissimum</i>	40	400	2.6
13	New variant (dwarf plant)	48	200	3.4
14	<i>O. viride</i>	40	450	2.0
15	F_1 hybrid	42	685	3.0
16	New variant	48	500	2.8
17	F_1 hybrid	42	1150	1.3
18	<i>O. gratissimum</i>	45	310	1.0
19	<i>O. viride</i> (highly dwarf)	40	125	3.5
20	<i>O. gratissimum</i>	44	450	1.7

*=Promising plants selected for detail investigation and further improvement.

MFB=Moisture free basis.

Table 2. Screening of F_2 population (12 plants) of the interspecific F_1 hybrid of *O. viride* ($2n=40$) $\times$ *O. gratissimum* ($2n=40$)

Plant number	Morphological appearance like	Chromosome number ($2n$)	Herbage per cutting (gm)	Oil yield (MFB)
1	F_1 hybrid	42	750	2.5
2	F_1 hybrid	40	885	3.1
3	<i>O. viride</i>	44	380	2.8
4	<i>O. viride</i>	42	350	2.7
5	<i>O. gratissimum</i>	42	550	3.3
6	<i>O. gratissimum</i>	48	425	1.8
7	F_1 hybrid	45	850	3.2
8	<i>O. viride</i>	40	375	1.2
9	<i>O. gratissimum</i>	48	400	1.1
10	<i>O. vitride</i>	40	550	0.9
11	New variant	48	600	1.3
12	New variant	80	3650	3.0

spores are of different sizes and show varying degree of stainability thereby rendering these plants highly sterile.

The meiosis in plant no. 6 is more or less normal with most frequent chromosome association at diakinesis–metaphase I having the formation of 40 bivalents. However, few univalents

Table 3. A comparative morphology of the six plants selected from the F₂ generation of *O. gratissimum* × *O. viride*

Characters	Plant no. 1	Plant no. 2	Plant no. 3	Plant no. 4	Plant no. 5	Plant no. 6
Habit	Perennial woody undershrub 265	Perennial woody undershrub 185	Perennial woody undershrub 215	Perennial woody undershrub 165	Perennial woody undershrub 195	Perennial woody undershrub 260
Height	4.5 to 9.5 (7.03)	4.0 to 8.5 (5.90)	3.0 to 9.5 (6.23)	1.5 to 7.0 (4.07)	3.5 to 8.0 (5.88)	4.0 to 9.0 (5.87)
Length of petiole (cm)	11.5 × 6.5 × 19.0 × 9.0 (16.53 × 7.95) (=2.1)	9.0 × 6.5 to 18.0 × 9.5 (12.73 × 7.36) (=1.73)	11.0 × 4.0 to 17.5 × 7.5 (14.6 × 6.6) (=2.21)	4.5 × 4.3 to 14.0 × 6.0 (10.43 × 4.24) (=2.46)	6.0 × 5.5 to 16.0 × 8.0 (13.53 × 7.07) (=1.91)	11.0 × 4.8 to 17.2 × 10.0 (=1.56)
Length of inflorescence (cm)	16.0 to 25.0 (20.3)	12.0 to 18.0 (14.1)	12.0 to 16.0 (15.1)	7.0 to 13.0 (11.2)	10 to 20 (17.5)	15.0 to 22.0 (18.4)
Size of bract (mm)	4.5 × 4.0 to 9.0 × 4.0 (7.5 × 4.08) (=1.84)	6.0 × 4.5 to 8.0 × 5.0 (6.71 × 4.4) (=1.53)	6.8 × 4.5 to 8.1 × 5.0 (7.2 × 4.81) (=1.49)	6.1 × 4.5 to 8.0 × 4.0 (6.96 × 4.69) (=1.48)	4.5 × 3.8 to 8.0 × 4.5 (6.55 × 3.91) (=1.67)	6.0 × 5.0 to 9.5 × 6.3 (8.12 × 5.59) (=1.45)
Length of calyx (mm)	4.5 to 5.5 (5.0)	4.0 to 5.0 (4.6)	5.0 to 6.0 (5.39)	4.9 to 5.2 (5.07)	4.0 to 5.0 (4.6)	5.0 to 6.0 (5.40)
Length of corolla (mm)	4.0 to 5.5 (4.88)	5.0 to 6.0 (5.33)	5.5 to 6.0 (5.74)	5.5 to 6.0 (5.73)	5.0 to 5.5 (5.20)	5.5 to 7.0 (6.30)
Length of stamen (mm)	3.5 to 4.5 (4.05)	4.0 to 4.5 (4.25)	4.0 to 5.0 (4.46)	4.4 to 5.0 (4.76)	4.0 to 5.0 (4.15)	5.0 to 6.0 (5.57)
Length of style (mm)	7.0 to 8.0 (7.4)	6.5 to 7.5 (7.02)	7.2 to 8.2 (7.78)	7.1 to 7.5 (7.36)	6.0 to 6.5 (6.25)	7.5 to 9.2 (8.66)
Length of fruiting calyx (mm)	5.0 to 6.0 (5.6)	4.8 to 6.0 (5.43)	5.5 to 7.0 (6.40)	5.0 to 5.5 (5.28)	5.0 to 6.0 (5.85)	8.0 to 9.0 (8.70)
Chromosome number	2n=40	2n=42	2n=44	2n=45	2n=48	2n=80
Pollen fertility (%)	2.94	12.3	83.41	24.25	11.61	90.10
Size of pollen (diameter in micron)	37.50 to 50.0 (42.63)	40.62 to 50.0 (47.06)	37.50 to 53.12 (46.31)	48.75 to 62.50 (52.69)	37.5 to 56.25 (45.75)	50.0 to 65.0 (59.85)
Seed set (%)	1.35	3.64	3.95	0.79	3.15	65.70

