Atatürk Üniv. Ziraat Fak. Derg.
33 (4), 405-412, 2002

Comparison of Individual Randomisation and Plot Design For Assessing Genetic and Environmental Variation in *Nicotiana rustica*

İrfan OZBERK
Field Crop Dept. Of Fac. Of Agriculture,.63040 Şanlıurfa.,Turkey

Harpal Sing POONI
The School of Biological Science, Univ.of Birmingham,UK

Fethiye OZBERK
Training, Extension and Research Center. Şanlıurfa., Turkey

Geliş Tarihi : 05.04.2002

ABSTRACT : This study aimed to investigate the effects of individual plant and plot randomizations on the expression of genetic variability for five characters among the generations of a selfing series which have been derived from a cross between V_2 and V_{12} pure breeding varieties of *Nicotiana. rustica.* A hierarchical analysis of variance performed on the generations of the selfing series for each of the five characters (Plant height in fourth week of growing=H_4, Plant height in sixth week of growing = H_6, Plant height in seventh week of growing=H_7, Lenght of the longest leaf =LL, Width of the widest leaf = LW) detected the absence of any maternal and paternal effects and the presence of genetic differences between families of F_3 and $F\infty$ (purely inbreed) generations in both designs. The means of the F_1 generation fell within the parental range for H_4, H_6 and H_7 suggesting absence of heterosis while it fell outside of the parental range for LL and LW suggesting presence of heterosis and non-additive effects. C and D scaling tests suggested the absence of non-allelic interactions for almost all the characters. Bartlett's test showed the presence of genotype x micro environmental interactions for almost all the characters. Heritability estimates varied between traits but not between designs. The weighted least squares method was used to estimate parameters from both the first and second-degree statistics. In most cases, simple m, [d], [h] and D, E and Ep (plot effects) models explained the variation adequately. Variance ratio tests showed that within plot variances were significantly smaller than the comparable within family variances of individual randomization for most trials. When tested by model fitting, E and Ep adequately explained the within family variances of $F\infty$ (fully inbreed) generation. In general the genetic components of variation were not found to be differing critically between the two designs.

Key Words: *N. rustica*, plot and individual randomization, first and second degree statistics

Tönbekilik Tütünde Genetik ve Çevresel Varyasyonun İncelenmesinde Tek Bitki ve Parsel Randomizasyonun Karşılaştırılması

ÖZET :Bu çalışmada V_2 ve V_{12} isimli tömbekilik tütünden türetilen kendileme generasyonlarında 5 değişik karakterin ortaya çıkması üzerine tek bitki ve parsel randomizasyonunun etkilerinin araştırılması amaçlanmıştır. H_4, H_6, H_7 , LL ve LW karakterleri için kendileme generasyonlarında yapılan hiyerarşik varyans analizleri sonunda anaya ve babaya bağlı etkilerin olmadığı, F_3 ve $F\alpha$ generasyonlarında aileler arası genetik farklılıkların bulunduğu anlaşılmıştır. F_1 ortalama değerleri H_4, H_6, ve H_7 için ana ve babaya ait değerlerin arasında bulunurken LL ve LW için ise ana ve baba değerleri dışında değerler vererek heterosisin varlığına işaret etmiştir. C ve D skala testleri bütün karakterler için allelik olmayan interaksiyonların olmadığını göstermiştir. Barttlet's testi ise tüm karakterler için genotip x çevre interaksiyonlarının varlığını tespit etmiştir. Dar anlamda kalıtım dereceleri tahminleri tüm karakterler için farklı tespit edilmiş ancak randomizasyona bağlı bir değişme olmamıştır. Ağırlıklı en küçük kareler yöntemi her karakter için 1. ve 2. derece istatistiklerin tahmininde kullanılmış, basit eklemeli- dominans model (m, [d], [h]) ortalama komponentlerini yeterince açıklamıştır. D ,E ve Ep den oluşan model de varyans komponentlerini yeterince açıklamıştır. En uygun model tespitinde E ve Ep , $F\alpha$ generasonunda aile içi varyansı yeterince açıklamışlardır. Genel olarak genetik varyans komponentleri her iki randomizasyon modelinde de değişmemiştir.

