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The nature of gene action in a *Nicotiana rustica* cross revealed by the recombinant inbred and second-cycle hybrid analysis

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Abstract A unique set of data recorded on 60 randomly extracted single-seed-descent (F_∞) lines of a highly heterotic cross between two varieties of *Nicotiana rustica* and their 870 reciprocally produced pairwise crosses, the second-cycle hybrids (SCH), are analysed to investigate the true nature of genetical control in the cross and the results are compared with those in earlier publications. The analysis revealed that epistasis, genotype-by-micro-environmental interaction, maternal effects and linkage are significant for several characters and the additive and non-additive components of variation take large values for all of the traits. Epistasis is predominantly duplicate and not complementary. Dominance is high but partial, all estimates of dominance ratio lying between 0.5 and 0.9. Dominance is predominantly unidirectional for leaf length, leaf width and final height, while for the remaining traits, some genes show ambidirectional dominance, although the incidence of unidirectional dominance is much higher throughout. The direction of dominance is predominantly for the increased score, except for flowering time where alleles conferring earliness are up to five times more frequently dominant. The present study has also confirmed that the F_2 and SCH_i distributions are very similar and that the former can be used to predict the transgression in the latter with confidence. The reduced range of the SCH_i families compared to the recombinant inbreds, further indicated that heterosis among many of the SCH_i is due to gene dispersion and there is little evidence for the presence of over-dominance.

Key words Dominance ratio · Genetic variation · Heritability · Non-allelic interaction · Transgression

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Introduction

The V2 × V12 cross of *Nicotiana rustica* was initially chosen for quantitative genetic analysis by Jinks (1954) because it displayed a highly complex inheritance pattern; it showed a high level of heterosis, epistasis, unidirectional dominance and maternal effects, while the parents displayed what was then considered to be a high gene association and an unusual combination of early flowering with increased height and low environmental sensitivity (Brumpton et al. 1977; Jinks 1954; Jinks et al. 1977). It was thus considered an important model cross with which to study the genetical control of heterosis. Analyses of the six basic generations (Pooni and Jinks 1981, 1982), triple-test-cross families (Jinks and Perkins 1970; Pooni and Jinks 1983 a, b) and selfed generations (Jinks and Pooni 1980), not only illustrated the power of the quantitative genetic techniques in revealing the underlying genetic basis of metrical variation but also allowed predictions to be made of the inbreeding and outcrossing potentials of this cross (Toledo et al. 1984 a, b; Pooni et al. 1985). However, results of many of these studies are yet to be validated from the products derived from this cross, viz., its descendent recombinant inbred lines ($F_{\infty i}$) and their pairwise crosses, the second-cycle hybrids (SCH_i). In this paper, we undertake a comprehensive analysis of the genetic variability displayed by the V2 × V12 cross by analysing data which are rarely available to the quantitative geneticist. These data were recorded on 946 families, which included large samples of the $F_{\infty i}$ and SCH_i families as well as all reciprocals of the six basic generations (P_1, P_2, F_1, F_2, $BC_{1.1}$ and $BC_{1.2}$) of the V2 × V12 cross, all evaluated in a single experiment. In addition to the estimation of various genetic components of variation and the detection of complications like epistasis, maternal effects, linkage and genotype by environment interactions, the nature of dominance variation is studied by several methods and

the transgressive characteristics of the $F_{\infty i}$ and SCH_i families are investigated in detail.

The experiment

The second-cycle hybrids were produced by the pairwise (diallel) crossing of 30 recombinant ($F_{\infty i}$) inbred lines. These lines were a random sample from the 60 $F_{\infty i}$ families that were extracted from the V2 × V12 cross during the early seventies by the method of single-seed descent (Jinks et al. 1977) and have been selfed continuously every year since then. Seeds of all 870 SCH_i and 60 $F_{\infty i}$ were produced during the same season by hand pollination to avoid the effects of seed age/maturity. Two plants of each line were used in the crossing programme; one was arbitrarily designated the female parent and therefore bore the capsules of 29 crosses and one self, and the second provided pollen for crossing with other lines. Multiple pollinations on a single plant are possible in *N. rustica* because each plant produces several flushes of excellent-quality flowers and capsule size, and pollination times generally do not significantly affect the performance of the progeny (Pooni et al. 1996).

The crosses were divided into two reciprocal sets of 435 families each and each set was raised in a separate block with 60 $F_{\infty i}$ lines plus 16 reciprocally produced families of the six basic generations [P_1, P_2, F_1(1x2), RF_1(2x1), $4F_2$s, $4Bc_{1.1}$s and $4Bc_{1.2}$s) of the V2 × V12 cross. Each block had 3290 individually randomised plants consisting of six sibs of each SCH_i and $F_{\infty i}$ family and 20 each of 16 families of the basic generations. The experiment was conducted during the summer of 1985 and both blocks were raised side by side. All plants were scored for the following seven characters.

