



Diallel Studies and Heritability Estimates Using Hayman's Approach in *Ocimum* spp.

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Abstract

Diallel analysis of Hayman was performed removing reciprocal in basil, the experimental material consisted of morphological diverse genotypes and their 21 direct crosses i.e. F1 populations. The traits days-to-flowering (50%), plant height (cm), no of branches/plant, inflorescence length (cm), fresh herb yield (g)/plant, oil content (%), oil yield (g)/plant, days-to-maturity, methyl-chavicol content (%), linalool content (%), citral content (%), eugenol content (%) explored additive type of gene effects. t^2 for all the characters are non-significance so its indicate validity of the hypothesis postulated by Hayman. Regression line for days-to-flowering (50%), days-to-maturity were below the origin showing the presence of over-dominance while for branches/plant, ocimene content, linalool content, camphor content and methyl-chavicol it was above the origin suggesting the presence of partial-dominance. Regression line for plant height, inflorescence length, fresh herb yield, oil content and oil yield were through the origin indicating the presence of complete-dominance. Narrow sense heritability estimates \hat{h}^2 (ns)% ranged from 7.9% (lowest) for inflorescence length to 59% (highest) for oil content. Genetic advance over mean ranged from 0.54 (days-to-maturity) to 53.11 (branches/plant).

Keywords: Diallel; Genetic Advance; Heritability; *Ocimum*

Introduction

Ocimum (family Lamiaceae), comprising of more than 150 species grows widely and is distributed throughout temperate regions of the world [1,2]. *Ocimum* are frequently cultivated in several countries of East Asia, Europe, America and Australia for the production of essential oils [3,4]. A high degree of polymorphism in the genus *Ocimum* determines a large number of subspecies, different varieties and forms producing essential oils with varying chemical composition offering variable level of medicinal potential [5]. Essential oils extracted from *Ocimum* plants have been reported to possess interesting biological properties. These volatile oils have been applied in perfumery, to inhibit growth of microorganisms, in food preservation and in aromatherapy. The uses of *O. basilicum*, *O. africanum*, *O. gratissimum* and *O. sanctum* essential oils, particularly as antioxidant and antimicrobial agents have also been explored [6-9]. The improvement of essential oil of better quality and high yield is one of the important goals of plant breeders in the *Ocimum*. Achievement in crop improvement has over the years ari-

used through a sensible application of a number of genetic principles. One of the important reasons for nearly stagnant essential oil yield levels of basil may be the limited genetic improvement. Most probably it have been due to narrow genetic base and uses of conventional breeding techniques with small or no understanding of the genetic architecture of the selected populations. Looking of the increasing demand of *Ocimum* essential oil widely used for various perfumery industries, medicines, pharmaceutical industry and cosmetics. The genetic improvement of basil for both quality and quantity of essential oil is imperative in the interest of national economy.

In diallel mating design has been mostly used in both self and cross pollinated varieties to understand the nature of gene action involved in the quantitative traits expression. In the plant breeding of high yielding varieties of plants, the plant breeder is challenged with the problem of the selection of parents. Rejection of poor yielding crosses on the basis of their performance in early

generation had been recommended, but the knowledge of the genetic architecture of yield will help to sort out the better crosses much efficiently. The analysis given by Griffing [10] do not provide about test to detect epistasis or linkage. Hayman and Jinks' analysis provided such test.

Experimental Materials

The research work comprises genetic analysis, heritability and genetic advance for various quantitative traits in 7 × 7 F1 diallel

cross of *Ocimum* spp. carried out at CSIR-CIMAP, Lucknow. The data obtained from half diallel with nine viz. days to flowering (50%), plant height (cm), branches/plant, Inflorescence length (cm), fresh herb yield (g)/plant, oil content (%), oil yield (g)/plant, camphor content (%), methyl chavicol content (%) (Table 1). Analysis of diallel data for graphical approach (Wr-Vr graph) and genetic components of variation was method of Hayman. Plotting of Wr-Vr graph had done with the help of sigma plotting package.