Anahtar kelimeler: Tönbekilik tütün, tek bitki ve parsel randomizasyonu, 1. ve 2. Derece istatistikler

INTRODUCTION

Several researchers have discussed genetic variance in populations of predominantly inbreeding species. Hillel, et al., (1971) developed the expectations for variances and covariance's of genetic parameters. No assumption has been made in their study concerning the rate of inbreeding and the gene frequencies in the population. Technically, the simplicity of the selfing series scheme allows larger experimental size than any other breeding scheme. But is was also pointed out that this scheme was less sensitive for detecting and estimating the genetic parameters, especially the dominance variance than some other schemes such as diallel and test cross series. Estimates of the heritable and non-heritable variations can be obtained from the means and variances of families generations that have been produced by selfing (Mather and Jinks, 1982). The contribution of the heritable source of variation are determined solely by gene and genotype frequencies and those of the non heritable sources by the experimental design and in particular the unit of randomization (Jinks, 1979)

This study aimed at investigating the effects of individual plant and plot randomizations on the expression of genetic variability among the generations of a selfing series that have been derived from a cross between a pair of pure breeding varieties of *N. rustica.*

The statistics, obtained from the two design will be compared using t-test, F-test or Bartlett's test; as required and components of the heritable and non-heritable variations will be estimated.

MATERIALS AND METHODS

Experimental Procedures

P_1, P_2, F_1, F_2, F_3 and $F\infty$ generations derived from highly inbred V_2 and V_{12} parents of *N. rustica* were raised in two blocks. Individual plant randomization in block 1 and plots of 5 plants in block 2 were tested.

In both randomizations, name of generations, number of families, family size, number of plot/family and total number of plants were given in Table1.

The experiment was carried out in the main experimental field of the school of Biological Sciences of University of Birmingham, UK in 1991.Seeds were sown in a peat pots, initially. The pots with seeds were kept covered with thin later of muslin. After thinning, they were taken in to cold frame and hardened. After hardening, experiments were transplanted to the field. Plants were planted at 25 cm. distance within rows and rows kept 50 cm. apart. All other experimental procedures were followed as required. Plant heights 4, 6 and 7 weeks after transplanting, leaf length and width were scored.

Analysis Procedures

A hierarchical analysis of variance was performed on the various generations to detect the presence or absence of genetic and environmental variations.

The scaling tests of Mather (1949) with C= 4 $\bar{F}_2$ - 2 $\bar{F}_1$ - $\bar{P}_1$ - $\bar{P}_2$ and D= 4 $\bar{F}_3$ - 2 $\bar{F}_2$ - $\bar{P}_1$ - $\bar{P}_2$ were performed to detect the presence of non-allelic interactions on generation means In the absence of back cross families A and B scaling tests were not used.

A joint scaling test attributed to Cavalli (1952) as well as Mather and Jink (1971) was also conducted to test adequacy of 3 parameters model.

Generation means for each character were further investigated by the method of Mather and Jinks,(1982) to fit a 6-parameter model.

The statistics used in model fitting were given in Table 2.

Bartlett's test was performed to test for presence of micro environmental interactions.

Presence of micro environmental interactions was detected through Bartlett's test., there fore F_3 and $F\infty$ generations with homogenous within family variances items were taken into account for estimates of variance components (D, H, E). In model fitting by weighted least squares of Hayman (1960 a). Second degree statistics,used in model fitting were given in Table 3.

Table 1.Name of Generations, Number of Families, Family Size, Number of Plot/ Family and Total Number of Plants

Generations	Number of Family (I.R and P.R)*	Family size (I.R)	Number of Plot/Fam. (P.R)	Family size (P.R)	Total Number of Plants (I.R and P.R)
P_1	1	20	4	5	20
P_2	1	20	4	5	20
F_1	1	20	4	5	20
RF_1	1	20	4	5	20
F_2	2	40	8	5	40
RF_2	2	40	8	5	40
F_3	10	10	2	5	100
RF_3	10	10	2	5	100
$F\infty$	10	10	2	5	100
$RF\infty$	10	10	2	5	100

I. R: Individual Randomization, P. R: Plot Randomization

Table 2. The First Degree Statistics, used in Model Fitting

Generations	Parameters				
	m	[d]	[h]	[i]	[l]
P_1	1	1	0	1	0
P_2	1	-1	0	1	0
F_1	1	0	1	0	1
F_2	1	0	½	0	¼
F_3	1	0	¼	0	1/16
$F\infty$	1	0	0	1	0