() : Mean, (=): Index = $\frac{\text{Length}}{\text{Width}}$, M.F.B. = Moisture free basis.

Figs. 2-7. Mitotic chromosomes of F₂ *O. gratissimum* × *O. viride* plant nos. 1-6, showing 2n=40, 42, 44, 45, 48 and 80 chromosomes.

Table 4. Karyotype comparison of the six selected F₂ plants of *O. gratissimum* (2n=40) × *O. viride* (2n=40)

Plant no.	Chromosome number (2n)	Length range of individual chromosome (micron)	Karyotype (2n)	Total chromatin length (micron)
1	40	0.7 to 1.6	1B+5C+22D+12E	39.15
2	42	0.6 to 1.65	3B+2C+25D+12E	39.60
3	44	0.6 to 1.8	1A+2B+14C+22D+5E	48.0
4	45	0.6 to 1.25	4C+29D+12E	41.15
5	48	0.65 to 1.5	3B+16C+15D+14E	52.20
6	80	0.6 to 1.75	8A+8B+16C+34D+14E	81.40

Table 5. Summary of meiotic analysis of six *F*₂ plants of *O. gratissimum* ($2n=40$) $\times$ *O. viride* ($2n=40$)

Meiotic stage	Diakinesis-metaphase I 30*				Anaphase I 40*			Anaphase II 50*			
	I	II	III	IV	Segregation	Laggards	Restitution nuclei (%)	Number of chromosome sets	Number of micro-nuclei	Number of laggards	Number of restitution nuclei
Plant no. 1 $2n=40$	8-23 (17.30)	6-15 (9.67)	0-3 (0.64)	0-2 (0.37)	14+13-24+16 (19.21+16.44)	0-13 (4.13)	2.5	2-4 (3.82)	0-5 (0.3)	0-8 (3.16)	0-2 (0.40)
Plant no. 2 $2n=42$	6-30 (17.10)	5-15 (10.50)	0-3 (0.87)	0-2 (0.33)	18+17-27+15 (21.10+17.95)	0-8 (2.95)	—	2-5 (3.76)	0-3 (0.32)	0-5 (1.54)	0-2 (0.46)
Plant no. 3 $2n=44$	13-33 (22-60)	4-14 (9.77)	0-2 (0.54)	0-1 (0.07)	14+16-26+14 (21.48+18.93)	0-14 (3.60)	—	2-5 (4.02)	0-3 (0.30)	0-4 (0.30)	0-2 (0.24)
Plant no. 4 $2n=45$	9-29 (17.83)	8-18 (12.67)	0-2 (0.57)	0-1 (0.03)	19+18-27+15 (22.18+19.03)	0-8 (3.53)	—	3-4 (3.82)	0-1 (0.02)	0-6 (1.94)	0-1 (0.18)
Plant no. 5 $2n=48$	12-28 (21.23)	9-18 (12.47)	0-2 (0.43)	0-1 (0.52)	20+18-27+21 (23.38+20.43)	0-10 (4.15)	—	3-5 (3.80)	0-4 (0.64)	0-4 (1.32)	0-1 (0.46)
Plant no. 6 $2n=80$	0-8 (1.53)	32-40 (38.63)	—	0-4 (0.27)	40+40 (normal)	—	—	4 (normal)	—	—	—

(): Average,

* Number of PMCs scored.