H6	Plant height (cm) at 6 weeks after planting in the field
H7	Plant height (cm) at 7 weeks after planting in the field
LL	Length (cm) of the largest leaf petiole recorded at the end of growing season
LW	Width (cm) of the largest leaf petiole recorded at the same time as LL
FT	Days taken (from 1st July) to the opening of the first flower on the plant
HFT	Plant height (cm) at the time of flowering
FH	Plant height (cm) at the end of the season.

Analysis and results

Tests of genetic variation and micro-environmental sensitivity

Tests of genetic variation were based on the separate analysis of variance (anova) of the $F_{\infty i}$ and the SCH_i families. Each anova had four items, viz., blocks, families, families × blocks and within families/within blocks. The results (Table 1) showed that both $F_{\infty i}$ and SCH_i differ significantly for all seven characters. The between-$F_{\infty i}$ mean square (ms) provides a direct test of the additive genetic variance ($2V_A^*$) while differences between crosses can be due to both additive and non-additive effects. In fact, the variance (σ_b^2) component of the SCH_i families is equal to $V_A^* + V_D^*$ when both additive and non-additive effects are present (see Kearsey and Pooni 1996 for definitions), and this and other components could be used to estimate the heritability of each trait as $1/2\sigma_b^2(F_{\infty i})/[\sigma_b^2(SCH_i) + \sigma^2_{within}(SCH_i)]$, also given in Table 1. The block and families × blocks interaction components were excluded from these calculations, even when they were significant among the SCH_i families for almost all the traits, because blocks and families × blocks represent primarily the reciprocal effects among the SCH_i while interaction is not significant among the $F_{\infty i}$ families (see next section).

The SCH_i and $F_{\infty i}$ families are not expected to show any segregation because the $F_{\infty i}$ families are inbred beyond the F_{30} generation. Thus, variation within each family can be treated as purely environmental (V_E) and in the absence of micro-environmental interactions these variances are expected to be homogeneous over all the families. Pooni, Jinks and Jayasekara (1978) have shown that Bartlett's test can be used to detect the

Table 1 Analysis of variance, heritability and test of genotype × micro-environment interaction for the various traits

Source	*df*	Characters						
		H6	H7	LL	LW	FT	HFT	FH
		$F_{\infty i}$ families						
Blocks	1	2400.05***	5029.06***	1.60 ns	11.40 ns	1911.32***	343.00 ns	225.78 ns
Families	59	1471.68***	3239.52***	116.51***	114.11***	1173.70***	3096.95***	4559.76***
Fams × blocks	59	20.67 ns	64.62 ns	5.75 ns	6.40 ns	21.40 ns	111.80 ns	44.92 ns
Within	560	48.90	84.73	6.81	7.35	29.95	100.45	138.57
		SCH_i families						
Blocks	1	19 890.75***	67 107.74***	35.07***	193.55***	14 148.19***	2890.03***	2965.11***
Families	434	1064.64***	2276.41***	60.17***	65.53***	552.05***	2338.38***	3097.89***
Fams × blocks	434	94.99***	185.73***	6.77 ns	9.68*	42.70***	160.83***	183.62*
Within	4225	61.78	117.35	6.47	8.36	16.73	108.75	152.62
		Heritability						
		0.45	0.48	0.46	0.37	0.85	0.46	0.50
		Genotype × micro environment interaction						
Variance ratio		1.26***	1.38***	1.05 ns	1.14*	1.79***	1.08 ns	1.10 ns

*** $P < 0.001$; ** $0.01 \geqslant P > 0.001$; * $0.05 \geqslant P > 0.01$; ns $P > 0.05$

heterogeneity of such variances and the significance of the test provides a direct test of the presence of micro-environmental interactions. In the present case, a comparison of the "between-plants within-families" components of the $F_{\infty i}$ and SCH_i families provided an efficient test of the relative stability of the two types of families and the variance ratios in Table 1 show that the within-variances of inbreds and hybrids differ significantly for H6 and H7, where the SCH have the larger V_E, and for LW and FT the F_∞ have the larger V_E. In general, all within-family variances show scalar effects and their magnitudes go up with a corresponding increase in the overall means. Thus, the direction and magnitude of differences between the $F_{\infty i}$ and SCH_i variances are largely dependent on the means of the two sets of families.

Non-allelic interaction, linkage and maternal effects

Both means and variances provide tests of epistasis and linkage. Using family means, a comparison of the mid-parental value ($1/2P_1$ mean + $1/2P_2$ mean) with the overall mean of the $F_{\infty i}$ families tests the presence of the additive × additive type of epistasis, [*aa*], while significance of the F_2 mean − $1/2(F_1$ mean + overall $F_{\infty i}$ mean) detects dominance × dominance interaction, [*dd*] (see Kearsey and Pooni 1996 for definitions of the epistatic components). Further, a comparison of the F_2 mean with the overall mean of the SCH_i families detects linkage between those genes which may be showing epistasis, while the variance ratio of the F_2 and the SCH_i variances detects linkage disequilibrium. Table 2 shows that [*aa*] is significant for three (LW, FT and FH), [*dd*] for two (HFT and FH), and linkage between epistatic genes for three (H6, LL and LW) characters, respectively.