Parents	Accession Nos.	Cluster	Salient features
P ₁	OC 6	VIII th	Plant dwarf, short duration plant, early maturity, oil content (0.8%), citral rich (72%)
P ₂	OC 15	III rd	Plant dwarf, early flowering, oil content (0.68%), methyl chavicol rich (62.54%)
P ₃	OC 5	III rd	Plant tall, oil content (0.8%), methyl chavicol rich (75.21%)
P ₄	OC 17	I st	Evergreen, long duration plant, late maturity, oil content (0.7%), methyl chavicol rich (85%)
P ₅	OC 10	IV th	Medium height plant, late maturity, methyl chavicol 45% and linalool 32%
P ₆	OC 7	V th	Medium dwarf plant, cold tolerant, linalool rich 71% and oil content 0.8%
P ₇	OC 22	II nd	Tall plant, cold tolaent ever green plant, oil content 0.5%, champhor rich plant

Table 1: Salient features of seven parents of *Ocimum*.

Statistical analysis

For statistical analysis using the Statistical Software 4.0 version, available in the Division of Genetics and Plant Breeding of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India, that is based on the standard methods in Panse and Sukhatme [11] and Singh and Chaudhary [12]. The pooled mean values of two years for all nine characters were subjected to Hayman diallel analysis [13,14].

Estimation of components of variation (Griffing's model and Hayman's model)

Analysis of genetic variance components was carried out by numerical analysis as per Hayman's model. The following statistics were computed.

$$D=V_{OLO}-E \text{ (due to additive gene effect)}$$

$$F=2V_{OLO}-4W_{OLO1}-2(n-2) E/n \text{ (The mean of 'Fr' over the arrays)}$$

$$\hat{H}_1=V_{OLO}-4W_{OLO1}+4V_{1L1}-(3n-2) E/n \text{ (due to dominance effects of genes).}$$

$$\hat{H}_2=4V_{1L1}-4V_{OL1}-2E=H_1[1-(u-v)^2] \text{ (Proportion of positive and negative genes in parents where, u was the proportion of positive genes and v was the proportion of negative genes and } u+v=1).$$

$$\hat{h}^2=4(M_{L1}-M_{LO})^2-4(n-1)E/n^2 \text{ (Dominance effect as the algebraic sum over all loci in heterozygous phase in all crosses).}$$

To test each of these components standard error for each was also calculated. For this first of all common multiplier or variance (=s²) was calculated as per following formula:

$$s^2=(1/2)[\text{Var (Wr-Vr)}]$$

$$s^2=(1/2)[1/n-1\{\sum(Wri-Vri)\}^2-[\sum(Wri-Vri)]^2/n}$$

Now from Hayman's the specific multiplier for each component was calculated, considering n = 7, these are for:

$$D=(n^5+n^4)/n^5$$

$$F=(4n^5+20n^4-16n^3+16n^2)/n^5$$

$$H_1=(n^5+41n^4-12n^3+4n^2)/n^5$$

$$H_2=(36n^4)/n^5$$

$$h^2=(16n^4+16n^2-32n+16)/n^5$$

$$H_1=n^4/n^5$$

Graphical analysis (Wr-Vr graphs)

The validity of the assumptions of diallel theory was tested by 't²' test for uniformity of Vr, Wr values.

$$t^2 = \frac{(n - 2)}{4} \times \frac{(Var Vr - Var Wr)^2}{Var Vr \times Var Wr - Cov^2 (Vr, Wr)}$$

Results and Discussion

Graphical analysis was completed of the experimental data to provided the information about allelic constitution of the parents used in the diallel cross. Confirmation of validity of hypothesis was tested by unit slope of regressions of Wr and Vr and by non-significant value of t^2 as given by Hayman. In this experiment, regression coefficient values (b, wr, vr) for nine characters viz. days to flowering (50%), plant height (cm), branches/plant, inflorescence length (cm), fresh herb yield (g)/plant, oil content (%), oil yield (g)/plant, camphor content (%), methyl chavicol content (%) did not differ significantly from unity indicating the absence of epistasis. This indicated the confirmation of epistasis is absent for all these characters.