Table 3. Second Degree Statistics, Used in Model Fitting

Individual Randomization					Plot Randomization					
Gen.Means Square	DF	D	H	E	Gen.Means Square	DF	D	H	E	Ep
F_3					Bet. Fam	18	5.25	0.75	1	5
Bet. Fam	18	5.25	0.75	1	Bet Plot	20	0.125	0.0625	1	5
Within Ind	180	0.25	0.125	1	Bet Ind.	160	0.125	0.0625	1	0
$F\infty$					Bet. Fam	18	10	0	1	5
Bet. Fam	18	10	0	1	Bet Plot	20	0	0	1	5
Within Ind	180	0	0	1	Bet Ind.	160	0	0	1	0

The heritability estimates were calculated as described below;

Ind. Randomizations

$h_2n = ½D/(½D + ¼H + E) = VA/VF_2$

Plot Randomization

$$h_2n = ½D/(½D+¼H + Ew + Ep) = \frac{VA}{VF_2+Ep}$$

Where;
h_2n: narrow heritability
D: Additive comp of variation
H: Dominance comp of variation
E: Environmental comp of variation
Ew: Environmental variance within plots
Ep: Environmental variance due to plots

Comparisons of the first degree and second-degree statistics, obtained from two different randomizations were tested employing t-test and variance ratio test.

RESULTS AND DISCUSSION

Preliminary Analysis of Variance

The results of analysis of variance performed on each character for individual and plot randomization were given in Table 4 a and b respectively.

There was no significant difference between reciprocals in all generations except for a few case-for all characters. This meant the absence of any maternal or paternal effects.

Between families/within reciprocals item for F_3 and $F\infty$ generations were found to be significant for all characters.

The results indicated that there were genuine genetic differences between families.

For H_6 and H_7, means of F_1 generations fell out of parental range suggesting the presence of some dominance effects. Means of the F_1 generations for the rest of the characters fell within parental range indicating absence of dominance effects.

There was no significant difference between reciprocals suggesting the absence of any maternal effects for all characters in all generations.

Between fam./within reciprocals item for F_3 and $F\infty$ generations turned out to be significant indicating the presence of genuine genetic differences among families for all characters.

Between plot/between family/within reciprocals items turned out to be either significant or in significant for all characters and generations indicating presence of some environmental effects to the plots.

For LL and LW, means of F_1 generation fell out of parental range indicating presence of some dominance effects.

Table 4 a. Mean Squares From The Analysis of Variance of Selfing Series for 5 Characters of V_2 x V_{12} Cross of *N. rustica* ,Individual Randomization

Source	df	H_4	H_6	H_7	LL	LW
F_1						
Bet reciprocals	1	0.015	0.254	8.750	26.020	16.004
within ind.	37	4.889	4.889	149.050	4.090	11.323
F_2						
Bet reciprocals	3	6.415	288.285*	593.229**	24.747	13.74
within ind.	76	4.032	89.9	202.935	10.342	10.719
F_3						
Bet reciprocals	1	5.445	39.205	386.420	186.245	14.045
Bet fam./within rec	18	24.74**	563.117**	1130.76**	74.866**	94.646**
Within ind./b.fam/w. rec.	180	4.162	91.396	178.016	7.969	10.427
$F\infty$						
Bet reciprocals	1	0.605	30.504	1180.98	419.465	347.737
Bet fam./within rec.	18	53.50**	956.954**	1432.129**	217.549**	246.815**
within ind./b.fam/w. rec.	180	3.73	59.817	88.64	7.620	8.44

C and D Scaling Test

Results of C scaling test except for one character (LL) turned out to be non-significant indicating absence of any epistatic effects on generation means. D scaling test confirmed above results giving non-significant "t" values for all characteristics (Table 5 a and b).

Table 4 b Mean Squares From The Analysis of Variance of Selfing Series for 5 Characters of V_2 x V_{12} Cross of *N. rustica* ,Plot Randomization