Another pertinent test of epistasis and/or linkage is based on the Wr/Vr analysis of Jinks (1954). This analysis was applied to the means of individual families calculated as averages over the sibs and blocks. The resulting 435 SCH_i and 30 $F_{\infty i}$ means were used to obtain the array variances (Vr_i) and array co-variances (Wr_i) and these statistics were then subjected to regression analysis (see Jinks 1954 for details).

An alternative method of testing epistasis in a diallel set of second-cycle hybrids is the regression of array means (AM_i) on the parental ($F_{\infty i}$) means. Theoretical calculations revealed that the range of deviations between the array means is exactly half that of the corresponding $F_{\infty i}$ means when an additive/dominance model is adequate (see Kearsey and Pooni 1996 for symbols and definitions of the gene effects). It is further apparent from Table 3 that the co-variance of AM_i and $F_{\infty i}$ means is exactly half the variance of $F_{\infty i}$ means, but this relationship holds only when epistasis is absent. Thus the regression of AM_i on $F_{\infty i}$ will be significant and the regression coefficient (b) will be equal to 0.5 ($\pm$ SD) in the absence of epistasis.

Epistasis can also be tested among the L_{1i} and L_{2i} families of a triple test cross (Kearsey and Jinks 1968) and their non-recurrent $F_{\infty i}$ parents using the procedure of Jinks et al. (1969) (see their paper for definitions of L_{1i} and L_{2i} etc.). In the absence of epistasis, the magnitude of the $L_{1i} + L_{2i}$-$F_{\infty i}$ comparison is expected to remain constant across i = 1, *n* sets and consequently the variance of the *n* values (for *n*-1 *df*) is not expected to be significantly larger than the appropriate error ms. In the present case, however, we use the regression of $1/2(L_{1i} + L_{2i})$ on $F_{\infty i}$ to determine the presence of epistasis. The expectations in Table 4 show that b $[1/2(L_{1i} + L_{2i})/F_{\infty i}]$ will also be equal to 0.5 when an additive/dominance model is adequate, and is expected to deviate significantly from 0.5 when epistasis is present.

The 30 × 30 tables of means constructed for the Wr/Vr regressions also provided relevant data for conducting the above tests, and the results (Table 5) reveal that only one of three tests is significant for H6, LL and LW, while two detect epistasis for FT and FH.

The regression of $1/2(L_{1i} + L_{2i})$ on $F_{\infty i}$ assumes that the tester genotypes differ for all the genes for which the $F_{\infty i}$ families show segregation. While the failure of this assumption can affect the efficiency of the test, it does

Table 2 Tests of epistasis and linkage on the generation means

Test	Characters						
	H6	H7	LL	LW	FT	HFT	FH
$1/2(\bar{P}_1 + \bar{P}_2) - \bar{\bar{F}}_{\infty i}$	1.74 ns[a] ± 1.67	3.24 ns ± 2.41	− 0.20 ns ± 0.52	− 1.14** ± 0.51	4.72*** ± 1.52	0.12 ns ± 2.29	− 6.62** ± 2.92
$1/2(\bar{F}_1 + \bar{\bar{F}}_{\infty i}) - \bar{F}_2$	1.64 ns ± 1.29	2.84 ns ± 1.88	0.16 ns ± 0.36	0.30 ns ± 0.38	− 1.57 ns ± 0.96	− 5.70*** ± 1.80	− 5.63** ± 2.03
$\bar{F}_2$-$\bar{\bar{S}}\bar{\bar{C}}\bar{\bar{H}}_i$	− 2.23** ± 1.06	− 2.39 ns ± 1.55	− 1.26*** ± 0.29	− 1.46*** ± 0.30	1.03 ns ± 0.75	1.27 ns ± 1.50	− 0.82 ns ± 1.63

[a] See Table 1 for probability

Table 3 Genetic variance of the recombinant ($F_{\infty i}$) inbreds and co-variance between the array (AM) and $F_{\infty i}$ means for the four situations defined by the absence/presence of linkage and non-allelic interaction