The Vr-Vr graphs for all the nine traits have been presented in figures 1-9. The parental array points were scattered along the regression line for approximate all the nine traits. This represents the genetic diversity among all the seven parents for all the character studied. The recessive and dominant alleles present in parent was represented by the distribution of array points by relative proportion.

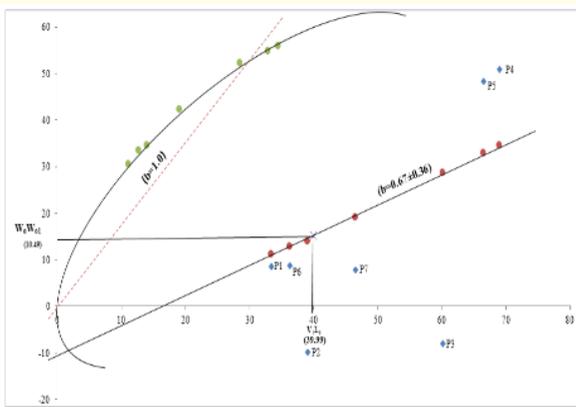


Figure 1: Vr - Wr graph for days to flowering (50%).

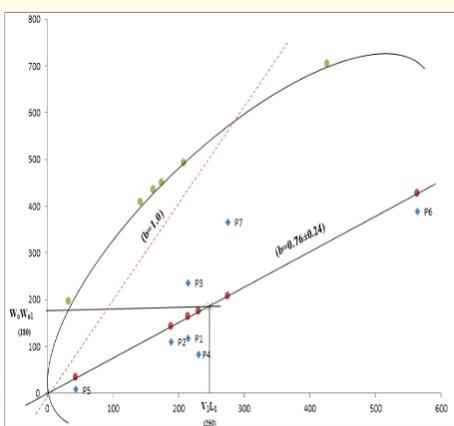


Figure 2: Vr - Wr graph for the plant height.

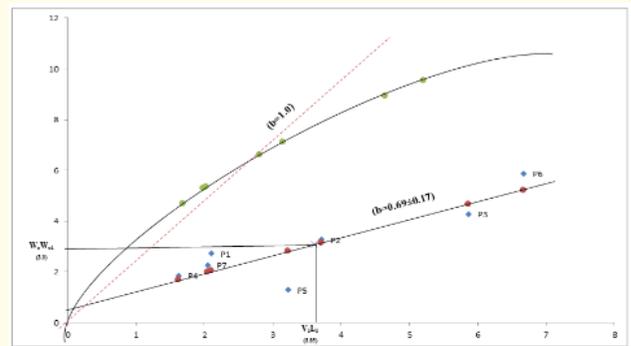


Figure 3: Vr - Wr graph for the branches/plant.

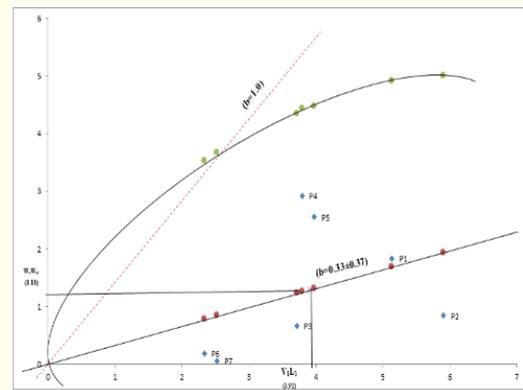


Figure 4: Vr - Wr graph for inflorescence length.