Figs. 8–13. Meiosis in F₂ *O. gratissimum* × *O. viride*. 8, plant no. 1 showing 1 tetraivalent, 11 bivalents and 14 univalents. 9, plant no. 2 showing 1 trivalent, 15 bivalent and 9 univalents. 10, plant no. 3 showing 1 trivalent, 12 bivalents and 17 univalents. 11, plant no. 4 showing 1 trivalent, 11 bivalents and 20 univalents. 12, plant no. 5 showing 11 bivalents and 26 univalents. 13, plant no. 6 showing 39 bivalents and 2 univalents.

Table 6. Variation in the number of spores in the tetrad produced by PMCs of the six selected F₂ plants of *O. gratissimum* (2n=40) × *O. viride* (2n=40)

Plant no.	Monad (%)	Diad (%)	Triad (%)	Tetrad (%)	Pentad (%)	Hexad (%)
1	—	1	38	58	3	—
2	—	3	33	55	6	3
3	—	3	31	66	—	—
4	—	—	28	72	—	—
5	—	3	17	65	13	2
6	—	—	4	96	—	—

and tetravalents are encountered in few PMCs. The mean pairing of chromosomes per PMC therefore, comes to be $1.53 \text{ I} + 38.63 \text{ II} + 0.27 \text{ IV}$. 96% of the tetrad cells showed the presence of four spores and the remaining showed triads. The pollens are almost of uniform size and are deeply stained and hence the plant is fertile with 65–70% seed set.

Discussion

The F_2 populations raised from selfed partially fertile F_1 hybrids involving *O. gratissimum* and *O. viride* showed a great array of morphological and cytological variation. On the basis of morphological appearance, these F_2 plants could be grouped into four different categories i.e. extra ordinary gigantic plants, plants resembling F_1 hybrids, plants resembling either of the parents and plants with new combination of characters (new variants having characters of both the parents as well as some new characters which were not present in either parent). The six selected plants of the F_2 populations were quite distinct morphologically from each other which were robust and having high herb and oil yield. A complete reshuffling of morphological characters of the two parents has taken place which has resulted in the development of new forms of which some had less chance to survive while some others had great potential to survive in more diverse conditions.

Mitotic studies of the six selected F_2 plants showed chromosome numbers ranging from $24=40$ to $2n=80$. The chromosome number in plant no. 1 is $2n=40$ which is the same as in both the parents of F_1 hybrids. Plant nos, 2, 3, 4, 5 and 6 have chromosome numbers $2n=42, 44, 45, 48$ and 80 respectively. These numbers excepting $2n=48$ are new counts in *Ocimum* species presently investigated. Detailed morphological studies carried out of the individual chromosome of each of F_2 selectants have shown that these plants have each a different chromosome number as well as chromosome combinations belonging to different groups. The formation of different chromosome numbers in these F_2 plants was due to the formation of gametes with different chromosome numbers in the F_1 plants and their chance fertilization resulted in the occurrence of plants with varying chromosome numbers. It was also interesting to note that no plant with chromosome number less than $2n=40$ was found in F_2 population which perhaps was due to nonviability of gametes having less than $n=20$ chromosomes. The formation of $2n=80$ chromosomes in plant no. 6 was attributed to a chance fertilization of two unreduced gametes arose as the result of restitution nuclei formed during their course of meiosis. Grant (1963, 1966), Mayer (1963) and Stebbins (1966) suggested the catalytic effect of hybridization on the biological evolution of two species. In the first place, the genetic recombination which inevitably takes in wholly and partially fertile progeny of hybrids gives rise to large quantitative increase in size of the gene pool. Such a kind of evolutionary jumps have been reported in a number of plant genera like *Elymus*, *Potentilla*, *Lianthus*, *Galeopsis*, *Gilia*, *Nicotiana* by Stebbins (1950, 1966, 1971).