	Category	Expectation
Genetic variance of the $F_{\infty i}$ means		
1	Linkage equilibrium and no epistasis	$a_A^2 + a_B^2$ [a]
2	Linkage disequilibrium and no epistasis	$a_A^2 + a_B^2 \quad \begin{matrix} +\,C\ \dfrac{2(1-2p)}{(1+2p)}\ a_A a_B{}^* \\ -\,R \end{matrix}$
3	Linkage equilibrium and epistasis	$a_A^2 + a_B^2 + aa_{AB}^2$
4	Linkage disequilibrium and epistasis	$a_A^2 + a_B^2 \quad \begin{matrix} +\,C\ \dfrac{2(1-2p)}{(1+2p)} a_A a_B + \dfrac{8p}{(1+2p)^2} aa_{AB}^2 \\ -\,R \end{matrix}$
Co-variance of the array means with the $F_{\infty i}$ means		
1	Linkage equilibrium and no epistasis	$1/2\,(a_A^2 + a_B^2)$
2	Linkage disequilibrium and no epistasis	$1/2(a_A^2 + a_B^2) \quad \begin{matrix} +\mathrm{C}\dfrac{(1-2p)}{(1+2p)} a_A a_B \\ -\mathrm{R} \end{matrix}$
3	Linkage equilibrium and epistasis	$1/2(a_A^2 + a_B^2) + 1/2(aa_{AB}^2 + a_A ad_{AB} + a_B ad_{BA})$
4	Linkage disequilibrium and epistasis	$1/2(a_A^2 + a_B^2) \quad \begin{matrix} +\,C\ \dfrac{(1-2p)}{(1+2p)} a_A a_B + \dfrac{2p}{(1+2p)^2}\,(a_A ad_{AB} + a_B ad_{BA}) \\ -\,R \end{matrix}$
		$+\ \dfrac{(2p)}{(1+2p)^2}\, aa_{AB}^2 + \dfrac{2p(1-2p)}{(1+2p)^3} aa_{AB} dd_{AB}$

[a] See text for symbols

Table 4 Covariance of the $1/2(\bar{L}_{1i} + \bar{L}_{2i})$ values with $F_{\infty i}$

	Category	Expectation
1	Linkage equilibrium and no epistasis	$1/2(a_A^2 + a_B^2)$ [a]
2	Linkage disequilibrium and no epistasis	$1/2(a_A^2 + a_B^2) \quad \begin{matrix} +\,C\ \dfrac{(1-2p)}{(1+2p)}\ a_A a_B \\ -\,R \end{matrix}$
3	Linkage equilibrium and epistasis	$1/2(a_A^2 + a_B^2) + 1/4\{aa_{AB}^2 + (a_A - a_B)(ad_{AB} - ad_{BA}) + aa_{AB} dd_{AB}\}$
4	Linkage disequilibrium and epistasis	$1/2(a_A^2 + a_B^2) \quad \begin{matrix} +\,C\ \dfrac{(1-2p)}{(1+2p)}\ a_A a_B \\ -\,R \end{matrix}$
		$+\ \dfrac{p}{(1+2p)}\,(a_A - a_B)(ad_{AB} - ad_{BA})$
		$+\ \dfrac{2p}{(1+2p)^2}\,(aa_{AB}^2 + aa_{AB} dd_{AB})$

[a] See text for symbols

not negate its outcome, although the test applies only to those genes for which the testers differ (Kearsey and Jinks 1968; Jinks et al. 1969). The validity of the Wr/Vr regression, on the other hand, depends on the existence of non-additive effects and we have tested their significance using Hayman's (1954) analysis of diallel tables.

In the present case, the families × blocks interaction is confounded with the reciprocal effects and, therefore, we do not have an independent test of the maternal effects. However, non-significance of the $F_{\infty i}$ families × blocks interaction ms in Table 1 indicates that families × blocks interactions are not important in the present experiment. The significance of item d, thus, suggests that reciprocal effects are present for at least four characters (H6, H7, FT and HFT). Consequently, we have used the error ms to test the significance of items a, b and c, and Table 6 shows that the additive and the non-additive effects, and the various sub-components of the latter, are highly significant for all the traits.

Table 5 Linear regressions of array co-variance (Wr) on array variance (Vr), array means (AM_i) on parental means ($\bar{F}_{\infty i}$), and test-cross means ($1/2(\bar{L}_{1i} + \bar{L}_{2i})$) on the $\bar{F}_{\infty i}$, and the significance of their difference from the expected values based on the adequacy of the additive-dominance model

Source	Characters						
	H6	H7	LL	LW	FT	HFT	FH
	Regression of Wr on Vr						
b	1.22***[a]	1.14***	0.90***	0.88***	0.89***	1.02***	1.05***
	± 0.08	± 0.11	± 0.07	± 0.11	± 0.04	± 0.09	± 0.08
1.0-b	**	ns	ns	ns	**	ns	ns
	Regression of AM_i *on* $\bar{F}_{\infty i}$						
b	0.45***[a]	0.44***	0.39***	0.38***	0.36***	0.46***	0.40***
	± 0.05	± 0.04	± 0.04	± 0.05	± 0.03	± 0.04	± 0.05
0.5-b	ns	ns	**	ns	***	ns	*
	Regression of $1/2(L_{1i} + L_{2i})$ *on* $\bar{F}_{\infty i}$						
b	0.54***	0.49***	0.48***	0.33***	0.45***	0.51***	0.38***
	± 0.04	± 0.06	± 0.05	± 0.06	± 0.03	± 0.05	± 0.06
0.5-b	ns	ns	ns	*	ns	ns	*