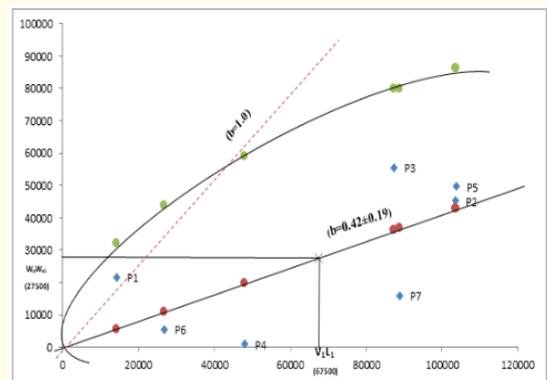


Figure 5: Vr - Wr graph for the fresh herb yield (g)/plant.

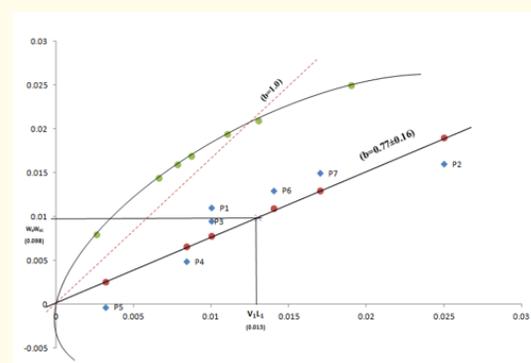


Figure 6: Vr - Wr graph for oil content (%).

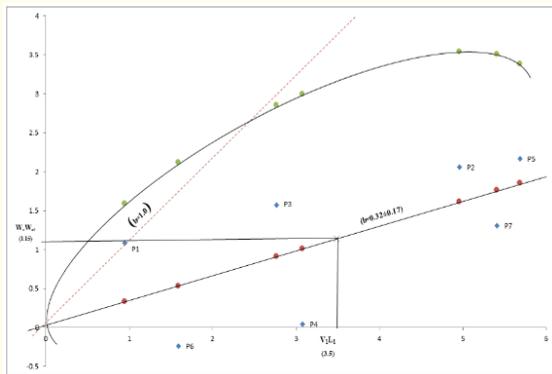


Figure 7: Vr - Wr graph for oil yield (g)/plant.

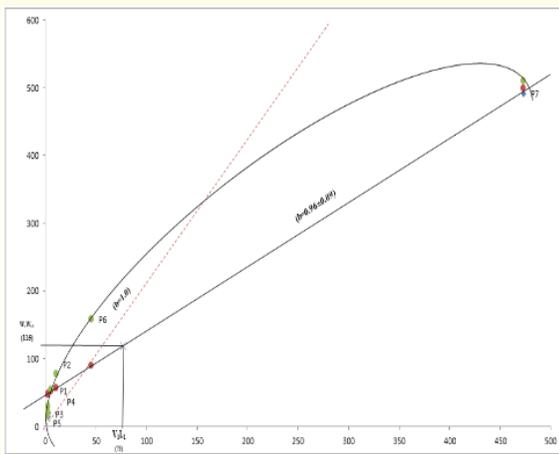


Figure 8: Vr - Wr graph for camphor content (%).

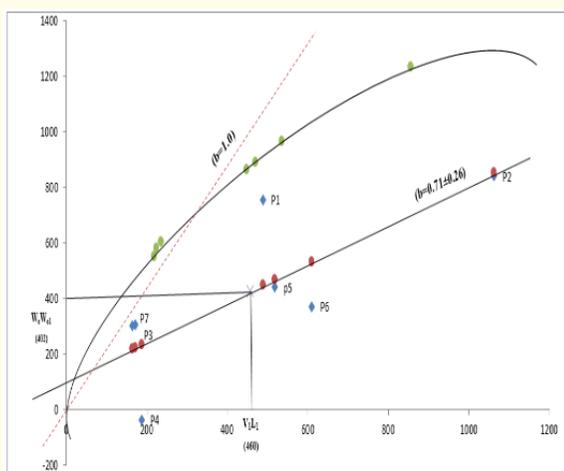


Figure 9: Vr - Wr graph for methyl chavicol (%).

The regression line crossed through the Wr-axis above the origin showing partial dominance for branches/plant, camphor content (%) and methyl chavicol content (%). While for characters like days to flowering 50% and days to maturity the regression line cut the Wr-axis just below the origin pointed out over dominance effect. Whereas, plant height, inflorescence length, fresh herb yield, oil content and oil yield indicate complete dominance. The scattered array point on the regression indicates diversity along with parents.