The data on meiotic studies of these F_2 selected plants excepting plant no. 6 showed many abnormalities. During the course of meiosis, some of the chromosomes were completely paired while other showed partial or loose pairing or behaved as univalents. The bivalents which showed complete pairing with chiasma formation were probably homologous. The number of such bivalents usually corresponds to the lowest number of bivalents recorded in these plants. The PMCs with higher degree of pairing usually had many loose bivalents which were with terminal association and without chiasma formation. There were observed certain rod like bivalents which failed to terminalize and during anaphase I were stretched to form chromatin bridge.

The presence of unpaired chromosomes resulted in irregular chromosome behaviour at the later stages of meiosis led to the formation of microspores with different size, number having

variable chromosome numbers. The formation of few seeds in these selectants (except plant no. 6 which is a fertile) suggests that occasionally a few gametes with balanced chromosome numbers are produced in these hybrid derivatives and that a chance fertilization of these gametes gives rise to seed set. Formation of restitution nuclei suggests the possibility of unreduced gametes being formed and their chance fertilization results in complete fertile plants as in case of plant no. 6.

The meiosis in plant no. 6 was basically normal with regular formation of 40 bivalents and equal anaphase segregation. Though there were rare occurrence of few univalents, and tetra-valents during early stages of meiosis which gets regularised in the later stages rendering the plants fertile with 65–70% seed set and hence is a new derivative with chromosome number $2n=80$.

Thus from the foregoing discussions on the phenotypic appearance, chromosome number, pollen fertility and essential oil chemistry of F_2 hybrid derivatives of *O. gratissimum* and *O. viride*, it is quite apparent that there exists an array of complex assemblage of characters with a good deal of variability. Morphological analysis of F_2 population showed great diversity from extra ordinary gigantic plants to highly diminutive ones. A study of their chromosomes also revealed various chromosome numbers ranging from $2n=40$ to $2n=80$. It was very interesting to note that these F_2 segregants with same chromosome number showed different morphological features. For example plant nos. 2, 11, 12, 14 and 19 of F_2 progeny of *O. gratissimum* $\times$ *O. viride* and 2, 8, 10 nos. of the reciprocal hybrid had $2n=40$ chromosomes but surprisingly showed different morphological features. Such new combinations suggest that the gene regulating various characters in these plants are present on different chromosomes and the presence of different morphological characters depends upon the genotypes. Hence, to establish any correlation between the phenotypic features and chromosome number in these F_2 variants is not possible as there exists no uniformity with regard to their chromosome numbers and morphological characters in these F_2 plants.

Summary

F_2 populations were raised from the partial fertile F_1 hybrid of *O. gratissimum* $\times$ *O. viride* and their reciprocal hybrids which showed a great diversity from extra ordinary gigantic plants to highly diminutive ones. F_2 segregants were grouped into six categories having chromosome numbers $2n=40, 42, 44, 45, 48$ and 80 respectively. Six plants (one from each category) with promising combinations of desirable characters have been selected. All the F_2 selectants especially plant no. 6 were higher yielders. The F_2 selectants exhibited highly irregular meiosis excepting plant no. 6 which showed normal meiosis. Various meiotic irregularities investigated have been discussed. The detailed morphological and cytological studies of F_2 segregant have shown that there is hardly any correlation between the phenotypic features and the chromosome number present in these variants.

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References

- Grant, V. 1963. The Origin of Adaptations. Columbia University Press, New York.
— 1966. The origin of new species of *Gilia* in a hybridization experiment. *Genetics* **54**: 1189–1199.

- Khosla, M. K. and Sobti, S. N. 1984. Hybridization between different geographical races of *Ocimum gratissimum* L. *The Nucleus* **27**(3): 156–159.
- and — 1985. Karyomorphological studies in genus *Ocimum* II. "Sanctum group". *Cytologia* **50**(2): 253–263.
- and — 1986. Cytogenetic studies in the genus *Ocimum*: Interspecific hybrids and induced amphiploids of *O. gratissimum* L. ($2n=40$) $\times$ *O. viride* Willd. ($2n=40$). *Cytologia* **51**(1): 225–234.
- Mayer, E. 1963. *Animal Species and Evolution*. Harvard Univ. Press, Cambridge.
- Stebbins, G. L. 1950. *Variation and Evolution in Plants*. Columbia Univ. Press, New York.
- 1966. Chromosomal variation and evolution. *Science* **152**: 1463–1469.
- 1971. *Chromosomal Evolution in Higher Plants*. Edward Arnold Ltd., London.
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