[a] See table 1 for probability

Table 6 Hayman's anova of the 30 $F_{\infty i}$ families and their 435 F_1 pairwise crosses (SCH_i)

Source	*df*	Mean squares						
		H6	H7	LL	LW	FT	HFT	FH
a[a]	29	2619.06#	5433.13#	135.98#	145.40#	1427.65#	5414.29#	6517.02#
b	435	24.40#	61.76#	2.36**	2.92#	10.80#	72.65#	159.43#
b1	1	1137.92#	4152.95#	196.36#	301.59#	455.41#	3906.03#	15228.58#
b2	29	45.46**	89.08#	2.90**	4.05**	31.58#	57.29**	151.72#
b3	405	20.14**	49.71**	1.84**	2.10**	8.21**	64.29#	122.78#
c	29	124.46#	360.19#	2.70**	4.97**	77.87#	104.61**	125.72#
d	406	17.44*	36.38**	1.08 ns	1.53 ns	8.32**	24.30**	28.62 ns
	(59)[b]	(8.72)	(15.13)	(1.22)	(1.31)	(5.36)	(17.94)	(24.77)
Error	4420	10.22	19.23	1.09	1.38	3.67	18.23	25.51

ns $P > 0.05$; * $0.05 \geqslant P > 0.01$; ** $0.01 \geqslant P > 0.001$; # $P \leqslant 0.001$
[a] See text for symbols
[b] ms for the $F_{\infty i}$ fams × blocks interaction (see text for further details)

Average dominance and the degree of gene dispersion

Direct estimates of average dominance were obtained from the test crosses of the most associated $F_{\infty i}$ families mentioned earlier. The additive (σ_s^2) and dominance (σ_d^2) components were estimated for each character from the sums $[1/2(L_{1i} + L_{2i})]$ and differences $[1/2(L_{1i} - L_{2i})]$ comparisons, and the average dominance was obtained as $\sqrt{(\sigma_d^2/\sigma_s^2)}$. Wr/Vr analysis, on the other hand, provided a qualitative measure of average dominance because the intercept of the regression equation takes a positive value when dominance is partial, is zero for complete dominance, and becomes negative when there is overdominance (see Jinks 1954). In addition, the following relationships could also be utilized for estimating the dominance ratio, where $(dev)_i$ represents the deviation of the SCH_i mean from the average score of its parental lines [$= 1/2(P_1$ mean + P_2 mean)]:

(1) (F_1 mean − m)/1/2(range of the $F_{\infty i}$ means)
(2) [Maximum range of $(dev)_i$]/(range of the $F_{\infty i}$ means).

Method (1) will provide a more realistic estimate of the average dominance ratio when the dominance effects of all the genes are unidirectional and the extreme $F_{\infty i}$ families show complete association for the + and − alleles at all the loci for which V2 and V12 differ for the trait. Method (2) will give a better estimate of average dominance when there is ambidirectional dominance, because the maximum range of $(dev)_i$ will provide a more reliable estimate of $\Sigma|d_i|$ and the dominance ratio will be calculated as $\Sigma|d_i|/\Sigma a_i$, and not as

$\Sigma d_i/\Sigma a_i$where Σd_i is subjected to internal cancellation. But, the latter estimate of dominance ratio will be more realistic when the additive and dominance effects are estimated from similar numbers of $F_{\infty i}$ and SCH_i families. As this is not true in the present case, i.e. there are many more SCH_i than $F_{\infty i}$, we have used the combined range of the $F_{\infty i}$ and SCH_i families as the denominator for estimating the dominance ratio by the latter method, assuming that the best SCH_i scores can also be obtained through inbreeding.

The ratio of the average difference between the V2 and V12 means and the combined range of the $F_{\infty i}$ and SCH_i families provided a conservative estimate of the coefficient of gene association, r_a, for the parental varieties and these estimates are given in Table 7 together with those of the dominance ratio obtained from the various methods described above.

The results in Table 7 show that various methods provide highly consistent estimates of average dominance. For example, estimates from the test crosses and the "range of $(dev)_i$/ range of the $F_{\infty i}$ means" are very close to each other and their values are always less than unity for each trait. Partial dominance is also confirmed by the Wr/Vr and (F_1 mean $- m$)/1/2(range of the $F_{\infty i}$ means) methods, although the last method is comparatively less reliable because the critical assumption of complete unidirectional dominance is rarely met in practice (see next section) and maternal effects may also influence the F_1 performance.

Gene dispersion is shown to be high in the V2 × V12 cross and V2 seems to contain from 19% to 83% of the + alleles for the various traits [calculated as $50(1 - r_a)$ from the r_a values in Table 7].