In the present study, the nature of genetic variance component and type of gene actions were determined. GCA mean squares were invariably higher than SCA mean squares for all the characters except days to maturity. The additive gene action seemed to be mainly responsible for the expression of yield and its components. But a different picture emerged when the estimates of variance due to GCA ($\hat{\sigma}^2_g$) and SCA ($\hat{\sigma}^2_s$) were computed and $\hat{\sigma}^2_g/\hat{\sigma}^2_s$ was compared with GCA/SCA. The magnitude of $\hat{\sigma}^2_s$ was higher than that of $\hat{\sigma}^2_g$ for all the characters indicating the preponderance of non-additive gene action of greater significance than additive action for these characters (Table 2). Apparently, GCA/SCA did not always project the true picture of the gene action for a particular character.

Nevertheless, by use of Hayman's model, the Additive component (\hat{D}) was significant for all the characters. The estimates of dominance component (\hat{H}_1) were also significant for all the characters (Table 3). This suggested the importance of both additive and non-additive components [15]. The overall dominance effect (\hat{h}_2) indicated that substantial heterozygosity in the parents was present for the traits (days to flowering (50%), branches/plant, linalool content and camphor content) showing significant \hat{h}_2 estimates. Symmetry of distribution of dominant and recessive alleles in parents indicated $F > 0$ for days to flowering 50%, plant height, branches/plant, inflorescence length, fresh herb yield, days to maturity, linalool content, camphor content and methyl chavicol content meaning thereby prevalence of dominant alleles whereas $F < 0$ for oil content, oil yield and ocimene content means recessive alleles were more prevalent than dominant alleles [16-18].

Narrow sense heritability estimates \hat{h}^2 (ns) % ranged from 7.9% (lowest) for inflorescence length to 59% (highest) for oil content. It was high for camphor content (52%), methyl chevicol content (50%), fresh herb yield (45%) plant height (39%), ocimene content (37%), branches/plant (34%) and days to flowering 50% (30%). Other characters oil yield (29%), linalool content (25%) and days to maturity (17%) were moderately heritable.

Variance component	DF	PH	NB	IL	HY	OC	OY	DM	OM	LI	CA	MC
$\hat{\sigma}_g^2$	2.72	34.23	0.83	0.048	19315.75	0.004	0.49	-1.29	0.67	2.59	19.59	111.17
$\hat{\sigma}_s^2 = \hat{\sigma}_D^2$	36.63	192.53	1.17	2.39	32170.03	0.007	1.91	44.69	4.58	122.87	57.09	345.76
$\hat{\sigma}_r^2$	35.05	162.71	2.49	10.06	62166.41	0.016	4.73	45.19	5.86	71.82	11.65	425.8
$\hat{\sigma}_e^2$	1.13	35.88	2.79	2.57	28112.18	0.001	1.89	1.95	0.10	0.23	0.05	0.61
$\hat{\sigma}_A^2$	5.44	68.44	1.67	0.09	38631.51	0.009	0.99	-2.59	1.35	5.19	39.18	222.34
$\hat{\sigma}_g^2 / \hat{\sigma}_s^2$	0.07	0.18	0.71	0.02	0.60	0.57	0.26	-0.03	0.15	0.02	0.34	0.32
$(\hat{\sigma}_s^2 / \hat{\sigma}_g^2)^{0.5}$	3.73	2.37	1.19	7.06	1.29	1.08	1.97	5.89	2.61	6.89	1.71	1.76

Table 2: Estimates of genetic components of variance in 7 × 7 diallel crosses for twelve characters in *Ocimum* (Griffing's model, 1956). Where, DF: Days of flowering (50%), PH: Plant height; NB: Number of branches; IL: Inflorescence length; HY: Fresh herb yield; OC: Oil content; OY: Oil yield/plant; DM: Days to maturity; OM: Ocimene content; LI: Linalool content; CA: Camphor; MC: Methyl chavicol content.