Source	df	H_4	H_6	H_7	LL	LW
P_1						
Bet Plots	3	1.383	17.93	4.932	1.40	0.85
Within ind.	16	0.675	5.825	11.125	1.85	1.4
P_2						
Bet Plots	3	8.80*	115.065**	188.069*	0.315	9.25
Within ind.	16	1.80	16.72	42.10	3.375	4.175
F_1						
Bet reciprocals	1	0.90	11.023	19.50	2.023	0.023
Bet Plots/within rec.	6	1.25	31.157	146.747**	15.75*	28.080**
Within ind./b. plot /w. rec.	32	2.00	37.43	85.25	5.4	7.95
F_2						
Bet reciprocals	3	0.653	115.709	460.6	19.14	2.591
Bet Plots/within rec.	12	6.98*	186.07**	490.33**	27.18	39.590
Within ind./ b plot /w. rec.	63	2.29	68.781	158.95	17.48	22.189
F_3						
Bet reciprocals	1	41.60	1416.344	3956.356	149.534	1416.35**
Bet fam./within rec.	18	10.819*	627.53**	1715.59**	71.812**	92.44**
Bet P./bet fam/w.re	20	4.43**	101.03**	205.658*	7.363	6.9
Within ind./b. fam/w. rec.	159	2.264	54.00	124.303	8.88	10.02
F_∞						
Bet reciprocals	1	8.160	9.0	298.005	272.25	308.755
Bet fam./within rec.	18	39.308**	1060.220**	2230.49**	198.78**	211.178**
Bet P./ bet fam/w.re	20	3.625**	60.618**	80.479	8.64	12.646
Within ind./B. Fam/W. rec.	156	1.74	33.294	75.85	5.56	8.156

Table 5a. C and D Scaling Tests: Individual Randomization

C: $4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2$ VC: $16V\bar{F}_2 + 4V\bar{F}_1 + V\bar{P}_1 + V\bar{P}_2$

D: $4\bar{F}_3 - 2\bar{F}_2 - \bar{P}_1 - \bar{P}_2$ VD: $16V\bar{F}_3 + 4V\bar{F}_2 + V\bar{P}_1 + V\bar{P}_2$

$t_{1\,(76)} = C/\sqrt{VC}$ $t_{2\,(147)} = D/\sqrt{VD}$

CHARACTERS	C	D	VC	VD	t_1	t_2
H_4	-1.89	1.366	9.201	2.725	ns	ns
H_6	-8.872	5.46	36.125	59.293	ns	ns
H_7	-13.658	4.65	67.800	112.96	ns	ns
LL	-3.60	-2.74	3.160	7.096	2.030*	ns
LW	0ç898	2.74	4.017	8.793	ns	ns

Table 2b C and D Scaling Tests: Plot Randomization

CHARACTERS	C	D	VC	VD	t_1	t_2
H_4	0.210	0.372	2.216	1.696	ns	ns
H_6	1.686	-4.598	48.292	66.260	ns	ns
H_7	-5.588	-9.536	116.580	166.060	ns	ns
LL	-6.628	-4.542	5.66	6.988	2.786**	ns
LW	-2.800	-0.040	8.005	8.99	ns	ns

Estimates of Parameters from Generation Means

The components of generation means were estimated by weighted least squares method. Data used in that analysis is given in Table 6. Results of the two randomizations are given in Table 7.

The objective of the model fitting was to obtain simplest model to adequately describe the generation means for a particular character and determine the importance and magnitude of the various genetic components.

Results indicated that simple additive-dominance model (m, [d], [h]) except for one case (LL for plot ran.) was found to be adequate with a non-significant χ^2 value for all characteristics. Results confirmed the absence of any epistatic effect in both randomizations.

Table 6. Data, Used for Weighted Least Squares Method for Either Randomisation.

	Individual Randomization			Plot Randomization		
H_4	n	$\bar{X}$	W= 1/V $\bar{X}$	n	$\bar{X}$	W= 1/V $\bar{X}$
Generations						
P_1	20	3.50	18.818	20	3.050	25.412
P_2	20	8.20	2.008	20	6.200	2.272
F_1	39	6.769	8.191	40	5.700	12.277
F_2	80	5.837	19.450	79	5.215	11.318
F_3	200	6.185	8.074	199	5.013	18.393
$F\infty$	200	6.365	3.738	196	5.020	4.986
H_6						
Generations						
P_1	20	22.50	0.615	20	18.500	2.583
P_2	20	53.000	0.122	20	43.400	0.173
F_1	39	47.026	0.476	40	39.725	0.890
F_2	80	39.425	0.889	79	35.759	0.424
F_3	200	39.205	0.355	198	32.205	0.315
$F\infty$	200	35.615	0.208	196	28.445	0.184
H_7						
Generations						
P_1	20	42.5	0.451	20	34.60	1.971
P_2	20	90.45	0.097	20	80.100	0.475
F_1	39	84.487	0.268	40	76.000	0.272
F_2	80	72.063	0.394	79	65.278	0.161
F_3	200	70.430	0.174	198	58.930	0.115
$F\infty$	200	62.72	0.139	196	52.630	0.087
LL						
Generations						
P_1	20	26.95	7.176	20	27.100	11.242
P_2	20	28.95	2.331	20	30.450	6.915
F_1	39	33.154	9.535	40	33.375	2.539
F_2	80	29.65	7.346	79	29.418	4.148
F_3	200	28.115	2.671	199	27.961	2.757
$F\infty$	200	27.078	0.914	196	27.420	0.986
LW						
Generations						
P_1	20	21.10	4.051	20	21.450	15.232
P_2	20	23.85	2.283	20	27.150	4.019
F_1	39	31.026	3.407	40	31.025	1.484
F_2	80	26.975	7.383	79	26.962	3.276
F_3	200	25.415	2.113	199	25.622	2.141
$F\infty$	200	22.938	0.806	196	23.408	0.928