Test and measurement of ambidirectional dominance

Another facet of dominance which the present experiment allows us to investigate is the direction of the non-additive effects at the various loci, i.e. whether dominance is completely unidirectional or not. For example, when + alleles are consistently dominant, all significant $(dev)_i$ will have positive values and none would be significant and negative. Ambidirectional dominance, on the other hand, will make some crosses show negative dominance while others will display positive dominance. Further, both types of dominance will be detected and, from the largest positive and the largest negative $(dev)_i$ values, we can estimate the degree of dominance for the + and − alleles as:

(3) [The largest positive $(dev)_i$ value]/ (the highest $F_{\infty i}$ mean $- m$)
(4) [The largest negative $(dev)_i$ value]/ (the lowest $F_{\infty i}$ mean $- m$).

In addition, we can measure the extent of unidirectionality of dominance by counting the positive and negative $(dev)_i$ values and converting these counts into a scale-free coefficient, r_d, which is calculated as [number of positive $(dev)_i$] − [number of negative $(dev)_i$]/total. Coefficient r_d will be 1 or -1 when dominance is completely unidirectional for + or − alleles respectively, and $1 > r_d > -1$ will indicate ambidirectional dominance. Further, $r_d = 0$ when positive and negative dominance effects are equal in magnitude.

The present experiment also allows us to investigate the distribution of + and − alleles among the parents of those SCH_i which have the largest positive or the largest negative $(dev)_i$ values. This is achieved by comparing the parental means with m and with the range of the $F_{\infty i}$ means.

The results in Table 8 reveal that only positive dominance is significant for the leaf measurements (LL and LW) and final height (FH). Consequently, the coefficient of unidirectionality is almost 1 for these traits. For the remaining traits, ambidirectional dominance is important and a higher number of crosses show dominance towards the lower score, compared to LL, LW or FH. The lowest r_d value of -0.74 is observed for FT and its negative sign indicates that dominance is predominantly for the lower score, i.e. early flowering.

Perusal of the parental means reveals that the crosses showing the largest negative values of $(dev)_i$ involve much higher allele dispersion compared to those which have the largest positive $(dev)_i$. Furthermore, mid-parental values of the crosses with the largest negative

Table 7 Estimates of average dominance and degree of gene dispersion

Source	Characters						
	H6	H7	LL	LW	FT	HFT	FH
	Dominance ratio						
Test crosses	0.54	0.50	0.68	0.53	0.60	0.54	0.76
Wr/Vr Intercept	> 0	> 0	> 0	> 0	> 0	> 0	> 0
$(\bar{F}_1 - m)/1/2$(range of $\bar{F}_{\infty i}$)	0.48	0.60	0.70	0.82	− 0.28	0.56	0.83
[Range of $(dev)_i$]/(range of $\bar{F}_{\infty i}$ and $\overline{SCH_i}$)	0.61	0.63	0.46	0.56	0.61	0.57	0.52
	Degree of gene dispersion in the ancestral (V 2 × V12) cross						
$(\bar{P}_1 - \bar{P}_2)$/(range of $\bar{F}_{\infty i}$ and $\overline{SCH_i}$)	0.62	0.65	0.20	0.36	0.66	0.26	0.34

Table 8 Significance of the dominance deviations [$(dev)_i$] with the extreme positive or negative values, the degree of dominance of the plus and minus alleles, and the genotypic relationships between the parents of those SCH_i which display the extreme dominance deviations. See Table 1 for probability

Source	Characters						
	H6	H7	LL	LW	FT	HFT	FH
	Extreme values of $(dev)_i$						
Largest + ve $(dev)_i$	32.93***	49.92***	7.64***	9.15***	6.88***	38.60***	61.50***
Largest − ve $(dev)_i$	− 8.45**	− 11.35**	− 1.15 ns	− 0.87 ns	− 23.18***	− 9.80**	− 1.96 ns
	Degree of directional dominance						
(Largest + ve $(dev)_i$)/(highest $F_{\infty i} - m$)	0.81	0.88	0.66	0.87	0.79	0.81	0.87
(Largest − ve $(dev)_i$)/(lowest $\bar{F}_{\infty i} - m$)	− 0.25	− 0.28	− 0.15	− 0.11	− 1.19	− 0.27	− 0.04
	The extent of ambidirectional dominance						
Number (n1) of + ve $(dev)_i$	406	423	425	430	57	415	434
Number (n2) of − ve $(dev)_i$	29	12	10	5	378	20	1
Coefficient of unidirectionality r_d = (n1 − n2)/total	0.87	0.94	0.95	0.98	− 0.74	0.91	1.00
	Parental scores of the SCH_i *with extreme* $(dev)_i$ *values*						
Largest + ve $(dev)_i$ $\bar{P}_1$	34.17	56.33	30.50	27.58	75.29	122.73	165.10
$\bar{P}_2$	18.14	29.50	17.88	17.13	51.92	75.18	89.55
$(\bar{P}_1 - \bar{P}_2)$/(range of $\bar{F}_{\infty i}$)	0.31	0.31	0.86	0.70	0.48	0.61	0.74
Largest − ve $(dev)_i$ $\bar{P}_1$	50.73	84.45	30.42	28.17	44.58	133.27	132.50
$\bar{P}_2$	49.17	84.25	28.55	27.91	28.50	105.00	132.08
$(\bar{P}_1 - \bar{P}_2)$/(range of $\bar{F}_{\infty i}$)	0.03	0.00	0.07	0.00	0.33	0.36	0.00
$\bar{F}_{\infty i}$ $(= m)$	40.74	66.74	25.46	22.14	45.70	100.49	136.51