$\hat{\sigma}_g^2$ = g.c.a. variance

$\hat{\sigma}_s^2 = \hat{\sigma}_D^2$ = s.c.a. variance

$\hat{\sigma}_r^2$ = reciprocal variance

$\hat{\sigma}_e^2$ = environmental variance.

Variance component	DF	PH	NB	IL	HY	OC	OY	DM	OM	LI	CA	MC
\hat{D} (Additive component)	43.7**± 15.59	837.6**± 70.78	10.82**± 0.72	2.68**± 1.04	43018.61**± 20323.7	0.023**± 0.002	1.98**± 1.11	49.42**± 16.90	1.86**± 3.59	160.36**± 85.17	549.22**± 27.62	1531.25**± 158.33
\hat{E} (Environmental component)	1.47± 5.51	38.76± 25.02	2.8± 0.25	2.66± 0.37	28528.98± 7185.51	0.001± 0.0008	1.89± 0.39	1.94± 5.98	0.11± 1.27	0.27± 30.11	0.053± 9.77	0.66± 55.98
\hat{F} (Mean cov. of D and \hat{h}_1)	27.43± 37.41	949.07**± 169.79	10.93**± 1.75	1.71± 2.49	-8599.25**± 4876.14	0.006± 0.005	0.46± 2.67	102.45**± 40.55	0.94± 8.61	168.89± 204.32	621.37**± 66.27	1356.8**± 379.83
\hat{h}_1 (Dominance component)	140.35**± 37.54	1011.92**± 170.39	8.11**± 1.76	8.59**± 2.5	153372.8**± 48929.73	0.032**± 0.005	8.14**± 2.68	226.67**± 40.69	20.06**± 8.64	510.27**± 205.05	378.89**± 66.50	1653.15**± 381.18
\hat{h}_2 (Proportion of +/- genes)	127.91**± 33.08	670.07**± 150.14	4.09**± 1.55	8.22**± 2.2	112090**± 43113.08	0.026**± 0.005	6.73**± 2.36	156.88**± 35.86	16.05**± 7.61	431.19**± 180.68	200.41**± 58.59	1213.58**± 335.87
\hat{h}_2 (Overall dominance effect)	69.86**± 22.21	77.49± 100.84	2.8**± 1.04	-0.66± 1.48	-8011.764± 28956.73	0.0004± 0.003	-0.67± 1.58	13.00± 24.08	3.01± 5.11	203.58± 121.35	145.49**± 39.35	30.27± 225.59

Table 3: Estimates of genetic components of variance in (7 × 7) diallel crosses for twelve characters in *Ocimum* (Hayman's model).

Where, DF: Days of flowering (50%); PH: Plant height; NB: Number of branches; IL: Inflorescence length; HY: Fresh herb yield; OC: Oil content; OY: Oil yield/plant; DM: Days to maturity; OM: Ocimene content; LI: Linalool content; CA: Camphor; MC: Methyl chavicol content. * = p < 0.05; ** = p < 0.01, respectively.

Genetic advance over mean ranged from 0.54 (days to maturity) to 53.11 (branches/plant). It was high for herb yield (50.33), oil yield (28.33), ocimene content (18.09) and camphor content (14.43). Other characters like oil con-

tent (9.69), plant height (9.57), methyl chevicol content (3.44), inflorescence length (3.23), were moderate. Remaining traits like days to flowering (50%) (1.76), and linalool content (0.61) were low (Table 4).