Table 7. The Estimates of Components of Mean of Generations Plot and Individual Randomization

PARAMETERS	m	[d]	[h]	[i]	df	χ^2 (chi sg)
H_4						
Ind. Rand.						
Value	6.100	2.550	0.593 ns		4	5.783 ns
Std. Error	0.140	0.250	0.445 ns			
Plot Rand.						
Value	4.770	1.690	0.920		3	0.490 ns
Std. Error	0.200	0.250	0.370			
t test	4.64***	2.432**	0.556 ns			
H_6						
Ind. Rand.						
Value	35.53	13.270	10.440		3	2.709 ns
Std. Error	1.090	1.030	1.870			
Plot Rand.						
Value	30.450	12.010	9.360		3	0.980 ns
Std. Error	0.950	1.030	1.490			
t test	3.62**	0.74 ns	0.45 ns			
H_7						
Ind. Rand.						
Value	67.79	22.940	18.42		3	3.610 ns
Std. Error	1.270	1.560	2.36			
Plot Rand.						
Value	59.90	22.48	18.540		3	2.900 ns
Std. Error	0.760	0.79	1.980			
t test	5.33***	0.26 ns				
LL						
Ind. Rand.						
Value	27.270	0.650	5.66	2.815 ns	3	6.452 ns
Std. Error	0.220	0.348	0.390	1.581 ns		
Plot Rand.						
Value	25.930	1.650	7.33	2.760	2	1.980 ns
Std. Error	0.710	0.240	1.130	0.750		
t test	1.80 ns	2.40**	1.39 ns	1.950		
LW						
Ind. Rand.						
Value	22.69	1.43	8.46		3	1.178 ns
Std. Error	0.33	0.4	0.64			
Plot Rand.						
Value	24.17	2.77	6.34		3	1.630 ns
Std. Error	0.25	0.27	0.75			
t test	3.57***	2.77**	2.15*			

ns: non-significant *: $p \leq 0.05$ significant **: $p \leq 0.01$ significant ***: $p \leq 0.001$

Analysis of Second Degree Statistics

Bartlett's Test

Bartlett's test was used to detect heterogeneity among the variance of the non-segregating generations.

Results, obtained from significant χ^2 values indicated the presence of micro environmental variations for H_4, H_6, H_7 and LL characteristics.

Whereas variances of non-segregating generations of LW were found to be homogenous in individual randomization.

Table 8. χ^2 Values of Bartlett's Test for Either of Two Randomization

CHARACTER	df	χ^2 (Ind. Ran.)	χ^2 (Plot Ran.)
(P_1, P_2, F_1) H_4	2	19.264***	5.40 ns
H_6	2	11.267***	14.779***
H_7	2	10.226***	16.510***
LL	2	6.6**	5.42 ns
LW	2	3.940 ns	12.670***

These tests showed significant chi-squared values for H_6, H_7 and LW while those for H_4 and LL turned out to be non-significant in plot randomization.

χ^2 values of Bartlett's test for either of two randomization were given in Table 5.

Estimates of Components of Variance

The weighted least squares analysis were performed and the results were presented in Table 9.

(i) Individual Randomization;

For the H_4, H_6, LL and LW, a model, containing D and E components was adequate. For H_7, the full D, H and E model was found to be adequate with a non-significant χ^2 value.

(ii) Individual Randomization;

For H_4 and H_6, the model with D, E and Ep parameters was adequate suggesting the presence of plot variation in addition to additive and environmental variation. For H_7 LL and LW, the model, containing D and E parameters was found to be adequate.

Heritability

In individual randomization, in most cases, estimates of H were non significant, therefore variance of F_2 was taken into account for heritability calculations in the plot randomizations.