Table 9 Comparative statistics of the F_2 generation and second-cycle hybrids together with the predicted and observed transgressions for the various traits. The % difference is a proportion of the F_2 statistic

Source	Characters						
	H6	H7	LL	LW	FT	HFT	FH
	Mean						
F_2	45.40	76.51	27.86	25.56	43.61	116.54	162.70
SCH_i	47.56	78.85	29.12	26.35	42.54	115.14	163.39
% Difference	+ 5	+ 3	+ 4	+ 3	− 2	− 1	0
	Genotypic SD						
F_2	8.85	13.50	2.28	2.48	6.21	15.54	14.49
SCH_i	9.39	14.10	2.32	2.21	7.05	14.26	16.69
% Difference	+ 6	+ 4	+ 2	− 12	+ 14	− 8	+ 15
	Phenotypic SD						
F_2	12.14	17.82	3.39	3.83	8.53	16.95	18.19
SCH_i	12.17	17.41	3.32	3.64	8.20	17.31	20.04
% Difference	0	− 2	− 2	− 5	− 4	+ 2	+ 10
	Percent transgression $(\overline{SCH_i} \geqslant \bar{F}_1)$						
F_2 predictions	19	15	11	0.0	23	37	14
SCH_i observations	22	11	19	0.0	21	27	8

$(dev)_i$ are generally larger than m but the reverse is not true for the SCH_i with the largest positive $(dev)_i$ (Table 8).

Comparison of the F_2 and the SCH_i generations

Toledo, et al. (1984 a) showed that in theory the distribution of the SCH_i families will be identical to that of the F_2 generation in all circumstances except when there are strong linkages and the number of segregating loci is small. The present experiment has large enough samples from both generations for valid comparisons to be made between the generations and their various statistics are given in Table 9. The two distributions are very similar and the small differences in mean and standard deviation fall well within the margins of

Table 10 Phenotypic diversity, transgression and the summary statistics of the $F_{\infty i}$ and SCH_i families. The parental data is given for comparison. See Table 1 for probability

Source	H6	H7	LL	LW	FT	HFT	FH
	Parental and F_1 means						
V2	21.49	38.36	23.36	17.61	66.59	89.53	109.29
V12	63.40	101.60	27.15	24.13	34.25	111.68	150.48
F_1	53.58	91.94	30.58	28.38	38.36	121.15	177.58
	Variance of the $F_{\infty i}$ and SCH_i means						
$F_{\infty i}$	134.74	294.09	10.64	10.42	112.69	287.43	433.39
SCH_i	92.85	195.71	5.11	5.60	47.69	200.77	264.20
	Skewness of the $F_{\infty i}$ and SCH_i means						
$F_{\infty i}$	− 0.09	− 0.02	− 0.19	0.08	0.48	0.18	− 0.20
SCH_i	0.09	0.03	0.02	− 0.34**	0.27*	0.18	− 0.16
	Kurtosis of the $F_{\infty i}$ and SCH_i means						
$F_{\infty i}$	− 0.82*	− 0.22	− 0.51	− 0.31	− 0.24	0.22	0.06
SCH_i	− 0.24	− 0.20	− 0.21	− 0.30	− 0.47*	− 0.58**	− 0.23
	Minimum score among the $F_{\infty i}$ and SCH_i family means						
$F_{\infty i}$	13.40	26.90	17.40	14.00	27.17	61.11	81.22
SCH_i	23.40	43.90	22.75	19.66	26.50	83.25	115.91
	Maximum score among the $F_{\infty i}$ and SCH_i family means						
$F_{\infty i}$	65.92	110.33	32.55	28.17	75.29	144.36	183.00
SCH_i	74.17	117.92	36.20	31.58	62.28	151.00	199.78
	Range of the $F_{\infty i}$ and SCH_i family means						
$F_{\infty i}$	52.52	83.43	15.15	14.17	48.12	83.25	101.78
SCH_i	50.77	74.02	13.45	11.92	35.78	68.33	88.83
	Transgression from P_2 (family mean $\leqslant P_2$ mean)						
$F_{\infty i}$ (out of 60)	4	3	16	4	8	16	6
SCH_i (out of 435)	0	0	1	0	56	8	0
	Transgression from P_1 (family mean $\geqslant P_1$ mean)						
$F_{\infty i}$ (out of 60)	1	2	20	17	1	13	15
SCH_i (out of 435)	25	23	353	360	0	244	337
	Transgression from F_1 (family mean $\geqslant F_1$ mean)						
$F_{\infty i}$ (out of 60)	8	3	1	0	42	8	2
SCH_i (out of 435)	123	71	121	98	311	146	85

sampling error. The transgression rates predicted from the F_2 population are very close to the observed performance of the SCH_i families for all the traits.