Proportion	DF	PH	NB	IL	HY	OC	OY	DM	OC	LI	CA	MC
$\left(\hat{H}_1/\hat{D}\right)^{0.5}$	1.79	1.09	0.87	1.79	1.89	1.18	2.03	2.14	3.28	1.78	0.83	1.04
$\hat{H}_2/4\hat{H}_1$	0.22	0.17	0.13	0.24	0.18	0.21	0.21	0.17	0.20	0.21	0.13	0.18
$\left\{\frac{1}{4}(4DH_1)^{\frac{1}{2}} + \left(\frac{1}{2}\right)F\right\} / \left\{\frac{1}{4}(4DH_1)^{\frac{1}{2}} - \left(\frac{1}{2}\right)F\right\}$	1.42	3.13	-0.85	1.44	0.89	1.27	1.12	2.88	1.17	1.84	5.27	2.49
Coor(r)=(Wr+Vr).Yr	-0.57	-0.55	3.8	0.24	-0.29	-0.41	-0.12	0.85	-0.61	-0.25	0.98	0.37
r ²	0.33	0.31	0.71	0.056	0.08	0.17	0.015	0.71	0.38	0.062	0.96	0.14
\hat{h}^2/\hat{H}_2	0.55	0.12	0.69	0.08	0.07	0.02	0.09	0.08	0.19	0.47	0.73	0.03
Σ Fri	192.03	6643.5	76.52	12.02	-60194.69	0.045	3.23	717.14	6.56	1182.19	4349.62	9497.59
t ²	0.01	0.073	1.11	0.12	2.88	0.82	5.08	0.035	3.41	3.54	0.0059	0.11
\hat{h}^2 (ns)%	30.00	39.00	34.00	7.90	45.00	59.00	29.00	17.00	37.00	25.00	52.00	50.00
G.A. over mean (%)	1.76	9.57	53.11	3.23	50.33	9.69	28.33	0.54	18.09	0.61	14.43	3.44

Table 4: Proportion of the genetic components of variance, t² values heritability in narrow sense (%) and genetic advance over mean in percent in 7 × 7 diallel crosses for twelve characters in *Ocimum* (Hayman's Model, 1954).

Where, DF: Days of flowering (50%); PH: Plant height; NB: Number of branches; IL: Inflorescence length; HY: Fresh herb yield; OC: Oil content; OY: Oil yield/plant; DM: Days to maturity; OM: Ocimene content; LI: Linalool content; CA: Camphor; MC: Methyl chavicol content.

$\left(\hat{H}_1/\hat{D}\right)^{0.5}$ = Mean degree of dominance.

$\hat{H}_2/4\hat{H}_1$ = Proportion of genes with positive and negative effect in parents.

$\left\{\frac{1}{4}(4DH_1)^{\frac{1}{2}} + \left(\frac{1}{2}\right)F\right\} / \left\{\frac{1}{4}(4DH_1)^{\frac{1}{2}} - \left(\frac{1}{2}\right)F\right\}$ = Proportion of dominant and recessive genes.

Coor (r) = (Wr+Vr).Yr = Coefficient of correlation (r) between the parental order of dominance (Wr+Vr) and parental measurement Yr

r² = Prediction for measurement of completely dominant and recessive parents,

\hat{h}^2/\hat{H}_2 = Set of genes that control a character and exhibit dominance.

Fr =The covariance of additive and dominance and dominance effects in a single array

t² = Test of validity of hypothesis

\hat{h}^2 (ns)% = Heritability in narrow sense in percent

G.A. = Genetic advance.

Conclusion

Hayman's graphical approach to diallel analysis is based on monogenic additive model. Hence, the first requirement is to test the adequacy of this model by detecting whether non-allelic interaction (epistasis) is present or absent. The t^2 for all the characters was non-significance indicated validity of the hypothesis given by Hayman (1954). The regression line for days to flowering (50%), days to maturity were below the origin indicating the presence of over-dominance while for branches/plant, ocimene content, linalool content, camphor content and methyl chavicol it was above the origin suggesting the presence of partial dominance. The regression line for plant height, inflorescence length, fresh herb yield, oil content and oil yield were through the origin indicating the presence of complete dominance. genetic advance was highest for branches/plant and herb yield. Based on this genetic information in this study, hybridizations, evaluation and isolation of superior genotypes by sib selection and recurrent selection, exploitation of hybrid vigor in specific parent cross-combinations are suggested for *Ocimum* crop improvement.

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