Ep was also considered (if significant in model fitting).

h^2n for H_4 (Ind. Rand.)= ½ D/VF_2 = 0.475
h^2n for H_4 (Plot Rand.)= ½ D/(VF_2 + Ep) = 0.338
h^2n for H_6 (Ind. Rand.)= ½ D/VF_2 = 0.517
h^2n for H_6 (Plot Rand.)= ½ D/(VF_2 + Ep) = 0.500
h^2n for H_7 (Ind. Rand.)= ½ D/VF_2 = 0.31
h^2n for H_7 (Plot Rand.)= ½ D/VF_2 = 0.512
h^2n for LL (Ind. Rand.)= ½ D/VF_2 = 0.443
h^2n for LL (Plot Rand.)= ½ D/VF_2 = 0.375
h^2n for LW (Ind. Rand.)= ½ D/VF_2 = 0.661
h^2n for LW (Plot Rand.)= ½ D/VF_2 = 0.297

Table 9. The Estimates of Components of Variance in Both Design.

PARAMETERS	D	H	E	Ep	df	χ^2(Chi.Sq)
H_4						
Ind. Rand.						
Value	3.910		3.49		2	1.377
Std. Error	1.014		0.303			
Plot Rand.						
Value	2.482		1.706	0.388	3	2.529
Std. Error	0.631		0.165	0.177		
t test	ns		5.170***			
H_6						
Ind. Rand.						
Value	100.766		61.898		2	0.489
Std. Error	21.465		5.399			
Plot Rand.						
Value	95.588		32.491	6.900	3	0.512
Std. Error	18.969		3.397	3.467		
t test	ns		4.61**			
H_7						
Ind. Rand.						
Value	134.442	446.347	88.634		1	0.000
Std. Error	41.369	182.275	8.804			
Plot Rand.						
Value	226.908		75.034		4	2.270
Std. Error	44.387		7.598			
t test	ns		ns			
LL						
Ind. Rand.						
Value	9.450		6.673		2	6.806
Std. Error	2.901		0.656			
Plot Rand.						
Value	18.315		5.634		4	4.693
Std. Error	2.711		0.542			
t test			ns			
LW						
Ind. Rand.						
Value	14.342		7.817		2	3.065
Std. Error	3.527		0.749			
Plot Rand.						
Value	12.086		7.488		4	6.983
Std. Error	2.492		0.780			
t test	ns		ns			

Comparison of the First and Second Degree Statistics, Obtained From Either of Two Randomization.

Within family variances of both designs were compared by variance ratio tests. Highly significant variance ratio values agreed that E component of plot randomization was splitted in to E and Ep for H_4, H_6 and H_7 but not LL and LW.

Estimates of the components of generation means obtained from either of design were compared by "t" test. m and [h] differed significantly for all characters. [d] differed significantly for H_4, LL and LW only.

The estimates of D and E obtained from two designs were also compared by t test. None of D values differed significantly. Estimates of E differed significantly for H_4 and H_6 only.

Differences between E values were further investigated by model fitting to determine of E of ind. rand. was indeed sum of E and Ep in F∞ generation. It was found that E and Ep were both significant for H_4 and

H_6. It was concluded that E and Ep components of plot rand. did not add up to the E of individual randomization.

REFERENCES

Cavalli, L.L. (1952). In Quantitative Inheritance (E.C.R. Reeve and C.H Waddington, Eds) pp. 135-144 H.M.S.O

Hayman, B.I. , (1960 a) Maximum likelihood estimates of genetic components of variation. Biometrics16;369-381.

Hillel, J., Simchen, G. and Jinks, J.L. (1971). Generalized Selfing as an Experimental Design, Heredity 1972, Vol. 3, no 4.

Jinks, J.L. (1979). The Biometrical Approach to Quantitative Variation Heredity 1979, (1,3)

Jinks, J.L. , Pooni, H.S. (1984). Non Heritable Source of Variation .Heredity 53, 299-308.

Mather, K. (1949). Biometrical Genetics. Methuen, London.

Mather, K.., Jinks, J.L. (1982). Biometrical Genetics. Chapman and Hall London.

Montgomery, D.C. (1984). Design and Analysis of Experiments, John Wiley And Sons.

Ozberk, I. (1991). Comparison of individual and plot randomizations for Assessing genetic and environmental variation in N. Rustica. M. Phil. Thesis. Univ. Of Birmingham, U.K.

Steel, R.G.D. ,Torrie, J. H. (1981). Principles and Procedures of Statistics, Mc. Graw-Hill Book Company.