Transgression and phenotypic diversity among the $F_{\infty i}$ and SCH_i means

Finally, the comparative data of the SCH_i and $F_{\infty i}$ families, presented in Table 10, show that the range covered by the SCH_i means is consistently smaller than that of the $F_{\infty i}$ means, even when the sample size (435) favours the SCH_i by a margin of 7.25:1. The same relationship is also observed among the variances of family means. However, the SCH_is are more vigorous and early flowering, and therefore show markedly more transgression for increased growth rate, height and leaf size, and early flowering. Furthermore, the distributions of the $F_{\infty i}$ and SCH_i are significantly kurtosed for one (H6) and two (LW, FT) characters, respectively, and skewness is detected among the SCH_i for FT and HFT.

Discussion and conclusions

The present study reveals the abundance of genetic variation in the V2 × V12 cross for all the traits studied. The high values of narrow heritability, h^2_n, for all the traits confirm what has already been observed on many occasions, i.e. *N. rustica* as a species, and the V2 × V12 cross in particular, show high levels of genetic variation for all the metrical traits that have been investigated so far (Virk 1976; Pooni et al. 1978). The inheritance of various traits is also very complex. In addition to the additive genetic and dominance variation, segregating genes display maternal effects for several traits, and linkage disequilibrium is significant for H6, LL and LW. That epistasis is significant, but less important

than dominance or maternal effects, is clear from its less frequent detection for all the traits and its complete absence for H7. Micro-environmental sensitivity, on the other hand, seems to be much less important than was reported earlier and the scalar effects are equally important in inbreds as well as the second-cycle hybrids (Pooni et al. 1978).

Although epistasis is present in six characters its nature could only be determined for those traits where significant dominance × dominance ([*dd*]) interaction is detected. Opposite signs of [*dd*] and (*d*) components for both HFT and FH suggest that genes controlling these traits display predominantly duplicate epistasis. Component [*aa*] also shows the same relationship with [*d*] for LW, FT and FH, but its sign can not be relied upon for determining the nature of epistasis. Nevertheless, various tests provide convincing evidence that all three components, viz., [*aa*], [*ad*] and [*dd*], are contributing to the epistatic variation in the V2 × V12 cross.

Dominance is also an important source of genetic variation in all the traits, but average dominance is invariably partial and all estimates of dominance ratio are less than one. The presence of heterosis in the ancestral cross is therefore more likely to be caused by allele dispersion rather than by over-dominance. In fact, r_a is always much smaller than unity (maximum $r_a = 0.66$), indicating clearly that + and − alleles are highly dispersed in the V2 × V12 cross.

The above estimates of dominance ratio, however, do not preclude the presence of over-dominance at some loci, particularly for those traits for which dominance ratio is high and heterosis is significant (LL, LW and FH, see Tables 7 and 10). A reduced range of the $(dev)_i$ compared to the $F_{\infty i}$ means (Table 10), on the other hand, indicates strongly that such loci are not likely to be many and hardly any of the SCH_i, therefore, will be unconditionally superior to the second-cycle inbreds which can be extracted from the best of the second-cycle hybrids (see Kearsey and Pooni 1996).

The present study further reveals that dominance is significantly ambidirectional for all the heights except FH. However, even for these traits there is a high degree of unidirectional dominance and the largest positive $(dev)_i$ is approximately four-times as large as the largest negative $(dev)_i$. For flowering time the opposite is of course true because early flowering is dominant to late flowering at the majority of the loci which control flowering. A lower level of ambidirectionality is also indicated by the comparatively small number of SCH_i which show dominance in the opposite direction to the predominant phase of the dominance effects (Table 9).

Toledo et al. (1984 a, 1985) postulated that the genotypic and phenotypic distributions of the F_2 and SCH_i should be identical in most situations and the present study lends overwhelming support to this postulate. The overall means and phenotypic standard deviations of the F_2 and the SCH_i generations are indeed very similar and only small differences exist between them. On the other hand, improved vigour and reduced diversity which increase positive transgression among the SCH_i compared to the $F_{\infty i}$, particularly towards increased height, large leaf size and late flowering, also point out clearly that the best RILs are yet to be produced from the V2 × V12 cross for most of the traits under study.

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